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Trichloroethylene Issue Paper 1:

Issues in Trichloroethylene Pharmacokinetics

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LIST OF ABBREVIATIONS AND ACRONYMS

ACSL	Advanced Continuous Simulation Language
AUC	Area under the concentration curve
BL	Cysteine conjugate β-lyase
CDH	Chloral dehydrogenase (aldehyde oxidase)
CGDP	Cysteinyl-glycine dipeptidase
CH	Chloral hydrate
CHL	Chloral
CYP	Cytochrome P450
DCA	Dichloroacetic acid
DCVC	Dichlorovinylcysteine
DCVCS	DCVC sulfoxide
DCVG	Dichlorovinyl glutathione
DCVSH	Dichlorovinyl mercaptan
EHR	Enterohepatic recirculation
ERDEM	Exposure Related Dose Estimating Model
EPA	U.S. Environmental Protection Agency
FA	Formic acid
FMO	Flavin-containing monooxygenase
GA	Glyoxylic acid
GC/MS	Gas chromatography/mass spectroscopy
GGTP	γ-glutamyl transpeptidase
GSH	Glutathione
GST	Glutathione-S transferase
iv	Intravenous
K_M	Michaelis-Menten affinity parameter
MCA	Monochloroacetic acid
MFO	Mixed-function oxidase (P450)
MOA	Mode of action
NADCVC	N-acetyl dichlorovinylcysteine
NAS	National Academy of Sciences
NAT	N-acetyl transferase
NCEA	National Center for Environmental Assessment
NERL	National Exposure Research Laboratory

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

NRC	National Research Council
OA	Oxalic acid
ORD	Office of Research and Development
PBPK	Physiologically based pharmacokinetic
PBN	Phenyl-tert-butyl nitroxide
PD	Pharmacodynamic
RfD	Reference dose
TCA	Trichloroacetic acid
TCE	Trichloroethylene
TCE-O-P450	Oxygenated TCE-cytochrome P450 transition state complex
TCOG	TCOH glucuronide
TCOH	Trichloroethanol
UDP	Uridine diphosphate
UGT	UDP glucuronosyl transferase
V_{max}	Michaelis-Menten maximum velocity of reaction parameter

PREFACE

Publication of these issue papers is a part of EPA's effort to develop a trichloroethylene (TCE) human health risk assessment. These issue papers were developed to provide scientific and technical information to the National Academy of Sciences (NAS) for use in developing their advice on how to best address the important scientific issues surrounding TCE health risks. As such, these papers discuss a wide range of perspectives and scientific information (current through Fall 2004) on some of these important issues, highlighting areas of continuing uncertainty and data that may be relevant. They are intended to be useful characterizations of the issues, not a presentation of EPA conclusions on these issues. The papers have undergone internal review within EPA, but they have not been externally reviewed. The concepts presented in these papers will eventually be addressed in EPA's revised risk assessment of TCE, after the advice from the NAS, along with comments from the EPA Science Advisory Board and the public, as well as recently published scientific literature, have been incorporated.

AUTHORS AND CONTRIBUTORS

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THE TCE ISSUE PAPERS

BACKGROUND

In August 2001, a draft, *Trichloroethylene (TCE) Health Risk Assessment: Synthesis and Characterization,* was released for external review. This draft assessment drew on 16 "state-of-the-science" papers published as a supplemental issue of *Environmental Health Perspectives* (Volume 108, Supplement 2, May 2000). Subsequent to its release, EPA's 2001 draft assessment underwent a peer review by a panel of independent scientists through EPA's Science Advisory Board (SAB), which provided a peer review report in December 2002. In addition, the public submitted more than 800 pages of comments to EPA during a 120-day public comment period.

There are a number of important issues that EPA will need to examine as it moves forward in revising the draft TCE assessment. These include issues raised not only in the SAB peer review and public comments, but also by new scientific literature published since the release of the state-of-the-science papers and EPA's 2001 draft assessment. Some of this research is specific to the study of TCE or its metabolites while some of it describes advances in scientific fields more generally but which have potential relevance to characterizing the human health risks from TCE.

In February 2004, EPA held a symposium so that authors of some of the TCE-specific research that had been published since the release of the draft assessment could present their findings in more detail. This symposium represented only a limited cross section of recently published research, but was reflective of the breadth of new relevant science that EPA will consider in revising the assessment (the presentation slides and a transcript of the meeting are available separately on EPA's website and have already been sent to the NAS).

In 2004, EPA, in cooperation with a number of other federal agencies, initiated a consultation with the National Academy of Sciences (NAS) to provide advice on scientific issues related to the health risk assessment of TCE. It was recognized that a review by an NAS panel of the important scientific issues would be beneficial and informative to clarify the state-of-the-science as EPA moves forward in completing its health risk assessment. A charge was developed for the NAS through an Interagency Workgroup led by the White House Office of Science and Technology Policy.

PURPOSE OF THE TCE ISSUE PAPERS

Although EPA will need to address all of the issues identified in the charge to the NAS panel in updating its assessment, EPA would like to focus the NAS panel's attention on a subset of issues that EPA believes to be most critical in developing a revised risk assessment, as summarized in four issue papers developed by EPA staff:

1. Issues in trichloroethylene pharmacokinetics;
2. Interactions of trichloroethylene, its metabolites, and other chemical exposures;
3. Role of peroxisome proliferator-activated receptor agonism and cell signaling in trichloroethylene toxicity; and
4. Issues in trichloroethylene cancer epidemiology.

Each paper provides an overview of the science issues, a discussion of perspectives on those issues (including the SAB and public comments), and an outline of some of the recently published scientific literature. The pharmacokinetics issue paper also summarizes results from a recent collaboration with the U.S. Air Force on TCE pharmacokinetics, as well as EPA's planned approach for further refinement of the pharmacokinetic modeling of TCE and its metabolites. These scientific areas were selected because they are (a) critical to the hazard and/or dose-response characterization of TCE; (b) scientifically complex and/or controversial; and (c) areas in which substantial important scientific literature has been recently published. The input from the NAS on the topics described in the issue papers, as well as other topics put forth in the charge to the NAS, should help to strengthen EPA's revised TCE assessment.

NEXT STEPS

The advice from the NAS, along with comments already received from the EPA SAB and the public, as well as recently published scientific literature, will be incorporated into a revised EPA risk assessment of TCE, strengthening its scientific basis. Because of the substantial amount of new information and analysis that is expected, the revised draft of the assessment will undergo further peer review and public comment prior to completion.

1. INTRODUCTION AND PURPOSE

Understanding trichloroethylene (TCE) pharmacokinetics—absorption, distribution, metabolism, and elimination—is critical to both the qualitative and quantitative assessment of human health risks from environmental exposures. On a qualitative level, pharmacokinetic information can help to identify the chemical species that may be causally associated with observed toxic responses. In addition, the delineation of inter- and intraspecies differences can provide insights into how laboratory animal and epidemiological data may inform overall human health risks and into how individuals may differ in their susceptibility. Quantitatively, this information may allow the development of physiologically based pharmacokinetic (PBPK) models to describe the relationship between external measures of exposure and internal measures of toxicologically relevant dose. However, it should be noted that the selection of appropriate dose metrics depends not only on the reliability of PBPK data and models but also on the understanding of the mode of action (MOA) for a particular toxic effect (issues regarding the interactions of metabolites are discussed in a separate issue paper). With an adequate database, testing, and evaluation, quantitative results from PBPK models may then be used along with MOA information to develop appropriate alternatives to default procedures for addressing the pharmacokinetic component of a number of risk assessment issues, including extrapolation to different exposure conditions (e.g., exposure routes, time-concentration patterns, co-exposures); extrapolation across species (e.g., developing human equivalent doses or concentrations); dose-response relationships (e.g., low-dose extrapolation, use in pharmacodynamic modeling); uncertainty (e.g., due to pharmacokinetic complexity and/or data limitations); and variability (e.g., differences in metabolism or clearance).

This document is intended to provide an overview of TCE metabolism and PBPK modeling to focus the National Research Council (NRC) committee's advice on specific scientific issues and approaches to addressing TCE pharmacokinetics for the purposes of risk assessment. This document, in discussing issues in TCE pharmacokinetics, refers to both past work on TCE pharmacokinetics and to some of the recent studies that may be relevant for risk assessment, but it is not intended to provide a complete survey and synthesis of the scientific literature. Sections 2 through 4 summarize the issues and uncertainties surrounding TCE metabolism and PBPK models with particular attention to information from recent studies and modeling efforts. Section 5 describes the U.S. Environmental Protection Agency's (EPA's) plans for continued development of a TCE PBPK model for use in a revised TCE risk assessment. Input from the National Academy of Sciences (NAS) regarding interpretation of the information presented in the following sections, the availability of additional data sources, and

possible improvements to EPA's planned approach to PBPK modeling would help strengthen the basis of EPA's revised TCE assessment.

2. TCE METABOLISM

Lash et al. (2000a) presented a comprehensive review of the absorption, distribution, metabolism, and elimination of TCE and its metabolites as part of the State of the Science series on TCE published in 2000, and a brief summary was presented in EPA's 2001 draft risk assessment (U.S. EPA, 2001). TCE is rapidly and extensively absorbed by all routes of environmental exposure—ingestion, inhalation, and dermal contact. Once absorbed, TCE distributes via the circulatory system throughout the body. Because it is lipophilic, it can accumulate in fat and other tissues, although the half-life of TCE in fatty tissues (on the order of hours or days) is still much shorter than more persistent substances such as dioxins (on the order of years). The majority of TCE taken into the body is metabolized; direct exhalation is the other major route of elimination of the parent (Lash et al., 2000a). There are a number of complexities regarding the full spectrum of metabolic pathways. An understanding of the formation of TCE metabolites and the pharmacokinetics of each of them is motivated by the potential toxicological significance of many of these metabolites. In particular, for many endpoints, the toxicity of TCE is hypothesized to be attributable to one or more of these metabolites. (The toxicology of TCE metabolites and their interactions is discussed in more detail in a separate issue paper.) Figure 2-1 presents a postulated scheme for the pathways of TCE metabolism, adapted from the work of Lash et al. (2000a) and Clewell et al. (2000). As shown in the figure, TCE metabolism occurs through two main pathways—oxidation via the microsomal mixed-function oxidase (MFO) system (i.e., P450s) and conjugation with glutathione (GSH) by glutathione-S transferases (GSTs). Several important issues related to these metabolic pathways are discussed below. Particular attention is given to recently published literature that may be informative. Input from NAS regarding the interpretation of this new information and its potential utility for quantitative analysis would be helpful to EPA as it revises its draft assessment. Of particular importance across all the issues described below is whether sufficient information exists both within and across species to quantify rates of TCE metabolism as well as the factors that may influence differential flux through the various metabolic pathways. These issues are critical to PBPK model development and use because they inform the formation and relative concentrations of metabolites in potential target organs. Issues more directly related to pharmacokinetic modeling are discussed in Sections 3 and 4.

2.1. DCA FORMATION AND THE ROLE OF TCE OXIDE (EPOXIDE)

As noted in the Lash et al. (2000a) review, considerable uncertainty exists as to the extent of DCA formation from TCE exposure. Two potential sources of dichloroacetic acid (DCA) formation, from trichloroethanol (TCOH) and from dechlorination of trichloroacetic acid (TCA), were discussed in Lash et al. (2000a). As reviewed in Bull (2000), DCA is one of the TCE metabolites that has been hypothesized to be involved in liver tumor induction. Although some researchers have suggested that DCA levels are too low in mice and humans after TCE exposure to play a significant role in TCE toxicity (e.g., Barton et al., 1999), it has also been noted that DCA-induced toxicity has been observed at exposure levels for which DCA cannot be detected in vivo (Bull et al., 2002). Pharmacokinetic issues related to DCA formation are discussed below; recent toxicological information regarding the potential role of DCA in TCE toxicity is discussed in a separate issue paper.

Detection of DCA production in vivo after TCE administration has been complicated by reported problems with analytical methodologies that have led to artifactual formation of DCA ex vivo when samples contain significant amounts of TCA (Ketcha et al., 1996). Following the discovery of these analytical issues, Merdink et al. (1998) re-evaluated the formation of DCA from TCE, TCOH, and TCA in mice, with particular focus on the hypothesis that DCA is formed from dechlorination of TCA. They were unable to detect blood DCA in naive mice following administration of TCE, TCOH, or TCA, and, even with pretreatment with DCA to reduce clearance rates, they were unable to detect DCA following TCA administration. They concluded that "[a]lthough there is significant uncertainty in the amount of DCA that could be generated from TRI [TCE] or its metabolites, our experimental data and pharmacokinetic model simulations suggest that DCA is likely formed as a short-lived intermediate metabolite." However, it has been noted that when directly administered, DCA can produce significantly elevated liver tumor incidence in mice at doses for which DCA blood levels remain below analytical detection limits owing to DCA's rapid metabolism (Bull et al., 2002; Kato-Weinstein et al., 1998; Merdink et al., 1998). Fisher et al. (1998) reported the results of a controlled human exposure study in which DCA was detected in some, but not all, human blood samples. To minimize ex vivo formation of DCA resulting in chemical artifacts, the investigators analyzed plasma rather than whole blood. However, it is still difficult to determine whether the observed inter-individual differences are due to intrinsic variability (e.g., differences in DCA degradation via GST-zeta), measurement errors, or a combination of each.

Much of the focus on DCA formation following TCE administration has been on dechlorination of TCA. For instance, Merdink et al. (2000) report trapping of a DCA radical with the spin-trapping agent phenyl-tert-butyl nitroxide (PBN), identified by gas chromatography/mass spectroscopy (GC/MS), in both a chemical Fenton system and rodent

microsomal incubations with TCA as substrate. On the other hand, the work by Guengerich and colleagues has suggested that the source of DCA may be through a TCE oxide (epoxide) intermediary. Although oxidation of TCE by P450s results predominantly in chloral (CHL) (Lash et al., 2000a), previous work of Miller and Guengerich (1983) had reported evidence of formation of the epoxide as an independent oxidative pathway (i.e., not leading to formation of CHL). The epoxide itself may be of toxicological importance, either by itself through formation of protein or DNA adducts or through subsequently formed reactive species (Guengerich, 2004). In addition, Cai and Guengerich (1999) recently reported that a significant amount of DCA (about 35%) is formed from aqueous decomposition of TCE oxide via hydrolysis in an almost pH-independent manner. Because this reaction forming DCA is a chemical process rather than mediated by enzymes, and because evidence suggests that some epoxide was formed from TCE oxidation, Guengerich (2004) noted that DCA would be an expected product of TCE oxidation.

2.2. PATHWAYS OF GSH CONJUGATION AND SUBSEQUENT METABOLISM

As discussed in Lash et al. (2000b), TCE metabolism through the GSH pathway is hypothesized to be involved in renal toxicity, but processing of GSH conjugates is complex and poorly understood relative to the processing of oxidative metabolites. The first stable product of the conjugation of TCE is S-(1,2 dichlorovinyl)glutathione (DCVG). A postulated scheme for subsequent processing to dichlorovinylcysteine (DCVC), corresponding mercapturates (N-acetyl-DCVC), and other compounds, is shown in Figure 2-1. Evidence for the in vivo activity of the GSH pathway in humans comes from Lash et al. (1999a), who reported detection of DCVG in the blood of human volunteers exposed to TCE. However, DCVC was not detected in blood, and the mercapturates were detected only sporadically in urine. Bloemen et al. (2001) measured GSH pathway metabolites in the urine of human volunteers and occupationally exposed workers and reported that levels were below detection limits in all cases.

DCVC is thought to be a critical intermediate in the fate of GSH conjugates of TCE. Although one potential fate of DCVC is detoxification via N-acetylation to yield mercapturates, bioactivation to a toxic form is a potential parallel pathway. Thus, data on detoxification (e.g., urinary mercapturates) do not capture the total flux through the GSH pathway, and, in particular, the data are not informative regarding the amount bioactivated (Lash et al., 2000a). It has been hypothesized that bioactivation is through the renal beta-lyase metabolism of DCVC, producing reactive metabolites that may contribute to renal toxicity. Recent in vitro data (Krause et al., 2003; Lash et al., 2003) indicate that flavin-containing monooxygenases (FMO) also may be toxicologically important for the bioactivation of DCVC, particularly in the human kidney. Moreover, there are multiple ways in which DCVC may become available in the kidney for bioactivation. GSH conjugates produced in the liver may be exported directly to the blood into

systemic circulation, or to the bile, where they can be reabsorbed through the gut. Although the liver is the primary site of GSH conjugation, most tissues, including the kidney, contain GSTs (Lash et al., 2000a). In vitro studies (Cummings et al., 2000a, b; Cummings and Lash, 2000) have reported GSH conjugation of TCE in rat and human kidney cells, suggesting a potential role for local metabolism. This work has also identified several GST isoforms in kidney cells that may be involved in TCE metabolism.

Although the work cited above may help lead to a better understanding of complex pathways and the metabolism that results from TCE exposure, it appears to be limited for developing a firm quantitative understanding of the relative rates of in vivo processing and the bioactivation of conjugative metabolites.

2.3. EXTRAHEPATIC METABOLISM

Although it is generally thought that the liver is the major site of TCE metabolism, P450s, GSTs, and other metabolizing enzymes are distributed at varying levels of activity throughout other tissues (Lash et al., 2000a). Although extrahepatic metabolism may not contribute significantly to overall mass balance (Lash et al., 2000a), it may be important locally in terms of the toxicological effects from in situ production of metabolites. Two sites of potential importance are the lung and the male reproductive system (metabolism in the kidney was discussed above).

2.3.1. Oxidative Metabolism in the Lung

As discussed in Green (2000), the oxidative pathway of TCE metabolism in mouse lung Clara cells is hypothesized to be responsible for the accumulation of CHL in mouse lungs, leading to cytotoxicity. Forkert and colleagues had previously reported cytotoxicity in mouse lung Clara cells from TCE exposure (Forkert and Forkert, 1994; Forkert and Birch, 1989; Forkert et al., 1985). Green (2000) suggested that although the activity of enzymes is lower in the lung as a whole than in the liver, the activity of P450 in the lung appears to be *relatively* higher than the activity of enzymes involved in clearing CHL and TCOH (believed to be alcohol dehydrogenase and uridine diphosphate (UDP)-glucuronosyl transferase [UGT]). Hence, these two metabolites may accumulate in the mouse lung and lead to toxicity. Green (2000) suggests that such a mechanism in mice may not be relevant to humans because there is little CYP2E1 activity in the human lungs as a whole. In the draft TCE assessment, it was noted that metabolic activity from whole lungs may give misleading results because of the variety of cell types in which high activity in a few may be diluted by others with low activity. Boers et al. (1999) reported the number of Clara cells in the human lung and indicated that Clara cells both contribute substantially to cell renewal and are important in the development of lung

adenocarcinoma in humans. In addition, the differential activities of the relevant enzymes in human lung tissues and cell types have not been examined to date.

2.3.2. Metabolism in the Male Reproductive System

Reports of TCE exposure affecting the male reproductive system (reviewed in the 2001 draft TCE assessment), including the observation of Leydig cell tumors in rats exposed to TCE (Maltoni et al., 1988, 1986), have led to the investigation of metabolism and toxicity of TCE and its metabolites in the male reproductive system. Forkert et al. (2003, 2002) report several studies that indicate TCE oxidative metabolism occurs in the male reproductive tract. They detected CYP2E1 activity in the epididymal epithelium and testicular Leydig cells in mice, monkeys, and humans. Analysis of seminal fluid from eight human subjects diagnosed with clinical infertility and exposed to TCE occupationally was also performed and showed the presence of TCE, CHL, and TCOH in all eight subjects; DCA in two subjects; and TCA in one subject. TCA and/or TCOH were identified in urine samples from only two subjects. Although the lack of detailed exposure information limits the use of these data for development of a quantitative pharmacokinetic understanding, this evidence is qualitatively informative regarding the potential for local metabolism of TCE in the male reproductive tract.

3. EXISTING TCE PBPK MODELS

Multiple PBPK models published for TCE and its metabolites show varying levels of detail and data consistency. The focus of most of these models has been on the oxidative pathway and the major oxidative metabolites TCA, TCOH, and TCOH glucuronide (TCOG), reflecting the limited quantitative understanding described above for the other metabolic pathways. Section 3.1 briefly describes the models previously used in developing EPA's 2001 draft risk assessment. The draft assessment noted a substantial amount of model uncertainty because the models sometimes provided disparate internal dose predictions, differing in some cases by an order of magnitude. Section 3.2 describes recent efforts sponsored by EPA and the U.S. Air Force to develop a revised interim TCE PBPK model.

3.1. PUBLISHED TCE PBPK MODELS USED IN EPA'S 2001 DRAFT RISK ASSESSMENT

The PBPK models used in EPA's 2001 draft risk assessment were described in detail elsewhere (Fisher, 2000; Clewell et al., 2000; Bois, 2000a, b, and references therein) and are briefly summarized below.

3.1.1. Models Had a Common Basis

All the models described below are extensions of the earlier published models of Fisher et al. (1991) for rats and mice and Allen and Fisher (1993) for humans. The structures of these "first generation" models were similar to that for styrene, reported by Ramsey and Andersen (1984), which has been used as the basis for a number of PBPK models for volatile organic solvents. Common characteristics of these models include the following:

- Physiological compartments included the liver and fat, lumped rapidly, and slowly perfused tissues, where transport in and out is perfusion limited with rapid equilibrium partitioning between the tissues and the venous blood leaving the tissues.
- Gas exchange in the lung occurring via rapid equilibrium partitioning between alveolar air and arterial blood.
- Oxidative metabolism, modeled as a saturated (Michaelis-Menten) process, occurring in the liver, with the metabolite TCA lumped into an equivalent volume of distribution.

The models described below built on this early work to include additional physiological compartments and a more detailed description of metabolism.

3.1.2. "Second Generation" Fisher Models

3.1.2.1. *Updated Mouse PBPK Model*

Abbas and Fisher (1997) and Greenberg et al. (1999) developed updated PBPK models for TCE and its metabolites in mice for both oral (corn oil gavage) and inhalation exposure, respectively. The number of parent compartments was expanded with the addition of a lung, kidney, and gut compartment. Additional metabolites included in the model were CHL/chloral hydrate (CH), TCA, TCOH, TCOG, and dichloroacetic acid (DCA). Physiological submodels for each metabolite, with liver, kidney, lung, and a lumped body compartment, were linked to the parent model and to each other through liver metabolism. Physiological parameters were taken or derived from the literature; chemical-specific parameters were measured experimentally or inferred from the experimental data. New time-course data, which included extensive metabolite measurements in multiple tissues, were used for calibration and/or validation. For the inhalation experiments, previously collected time-courses (Fisher et al., 1991) were also used for validation purposes. Fisher (2000), in reviewing these efforts, noted several unresolved discrepancies in model parameters, including the following:

- In the inhalation experiments, a fractional uptake of 53% in the lung was hypothesized that significantly improved the model fit to the data.

- Several metabolic rate constants derived from model calibration differed significantly between oral and inhalation exposures.

3.1.2.2. *Updated Human PBPK Model*

Fisher et al. (1998) developed an updated human PBPK model for TCE and its metabolites. The model structure was similar to the mouse model, with an expanded physiological model for TCE and physiological submodels for metabolites. However, the metabolic scheme included only TCA, TCOH, and TCOG (Figure 3-1) because those were the only metabolites consistently and reliably detected in experimental data. New time-course data were collected on human subjects exposed to 50 ppm and 100 ppm of TCE in air. Data included individual measurements of parent and metabolite concentrations in exhaled air, blood, and urine as well as covariates, such as age, body weight, and percentage of body fat. Physiological parameters other than those measured were taken or derived from the literature; chemical-specific parameters were measured experimentally or inferred through calibration to the experimental data. Urinary excretion parameters were optimized on a subject-specific basis owing to high observed variability. The observed variability and the sparseness of the data set (i.e., limited measurements relative to the model complexity) required that all the data be used for calibration, and traditional validation was not performed. Additional comparisons were performed against previously published data from Monster et al. (1976) and Muller et al. (1974), but these required adjustment of metabolic parameters to obtain adequate fits. Overall, there was some overprediction of the exhaled vapor concentration after cessation of exposure and some underprediction of TCE blood concentrations at 50 ppm.

3.1.3. Clewell Model

Clewell et al. (2000) reported on the development of an updated PBPK model for TCE in mice, rats, and humans. The same model structure was used for all three species. The primary extension of the model from the original Fisher et al. (1991) and Allen and Fisher (1993) models was the inclusion of additional metabolites. Like the updated Fisher models, TCOH, TCOG, and DCA were described. However, at the time the model was developed (circa 1996), the more recent data from Fisher's laboratory (Greenberg et al., 1999; Fisher et al., 1998; Abbas and Fisher, 1997) were not yet available. Clewell et al. (2000) also included CHL in the tracheobronchial region and DCVC production in the liver with bioactivation in the kidney. Unlike the updated Fisher models, all circulating metabolites were modeled using one-compartment (volume of distribution) descriptions. Biliary excretion of TCOG and

enterohepatic recirculation of TCOH were also included as part of the metabolite model structure. As was the case for the Fisher models, physiological parameters were taken or derived from the literature, and chemical-specific parameters were derived from previously published measurements or inferred through calibration to previously published data sets. Specifically, evaluations were made against a subset of measurements for mouse oral and inhalation studies (Fisher et al., 1991; Templin et al., 1993; Prout et al., 1985), rat oral studies (Templin et al., 1995; Larson and Bull, 1992b), and human inhalation studies (Monster et al., 1979; Muller et al., 1975, 1974; Stewart et al., 1970). In addition, in vitro data were used to estimate some metabolism parameters related to CHL in the lung and DCVC in the kidney. Note that these data sets did not include any of the data collected by Fisher and colleagues reported above as having been used in the second-generation Fisher models. Finally, allometric scaling was used extensively to convert parameters across species from those for which calibration data were available to those for which no calibration data existed.

Validation was not performed in the strict sense because not enough experimental data were available for all the metabolites across species. Clewell et al. (2000) visually inspected the model simulations and the data and concluded that simulation results were generally reasonable, although it was clear that complete agreement between the model and each study investigated could not be obtained with a single set of parameters for each species. The results of the sensitivity and uncertainty analyses that were performed indicated that dose metrics for TCE and for the major metabolites TCA and TCOH could be expected to be reasonably precise (Clewell et al., 2000). Dose metrics related to CHL in the lung and DCVC bioactivation in the kidney, on the other hand, were reported to be highly uncertain owing to a lack of adequate pharmacokinetic data across species (Clewell et al., 2000). The DCA metrics also were considered to be uncertain because known analytical errors (Ketcha et al., 1996) existed in some measurements.

3.1.4. Bois Reparameterizations of Fisher and Clewell Models

Bois (2000a, b) performed reparameterizations of the Fisher and Clewell models using a Bayesian statistical framework. The basic approach was to develop a hierarchical statistical model for the population distribution of each model's parameters rather than to use the single values determined by Fisher and Clewell. Therefore, population variability and the uncertainty surrounding that variability were incorporated into the analysis. A Markov Chain Monte Carlo technique was used to perform the high-dimensional numerical sampling and integration necessary to derive the joint probability distribution of the parameters given the available data. These analyses also provided estimates of the uncertainty and variability surrounding individual dose estimates, although, in many cases, uncertainty and variability could not be disentangled because the data were aggregated. For the analysis of rodent data, the population model

described “inter-lot” or “inter-lab” variability, which included measurement errors, rather than actual inter-individual variability.

The Bois analysis of the Clewell model was performed first (Bois, 2000b). The statistical analysis required some minor modifications of the Clewell model. The data used included most of those reported in Clewell et al. (2000) and some additional published data sets. Again, the data used in Fisher’s second-generation models were not included here. Overall, this formal statistical method led to predictions that were systematically better fitting than those (already reasonable) fits obtained by Clewell et al. (2000). However, as found in Clewell et al. (2000), a number of data sets still showed a relatively poor fit, even with allowance for variability supplied by the hierarchical statistical framework.

The marginal posterior distributions for a number of parameters generated from this analysis were used as input priors to the Bois analysis of the Fisher model (Bois, 2000a). Some minor modifications of the Fisher model were required to accommodate the population analysis, and the data sets used included most of the data used by Fisher. However, the analysis did not include the Greenberg et al. (1999) mouse inhalation data that show evidence of fractional absorption in the lung (see Section 4.2.3). The mouse model showed good fits to TCE and TCA, while residuals for other metabolites often reached one or two orders of magnitude. Some of the differences may have been due to variability, as there seemed to be substantial “noise” in the data for some metabolites, but systematic differences were still apparent. The human model showed a substantially better fit, particularly for TCA, with residuals generally of a factor of 2 or less.

Because the Clewell model posterior distributions were used as input priors here, the Bois analysis of the Fisher model actually incorporates to some degree all the data from both analyses. The incorporation is imperfect not only because the two model structures are different but also because the covariance structure of the “Bois-Clewell” posterior distributions was lost in the use of marginal posterior distributions for the new priors. Therefore, somewhat different results may have been obtained if each model were calibrated to the entire data set.

3.2. U.S. EPA/U.S. AIR FORCE-SPONSORED TCE PBPK MODEL DEVELOPMENT

Throughout 2004, EPA and the Air Force have jointly sponsored an initial attempt at combining elements from the TCE PBPK modeling efforts described above. The interim results of this effort are briefly summarized here. A more detailed description will be available in a separate interim report. The development of this model included a peer consultation conducted in June 2004, a summary report for which is also available separately (TERA, 2004).

The goal of this effort was to address several important issues for the first time, particularly the following:

- A single interim model structure combining features from both the Fisher and Clewell models was developed and used for all three species of interest (mice, rats, and humans). An effort was made to combine structures in as simple a manner as possible; the evaluation of alternative structures was left for future work.

- The Fisher and Clewell models, as well as the Bois analyses of those models, reflected substantially different databases of information. This effort evaluated the revised model against a combination of the data sets previously used, to the extent applicable. However, a comprehensive review of all published data was left for future work.

- Similar to the Bois (2000a, b) analyses, a hierarchical Bayesian population analysis using Markov Chain Monte Carlo techniques was performed on the combined model with the combined database of kinetic data to provide estimates of parameter uncertainty and variability.

- Species- and dose-dependent TCA plasma binding was implemented to evaluate its effects on the associated dose metrics (see discussion in Section 4.2.5). Equilibrium binding, as reported in the in vitro study of Lumpkin et al. (2003), was incorporated into the TCA metabolite submodel. It was assumed that the on/off rates were fast compared to transport, as existing TCA studies have not reported the time-scale of TCA binding kinetics.

Interim results from this effort seem to suggest that a single model structure can fit a variety of data evaluated for TCE and its major oxidative metabolites TCA, TCOH, and TCOG, although in some cases different parameter values, particularly for metabolism, are required for different studies. This interim model represents a major step in the development of TCE PBPK models, particularly because the model was evaluated against a larger database of kinetic data than was any previous model. However, a number of the issues described below remain to be investigated; therefore, EPA plans additional model development, evaluation, and characterization. The general approach for these continued efforts is described in Section 5.

4. CONTINUING SCIENTIFIC UNCERTAINTIES IN TCE PBPK MODELING

4.1. UNCERTAINTY AND VARIABILITY IN PBPK MODELING

Uncertainty and variability in PBPK modeling are discussed in this section. (See Bernillon and Bois, 2000, for a more detailed discussion of statistical issues in PBPK modeling.) Specific issues related to TCE are discussed in Section 4.2. The terms “uncertainty” and “variability,” as used here, refer to distinct concepts: uncertainty refers to a lack of knowledge that may be reducible with additional data or study, and variability refers to inherent

heterogeneity that is irreducible. Uncertainty in the characterization of variability may exist because the data are limited or because understanding of the interrelationships within complex biological systems is lacking. Although one would ideally like to separate uncertainty and variability, this is not always possible. For instance, when data from individuals are aggregated, then measurement error and inherent variability may not be separable, even assuming the underlying model is valid.

Given the complexity of TCE pharmacokinetics and the concomitant complexity of TCE PBPK models, it is important to characterize the uncertainty in the PBPK modeling results so as to inform the uncertainty in dose-response assessment. This characterization can have both qualitative and quantitative components. Model uncertainty—which is the uncertainty due to the structural features and assumptions that underlie a particular model—is usually addressed primarily qualitatively through a critical evaluation of model features. Parameter uncertainty—which is the uncertainty in the values of input parameters that are required by the model—lends itself to more quantitative analysis. Although the use of PBPK models in risk assessment is intended to provide more accurate (i.e., less uncertain) estimates of dose relative to default procedures, it should be recognized that PBPK modeling cannot be expected to completely eliminate pharmacokinetic uncertainty. In some cases, rigorous analysis of PBPK models may actually reveal pharmacokinetic uncertainties that were not previously understood or characterized, or that are greater in magnitude than assumed through default procedures. Therefore, it is possible that analysis using PBPK models may *appear* to increase uncertainty. Even in this case, however, PBPK models can help to identify data that may have the greatest impact on reducing these uncertainties.

Owing to their biological basis, PBPK model parameters may also exhibit significant inter-individual variability. Parameters for PBPK models fall into several categories, with varying levels of independent information as their variability. Physiological parameters (such as organ weights and blood flows) are generally measurable, with some having a priori information as to their variability. Chemical-specific parameters, such as partition coefficients, are also measurable using in vitro methods. Generally less information is available about variability for these parameters. However, in these cases, experiment-specific data (other than body weights) are generally not available—that is, the individuals for which physiological and chemical-specific parameters are measured differ from the individuals for which pharmacokinetic data are collected. Parameters such as metabolic and clearance rates are usually inferred through comparison of model predictions with pharmacokinetic data—i.e., the models are calibrated by changing these parameters to fit experimental data, such as time-courses of chemical concentrations. In addition, they may be inferred from in vitro measurements (e.g., microsomal preparations) or data from other chemicals, but with an additional uncertainty that is not easily

quantified. Pharmacokinetic data for both humans and laboratory animals show considerable variability, even within single experiments that use inbred rodent strains under identical conditions (e.g., Prout et al., 1985). Variability in parameters can thus be inferred from variability in the kinetic data and, in some cases, independent measures obtained from in vitro data on enzyme content and/or activities.

PBPK models may be useful in characterizing this variability through a population approach. The basic idea is to fit the variability in individual data by assigning different parameter values to different individuals. Although this is a relatively new approach in human health risk assessment, it has been applied routinely in the development of pharmaceutical drugs; however, the structural models used in pharmaceutical research are typically empirical (e.g., one or two compartments) rather than physiologically based (Ette and Williams, 2004a, b, c; Davidian and Giltinan, 2003; Yuh et al., 1994; Sheiner et al., 1972). One commonly used approach is the "two-stage" method, which involves fitting a model to each separate individual's data and then obtaining population parameter estimates in a second step based on the individual results from the first step. Because of the difficulty of parameter estimation for a complex model, it is common practice either to fix all but a few parameters to estimated values or to set up a very simple model with only a few parameters (as is common for pharmaceuticals). Fisher et al. (1998) used this two-stage approach in their analysis of human data on TCE, calibrating the urinary excretion separately for different individuals. However, this procedure may attribute too much variability to the parameter being adjusted rather than to other parameters that also may be uncertain and/or variable (Bernillon and Bois, 2000; Woodruff and Bois, 1993). In addition, this procedure is ill-suited for the situation where multiple data sets provide information on overlapping sets of parameters.

A second approach involves developing a statistical model for simultaneously characterizing population variability and overall parameter uncertainty. This approach has been variously described as nonlinear mixed-effect modeling (e.g., Davidian and Giltinan, 2003; Sheiner et al., 1977) and Bayesian population modeling (e.g., Gelman et al., 1996), depending on whether the analysis uses a frequentist (typically maximum likelihood) or Bayesian statistical framework. The use of population models (in both pharmaceutics and toxicology) has focused on single (mostly human) data sets where individual measurements are available (Jonsson and Johanson, 2003, 2002, 2001a, b; Jonsson et al., 2001a, b; Smith et al., 2001; Bois et al., 1999; Gelman et al., 1996; Bois et al., 1996a, b). The use of this methodology for simultaneously calibrating parameters using multiple data sets, including laboratory animal data that are typically aggregated, is a relatively new area with few published results other than those of Bois (2000a, b) for TCE, described above. However, because of the ability to incorporate prior information on the analysis of any particular data set, the Bayesian approach is conceptually well suited for the

situation with TCE in which multiple data sets are being analyzed together. A commonly used method to implement this Bayesian population approach has been the use of Markov Chain Monte Carlo techniques.

4.2. SCIENTIFIC UNCERTAINTIES RELATED TO TCE PHARMACOKINETIC MODELING

Although the EPA/Air Force-sponsored effort to combine elements of the Fisher, Clewell, and Bois models into an interim PBPK model has examined some of the differences between these previous approaches (see the separate interim report for additional details), a number of uncertainties remain as to model assumptions, structure, and parameters. These issues are briefly summarized below. It is unclear at this time which of these uncertainties are important with respect to fitting available data or predicting internal dose; our proposed approach to assessing these sensitivities is summarized in Section 5.

4.2.1. Remaining Parameter Uncertainties in Oxidative Metabolism

The analyses described above reported a number of parameter uncertainties. Uncertainties related to modeling of low-concentration metabolites, such as DCA, DCVC, and CHL, are discussed below, as they are intertwined with structural issues. Even for the "well calibrated" oxidative pathway, two significant issues surrounding the metabolic parameter remain:

- Fisher (2000) reported substantial differences in parameter values for TCE oxidative metabolism in mice between inhalation and gavage dosing. Of particular note was that the Michaelis-Menten (K_M) parameter used to fit the corn oil gavage data was greater by over an order of magnitude than that used to fit the inhalation data. Moreover, the estimate for K_M based on the gavage data was substantially greater than that based on previously published studies, a finding also reported in the Bois (2000a) analysis of the Fisher models. Fisher (2000) suggested that this adjustment reflected the model's oversimplification of the oral uptake of TCE in corn oil rather than a true change in the K_M for oxidation of TCE. Smaller changes of up to a factor of two in other parameters, such those for as glucuronidation of TCOH, were also noted by Fisher (2000) between gavage and inhalation studies.

- Fisher (2000) reported a substantially lower value for the $BW^{3/4}$-scaled Michaelis-Menten V_{max}, or maximum velocity of reaction, parameter (denoted "VmaxC") in humans based on the Fisher et al. (1998) data than was reported in previous studies using previously published data. This finding was also reported in the Bois analysis of the Fisher models, with Bois (2000a) noting that the difference could be due to differences in the data, rather than in the modeling.

Currently, it is not clear whether these differences are due to inherent variability or structural misspecification of the model.

4.2.2. Enterohepatic Recirculation

In the liver, chemicals can be secreted into the bile and circulated into the gut, where they are reabsorbed into the portal blood. Recirculation of metabolites increases their effective half-life in the body, and this is reflected in urinary output of the chemicals. TCOG and TCA have been measured in the bile of rats (Stenner et al., 1997), and bile-cannulated rats showed different blood profiles of the chemicals. A PBPK model developed based on this work (Stenner et al., 1998) included enterohepatic recirculation and showed a reasonable match to rat concentration profiles after oral doses of TCE (in 2% Tween 80) and intravenous doses of TCA and TCOH. Difficulties exist in extrapolating the rat data to other species because pharmaceutical studies have shown that biliary excretion does not scale uniformly (Mahmood and Sahajwalla, 2002).

The significance of recirculation on important dose metrics is uncertain, as existing PBPK models have generally shown reasonable fits to blood and urine data without recirculation. For instance, even though Clewell et al. (2000) implemented recirculation structurally, reabsorption in the gut was set to zero for comparison to most data. Bois (2000a) noted, however, that urinary excretion data for TCOG in mice was not well fit by the Fisher model, which did not include recirculation. Overall, model fit and the sensitivity of dose metrics with and without enterohepatic recirculation have not been evaluated. It is likely that TCA and TCOH metrics are sensitive to enterohepatic recirculation, but a quantitative characterization has not yet been reported.

4.2.3. Wash-In/Wash-Out for Inhalation

For different exposure routes in a particular species, the distribution, metabolism, and elimination of chemicals are expected to be the same, with the only difference being absorption and first-pass clearance for the particular route. Inhalation is commonly modeled as being complete, whereas for volatile chemicals, the blood:air partition coefficient determines both the uptake and elimination of a chemical.

As mentioned above, when the best-fit model parameters based on oral mouse data (Abbas and Fisher, 1997) were used in an inhalation exposure simulation (Greenberg et al., 1999), the model overpredicted the absorption of TCE as reflected in TCE blood and exhalation concentrations. The model could be made consistent with the data if fractional uptake were implemented, where only a fraction of the chemical is available for transfer to the plasma during inhalation exposure. Physiologically, this could occur if the lung epithelium were to act as a

reservoir for TCE during inhalation exposure, so only a fraction of the TCE inhaled reaches the alveolar region. Fractional uptake has been reported for other water-soluble solvents (Pastino et al., 1997; Perkins et al., 1995; Johanson, 1991), and because the lung tissue is exposed to high local concentrations, it has a potential impact on risk.

4.2.4. Diffusion Limited Fat and Liver Compartments

The PBPK models for TCE described above all assume perfusion-limited distribution of chemicals to the physiologic compartments. The representation assumes that the compartments are well mixed over the time-scales of blood flow, so that the compartment concentration can be described by the blood concentration and a partition coefficient. Some discrepancies have been noted, however. For instance, Bois (2000a) reported that the measured adiposity of the individual subjects from Fisher et al. (1998) did not correlate well with the posterior estimates for the model parameter for percentage body weight as fat. Bois suggested that one possible explanation was that the pharmacokinetic compartment for fat was not well estimated by external adiposity measurements. However, model error has also been proposed as an explanation.

The liver and fat are known to be heterogenous tissues (Albanese et al., 2002; Andersen et al., 1997), and based on their importance to PBPK models of volatile organics, investigations have been conducted on the impact of diffusion limitations in these tissues. For instance, Keys et al. (2003) recently developed a PBPK model for TCE parent kinetics in rats and mice that includes more complex descriptions of the fat and liver compartments. For the fat, transport between the blood and the compartment was changed from flow- to diffusion-limited. For the liver, a second “deep” compartment was added with transport via diffusion to and from the “shallow” liver, which is also the site of metabolism. Keys et al. (2003) concluded that TCE parent concentrations are better simulated by this more complex model, and that although other dose metrics were not evaluated, metabolite concentrations would not be expected to be significantly changed.

4.2.5. Plasma Binding

The binding of chemicals to proteins in plasma affects their availability to other tissues and ultimately their effective half-life in the body. Typical descriptions of relatively weak serum protein binding assume fast rates compared to the other relevant time-scales.

The TCE metabolites TCA and DCA are known to bind to plasma proteins. Templin et al. (1995) measured the extent of TCA binding in humans, mice, and rats over limited concentration ranges. More recently, Lumpkin et al. (2003) measured TCA binding in humans, mice, and rats over a wide concentration range that spans reported TCA plasma concentrations from experimental studies. These data showed significant species differences, with humans

exhibiting the most binding and mice exhibiting the least. As mentioned above, existing TCA studies have not reported the time-scale of TCA-binding kinetics. For modeling, one typical assumption is that the ratio of bound-to-free is in equilibrium in arterial blood, but only the free fraction is available for exchange with tissues.

Schultz et al. (1999) measured the extent of DCA binding in rats at a single concentration of about 100 µM and found a binding fraction of less than 5%. However, these data are not greatly informative for TCE exposure in which DCA levels are significantly lower, and limitation to a single concentration precludes fitting to standard binding equations from which the binding at low concentrations could be extrapolated. Furthermore, there is insufficient information on cross-species differences for the extrapolation of rat DCA data to other species.

4.2.6. Metabolites With Low Circulating Concentrations and/or Extrahepatic Metabolism

As mentioned in Section 2, significant uncertainty surrounds the metabolic pathways for metabolites with low circulating concentrations and for which extrahepatic metabolism may be important toxicologically. These include DCA, CHL, and the metabolites from GSH conjugation. Previous modeling of TCE metabolism indicates that the relative formation rates of these chemicals are small, so that they are not appreciably constrained by the total TCE mass balance (Clewell et al., 2000). However, it would be desirable to model the metabolism to these chemicals (e.g., Figure 4-1) because of their potential toxicological importance.

Pharmacokinetic studies of DCA exposure (Barton et al., 1999; Abbas et al., 1997; Larson and Bull, 1992a) provide a picture of the behavior of DCA once formed, but, as discussed in Section 2, the magnitude of the amount formed from TCE is uncertain. Circulating DCA in mice has been measured in a variety of TCE exposure studies (Greenberg et al., 1999; Merdink et al., 1998; Abbas and Fisher, 1997). The only DCA data for humans following TCE exposure are from Fisher et al. (1998). For all of these studies, the extent to which analytical artifacts of DCA remain (Ketcha et al., 1996) is unclear, so these data may be useful only as a maximum constraint.

As discussed in Section 2, a complication for CHL and GST metabolites is that local metabolism to these compounds can occur in the lung and kidney, respectively, and the contributions of local and liver metabolism to the concentration at the site of action are unknown. CHL (in equilibrium with CH) is rapidly metabolized into TCOH and TCA, so circulating CHL levels are expected to be low. Circulating CH/CHL was measured after high-dose TCE exposures in mice (Greenberg et al., 1999; Abbas and Fisher, 1997; Prout et al., 1985) and rats (Prout et al., 1985). In Abbas and Fisher (1997) and Greenberg et al. (1999), the concentration of CH was also measured in lung homogenate, although the concentration in the lung Clara cells, which are believed to be a site of local production and toxicity (Odum et al., 1992), was not

accessible. CH was administered to mice (Abbas et al., 1996) and humans (Muller et al., 1974) in controlled exposures, which may be useful for characterizing its behavior after formation from TCE. The relative contributions of hepatic and extrahepatic metabolism remain uncertain.

As mentioned in Section 2, Lash et al. (1999a) measured DCVG concentrations in blood following TCE exposure. In addition, the results of a rat study measuring both oxidative and GST metabolites are in preparation (Lash, 2004). However, the kidney is believed to be the site of action for metabolites of DCVG, and the contribution of circulating DCVG produced in the liver relative to local production of DCVG is uncertain. Bernauer et al. (1996) and Birner et al. (1993) have measured the urinary metabolites of DCVG, providing an indicator of the amount of metabolism that occurred, at least through the N-acetyl transferase (NAT) detoxification pathway. In vitro studies exist that measure metabolism by GST in liver and kidney cells (Lash et al., 1999b) and for activity of beta-lyase (Lash et al., 1990), which has been associated with the nephrotoxicity (Anders et al., 1988). The relative in vitro kinetics can be used to inform reasonable parameter values in the liver and kidney, although, as discussed in Section 2, the potential role of FMO complicates the quantification of GST metabolite bioactivation across species.

5. PLANS FOR CONTINUED TCE PBPK MODEL DEVELOPMENT

The issues raised in the previous sections suggest a number of potential approaches for PBPK model development. The models of Fisher, Clewell, and Bois provide a relatively consistent basis for development of a single base model structure for mice, rats, and humans. Additional structural features can then be evaluated against the database of existing data. The model parameters can then be refined and further characterized through a Bayesian statistical framework. Given the complexity of the model and the large database of studies with which to compare model predictions, this process will need to proceed in an iterative manner. An interim model developed through an EPA/Air Force collaboration, described above, will be used as a starting point for additional model development and evaluation. The following sections are intended to inform the NRC committee of EPA's plans for continued TCE PBPK model development. NRC feedback on this approach, and suggestions for improving it, would help to strengthen the basis of EPA's revised TCE risk assessment.

5.1. MODEL PURPOSE AND SCOPE

The main objective of EPA's TCE PBPK modeling effort is to estimate biologically relevant internal doses for use in risk assessment. These dose estimates may be used for a variety of extrapolations (e.g., high to low dose, inter- and intra-species, exposure route or regime) and

are subject to both uncertainty and variability. Selection of the appropriate dose metric for use in risk assessment is dependent not only on the reliability of the data and models but also on the understanding of the MOA for a particular toxic effect—i.e., what may be the "biologically relevant" internal doses. Because a revised assessment of TCE MOA hypotheses has not yet been completed, selection of the most appropriate dose metric(s) is not possible at this time (although metabolite interactions are discussed in a separate issue paper). Therefore, the approach EPA is planning concentrates first on characterization of the PBPK model structure and parameters. Previous PBPK modeling of TCE is used as a guide to develop a model that is both flexible and reliable to the extent scientifically supported by available data.

How PBPK modeling was used in the 2001 draft assessment provides an important first step in consideration of the scope of continued PBPK model development. Uses of PBPK modeling considered in the 2001 draft assessment included the following:

- Cross-species extrapolation of cancer risk estimates based on mouse and rat bioassays. Use of PBPK modeling was considered for analyzing mouse liver tumors (based on TCA and DCA area under the concentration curve [AUC] dose metrics), rat kidney tumors (based on bioactivation of DCVC), and mouse lung tumors (based on CHL). Because of the significant model and parameter uncertainty of these three endpoints, PBPK model-based cancer risk estimates were developed only for mouse liver tumors.

- Route-to-route extrapolation of human cancer risk estimates, based on either TCA or DCA AUCs.

- Development of noncancer risk estimates based on mouse and rat noncancer studies. Dose metrics considered included TCA and DCA AUC, TCOH peak concentration, and bioactivation of DCVC, depending on the study and endpoint. Because the draft reference dose (RfD) was based on liver weight changes, TCA and DCA AUC dose metrics were used. These dose metrics were used both for characterizing the pharmacokinetic component of cross-species extrapolation and human variability, based on the Bois analysis of the Clewell model.

Therefore, the basic toxicological database, as reviewed in the 2001 draft, suggests that the PBPK models of TCE be developed in mice, rats, and humans. These are also the species in which numerous pharmacokinetic studies have been conducted. EPA's modeling effort will consider the dose metrics that have been previously considered for use in risk assessment, although additional dose metrics may need to be evaluated if the MOA evaluation suggests alternatives not listed here. Regarding routes of exposure, oral and inhalation kinetic studies are most common in mice and rats, and nearly all controlled human kinetic studies are based on inhalation exposure. A few rodent studies were reported for injection exposures (Stenner et al.,

1997; Abbas et al. 1997, 1996; Lee et al., 1996; D'Souza et al., 1985), and only one group has reported results for controlled dermal exposure in rats and humans (Poet et al., 2000). However, the general human population may be exposed orally and dermally as well. Therefore, for risk assessment purposes, the model should ideally simulate inhalation and oral exposures for all species, as well as injection for rodents and dermal exposure for humans.

Although the 2001 draft assessment noted that co-exposures with other chemicals may modulate TCE toxicity, the PBPK modeling effort is not planning to include explicit modeling of chemical mixtures beyond the extent to which TCE metabolism leads to a mixture of circulating metabolites. Modeling additional exposure from some of the metabolites themselves would presumably be straightforward, but there appear to be insufficient data on more complex chemical mixtures with TCE to model with a PBPK model. Some exploratory analyses have been reported (Dobrev et al., 2002, 2001), but the data sets are sparse, where only a few chemicals and compartments were measured. Also, the previously mentioned uncertainties with TCE alone seem significant enough so that evaluation of a model of mixtures seems impractical at this time. However, the mechanistic framework of the PBPK model illustrates the parameters that have an important impact on the dose metrics and observable quantities, thereby facilitating experimental design for future investigations of mixtures.

The 2001 draft assessment also noted the potential for susceptible subpopulations with differential risks from TCE exposure. However, the data appear to remain largely qualitative, so potential subpopulations will not be explicitly modeled within the PBPK models, although they may be addressed through other means in the revised risk assessment. Although physiologic differences are modestly characterized for segments of the population, substantial uncertainties are associated with the TCE-specific biochemical differences associated with age, disease state, and genetic polymorphisms. These questions can to a degree be addressed in the individual model evaluation, where the sensitivity analysis will reveal the parameters that are sensitive with respect to toxicologically important dose metrics. These parameters can be compared to the knowledge of the represented biological process to identify potential subpopulations that may respond differently and to develop hypotheses for the identification and eventual quantification of risk metrics for those populations.

5.2. SOURCES OF PHYSIOLOGICAL AND KINETIC DATA

Parameters for PBPK models include three distinct types of data: physiological data, chemical-specific parameters, and parameters for determining the stochastic behaviors of models. The physiological data are independent of the chemical being modeled and refer to such areas as organ volumes and blood flows. Chemical-specific parameters include the partition coefficients,

metabolic rate constants, and coefficients for protein binding. Important stochastic behaviors to be modeled are derived from inter-individual and experimental variances.

The specific physiological compartments considered in EPA's modeling effort are selected based on information available for exposure, toxicology, and metabolic profile related to a particular chemical and potential active metabolites. Important information includes measurements of parent or metabolite concentrations, known metabolism or elimination, or a known toxic effect within specific tissues. Distribution within, between, and among organs, tissues, and fluid is modeled according to compartmental volumes, blood flow rates, and blood:tissue partitioning.

As in previous PBPK modeling efforts (Clewell et al., 2000; Fisher et al., 1998), physiological parameters and chemical-specific partition coefficients are estimated from independent studies, such as standard physiology references or in vitro experiments. The kinetic parameters associated with absorption, metabolism, and elimination are fit to kinetic data, although in some cases they may be informed by in vitro data as well.

Studies on the formal analysis of variability using PBPK models are limited (Bois, 2000a, b). There is no established method to characterize the variability in a heterogeneous population. Even physiologic references, such as ILSI-RSI (1994), Brown et al. (1997), and ICRP (2003), focus on determining the range and/or central tendency of reasonable values, not on developing the distributions that are necessary for probabilistic analyses. In addition, the covariance between parameters (e.g., between tissue volumes and blood flows) are not characterized but may need to be accounted for to avoid unrealistic combinations of parameter values.

For the chemical-specific parameters, the variability of parameters can be estimated from in vitro experiments (partition coefficients, metabolism) and individual pharmacokinetic data. As discussed previously, formal evaluation of variability against data can be performed using a hierarchical Bayesian population model employing a Markov Chain Monte Carlo algorithm. However, for much of the laboratory data on TCE, individual data were pooled, making it difficult to characterize inter-individual variation and to separate variability from measurement error. An additional challenge is that human data sets tend to be relatively homogeneous and performed under controlled conditions, and thus may not represent the range of inter-individual variability present in the full population. Therefore, in the evaluation of variability for the target populations of the risk assessment, posterior distributions from the Bayesian analysis could tend to underestimate full population variability and thus may not always be appropriate.

5.3. MODEL EVALUATION

Model evaluation involves the consideration of alternative model structures, the estimation of model parameters, and characterization of uncertainty and variability. The EPA effort will be conducted in three broad phases: exploratory model development, sensitivity analysis, and Bayesian population analysis of uncertainty and variability. The resulting individual models with "mean" parameter values estimated from the Bayesian population analysis will be made available to the public through the Exposure Related Dose Estimating Model (ERDEM) system, developed by the U.S. EPA (Appendix B). The Bayesian population analysis using the Markov Chain Monte Carlo algorithm will also be done using publicly available software, MCSim (Appendix B).

The first phase will involve exploratory analysis in which a single model structure (or set of structures) will be built with separate parameterization for each species. During this phase, the uncertainties described above surrounding alternative model structures will be investigated. These include enterohepatic recirculation, wash-in/wash-out in the lung, diffusion-limited fat and liver compartments, and plasma binding. In addition, as described above, considerable uncertainty exists regarding the metabolic pathways and parameters for low-concentration metabolites and those locally produced. The goal of this phase is to settle on one or a few model structure(s) that best fit(s) the available data while maintaining biological plausibility. These exploratory models will be calibrated with available kinetic data. The evaluation of model fit, performed either statistically and/or by visual inspection depending on the nature of the available data, will guide the refinement of model structure and parameters. Appendix A provides a table of the candidate pharmacokinetic studies against which the PBPK models are to be evaluated.

For simple models and a limited number of data sets, a typical "validation" after calibration would be to compare the fit model with data not used for calibration. However, the data sets for TCE include different exposure scenarios and measurements on different scales that do not present a clear metric to evaluate the performance of an individual model. Also, despite the number of studies, the data are sparse relative to the qualitatively important compartments and metabolites. Thus, traditional "validation" will not be feasible. An arguably more relevant question for risk assessment is a characterization of the confidence with which a model can predict dose metrics of interest. This is related to the characterization of uncertainty and variability, described below.

The second (intermediate) phase is sensitivity analysis of the individual models to determine the important parameters. Two types of analyses will be performed: sensitivity of the fit to data and sensitivity of the dose metrics. Sensitive parameters are critical to characterize the analysis of uncertainty and variability. The priors assigned to those parameters will reflect the certainty to which the value is known. For example, the tissue volumes for which we have

independent measurements will have greater precision than the kinetic rate constants that were fit to data. The parameters that impact the dose metrics but not the fit to data require other constraints to characterize their uncertainty. The model can also be analyzed around these parameters to determine the measurable quantities that would constrain the value; this provides the basis for future targeted experimental studies to reduce the uncertainty. The sensitivity analysis also highlights the biological processes that affect risk by identifying the corresponding model parameters. These parameters can be evaluated against the knowledge of the biological pathway to identify other chemicals that would impact the dose metrics (i.e., sharing the same saturable metabolic pathway) or to identify subpopulations that would exhibit different risks due to differences in a pathway.

The third phase of evaluation is a Bayesian population analysis (using a Markov Chain Monte Carlo algorithm) that incorporates the variability in the available data to characterize the uncertainty and variability in the model parameters and their impact on model predictions. Examples of the use of the Bayesian population approach with PBPK models were discussed earlier. The previous section described the sources of data for developing prior estimates of the population means and variances and their attendant uncertainties. Other published uncertainty analyses, including both the Bayesian analyses cited above and traditional Monte Carlo analyses (e.g., Clewell et al., 2000; Cronin IV et al., 1995) will also be used as a guide for reasonable prior distributions. As noted above, however, the populations for which kinetic data are available (i.e., Appendix A) may not be wholly representative of the target populations of interest for the risk assessment.

Before using the results of this analysis to perform inferences as to the uncertainty and variability in dosimetry predictions, it is necessary to confirm that the model is consistent with the data and existing biological knowledge. As was the case for the exploratory analysis, a formal model "validation" will not be performed owing to the need to use all the available data in calibrating the model. However, "model fit" does need to be assessed, particularly because posterior parameter estimates may be overconstrained if the model does not fit the data (e.g., discussion in Bois, 2000a). Gelman et al. (2004) provide an extensive discussion of techniques for "posterior predictive checking," or the assessment of whether the model is consistent with the data. The general basic idea is to generate simulated data from the posterior parameter distribution for comparison with the actual observed data. Bois (2000a, b) provided some examples of this type of analysis. In addition, posterior estimates of parameters and predictions should also be checked for biological plausibility because there is biological knowledge that is not formally included in either the prior estimates or in the likelihood. For instance, it can be difficult to translate biological knowledge quantitatively into formal prior distributions, particularly with respect to parameter covariances. Thus, parameter combinations that are

unrealistic biologically may be easier to check a posteriori. The results of these analyses may lead to additional refinement of the model and/or parameters and may thus necessitate iteration of the entire model evaluation process. It should be noted, however, that it may not be possible to completely disentangle the combination of data errors, model errors, and parameter variability.

Finally, the results of the Bayesian population analysis will be used along with available information on the target populations of interest (see Section 5.2) to characterize the uncertainty and variability in the pharmacokinetic modeling results. This characterization will be an important input, along with the appropriate mode-of-action and hazard characterization, into the selection of dose metrics as well as species- and route-extrapolation methods for use in quantitative dose-response analysis.

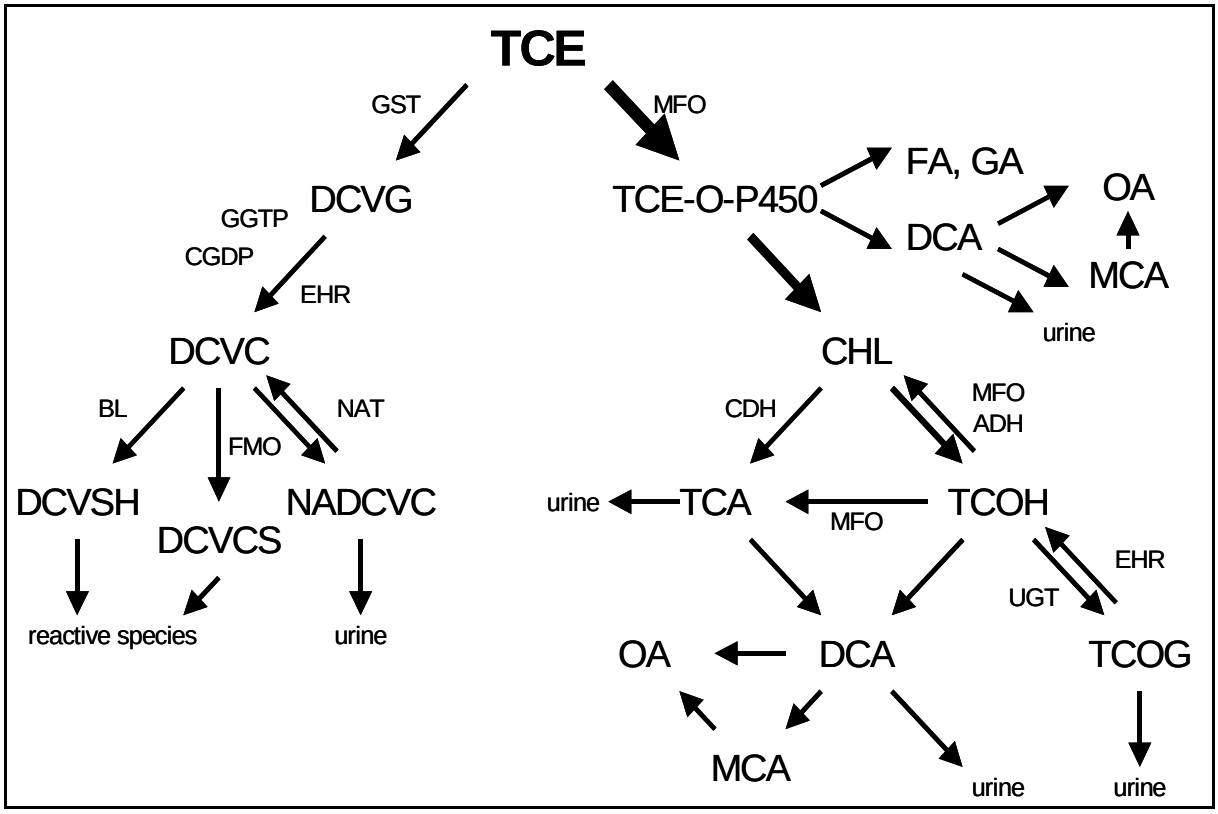

Figure 2-1. Metabolism of trichloroethylene (TCE).

CDH = chloral dehydrogenase (aldehyde oxidase); EHR = enterohepatic recirculation; FA = formic acid; FMO = flavin-containing monooxygenase; GA = glyoxylic acid; OA = oxalic acid; TCE-O-P450 = oxygenated TCE-cytochrome P450 transition state complex; TCOG = TCOH glucuronide; UGT = UDP glucuronosyl transferase; BL = cysteine conjugate β-lyase; CGDP = cysteinyl-glycine dipeptidase; DCVCS = DCVC sulfoxide; DCVG = dichlorovinyl glutathione; DCVSH = dichlorovinyl mercaptan; GGTP = γ-glutamyl transpeptidase; NADCVC = N-acetyl dichlorovinylcysteine; NAT = N-acetyl transferase.

Source: Adapted from Clewell et al. (2000) and Lash et al. (2000a).

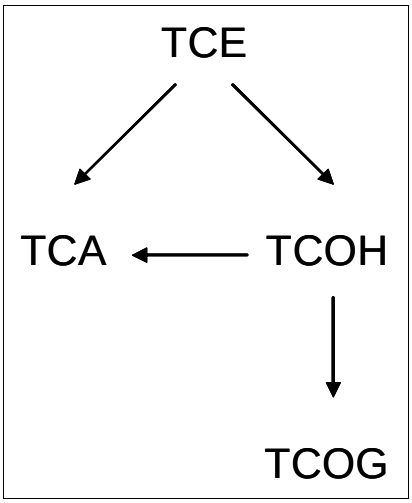

Figure 3-1. Typical simplified metabolism scheme for modeling.

TCE = trichloroethylene; TCA = trichloroacetic acid; TCOH = trichloroethanol; TCOG = TCOH glucuronide.

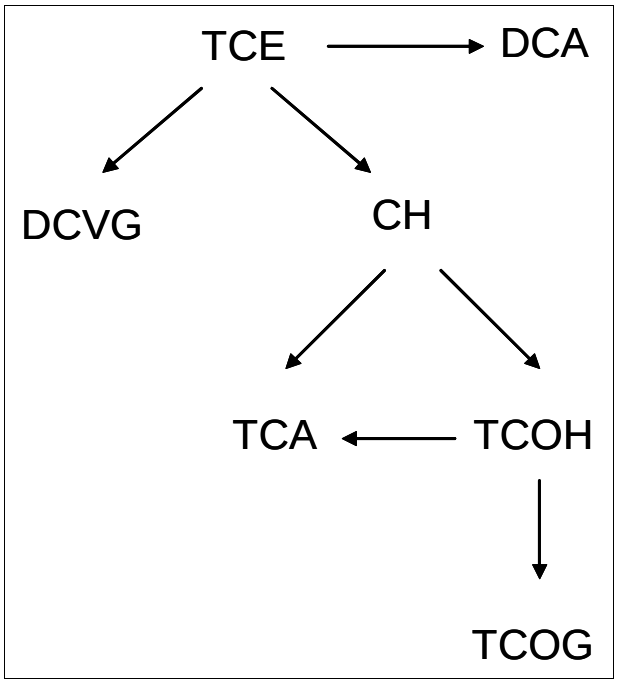

Figure 4-1. Possible liver metabolism scheme to model potential dose metrics.

TCE = trichloroethylene; DCA = dichloroacetic acid; DCVG = dichlorovinyl glutathione; CH = chloral hydrate; TCA = trichloroacetic acid; TCOH = trichloroethanol; TCOG = TCOH glucuronide.

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APPENDIX A: CANDIDATE STUDIES FOR MODEL EVALUATION

Lead author	Reference	Species	Exposure scenario	Measurements
R.R. Abbas	Toxicol Appl Pharmacol 147:15–30, 1997.	Mice-Male B6C3F1	TCE oral	TCE and metabolites in blood, liver, lung, kidney, fat, and urine
R.R. Abbas	Toxicologist 36:32–33, 1997.	Mice-B6C3F1	TCA, TCOH, DCA, CH iv	Compounds in blood and urine
R.R. Abbas	Drug Metab Dispos 24(12):1340–1346, 1996.	Mice-B6C3F1	CH iv	Compounds in blood and urine
H.A. Barton	Toxicol Appl Pharmacol 130:237–247, 1995.	Rats-Male SD	TCE inhalation	TCE closed chamber concentrations
H.A. Barton	Toxicol Lett 106(1):9–21, 1999.	Mice-Male B6C3F1	DCA iv and oral (aqueous)	DCA in blood
U. Bernauer	Arch Toxicol 70(6):338–346, 1996.	Humans-Male	TCE inhalation	Oxidation and GST metabolites in urine
U. Bernauer	Arch Toxicol 70(6):338–346, 1996.	Rats-Wistar	TCE inhalation	Oxidation and GST metabolites in urine
G. Birner	Environ Health Perspect 99:281–284, 1993.	Rats-Wistar, F344 Mice-NMRI	TCE oral gavage	NADC, TCA in urine
L.J. Bloemen	Int Arch Occup Environ Health 74:102–108, 2001.	Humans-Male	TCE inhalation	Oxidation and GST metabolites in urine
C.E. Dallas	Toxicol Appl Pharmacol 110:303–314, 1991.	Rats-Male SD	TCE inhalation	TCE in blood, breath
R.W. D'Souza	J Toxicol Environ Health 15:587–601, 1985.	Rats-Male SD	TCE iv	TCE in blood
J.G. Fernandez	Br J Ind Med 34(1):43–55, 1977.	Humans-Male	TCE inhalation	Alveolar, excreted TCE
J.W. Fisher	Risk Anal 13(1):87–95, 1993.	Mice-Male and female B6C3F1	TCE oral gavage in corn oil	TCE in blood; TCA in plasma
J.W. Fisher	Toxicol Appl Pharmacol 109(2):183–195, 1991.	Mice-Female B6C3F1	TCE inhalation	TCE and metabolites in blood and chamber
J.W. Fisher	Toxicol Appl Pharmacol 109(2):183–195, 1991.	Mice-Male B6C3F1	TCE inhalation	TCE and metabolites in blood and chamber
J.W. Fisher	Toxicol Appl Pharmacol 109(2):183–195, 1991.	Rats-Female F344	TCE inhalation	TCE and TCA in blood

APPENDIX A: CANDIDATE STUDIES FOR MODEL EVALUATION (continued)

Lead author	Reference	Species	Exposure scenario	Measurements
J.W. Fisher	Toxicol Appl Pharmacol 109(2):183–195, 1991.	Rats-Male F344	TCE inhalation	TCE and TCA in blood
J.W. Fisher	Toxicol Appl Pharmacol 152(2):339–359, 1998.	Humans-Female	TCE inhalation	TCE and metabolites in blood and urine; exhaled TCE
J.W. Fisher	Toxicol Appl Pharmacol 152(2):339–359, 1998.	Humans-Male	TCE inhalation	TCE and metabolites in blood and urine; exhaled TCE
M.S. Greenberg	Toxicol Appl Pharmacol 154(3):264–278, 1999.	Mice-Male B6C3F1	TCE inhalation	TCE and metabolites in blood, liver, lung, fat, and kidney; TCE chamber concentrations
I. Jakobson	Acta Pharmacol Toxicol (Copenh) 59(2):135–143, 1986.	Rats-Female SD	TCE inhalation	TCE in blood
T. Kaneko	Toxicology 143(2):203–208, 2000.	Rats-Male Wistar	TCE inhalation	TCE in blood; TCA, TCOH in urine
D.A. Keys	Toxicol Sci 76(1):35–50, 2003.	Rats-Male SD	TCE inhalation	TCE in blood and tissues
S. Lapare	Int Arch Occup Environ Health 67(6):375–394, 1995.	Humans	TCE inhalation	TCE in blood; TCA in urine
J.L. Larson	Toxicol Appl Pharmacol 115(2):278–285, 1992.	Mice-Male B6C3F1	TCE oral (aqueous)	TCE and metabolites in blood
J.L. Larson	Toxicol Appl Pharmacol 115(2):278–285, 1992.	Rats-Male Sprague-Dawley	TCE oral (aqueous)	TCE and metabolites in blood
J.L. Larson	Toxicol Appl Pharmacol 115(2):268–277, 1992.	Mice-Male B6C3F1	DCA, TCA oral	DCA, TCA in plasma
J.L. Larson	Toxicol Appl Pharmacol 115(2):268–277, 1992.	Rats-Male F344	DCA, TCA oral	DCA, TCA in plasma
L.H. Lash	J Toxicol Environ Health A. 56(1):1–21, 1999.	Humans-Female-1-BW 66.5	TCE inhalation	DCVG in blood
L.H. Lash	J Toxicol Environ Health A. 56(1):1–21, 1999.	Human-Male-1-BW 71.4	TCE inhalation	DCVG in blood

APPENDIX A: CANDIDATE STUDIES FOR MODEL EVALUATION (continued)

Lead author	Reference	Species	Exposure scenario	Measurements
K.M. Lee	Toxicol Appl Pharmacol 164(1):55–64, 2000.	Rats-Male SD	TCE stomach injection	TCE in arterial blood
K.M. Lee	Toxicol Appl Pharmacol 139(2):262–271, 1996.	Rats-Male SD	TCE arterial, venous, portal, stomach injections	TCE in arterial blood
J.L. Merdink	Toxicol Sci 45(1):33–41, 1998.	Mice-Male B6C3F1	CH, TCE iv	TCA, CH, TCOH in blood
J.L. Merdink	J Toxicol Environ Health A. 57(5):357–368, 1999.	Rat-Male F344	CH, TCOH iv	CH, TCOH in blood; TCOG, CH, TCA in bile
A.C. Monster	Int Arch Occup Environ Health 38(2):87–102, 1976.	Humans	TCE inhalation	TCE in breath; TCA and TCOH in blood and urine
A.C. Monster	Int Arch Occup Environ Health 42(3–4):283–292, 1979.	Humans	TCE inhalation	TCE in blood and breath; TCA and TCOH in blood and urine
G. Muller	Arch Toxicol 32(4):283–295, 1974.	Humans-Male	TCE inhalation; CH, TCA, TCOH oral	TCE in blood and breath; TCA and TCOH in blood and urine
G. Muller	Arch Toxicol 33(3):173–189, 1975.	Humans-Male	TCE inhalation	TCE in blood and breath; TCA and TCOH in blood and urine
Z.V. Paykoc	J Pharmacol Exp Ther 85:289, 1945.	Humans	TCA iv	TCA in blood and urine
T.S. Poet	Toxicol Sci 56(1):61–72, 2000.	Humans	TCE dermal	TCE in breath
T.S. Poet	Toxicol Sci 56(1):61–72, 2000.	Rats-Male F344	TCE dermal	TCE in chamber
M.S. Prout	Toxicol Appl Pharmacol 79(3):389–400, 1985.	Mice-Male B6C3F1 and Swiss Webster	TCE gavage	TCE and metabolites in blood; ^{14}C elimination
M.S. Prout	Toxicol Appl Pharmacol 79(3):389–400, 1985.	Rats-Male Osborne-Mendel and Alderley Park Wistar	TCE gavage	TCE and metabolites in blood; ^{14}C elimination

APPENDIX A: CANDIDATE STUDIES FOR MODEL EVALUATION (continued)

Lead author	Reference	Species	Exposure scenario	Measurements
S.A. Saghir	Environ Health Perspect 110:757–763, 2002.	Rats-Male F344	DCA iv, oral	DCA in plasma
A. Sato	Br J Ind Med 34(1):56–63, 1977.	Humans-Male	TCE inhalation	TCE in blood, expired air; TCA in urine
J.E. Simmons	Toxicol Sci 69(1):3–15, 2002.	Rats-Male long-evans	TCE inhalation	TCE in chamber, liver, blood, brain, fat
R.D. Stenner	Drug Metab Dispos 25(5):529–535, 1997.	Rats-Male F344	TCOH, TCA iv; TCE intraduodenal	TCA, TCOH in blood
R.D. Stewart	Arch Environ Health 20(1):64–71, 1970.	Humans	TCE inhalation	Exhaled TCE; TCA, TCOH in urine
M.V. Templin	Toxicol Appl Pharmacol 23(1):1–8, 1993.	Mice	TCE oral (aqueous)	TCE and metabolites in blood
M.V. Templin	J Toxicol Environ Health 44(4):435–447, 1995.	Rats-Male F344	TCE oral (Tween 80 solution)	TCE in blood; TCA and TCOH in blood and bile
K.D. Thrall	J Toxicol Environ Health A. 59(8):653–670, 2000.	Rats-Male F344	TCE iv	TCE in breath, chamber

APPENDIX B: COMPUTER IMPLEMENTATION

The general modeling strategy described above will be implemented in different languages in two separate modeling efforts. This activity provides a quality control check on the modeling software and the coding of the model so that outputs of the two models given the same input should be similar. Divergence of the two model outputs would indicate improper coding in at least one of the models. Since the two languages that are being used differ in syntax and in how the model code is structured, it is unlikely that a coding error would be made in both programs that would be similar enough to go undetected (i.e., both program outputs would be the same). Moreover, the capabilities of the two modeling languages differ, and features unique to each program add to the overall ability to develop and test the model. The two models are described below.

Model 1: Exposure Related Dose Estimating Model

The U.S. Environmental Protection Agency's (EPA's) National Exposure Research Laboratory (NERL) has developed the Exposure Related Dose Estimating Model (ERDEM) as a platform for the application of physiologically based pharmacokinetic (PBPK) and PBPK/pharmacodynamic (PD) models. The heart of ERDEM (http://www.epa.gov/heasdweb/erdem/erdem.htm) is a PBPK model that simulates the absorption, distribution, metabolism, and elimination of chemicals in mammalian systems.

Simulated chemicals are introduced into the physiological system by any of several routes, including injection, ingestion, inhalation, and/or dermal absorption. The ERDEM system contains a large set of potential compartments and processes, with over 30 physiological compartments, such as arterial and venous blood, brain, skin (surface and dermis), fat, kidney, liver, rapidly and slowly perfused tissues, lung, stomach, and intestine. Any given model is derived by selecting the compartments and processes that are most applicable to the kinetics of the chemical(s) and endpoint of interest. ERDEM is programmed using the Advanced Continuous Simulation Language (ACSL). Model-specific parameter values are entered into ERDEM based on the physiological, biological, and biochemical modeling data specific to the chemical and/or scenario of interest. Any PBPK model, including ERDEM, is made up of a series of the differential equations that describe the rates of inflow, distribution, metabolism, or outflow of a chemical and various metabolites in each separate biological compartment.

ERDEM consists of an ACSL-based model engine and a power builder front end. Both of these components will be made available to the public as executables from EPA's Office of Research and Development (ORD)-NERL. No special software is required. An ACSL software license is needed to recompile the code and cannot be provided by EPA. However ERDEM

should require no additional recompilation of code to run the model as described in the document.

Model 2: PBPK Development Using MCSim Language

A second model is being developed in the MCSim language. MCSim is an open-source statistical modeling package initially developed by Frederic Bois and others for the application of modern Monte Carlo statistical methods in complex nonlinear models. Since MCSim includes a sublanguage for describing dynamic models in terms of their component differential equations and typical time-varying inputs, it has been particularly valuable in the application of Markov Chain Monte Carlo methods to estimating Bayesian posterior distributions for parameters of PBPK models.

Dynamic models in MCSim are written in an algebraic language. Model specification includes predefining all the parameters for the model, declaring all the variables whose dynamics are governed by differential equations, declaring all the variables whose values need to be output, specifying input variables whose values will be determined by special functions that provide for periodic or episodic inputs, and specifying the differential equations for the model. This model specification file is translated by the MCSim software into the C programming language. Then it is compiled and linked to libraries that provide routines for integrating the differential equation system, carrying out the required Monte Carlo simulations, and doing the input and output functions. The resulting executable file is then run with specially formatted input files that can change parameter values and specify the nature of the desired simulation, whether it is a numerical integration of the differential equation system, a Monte Carlo simulation of parameter variability or uncertainty, or a Markov Chain Monte Carlo estimate of Bayesian posterior distributions for model parameters.

MCSim models are portable at several levels. At the lowest level, since MCSim itself is open source, and since open-source C-language compilers are available for almost all computing platforms (e.g., UNIX, Microsoft Windows, and Apple OS-X), models can be distributed as model source and recompiled and run with little additional cost to reviewers. Compiled models are also executable files and can be run without any additional software (although the executables are specific to particular operating systems and computing hardware). Thus, the compiled models can be distributed and their behavior evaluated without the installation of additional software.