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Chapter 8. Dose-Response Modeling for 2,3,7,8-TCDD

Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds

Part II: Health Assessment for 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and Related Compounds

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8. DOSE-RESPONSE MODELING

8.1. INTRODUCTION

8.1.1. Overview

This chapter describes concepts that embody the evaluation of dose-response relationships for the dioxins and related compounds and examines dose-response models for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCDD is the most potent form of a broad family of xenobiotics that bind to an intracellular protein known as the aryl hydrocarbon receptor (AhR) (Chapter 2). Other members of this family, in addition to the polychlorinated dibenzodioxins (PCDDs), include polyhalogenated hydrocarbons such as the polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and polychlorinated naphthalenes (PCNs). In addition, there are other classes of chemicals that bind to the AhR, such as polynuclear aromatic hydrocarbons and naturally occurring compounds. A detailed discussion of the interactions of these chemicals and the concept of TCDD equivalence is presented in Chapter 9. The biological and toxicological properties of dioxins have been investigated extensively in more than 5,000 publications and abstracts since the identification of TCDD as a chloracnogen (Kimmig and Schulz, 1957). Some data sets on members of this family of compounds other than TCDD are clearly amenable to dose-response modeling. However, this chapter focuses exclusively on studies in laboratory animals that can be used to evaluate dose-response for TCDD. In addition, it evaluates human data where exposure to TCDD has been estimated and dose-response can be modeled quantitatively.

Most of the information presented in this introduction is found in more extensive detail later in this chapter or in the other parts of this reassessment. This introduction sets the stage for discussion of dose-response modeling of TCDD by briefly answering the questions, “what is dose?” “what is response?” and “what is modeling?” It then goes on to describe and, to a limited degree, compare different modeling approaches. This introduction also shows the reader the types of data and information available for TCDD that may have an impact on the development of dose-response models. Both in the introduction and throughout this chapter, gaps in knowledge relating to the evaluation of TCDD dose-response are identified. Understanding these gaps and their impact on the conclusions of this chapter can guide the design of new experiments that will add to our knowledge of TCDD action and clarify issues related to its dose-response.

8.1.2. What Is Dose?

When performing dose-response analyses, it is critical to understand what is meant by dose and how it applies to the response. The dose, in dose-response modeling, is an inclusive term. Examples of dose include the amount of TCDD given to an experimental animal by some specific route at some specific frequency, measured tissue concentrations in laboratory studies, body burdens attained in these studies, or daily exposure seen by workers in an occupational setting. In general, units of dose should reflect the magnitude of the exposure and the frequency over which it applies. Dose can be expressed in a multitude of metrics. Some of these metrics include daily intake (ng/kg/day), total body burden (ng/kg), body burden averaged over a given period of time, or tissue concentration. Depending on the particular endpoints to be compared, and in consideration of the half-life of elimination of TCDD (see Section 8.2), it may be possible to express dose in a form that allows comparison of response across various endpoints and species. Specific issues relating to dosage and comparison across species and endpoints are discussed in Section 8.2.

Most, if not all, of the effects elicited by TCDD are mediated by the ability of this chemical to bind to and activate the AhR. The activation of this protein leads to a series of molecular and biochemical events that ultimately contribute to particular biological responses (see Part II, Chapter 2). It is clear from the available human and animal data that TCDD can elicit many types of responses depending on the species, the age of the animal at exposure, and whether the exposure is acute or chronic. These responses vary from biochemical alterations such as enzyme induction, which may require only acute exposures, to developmental effects, which may require a level of exposure at a particular window of tissue development, to more complex responses such as cancer, which may require prolonged exposures (Section 8.1.3). To determine what might be the most sensitive endpoints, the species variation in sensitivity to these endpoints, and how these differences or similarities might be extrapolated to effects in humans, requires a comparison of the amount, or dosage, of TCDD that is present in particular tissues and/or the whole organism.

Dose is not always a known quantity. For humans, the actual dose is rarely known and best estimates are made on the basis of several assumptions and observations made at only a few time points, often many years after what may be believed to be the period of highest exposures. For these cases, models of exposure linked to response data may be used to develop a dose-response model. However, limited knowledge of the events that control tissue distribution (especially in humans at low levels of exposure) and those molecular and biochemical processes that ultimately lead to particular responses contribute uncertainty in these analyses.

8.1.3. What Is Response?

Response, in this context, generally relates to an observation seen in an animal or a human following exposure to TCDD. These responses cover a broad range of observations, ranging from early responses such as biochemical alterations that are closely coupled to activation of the AhR to more complicated responses such as cancer and developmental defects. The responses are sometimes species- and/or tissue-specific and have different degrees of variation across individuals. However, there is some commonality across species and there are known linkages between some responses (e.g., mRNA serves as a precursor molecule for the synthesis of protein). Dose-response modeling can address endpoints separately, provide insight into their quantitative similarity across species and tissues, and link responses in a mechanistically reasonable manner.

The binding of TCDD to the AhR is similar, although not identical, to the interaction of many steroid hormones with their intracellular receptors (Poellinger et al., 1987; Cuthill et al., 1991; DeVito et al., 1991; Lucier et al., 1993). An overall hypothesis for the mode of action of TCDD, put forth by several groups, is based on the transcriptional activation of expression of specific genes. This hypothesis has been most well characterized for transcriptional activation of the cytochrome CYP1A1 gene. There is also some evidence to indicate that activation of the AhR by TCDD may elicit responses by mechanisms that may not involve direct transcriptional activation of genes. The biological basis for these models of AhR action is outlined in Part II, Chapter 2. It is accepted by most researchers that most, if not all, cellular responses to TCDD require the initial interaction between TCDD and the AhR.

Although gaps in our knowledge remain, evidence to date is consistent with the hypothesis that binding of TCDD to the AhR and inappropriate activation of this protein represent the first steps in a series of biochemical, cellular, and tissue changes that define the toxicity observed. These changes are defined as responses to TCDD. Evidence to support this theory has been reviewed in several sections of this document as well as in the peer-reviewed literature (Safe, 1990; Birnbaum, 1994; Poland and Knutson, 1982). Many of the known biological activities of related PCDDs and PCDFs also appear to follow their rank order of binding affinity of the congeners and analogues to the AhR (see Part II, Chapters 2 and 9). This rank order holds for toxic responses such as acute toxicity and teratogenicity and for changes in concentration of several proteins, including the induction of cytochromes P-450 1A1 (CYP1A1), 1A2 (CYP1A2), estrogen receptor, and epidermal growth factor receptor (EGFR). The direct relationship between AhR binding and carcinogenicity of TCDD is less clear, although limited structure activity relationship studies on tumor promotion demonstrate a rank order in potency similar to binding to the Ah receptor (see Part II, Chapter 9).

The AhR has been identified in numerous mammalian species including humans (Okey et al., 1994; Roberts et al., 1985, 1986; Abbott, 1995; Manchester et al., 1987; Lorenzen and Okey, 1991; Cook and Greenlee, 1989), several non-mammalian vertebrates including chicken embryos

(Denison et al., 1986) and newts (Marty et al., 1989), and several aquatic species from whales to teleosts and elasmobranchs (Hahn, 1998). The broad phylogenetic distribution in vertebrate evolution (Hahn, 1998) and the phylogenetic conservation of this receptor also suggest that it has an important role in regulating cellular function in vertebrate animals. However, the physiological role or function of this receptor has yet to be determined.

Although the human data are limited, there is relatively good concordance for the biochemical/molecular effects of TCDD between laboratory animals and humans, indicating that animal models are generally appropriate for estimating human responses. Where wide species differences exist, understanding the relative sensitivity of human responses may not be possible at this time. However, many of the biochemical effects produced by TCDD and its analogues in animals also occur in humans. Data on effects of TCDD and its analogues in humans are based on in vitro (i.e., in cell culture) as well as epidemiological studies. Placentas from Taiwanese women exposed to rice oil contaminated with dioxin-like PCBs and PCDFs have markedly elevated levels of CYP1A1 (Lucier et al., 1987). Comparison of these data with induction data in rat liver suggests that humans are at least as sensitive as rats to enzyme-inductive actions of TCDD and its structural analogues (Lucier, 1991). Consistent with this contention, the in vitro EC_{50} for TCDD-mediated induction of CYP1A1-dependent enzyme activities is ~1.5 NM when either rodent or human lymphocytes are used (Clark et al., 1992). The human AhR appears to have greater than a twentyfold range in TCDD affinity (Okey et al., 1994). This range is comparable to that of the sensitive and resistant mouse strains as well as that of rats (see Chapter 2). It does appear that humans contain a fully functional AhR (Cook and Greenlee, 1989), as evidenced by significant CYP1A1 induction in tissues from exposed humans, and that this response occurs with similar sensitivity as observed in experimental animals.

One of the biochemical effects of TCDD that might have particular relevance to toxic effects is the loss of plasma membrane EGF receptor. There is evidence to indicate that TCDD and its structural analogues produce the same effects on the EGF receptor in human cells and tissues as observed in experimental animals. Incubation of human keratinocytes with TCDD decreases plasma membrane EGF receptor, and this effect is associated with increased synthesis of transforming growth factor-α (TGF-α) (Choi et al., 1991; Hudson et al., 1985). Placentas from humans exposed to rice oil contaminated with PCDFs also exhibited markedly reduced EGF-stimulated autophosphorylation of the EGF receptor, and this effect occurred with similar sensitivity as observed in rats (Lucier, 1991; Sunahara et al., 1989). The magnitude of the effect on autophosphorylation was positively correlated with decreased birth weight of the offspring.

Chloracne, a well-known response observed in highly exposed humans, has also been shown to occur in several animal species including nonhuman primates, rabbits, and hairless mice. However, it should be noted that in populations exposed to similar amounts of TCDD (e.g., Seveso,

Italy), some humans may exhibit chloracne while others do not. In mice, responsiveness to TCDD and related chemicals can be modified by genes as well as the AhR. For example, mice congenic at the hairless (*hr*) locus demonstrate altered sensitivity to the chloracnegenic and tumor-promoting effects of TCDD (Poland et al., 1982). These data suggest that there may be multiple factors (e.g., genetics) that may contribute to the development of a particular response both within and between species.

Several reports in the literature suggest that exposure of humans to TCDD and related compounds may be associated with cancer at many different sites, including malignant lymphomas, soft tissue sarcomas, hepatobiliary tumors, hematopoietic tumors, thyroid tumors, and respiratory tract tumors. These studies are evaluated in Part II, Chapter 7a, including discussion of confounding factors and strength of evidence. TCDD is a carcinogen in several species of laboratory animals (mice, rats, hamsters, fish) and the tumor sites include liver, thyroid, and the respiratory tract, as well as others.

Several noncarcinogenic effects of PCDDs and PCDFs show good concordance between laboratory species and humans (DeVito et al., 1995). For example, in laboratory animals, TCDD causes altered intermediary metabolism manifested by changes in lipid and glucose levels. Consistent with these results, workers exposed to TCDD during the manufacture of trichlorophenol showed elevated total serum triglycerides and cholesterol with decreased high density lipoprotein (Walker and Martin, 1979), similar to results seen in Air Force personnel following exposure to Agent Orange (Wolfe et al., 1990; Fallon et al., 1994). Another interesting finding of these studies was a positive relationship between TCDD exposure and diabetes (see Part II, Chapter 7b).

There are also differences between human and animal effects associated with TCDD. For example, chloracne has been observed in exposed humans but in only some animal species. Similarly, increases in humans of certain cancers such as soft-tissue sarcoma have not been observed in animals (see Part II, Chapters 6 and 7). Also, immunotoxic endpoints consistently seen in animals have rarely been demonstrated, or looked for, in humans (see Part II, Chapter 4). The recognition of these similarities and differences is essential when using animal data to estimate human effects. Understanding of these similarities and differences can substantially improve dose-response analysis.

The human-to-experimental-animal comparison is also complicated by several other factors:

(1) for most toxic effects produced by dioxin, there is marked species variation. An outlier or highly susceptible species for one effect (i.e., guinea pigs for lethality or mice for teratogenicity) may not be an outlier for other responses;

(2) human toxicity testing is based on epidemiological data comparing "exposed" to "unexposed" individuals. However, the "unexposed" cohorts contain measurable amounts

of background exposure to PCDDs, PCDFs, and dioxin-like PCBs. Also, the results of many epidemiological studies are hampered by small sample size, and in many cases the actual amounts of TCDD and related compounds in the human tissues were not examined; and

(3) In addition, it is often difficult, if not impossible, to assess in humans the same endpoints that might be determined in experimental animals (e.g., some immunotoxic effects and altered liver enzymes).

In summary, for many of the biological responses elicited by TCDD, animal models appear to be reasonable surrogates for estimating human risks. However, it must be kept in mind that the animal-to-human comparison would be strengthened by additional mechanistic information, especially the relevance of specific molecular/biochemical precursors to toxic responses. It is also important to note that the key events leading to carcinogenesis may be quite different at different sites (see Part II, Chapter 6).

8.1.4. What Is Modeling?

In the sciences, a model is a representation of how something works. Models are of several types, such as conceptual (e.g., a mental image of how something works), biological (e.g., transgenic mice as a surrogate for a human system), physical (e.g., a three-dimensional model of the human heart) and mathematical (e.g., a physiologically based pharmacokinetic model [PBPK]). Any model is defined by a set of parameters that make up its key components, and usually has inputs (e.g., dose) and outputs (e.g., response) that correspond to its real-world counterparts. Mathematical models of dose-response generally can be classed into two broad areas: empirical models and mechanism-based or mode-of-action models; these are described in the next two sections.

Modeling involves the application of a mathematical model to data as a tool to allow for analysis and prediction. Any modeling exercise requires the estimation of model parameters. The tools used to estimate parameters range from very simple techniques, such as estimating a slope of a straight line (linear regression), to extremely complicated approaches, such as estimation by maximizing a statistical likelihood function comprising unknown model parameters. In some cases, estimation of parameters in a model involves choosing a value based upon scientific judgment. The quality of any parameter estimate is dependent on the available data to characterize the model. The quality of the data and information used to develop a mathematical model is the major component in determining the confidence placed in any conclusions or predictions from that mathematical model.

Dose-response models for receptor-mediated events should use information on the quantitative relationships among ligand concentration, receptor occupancy, and biological response.

For example, Roth and Grunfeld (1985) state: "At very low concentrations of hormone receptor, occupancy occurs but may be trivial; i.e., the curve approaches 0% occupancy of receptors. But if there are 10,000 receptors per cell (a reasonable number for most systems), the absolute number of complexes formed is respectable even at low hormone concentrations. One advantage of this arrangement is that the system is more sensitive to changes in hormone concentration; at receptor occupancy (occupied receptors/total receptors) below 10%, the concentration of occupied receptors is linearly related to the concentration of hormone, whereas at occupancies of 10 to 90%, the concentration of HR is linear with log hormone concentration, a given increase in the concentration is more effective in generating occupied receptors at the lowest part of the curve than at the middle."

It is clear that multiple dose-response models are possible when considering ligand-receptor mediated events. For example, when there is a proportional relationship between receptor occupancy and biological response, occupancy of any number of receptors would produce a response, although it would be unlikely that the response could be detected if the number of receptors occupied was very low. Given this proportionality, a simple model, describing the response as a linear function of dose, may be adequate. However, such a simple relationship is unlikely to explain the diversity of biological responses that can be elicited by a single hormone utilizing a single receptor. For example, low concentrations of insulin produce much greater effects on fat cells than on muscle cells because fat cells have more receptors. These differences are due to cell-specific factors that determine the qualitative relationship between receptor occupancy and response. Similarly, it is expected that there are markedly different dose-response relationships for different effects of TCDD.

Coordinated biological responses, such as TCDD-mediated increases in cell proliferation, likely involve other systems, which means that the dose-response relationships for relatively simple responses (i.e., CYP1A1 induction) may not accurately predict dose-response relationships for complex responses such as cancer. Thus, it is necessary to consider what is known and observed regarding a biological response before a reasonable mathematical model can be applied to the data. Responses that include coordination of multiple steps that have linear dose-response relationships may ultimately produce markedly nonlinear dose-response relationships.

The goal of mathematical modeling should be to use as much data as possible to reduce uncertainties and to identify the areas where data gaps exist. Several important concepts have been generally accepted that may determine the types of mathematical models one might apply to responses due to exposure to TCDD:

(1) TCDD is a member of a class of xenobiotics (and probably natural products) that is not directly DNA reactive, binds to a cellular receptor, alters gene expression, and alters cell growth and development;

(2) a significant amount of information is available for estimating risks from exposure to this compound, and these data should be used to their fullest extent; and

(3) the biology of receptor-mediated events should be included to the greatest extent possible in any modeling exercise for TCDD, empirical or mechanism-based.

8.1.5. Empirical Modeling

By its very nature, data applicable to dose-response modeling can generally be expressed through groups of individuals (cells, animals, humans) exposed to a common level of a toxic agent (TCDD) for which some response is measured. Given sufficient numbers of exposure groups, it is possible to see a pattern arise, which indicates a change of that response as a function of increasing dose. Empirical dose-response modeling attempts to find a simple mathematical model that adequately describes this pattern. Empirical models generally have little or no direct linkage to the underlying mechanisms driving a given response, but instead focus on flexible mathematical forms that can fit a broad spectrum of data and allow comparisons across individual data sets. However, empirical models should be interpreted in light of information available on the biology of the modeled response and, in doing so, can provide qualitative insights into underlying mechanisms.

Examples of empirical models include linear functions (such as those used in linear regression), log-linear models, Poisson regression (commonly used in epidemiology), and Hill models (commonly used to analyze ligand-receptor data). Empirical models have the advantage of ease of use, the existence of "user-friendly" software tools capable of fitting these models to dose-response data , and a formal framework for hypothesis testing and interpolation between data points. In addition, empirical models can be used to estimate a point of departure for extrapolation. The major disadvantage of empirical models is their inability to quantitatively link multiple data sets in a mechanistically meaningful manner.

8.1.6. Mechanism-Based and Mode-of-Action-Based Modeling

In contrast to empirical modeling, mechanism-based modeling attempts to use an understanding of the mechanistic relationship between exposure and multiple endpoints to simultaneously describe the observed response. Mechanism-based modeling can be a powerful tool for understanding and combining information on complex biological phenomena (Lucier et al., 1993). Mechanism-based modeling commences from a series of experiments with a xenobiotic agent. The experimental results (data) can indicate a mechanism supporting the creation of a mathematical model. The predictions of that model are tested for consistency with the existing knowledge base for the agent and effect under study. Defects in the fit can suggest new experiments that may permit refinement of the model. On each iteration of this process, the model either gains additional credibility by predicting the new experimental results or it is modified to fit the new as

well as previous results. In either case, subsequent iterations of this process increase our confidence in accepting or rejecting a final model, although it may be difficult or impossible to quantify this confidence.

Mathematical models that incorporate parameters that correspond to actual biological structures or processes do not automatically constitute "mechanism-based models." The types of data available for the model and the method by which these data are incorporated into the model determine if a model truly reflects the biology. A parameter that specifies the activity of a xenobiotic metabolizing enzyme, for example, should have a biologically realistic value. Without careful attention to the representation of biological detail, confidence in the model and use of its results is reduced.

Ideally, the parameters in a mechanism-based model are derived from first principles in a "bottom-up" fashion. In this case, the structure of the model is an accurate mathematical representation of the known properties of the system being modeled, and the mechanistic parameters in the model are estimated directly from data. Such a model can increase confidence in extrapolating outside the range of the data as long as attendant uncertainties are carefully evaluated. In practice, it is generally impossible to completely develop a mathematical model for biological processes. At some point, processes by which the mechanistic events elicit the observed toxic effects must be deduced in a "top down" approach that uses some curve fitting. The concept of mode of action has been developed in response to this difficulty in implementing the "bottom up" approach (U.S. EPA Guidelines for Carcinogen Risk Assessment, EPA/600/Z96001). The term *mode of action* is defined as a series of key events and processes starting with interaction of an agent with a cell, through operational and anatomical changes resulting in cancer formation and other toxicities. "Mode" is contrasted with "mechanism" of action, which implies a more detailed molecular description of events. Operationally, the description of the mode of action should convey enough information to characterize the shape of the exposure-response curve. A risk assessment model based on the mode of action is preferable to empirical modeling when making inferences outside of the range of the effects data.

Without data (as is the case with extrapolated predictions), the statistical issue of the accuracy of a prediction cannot be easily addressed. Thus, while there may be greater biological confidence in extrapolated results, it is unlikely that an increased statistical confidence can be demonstrated. However, for each level and type of data, there are ranges of exposure beyond which it is impossible to demonstrate an effect because of limitations in the sensitivity of those assays. In general, effects can be demonstrated at lower exposures for mechanistic data (e.g., gene expression) than for toxicity data. Hence, use of a true mechanism-based approach should enable reliable and scientifically credible extrapolations to lower exposures.

Risk assessment typically involves extrapolations between species, from high to low doses, and between different patterns of exposure. Uncertainty in risk assessment is reduced to the extent that these extrapolations are based on mechanistic considerations. For TCDD, the mechanisms of three processes are of primary interest: (1) the dosimetry of TCDD throughout the body and specifically to target tissues; (2) the molecular interactions between TCDD and tissue proteins, emphasizing the activation of gene transcription and increases in cellular concentrations of growth-regulatory gene products and metabolic enzymes; and (3) the progressive tissue-level alterations resulting from these interactions that lead, eventually, to toxicity. Mechanism-based modeling for TCDD is the quantitative description of the mechanisms that define these processes. A model based on mechanistic understanding of the biochemistry of TCDD-induced toxicity and that accurately reproduces observed effects would permit more confident extrapolations to low doses and more reliable resultant risk estimates. As previously stated (Greenlee et al., 1991), "Neither the position taken by U.S. EPA or by Environment Canada (and several other countries such as Germany and the Netherlands) is based on any detailed mechanistic understanding of receptor-mediated interactions between TCDD and target tissues. In addition to their use in risk assessment, models of these processes can aid in the design of future experiments to clarify understanding of TCDD toxicity and support further risk estimation."

Several models ranging from very simple to complex have been developed to describe the toxicity of TCDD. It is obvious that the biology governing the toxicity of TCDD, beyond a few initial critical events, is not straightforward. These critical events, the first of which is binding to the AhR, are generally response-independent. The response-dependent events are species-, sex-, organ-, tissue-, cell- and developmental stage-specific. If binding to the AhR is essential but not sufficient for effects to occur, then the dose-response curve for this event (as well as the rate equations) should be a better predictor of biological action than external dose as long as the shapes of the dose-response curves for these subsequent actions are similar to those of receptor binding curves. In general, the available data indicate that receptor involvement is necessary for most if not all low-dose actions of TCDD. However, it is clear that for many responses, the dose-response curves are different from receptor binding curves. Furthermore, although the AhR has been detected in many kinds of cells, not all of these exhibit toxic responses. These data suggest that there must be other factors that are necessary for TCDD-induced toxicity. The roles of these cell-specific factors and how they affect the ultimate response must be elucidated before there is a complete understanding of TCDD action. However, a model may be developed for specific endpoints by using available data and biologically plausible assumptions.

TCDD can be considered as a prototype for exploring and examining the ability of mechanism-based modeling to improve the accuracy of quantitative risk assessment. The database for a mechanistic modeling approach to TCDD is extensive and contains a considerable amount of

information on low-dose behavior. In addition, there is some concordance between human data and experimental evidence in animals (see Section 8.3). On the other hand, some aspects of the mechanism by which TCDD induces its effects, such as binding of the AhR to accessory proteins, have not been modeled extensively because of lack of data. Because of this deficiency, several alternative mechanistic hypotheses may agree with the existing data. The role of mechanism-based modeling in this case is to identify a set of candidate biologically plausible models, rather than to provide a final description. This outcome is inevitable for the application of the technology of mechanism-based modeling to a new area. Reduction in the size of the candidate set and, eventually, identification of the preferred model must await additional results from the laboratory.

To reiterate an earlier point, mechanism-based modeling can aid in explaining and understanding experimental results, beyond its proposed use in risk assessment.

8.1.7. Elements of Chapter 8

The following sections of this chapter discuss the underlying science related to selection of appropriate dose metrics for dose-response modeling, empirical modeling of individual data sets, and mechanism-based dose-response modeling for biochemical responses and tissue responses. This modeling effort follows a natural progression related to the kind of information available at the time these models were developed. In addition, knowledge gaps have been identified throughout the chapter and have been consolidated in a section related to data gaps and research needed to address critical uncertainties that remain in the dose-response modeling of TCDD. Discussion of the strengths and weaknesses, assumptions and uncertainties, and implications of these TCDD dose-response modeling efforts follows. Detailed tables containing the outputs of the empirical dose-response modeling efforts are appended to this chapter for the benefit of those readers who wish a more detailed view of the data and analyses supporting the discussion and conclusions of this chapter. General conclusions are presented in a short summary statement that is found toward the end of this chapter.

8.2. DOSE METRICS

8.2.1. Introduction

One of the more perplexing issues in toxicology is animal-to-human dose extrapolation. To provide significant insight into differences in sensitivity among species, an appropriate animal-to-human extrapolation of tissue dose is required. Chemicals can produce many different types of responses depending on the exposure scenario and the response. Some responses are reversible (enzyme induction) whereas others are irreversible (death, cancer). Some responses require prolonged exposures (porphyria and cancer). Others have unique windows of susceptibility where an adverse effect (e.g., cleft palate) occurs only after a critical window of exposure (e.g.,

during development). The processes leading to particular toxic responses are highly divergent, with some responses requiring a continued exposure over a prolonged period of time and some requiring an exposure over only several hours. It is unlikely that a single dose metric will be adequate for interspecies and intraspecies extrapolation for all of these endpoints.

Estimating risk to various human populations is complicated by differences in exposure scenarios. Human exposures to high levels of dioxins have occurred in several different scenarios. There have been industrial accidents that have resulted in high exposures over a very short period of time, such as the explosion at the ICMESA trichlorophenol plant near Seveso, Italy, in 1976 (Ghezzi et al., 1982) and the BASF chemical plant in Ludwigshafen, Germany, in 1953 (Zober et al., 1990). Increased daily exposures over background to dioxins have occurred in occupationally exposed populations using some herbicides, for example, during the Vietnam War (Verger et al., 1994) and in agricultural workers (Kogevinas et al., 1995). Routine occupational exposures have occurred in several manufacturing facilities around the world. The final type of human exposure occurs in the general population, which is exposed daily to TCDD in the diet at a dose rate of approximately 0.14 to 0.4 pg/kg/day[1] (see Part I). One of the difficulties in examining and comparing these different populations is that the actual dose or exposure is rarely known. Estimates are often based on present serum TCDD concentrations, with extrapolation back to the initial time of exposure based on the half-life of TCDD in humans (Fingerhut et al., 1991; Scheuplein and Bowers, 1995).

In contrast, the exposures in animal experimentation are controlled and well defined. Animal studies use multiple dosing regimens including single acute exposures, chronic daily exposures, and biweekly exposures. Comparison across species sometimes requires extrapolation from one exposure scenario to another. Large differences between species and the half-life of TCDD, and quantitative differences in the tissue distribution of TCDD, must be considered (van der Berg et al., 1994).

Determining the most appropriate dose metric represents an additional difficulty when different endpoints and species are compared. Comparison of responses across species requires the expression of dose using an equivalent metric. Dose can be expressed in a multitude of metrics (DeVito et al., 1995) such as daily intake (ng/kg/day), current body burden (ng/kg), average body burden over a given period of time, plasma concentration, concentration of occupied AhR (Jusko, 1995), induced CYP1A2 (Andersen et al., 1997a; Kohn et al., 1993), and reduced EGFR (Portier and Kohn, 1996).

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[1]Calculated from human daily dietary dose of 10 to 20 pg/day TCDD and human body weights between 50 and 70 kg; it should be noted that, on a total TCDD equivalents (TEQ) basis, total daily intake equals approximately 70 pg/day (see Part I) (see Chapter 9 for discussion of TCDD equivalents).

Different dose metrics can lead to widely diverse conclusions. For example, the lowest dose with an increased tumorigenic response (thyroid tumors) in a rat (NTP, 1982a) is 1.4 ng/kg/day and the daily intake in humans is approximately 0.14 to 4 pg/kg/day. This implies that humans are exposed to doses 3,500 to 10,000 times lower than the rat dose. However, 1.4 ng/kg/day in the rat leads to a steady-state body burden of approximately 25 ng/kg, assuming a half-life of TCDD of 23 days and absorption from feed of 50%[2]. The current body burden of TCDD in humans is approximately 5 ng/kg lipid or 1.25 ng/kg body weight (assuming about 25% of body weight is lipid), suggesting that humans are exposed to about 20 times less than the minimal carcinogenic dose for the rat. The difference between these two estimates is entirely due to the approximately 100-fold difference in the half-life between humans and rats. At least for this comparison, the most appropriate metric for comparison is the steady-state body burden. (Note that current daily intake for humans is likely lower than historical levels and is biased downward because of unknown sources, leading to a discrepancy between body burdens and daily intake.

In addition to the uncertainty in the half-life of TCDD in humans, such calculations assume exposure to TCDD at a constant rate rather than the actual episodic exposure scenarios generally seen in the studied populations. In principle, a reliable PBPK model for humans could be used to compute body burden, tissue dose, or any other desired dose metric for any dosing scenario. However, as outlined in Section 8.4, the existing data are inadequate for this extrapolation. If time courses of TCDD in human blood were available for widely different doses, metabolic parameters for humans could be estimated. Inclusion of these quantities in a PBPK model would permit the calculation of a tissue dose or body burden to be used for risk assessment.

The developing embryo represents a very different complication in choosing a correct dose measurement. The susceptibility of a developing embryo or fetus to TCDD insult may be dependent upon the stage of development. For example, susceptibility to TCDD-induced cleft palate has a specific window of sensitivity. Once the palatal shelves fuse, cleft palates cannot be induced by TCDD. These windows of susceptibility are on the orders of hours to days. One of the difficulties is that the time span is often too short to clearly discriminate among dose metrics such as peak concentration, steady-state body burden, or average body burden. When these types of comparisons for TCDD are attempted, it appears that they are of equivalent utility, provided the dose metric was determined only during the window of sensitivity. In both animals and humans, the biological half-life of TCDD is much greater than the time span of the window of susceptibility. Hence, an average measurement or a peak measurement can be used as an appropriate dose metric.

[2] Steady-state body burden (ng/kg) = daily dose (ng/kg/day) [(half-life/ln(2)] (f where f is the fraction absorbed from the exposure route (unitless) and half-life is the half-life in days.

The windows of susceptibility for some of the developmental toxicities of TCDD have been identified (i.e., induction of cleft palate and hydronephrosis). Peak body burden may be a more appropriate dose metric for developmental effects because the window of susceptibility is undefined for several endpoints.

Ideally, the best dose metric is that which is directly and clearly related to the toxicity of concern by a well-defined mechanism. For mechanism-based cancer modeling, instantaneous values of a dose metric are used because these can be used as surrogates for mutational rates and growth rates within a two-stage cancer model. For epidemiology studies of lung cancer and all cancers combined, there is not enough information to develop a mechanistic approach. In this case the chronic exposures generally thought to be associated with the cancer process can be described by metrics that integrate dose over a specific time period., and an average body burden dose metric is acceptable for steady-state conditions. However, difficulties arise when this metric is applied to accidental high acute exposures. To allow for comparison across studies, it is sometimes useful to find a constant daily exposure or steady-state body burden that yields the same total exposure. Comparability of response over multiple species for a given dose metric can be used to assess the adequacy of that metric. It should be noted that for compounds like TCDD with very long half-lives, relative differences between doses expressed as steady-state body burden versus those expressed as total exposure may be small for humans, although the same may not be true in experimental animals where the half-life is much shorter.

8.2.2. Selection of Effective Dose Levels.

Comparisons across multiple endpoints, multiple species, and multiple experimental protocols are too complicated to be made on the basis of the full dose-response curve. Comparisons of this sort can be made by either choosing a given exposure and comparing the responses, or choosing a particular response level and comparing the associated exposures. In the analyses for the presentations in this chapter, responses are compared using estimated exposures associated with a given level of excess risk or response. To avoid large extrapolations, this common level of excess risk or response was chosen such that for most studies, the estimated exposure is in or near the range of the exposures in the studies being compared (Murrell et al., 1998; Gaylor and Zheng, 1996; Barton and Das, 1996; Allen et al., 1994a,b; McGrath et al., 1995), with extra weight given to the human data. A common metric for comparison is the effective dose, or ED_p, which is the exposure dose resulting in a excess risk in the studied population. Although effective dose reporting for the 2%, 5%, and 10% increased risks has been the suggested approach, these latter two levels are actually higher than those typically observed in the exposed groups in studies in humans. To illustrate, lung cancer mortality has a background lifetime risk of approximately 4% (smokers and nonsmokers combined), so that even a relative risk of 2.0 represents approximately a 4% increased

lifetime risk. On the basis of this observation, and recognizing that many of the endpoints studied in the laboratory include 1% effect levels in the experimental range, the dose resulting in a 1% effect above controls (ED_{01}) is presented.

Different measures can be used to present risks above and beyond the background risks encountered in the general environment or through genetic variables. For simplicity, a common measure will be used; the excess risk, defined as the effective dose for risk (p*100%), satisfying the relationship in equation (1):

$$p = \frac{R(d_p) - R(0)}{R(\infty) - R(0)} \qquad (1)$$

where $R(d_p)$ represents the response (either risk or other measure) at p at a given exposure or dose level d, and $R(\infty)$ is the maximum response possible (e.g., $R(\infty)$ =1 for quantal responses, such as cancer). In this exercise p is equal to 0.01.

The relative risk commensurate with a one percent excess risk can be calculated by rearranging the above formula:

$$\text{Relative Risk } (ED_{01}) = 0.99 + \frac{0.01}{R(0)}$$

Multiplying the relative risk by R(0), the background risk, gives the value of the absolute risk. If the background risk is 0 then the absolute risk equals the excess risk.

In the present analysis, the benchmark effect level has been specified as a 1% increase in the extra risk. Quantal data is determined as a probability on a scale of zero to one. Hence the difference between the probability of an adverse response at a given benchmark response level and the probability of a response at background is already on a standardized scale. In contrast, estimating the extra risk for continuous data is challenging. The changes in a continuous response that are considered adverse depend on the nature of the response that is determined. The change in effect that results in a significant public health problem is different for every response determined. In addition, the study design can influence this value. In order to have a consistent response level between endpoints, the measurement of response must be standardized between endpoints.

As outlined in Murrell et al (1998), there are several methods proposed to standardize continuous data. One method uses a specified change relative to background and is calculated according to the following equation:

$$E_{relative} = \frac{F_\theta(d) - F_\theta(0)}{F_\theta(0)}$$

where $F_\theta(d)$ is the function relating the response to dose *d*. There are some problems with this approach. $E_{relative}$ is now highly sensitive to the background response. For example, a small change in a response with a small background may seem more important compared to the same effective change in response with a large background. In addition, it is not clear that a certain percent change from background is an equivalent risk for all endpoints. For example, a 30% change in a heart rate may not be an equivalent risk as a 30% change in serum porphyrin concentrations. Therefore, using $E_{relative}$ does not result in a standardized risk level by which one could compare across endpoints.

Another proposed standardization method is to divide the change in effect by the standard deviation of the control group or from an assumed distribution of the mean effect for a particular dose group (Crump, 1984; Slikker et al., 1996). Because the standard deviation may vary for a variety of reason independent of the health importance of the effect, this method does not necessarily standardize across a variety of endpoints and experimental conditions.

Ideally, the benchmark effect level is one that separates a normal or no-effect level from abnormal or adverse effect levels (Crump, 1995). One of the difficulties in applying this approach are the assumptions that are made in the determination of the benchmark level. Often, there is not a clear consensus as to when a change in a continuous response becomes adverse. Because of the lack of a consensus on adversity levels for many of the effects examined in this analysis, this method was deemed inappropriate to use as a means of standardization across endpoints.

In the present analysis, the continuous data is standardized by the dynamic range of response for each effect (Murrell et al., 1998). Similar to quantal data, continuous data also has maximal response levels. Thus one can define extra effect as the change in the effect from background as standardized to the total range of the response. Dividing the change in effect by the theoretical or observed maximum produces a quantity that is standardized across endpoints with respect to scale.

8.2.3. Dose Corrections for Species Differences in Half-Lives

Considering the very large difference between half-lives of TCDD in various species, it is best to compare across species using body burden rather than daily intake (DeVito et al., 1995). Under steady-state conditions, it is possible to calculate total body burdens (ng/kg) for TCDD in equation (2).

$$ED_{01}(\text{ng/kg body burden}) = ED_{01}(\text{ng/kg/day}) * \text{half-life}/\ln(2) * f \qquad (2)$$

where f is the fraction of dose absorbed and is assumed to be 50% for absorption from food (Kociba et al., 1976) and 100% for other routes. Half-lives for converting between daily exposures and steady-state body burden are presented in Table 8-1.

In summary, the unit(s) of dose should appropriately reflect the magnitude of exposure and the frequency of this exposure. Given the various types of exposure scenarios and different types of responses, it is difficult to determine a single dose metric for TCDD that can be used to compare all endpoints and species. Nevertheless, for several types of specific endpoints, it is possible to express the dose of TCDD in a form that allows for a comparison of responses across various endpoints and species. For the analysis contained in this chapter, various measures of body burden will be used.

8.3. EMPIRICAL DOSE-RESPONSE MODELING OF INDIVIDUAL DATA SETS

8.3.1. Introduction

TCDD has been previously classified by EPA as a probable human carcinogen, and has more recently been classified as a known human carcinogen by the International Agency for Research on Cancer (IARC, 1997). In the Ninth Report on Carcinogens, the U.S. Department of Health and Human Services describes TCDD as "known to be a human carcinogen" (HHS, 2001). Epidemiological data have suggested increases in all cancers combined, respiratory system tumors, and soft-tissue sarcomas (see Chapter 7 for a detailed discussion of these findings).

TCDD is a carcinogen in all species and strains of laboratory animals tested (e.g., mice, rats, hamsters) with tumors detected in the liver, thyroid, respiratory tract, and other organs and tissues (see Part II, Chapter 6). Long-term rodent carcinogenicity studies have shown that TCDD is a potent carcinogen, with the most seriously affected organ being liver in female rodents (NTP, 1982a,b; Kociba et al., 1978; Portier et al., 1984).

8.3.2. Human Dose-Response Models

Despite the increasing amount of epidemiological data available for TCDD, it is generally difficult to find human data with sufficient information to model dose-response relationships. Unlike laboratory studies, human data can be affected by factors that are difficult to control. There exists the possibility of disease misclassifications, and measurements of exposure are often imprecise. However, risks studied in human populations do not require assumptions concerning species extrapolation and, as such, should be used maximally in studying dose-response. TCDD is no different in this regard, with several epidemiological studies providing varying degrees of utility for dose-response assessment. This section discusses those studies and the models that have been applied to them.

8.3.2.1. *All Cancers Combined and Lung Cancer*

There exist three studies of human occupational exposure that provide enough information to perform a quantitative dose-response analysis. These are the NIOSH study (Fingerhut et al., 1991; Steenland et al., 1999; Steenland et al., 2001), the Hamburg cohort study (Manz et al., 1991;

Flesch-Janys et al., 1995, 1998a; Becher et al., 1998), and the BASF cohort study (Zober et al., 1990).

8.3.2.1.1. ***NIOSH study*****.** NIOSH conducted a cohort study of 5,172 male workers at 12 plants in the United States that produced TCDD-contaminated chemicals (Fingerhut et al., 1991). They reported increased mortality for total cancers and for respiratory cancers for workers with greater than 1 year of exposure and more than 20 years latency since start of employment.

Steenland et al. (1999) performed an analysis of male workers from eight of the twelve plants in the NIOSH study (the plants with sufficient information on work histories and TCDD levels on the job) who had no exposure to pentachlorophenol. Exposure was measured using a job-exposure matrix. Cumulative TCDD exposure scores per day were assigned by multiplying TCDD concentration in industrial materials, fraction of work day spent working with TCDD-containing materials, and a qualitative degree-of-contact measure. The exposure scores for each day were added to get a cumulative exposure score, which cannot be interpreted as units of TCDD. Workers were divided into septiles by levels of this exposure score (with or without a 15-year latency taken into account).

SMRs were calculated by septile for all cancers and for lung cancer. SMRs for all cancers for 0 or 15 years latency showed a statistically significant ($p<=.05$) positive trend with exposure score, as did the SMRs for lung cancer with no latency. Cox regression analyses were also performed to compare high-exposure groups to the lowest exposure group. For the data with zero latency, rate ratios for all cancers did not show a significant positive trend with exposure. For data with 15-year latency, the analysis was performed for all cancers combined, lung cancers, smoking-related cancers, and non-smoking-related cancers. The rate ratios for all cancers and for non-smoking-related cancers showed a significant positive trend; ratios for lung cancer and for smoking-related cancer showed no significant trend with cumulative exposure, though they did show a significant trend with logarithm of cumulative exposure. ("Smoking-related cancer" here means cancer that has historically been associated with smoking; the smoker or nonsmoker status of workers was not itself included in the analysis.)

Steenland et al. (2001) extended their analysis of these workers to include estimated dioxin exposures. Serum lipid levels of TCDD in 1988 were measured in 193 workers at one of the eight plants in the study. First-order kinetics with a constant 8.7 year half-life were used to extrapolate back to serum level at time of last exposure. These serum levels were regressed on the exposure scores, using a first-order model for exposure between first exposure and last exposure (the resulting predicted serum level and the observed levels have correlation coefficient of 0.62). The formula derived by regression was used to estimate serum TCDD levels for all 3538 workers in the

cohort, and then to estimate serum TCDD areas under the lipid adjusted serum level curves over time (AUC).

Several different dose-response models were fit to these data to provide estimates of risk for dose-response assessment. The best-fitting model used the log(AUC) lagged by 15 years as the exposure metric. The analysis used Cox regression and had date-of-birth as a categorical variable (4 categories). Excess risk of cancer for intake of 1pg/kg/day for 75 years of exposure was estimated as 0.0094 for males and 0.0080 for females. This analysis assumes a background exposure of 0.5 pg/kg/day as background. The analysis was also carried out using log of TEQ AUC as a dose variable. Lifetime excess cancer risk using the TEQ model with an intake of 10 pg/kg/day TEQ was 0.0018 for males and 0.0015 for females.

A piecewise linear model fit nearly as well as the model using log AUC. Its risk estimates for an intake of 1 pg/kg/day TCDD for 75 years were 0.0005 for males and 0.0004 for females. When the background exposure included TEQ's, the risk estimates for an intake of 10 pg/kg/day TEQ for 75 years were 0.0005 for both males and females. These numbers appeared low and after discussing this with Dr. Steenland, he noted an error in the calculation and the corrected numbers are 0.0071 for males and 0.,0060 for females; in line with the numbers for the TCDD only analysis.

Aylward et al. (1996) presented a dose-response analysis using data from Fingerhut et al. (1991), considering only cancers occurring after 20 years of exposure. This analysis is superceded by the extended follow-up and exposure matrix used in Steenland et al (2001).

8.3.2.1.2. ***Hamburg cohort study*****.** Another cohort studied consisted of 1,189 men who worked at a herbicide plant in Hamburg, Germany (Becher et al., 1998; Manz et al., 1991; Flesch-Janys et al., 1995, 1998a). Flesch-Janys et al. (1995) used an estimate of TCDD levels in workers in their analysis. Levels of TCDD were measured in blood or adipose tissue for 190 male workers in the cohort. Levels at the end of employment were estimated using a first-order kinetic model, and the contribution of each of several job areas was estimated by regression of the TCDD level on time worked in the job areas. The regression results were used to calculate TCDD concentrations (ng/kg of blood fat) at the end of the occupational exposure for each member of the entire cohort. The cohort was divided into the lower four quintiles and ninth and tenth deciles of the calculated value. Cox regression was used to calculate relative risks for cancer mortality. Relative risks were calculated using either an external reference group (control group of gas workers) or the lowest two quintiles of the Hamburg cohort combined as internal reference. Variables used in the regression were TCDD level (categorized by quintiles), total duration of employment, age, and calendar year of first employment. A test for trend of the relative risks with increasing TCDD concentration was conducted. In the calculations using either reference group, the trend test was significant at $p<0.05$. Standard mortality ratios (SMRs) were calculated on the basis of the national mortality data

available from the German Federal Office of Statistics using standard methods (Breslow and Day, 1987). The SMRs for the tenth decile of TCDD concentration were significantly elevated, whereas none of the SMRs for lower TCDD concentration categories were significantly elevated in the comparison with the lowest two quintiles combined. In the comparison with the gas worker controls, SMRs were 129 or higher. The increase was significant for three of the five categories.

Flesch-Janys et al. (1998a) extended this analysis using mortality up to 1992 and calculating time courses for TCDD concentration in blood lipid. Workers were divided into quartiles by integrated blood concentrations over time and SMRs were calculated. For total cancer mortality, the mortality was significantly increased for the highest quartile (SMR 173; 95% CI=121-240) and for all workers combined (SMR 141, 95% CI=117-168). The overall cancer SMR is increased over the results of Manz et al. (1991), which included mortality only up to 1989. For all workers combined, lung cancer mortality was significantly increased (SMR 151, 95% CI= 107-208), but the SMRs were not significantly over 100 for any of the individual quartiles. A linear trend test on the SMRs by quartile was significant for total cancer deaths (p=0.01) but not for lung cancer deaths.

Another recent article (Becher et al., 1998) gave a dose-response analysis of the Hamburg cohort for all cancers combined. A Cox regression was used for the dose-response modeling. Three response models were used: a multiplicative model, an additive model, and a power model. The response variable in the analysis was SMR for total cancer mortality. The dose variable was the integrated blood levels for TCDD concentration (AUC) as calculated by Flesch-Janys et al. (1998a). Year of entry into employment, age at entry, duration of employment, and an exposure metric for beta-hexachlorocyclohexane were also used as covariates in the model. The models were calculated with latency times of 0 and 10 years. The dose-response was modeled using three classes of models. The "multiplicative model" had relative risk (RR) equal to $\exp(\beta d)$, where the dose d is the AUC. The "additive model" had $RR=1+\beta d$, and the "power model" had $RR= \exp(\beta \log(kd+1))=(kd+1)^{\beta}$. The value β and k are estimated parameters. The multiplicative model gave the best fit, but the fits for the three classes of models were so close that Becher et al. found no statistical reason to select between them. In all cases, the value of β was significantly different from 0 ($p<0.05$). The model results were used to calculate unit risk estimates, i.e., estimates for (risk of cancer death through age 70 given a daily dose of 1 pg/kg body weight of TCDD) – (risk given no exposure to TCDD). These calculations were based on background German mortality rates. The unit risks for intake of 1pg/kg/day of TCDD ranged from 0.0011 (risk for females under the multiplicative model with 10-year lag) to 0.0084 (risk for males under the power model with no lag).

Becher also gave results for a Cox regression for lung cancer deaths using the multiplicative model. The resulting risk (value of β) was close to that for the model of all cancer deaths.

8.3.2.1.3. ***BASF cohort study*****.** Zober et al. (1990) studied a cohort of 247 workers from a 1953 accident at a BASF factory in Germany that released TCDD into the factory. Overall cancer mortality for all workers combined was not significantly increased. However, for the 127 workers who developed either chloracne or erythema, and for a 20+ year latent period, mortality from all cancers was increased (SMR=201; 90% CI=122-315). There was also an increase in cancer mortality with a 20+ year latency for a subcohort of 153 workers who were considered most likely to have been exposed to TCDD (SMR 198; 90% CI=122-305).

Another study of the BASF cohort (Ott and Zober, 1996a) included 243 male workers. Chloracne status and estimated TCDD concentration (μg/kg body weight) at time of exposure were used as metrics of exposure. The concentration was calculated by a first-order kinetics model using a regression procedure. Subjects were divided into 3 or 4 groups by concentration. SMRs were calculated by dose group. Standardized incidence ratios were calculated by dose group for all cancers and for cancers at various sites. Neither total cancer mortality nor respiratory system cancer mortality was significantly increased overall, although respiratory cancer mortality was increased in the highest of three TCDD concentration groups (SMR 240, 95% CI=100- 500). The incidence was not significantly increased for all cancers or respiratory cancers, either overall or in any concentration subgroup. This study also included a dose-response analysis by a Cox proportional hazard model, which calculated relative risks, with cigarette smoking, body mass index, exposure to asbestos, exposure to aromatic amines, age, and date of first exposure included as explanatory variables. TCDD dose was found to be marginally significantly related to total cancer deaths (conditional risk ratio for 1 μg TCDD/kg body weight = 1.22; 95% CI=1.00-1.50), but not significantly related to respiratory cancer deaths or to incidence of either. There also appeared to be a trend for increasing total cancer deaths by TCDD level in smokers and in all workers, but not in nonsmokers or ex-smokers.

8.3.2.1.4. ***Other studies.*** Hooiveld et al. (1998) studied former workers at an herbicide factory in the Netherlands. A back-calculation and regression method was used to estimate peak TCDD concentration for all workers. A total of 1,031 male workers were divided into groups of low, medium, or high estimated peak TCDD level (cutpoints were 7.7 and 124.2 ppt). These groups were approximately tertiles of the TCDD level. Relative risks (RR) of mortality were calculated for the high and medium groups versus the low group, with adjustment for age, time of follow-up, and time since first exposure. Relative risks for total cancer deaths were significantly increased for both medium (RR 1.9, 95% CI=1.2-2.8) and high (RR 1.9, 95% CI=1.3-2.8) exposure groups, but with no apparent trend. Some relative risks for specific cancer types were marginally significant, but with no apparent trend from medium to high exposure. Not enough information is given in this

study to calculate average body burden. In the cohort of residents from Seveso, Italy (Bertazzi et al., 1993), a single episode of exposure to TCDD occurred following an explosion at a local chemical plant. Men, women, and children from this community have been followed for cancer mortality for 15 years. However, this study could not be included in this analysis because the limited exposure information is not sufficient at present to calculate average body burden. Two other studies were also not included in this analysis for various reasons. Kuratsune et al. (1998) reported increased lung cancer mortality in male victims (SMR = 330, based on eight cases) from the Yusho PCB and PCDF contaminated rice-oil poisonings. Although there are serum measurements and 37 total TCDD equivalents (TEQ) estimates available for this cohort, there was no TCDD in the contaminants reported. Because this chapter has focused primarily on the effects of TCDD, this cohort will not be included in the modeling effort. In addition, Collins et al. (1993) reported increased mortality for both lung cancer and all cancers combined for a subcohort of 122 U.S. workers who developed chloracne following exposure to TCDD at a chemical plant during a 1949 accident. Their analysis, however, attributes this increase in mortality to co-exposure to 4-aminobiphenyl. As that chemical plant is included in the NIOSH study cohort (Fingerhut et al., 1991), it is discussed in Chapter 7.

8.3.2.2 *ED and Unit Risk Calculations*

Life table data (total death risk by age and percent of deaths due to cancer by age) from 1995-1997 were obtained from the National Center for Health Statistics (NCHS, 1999). Cancer deaths through age 75 due to TCDD exposure of 1 ppt body burden over background (background assumed to be a steady-state lipid concentration of 5 parts per trillion; 1 ppt body burden above background equals 4 ppt lipid concentration over background) were calculated using the best-fitting models for the NIOSH data (Steenland et al., 2001), the Hamburg data (Becher et al., 1998), and the BASF data (Ott and Zober, 1996). In these calculations, exposure was assumed to be at steady-state TCDD body burden.

The models literature used by Steenland et al. and Becher et al. use TCDD or TEQ lipid concentration in calculating the AUC; lipid concentrations were converted to body burdens by dividing by 4. For those models, the exposure levels were used to calculate AUCs with a time lag included as specified by the model.

The model used by Ott and Zober (1996) gives risk in terms of conditional risk ratio per unit TCDD dose. Units for TCDD were μg/kg body weight at time of initial exposure to TCDD. For purposes of the current analysis, it is necessary to convert from units of steady-state body burden to Ott and Zober's units of initial dose. Assuming a constant half-life of 2593 (approximately 7.1 years) as in Table 8-1, an initial body burden of B_0 will yield a body burden at time t of

$B(t) = B_0 e^{-k_e t}$, where k_e is an elimination constant equal to ln(2)/(half-life in years). This implies that the AUC at time T after initial exposure is $AUC = \frac{B_o}{k_e}\left(1 - e^{-k_e T}\right)$. T in this case will be 39 years (time from the accident in 1953 to the followup in 1992). Dividing by a lifetime of 71 years (mean age in 1954, 33 years, plus 38 years from 1954 to the followup in 1992) gives the lifetime mean body burden as:

$$B_{mean} = \frac{B_o}{71 k_e}\left(1 - e^{-k_e T}\right)$$

.

In the risk calculations, therefore, the steady-state body burden will be converted to units of equivalent initial dose by dividing by the constant $\frac{1}{71 k_e}\left(1 - e^{-k_e T}\right)$. With the given values for half-life and T, that constant is 0.1411 and 1/(the constant) is 7.0851. The model from Ott and Zober has risk proportional to $e^{\beta \times dose}$ with β=ln(1.22). The corresponding slope for the mean (steady-state) body burden is 7.0851*log(1.22)*0.001 (the 0.001 converts nanograms to micrograms) and the slope for steady-state lipid concentration is that value divided by 4.

ED_{01}, ED_{05}, and ED_{10} values were calculated by finding the dose giving the specified excess risk. All estimates were calculated using the same methods as the original author with estimates of risk from the background exposure subtracted from the mortality data.

ED values are given below in Table 8-2. The values are exposures above background exposure which will produce the given level of excess risk. The table also gives unit excess risks: the excess risk for a unit exposure above background, given for exposure of 1 ppt body burden above background. Steenland et al. (2001) provide sufficient information to develop confidence bounds for the calculations using their models. Confidence limits for the models using the Hamburg data could not be calculated due to insufficient detail in the manuscript. Because the lower confidence limit for the risk value in the Ott and Zober (1996) model is zero (conditional risk ratio of 1.00), the lower confidence limits for unit risk are zero and the upper confidence limits for the ED values are infinite. The power model from the Steenland et al. (2001) data predicted that an unrealistically large fraction of the tumors seen in humans was due to background dioxin exposure.

8.3.2.3. *Noncancer Endpoints*

8.3.2.3.1. ***Cardiovascular disease***. A pattern of increased risk of cardiovascular and ischemic heart disease mortality was observed by Flesch-Janys et al. (1995) across six exposure categories. There was a statistically significant trend (p=0.04) in relative risk for mortality for all cardiovascular diseases when gas workers were used as the reference population, but in no single class of TCDD exposure was there a significantly increased relative risk. There was no statistically significant trend for death from ischemic heart disease (p=0.1), but the highest TCDD group (344.7-3,890.2 ppt) showed a significant relative risk of 1.99 (CI=1.05-3.75). When national rates were used for the reference population, there were no statistically significant trends for either disease, and all confidence intervals included 1. Information about time-average body burden could be obtained from Flesch-Janys et al. (1998 a,b). With these data, an excess body burden over background (95% lower bound) for 1% excess risk was calculated as 11.2 ng/kg (3.1 ng/kg) for all cardiovascular disease, assuming a lifetime risk of 25%. No statistically significant increase of cardiovascular diseases was observed for the NIOSH cohort (Steenland et al., 1999) or for the BASF cohort (Zober et al., 1990, 1994).

8.3.2.3.2. ***Effects on infants***. One major public health concern is the potential effects of environmental chemicals on the developing fetus, infants, and children. TCDD and related chemicals produce a broad range of effects in experimental animals exposed in utero ranging from alterations in biochemical parameters to overt toxicity and lethality (see Chapter 5 for a review). Few studies have examined the effects of TCDD and related chemicals in humans following in utero exposures. Studies in the Netherlands (Huisman et al., 1995; Koopman-Esseboom, 1996; Weisglas-Kuperus et al., 1995) have examined infants for thyroid hormone status, mental and psychomotor development, and immunological status. Exposures were assessed by determining the concentrations of PCBs, PCDFs, and PCDDs in maternal and umbilical blood and maternal breast milk. Exposures were then categorized by total TCDD equivalents (TEQs), Planar-PCB TEQ, nonplanar-PCB TEQ and total dioxin-PCB TEQs. (For a discussion of the TCDD toxic equivalency concept, refer to Chapter 9.) These studies are discussed in greater detail (design, analysis, and limitations) in Chapter 7. There is an indication that these data would be amenable to dose-response analysis for complex mixtures of PCDDs, PCDFs, and PCBs, but not for TCDD exposure alone.

8.3.2.4. *Uncertainties in Estimates From Human Epidemiology*

There are many uncertainties associated with risk estimates derived from epidemiological studies, both in hazard identification and in dose estimation. The estimates of dose, although based on actual body measurements, may not be fully representative or precise. Although 253 subjects were sampled in the Fingerhut et al. (1991) study, the blood samples were all taken decades after

last exposure and were from 2 of a total of 12 plants. Subjects from the larger of these two plants had the higher TCDD levels but a lung cancer SMR=72 based on seven deaths, whereas the smaller plant had only one death from lung cancer (SMR=155). Thus, while serum TCDD levels correlated well with duration of occupational exposure for the 253 individuals sampled, and cancer response correlated well with duration of exposure for the 12 plants overall, correlation of serum TCDD levels with cancer response in this study is far less certain. Analysis by plant in the Fingerhut et al. (1991) study would have been possible if body measurements at these other 10 plants had been available.

The choice of half-life is another element of uncertainty. In the literature, average body burden was calculated on the basis of a one-compartment model with first-order elimination. Half-life assumptions in the literature vary. Some data, however, suggest a shorter half-life of as little as 5.8 years (Ott and Zober, 1996b) while others suggest a longer half-life of 11.3 years (Wolfe et al., 1994). A recent study (Portier et al., 1999) suggests a half-life of 9.5 years. However, the assumption of a single half-life is uncertain because it is possible that in humans the apparent half-life may be shorter at higher levels of exposure, as has been observed in rat liver (Walker et al., 2000). If this were the case, the actual initial exposure may have been higher than predicted using a single half-life. In addition, it is assumed that the apparent half-life for TCDD is independent of exposure to other dioxin-like compounds. In the rodent, apparent half-life is in part determined by binding to CYP1A2, which is inducible via the AhR. In humans, while neither the dose response for induction of CYP1A2 by TCDD nor the effect this may have on disposition of TCDD is known, it is likely that the half-lives for dioxin-like compounds are not independent.

The fraction of TCDD absorbed could have an impact on the risk estimates derived from the epidemiological data. In our calculations, we have either directly assumed a 50% absorption fraction or relied upon analyses by the original authors that used a 50% absorption fraction. In the analyses applied in this chapter, changes in absorption fraction result in a proportional change in steady-state body burden. Hence, a 10% change in absorption would result in a corresponding 10% change in steady-state body burden.

Another uncertainty is possible interaction or confounding between TCDD and tobacco smoking. In mice, TCDD and 3-methylcholanthrene (3-MC, one of the many polycyclic aromatic hydrocarbons in tobacco smoke) have been shown to be cocarcinogenic (Kouri et al., 1978). Other studies of mouse skin tumors have shown that TCDD can have anticarcinogenic properties when administered before initiation with either 3-MC or benzo(a)pyrene. Furthermore, dioxin's tumor-promoting ability suggests that two-stage models would be more appropriate if individual smoking histories were known. Smoking histories and analyses are presented only for the Zober et al. (1990) cohort; for the 37 cancer cases, only 2 were stated as being nonsmokers. Of the 11 men with lung cancer, only 1 reported never smoking. The Ott and Zober (1996b) analysis, which includes

smoking as a covariate, did appear to show an effect of smoking on TCDD dose-response. Although similar SMRs from other smoking-related diseases in the two subcohorts in Fingerhut et al. (1991) suggest similar smoking prevalence across this multifactory cohort, the effects with higher levels of TCDD could be synergistic for cancer. Steenland et al. (1999) point out that confounding by smoking is likely to be reduced in an exposure-response analysis comparing highly-exposed workers to workers with lower exposure.

Other potential confounders in all three studies include exposures concomitant with TCDD exposures, other chlorinated hydrocarbons in the case of Zober et al. (1990) and Manz et al. (1991) and miscellaneous chemicals including 4-aminobiphenyl, a known human bladder carcinogen, in the case of Fingerhut et al. (1991). These confounders raise the question of whether the increased SMRs are due to exposure to TCDD or to the confounders. However, it is important to note that within this context, 4-aminobiphenyl does not increase tumors overall, and there is no evidence that TCDD induces the incidence of bladder cancers.

Another source of uncertainty is the choice of model for analysis. The Becher et al. (1998) analysis of data from the Hamburg cohort used three models for dose-response for total cancer mortality, of which only one was linear. The risk estimates they derived using different models varied by as much as a factor of five. The risk estimates of Steenland et al. (2001) for their best-fitting model (Cox proportional hazards model) fall above the range of risk estimates given by Becher et al. The risk estimates for Steenland et al.'s second best fitting model (piecewise linear) are more than an order of magnitude lower than those for their best fitting model.

When interpreting the risk estimates presented in this section, a few additional caveats and potential biases must be kept in mind.

All observed risk is attributed to exposure to TCDD, even in the presence of exposure to other confounding chemicals. In particular, this analysis ignores exposure to PCDDs, PCDFs, and other dioxin-like chemicals. The extent to which exposure to other agents increases the total exposure on a TEQ basis (see Chapter 9) also increases the potential bias of calculated risk estimates. In general, exposure to these compounds is correlated with the exposure to TCDD, although differences in relative contribution of different dioxin-like compounds to the total TEQ have been observed and are briefly discussed for the epidemiological data. This issue is especially important for agents with shorter half-lives than TCDD (some will be longer; some shorter). Analysis of blood samples analyzed years after exposure may fail to adequately measure an initial exposure to dioxin-like compounds with shorter half-lives. For example, a current lipid level of 1 ppt for an agent with a half-life of 7 years, e.g., TCDD, would imply a lipid level of a little less than 8 ppt 20 years ago. On the other hand, an isomer with a current lipid level of 1 ppt and a half-life of 2 years would imply a lipid level of 1,024 ppt 20 years ago.

In any epidemiological study, misclassification can bias estimates of risk. In this case, recent exposures to TCDD, changes in the lipid fraction of body weight or presence/absence of genetic differences in humans that alter the distribution and metabolism of TCDD could cause misclassification bias, resulting in higher or lower risk estimates depending upon the direction of the misclassification.

Selection bias may be another factor. For example, it is possible that the subpopulation used for the biomonitoring of TCDD levels in human blood is not representative of the entire cohort used for risk estimation. There is also a potential bias due to a healthy worker effect in these occupational populations.

8.3.2.5. *Conclusions for Human Cancer Dose-Response Modeling*

Epidemiological studies of occupational exposure suggest a TCDD-mediated increase in all cancers and also suggest that the lung in the human male is a sensitive target for TCDD. Smoking and other factors (discussed above) may be modifiers for these cancers. Caution should be used in interpreting the overall risk estimates and care should be taken to understand them in the context of the entire weight-of-evidence concerning the potential toxicity of TCDD. The data obtained from two occupational studies were sufficient to calculate risk estimates. Estimates derived from the human data suggest an ED_{01} based on body burden in the range of 1.4-62 ng/kg for all cancers combined.

8.3.2.6. *Additional Knowledge Gaps in Human Cancer Dose-Response Modeling*

One major knowledge gap in the epidemiological data is a complete exposure history for each individual in the cohort. This includes lack of a realistic exposure matrix (areas and their exposure potency and time spent in such areas of occupational exposure) and TCDD concentrations measured over time during exposure. At present, only a few measurements per individual are available to estimate a time course ranging over many years of human life.

Different dose metrics have been discussed in Section 8.2, and others may arise if more information about the exposure process becomes available. Neither comparisons of the dose metrics applicable at present to available data sets nor simulation studies on artificial data sets have been performed to clarify the strengths and weaknesses of different metrics under different scenarios.

More information is needed on factors determining individual differences in half-life of TCDD such that these can be included into the calculation of individual time-average body burdens. Age, sex, and portion of body fat have been discussed and used as factors of influence. The existence of a more complex model for TCDD kinetics in humans may be possible, but no systematic usage of these factors in risk estimation has been made so far.

Information about confounders of human carcinogenesis, such as smoking or other behavioral cancer risk factors, was sparse in these studies. Future studies must reduce this lack of information by use of appropriate design measures, or by inclusion of appropriate biomarkers of coexposure. Exposure to related dioxin-like compounds clearly complicates the estimates of the effective dose of TCDD. For example, in the Hamburg cohort, the mean TCDD concentration for 236 males was 108.3 ppt, whereas the mean TEQ concentration based on all other PCDDs and PCDFs (except TCDD) was 142.0 ppt. Other coexposure-based confounders have been described above. Although TEQ values can be calculated for each person using half-life estimates of each individual PCDD and PCDF congener, it is unclear how an interaction of different congeners in the individual organism determines the concentration levels over a long time period in humans. Long-term studies, even of a small cohort of individual persons, would have the potential to clarify basic pharmacokinetics of these complex mixtures. One question to be addressed would be potential changes in half-life of TCDD in the presence of other dioxin-like compounds in different concentrations.

The ED_{01}s presented are based on simple dose-response models. The analyses uses the crude endpoint of all cancers combined. No mechanistic information was available for these cohorts to strengthen this analysis. This prohibited cancer modeling using parameters other than TCDD blood serum concentration. For a mechanism-based cancer risk estimation, such information would be required. If such information cannot be obtained for the entire cohort, investigators should consider statistically appropriate subcohort sampling as a possible source of information.

Risk estimates could not be calculated for infant or nonadult exposure. This is to some extent due to insufficiencies in study design for risk estimation for the total population and missing information in the reporting of the results. Similarly, it is not possible at present to identify subpopulations that may be at increased risk. Effects of limited but high exposure at an early age have not been investigated under conditions where dose-response analyses can be done. In addition, dose-response data are almost completely missing for human noncancer endpoints. Although the cohorts considered above are large (with a few thousand individuals), given the size of the effects to be expected, the statistical power of some analyses is quite small and larger studies with thorough epidemiological design consideration are required.

8.3.3. Rodent Dose-Response Models: Cancer Endpoints

8.3.3.1. *Animal Cancer Studies for Dose-Response Modeling*

Mathematical modeling can be a powerful tool for understanding and combining information on complex biological phenomena. Modeling of carcinogenicity can be accomplished using simple techniques (Portier et al., 1984) and can be improved by taking the results of an existing mechanism-based model on receptor-based effects of TCDD within the context of a physiologically based pharmacokinetic (PBPK) model (Kohn et al., 1993) and using these results in a detailed multistage model of carcinogenesis (Portier et al., 1996). Both approaches have been attempted. For a mechanism-based approach see Section 8.4.3.2.

Portier et al. (1984) used a simple multistage model of carcinogenesis with up to two mutation stages affected by exposure to model the five tumor types observed to increase in the 2-year feed study of Kociba et al. (1978) (Sprague-Dawley rats) and the eight tumor types observed to increase in the 2-year gavage cancer study conducted by the National Toxicology Program (1982a) (Osborne-Mendel rats and B6C3F$_1$ mice). The findings from this analysis are presented in Table 8-3. The ED_{01}s were calculated based on Portier et al. (1984). Excess risks were then calculated from the ED_{01} using equation (1) in Section 8.2.2. All but one of the estimated ED_{01} values are above the lowest dose used in the experiment (approximately 1 ng/kg/day) and are thus within the experimental range. The exception, liver cancer in female rats from the Kociba study, is very near the lowest dose used in this study. Steady-state body burden calculations were also used to derive doses for comparison across species (see Section 8.2). Absorption was assumed to be 50% for the Kociba et al. (1978) study (feed experiment) and 100% (Rose et al., 1976) for the NTP study (1982a) (gavage experiment). Also presented in Table 8-3 are the shapes of the dose-response curves as determined by Portier et al. (1984).

The predominant shape of the dose-response curve in the experimental region is linear; this does not imply that a nonlinear model such as the quadratic or cubic would not fit these data. In fact, it is unlikely that in any one case, a linear model or a quadratic model could be rejected statistically (Hoel and Portier, 1994). These studies had only three experimental dose groups; hence these shape calculations are not based upon sufficient doses to guarantee a consistent shape estimate; they should be viewed with caution. The body burdens at the ED_{01} values range from a low value of 14 ng/kg based upon the linear model associated with liver tumors in female rats, to as high as 1,190 ng/kg based upon a cubic model associated with thyroid follicular cell adenomas in female rats.

8.3.3.2. *Conclusions From Animal Cancer Dose-Response Modeling*

The animal studies show an increase in cancer incidence in rats and mice at various sites. The ED_{01} estimates of daily intake level obtained from an empirical linear model range from 0.8 to 43 ng/kg body weight/day depending on the tumor site, species, and sex of the animals investigated. These are equivalent to steady-state body burdens of 14 to 1,190 ng/kg body weight. By way of comparison, the ED_{01} estimate obtained from a linear mechanistic model of liver tumor induction in female rats (Section 8.4.3.2) was 0.15 ng/kg body weight/day, equivalent to a steady-state body burden of 2.7 ng/kg body weight (Portier and Kohn, 1996).

8.3.3.3. *Knowledge Gaps in Animal Cancer Dose-Response Modeling*

The dose-response data for cancer in animals following TCDD exposure are limited to three exposure groups. Although nonlinear models could be applied to these data (Portier et al., 1994), the estimates of the shape of the dose-response curve should be viewed with caution. Studies with more dose groups and sufficient animals per dose group are needed for distinguishing between different shapes of dose-response curves. Furthermore, mechanism-based cancer modeling could be improved if physiological, biochemical, and tissue response information were obtained from the same experiment.

Hepatocellular carcinomas have been the main focus for much of the research on the carcinogenicity of TCDD, although there has been increased tumor incidence in other organs. With respect to extrapolation to humans, the investigation of lung and thyroid cancer should be studied further. Animal cancer studies using other PCDDs, PCDFs, PCBs and complex mixtures reflecting human exposure patterns have rarely been done and may add information to the problem of complex human exposure.

8.3.4. Rodent Dose-Response Models: Noncancer Endpoints

8.3.4.1. *Methodology*

Risk assessments for noncancer endpoints traditionally have not used endpoint-specific mathematical models. Instead they have relied on safety assessment involving determination of a dose that is likely to be without risk, taking both data and model uncertainties into account. Although many of the same biochemical effects involved in carcinogenesis are also involved in many other toxicities, biologically based mathematical models for noncancer endpoints are not as developed as are the cancer risk models. In the interim, we will use a simple empirical modeling scheme to estimate effective doses and to discuss dose-response curve shape for the biological and toxicological effects induced by TCDD. The models and the statistical details follow similar analyses done by McGrath et al. (1995) and Murrell et al. (1998). In brief, two different models

were applied to the continuous data depending upon the number of dose groups used and the overall quality of the data. First choice was to use a Hill model of the form

$$R(d) = b + \frac{vd^n}{k^n + d^n} \quad (4)$$

where *R(d)* is the response at dose *d*, and *b*, *v*, *k*, and n are model parameters to be estimated from the data. The parameters each describe a different aspect of the dose-response curve: *b* is the background response, *v* is the maximum attainable response, *k* is the dose yielding half of *v*, and *n* is the Hill coefficient describing the curvature of the dose-response. As the shape of the dose-response curve is critical for risk assessment, it is of interest to consider important classifications based on *n*. When n is near or below 1, risk is predicted to be approximately proportional to dose or climbing more rapidly than proportional. When *n* is much larger than 1 ($n > 1.5$), the dose-response is sigmoidal and has been described as appearing to have a threshold. For these reasons, n will also be referred to as the shape parameter.

In the present exercise, *n* was not allowed to vary below 1, and thus the model as used does not predict supralinearity. Estimates of n were restricted to be greater than 1 to avoid instability. Estimates for the ED_{01} are sensitive to the slope of the dose-response curve evaluated at dose=0, and when $n<1$, this slope becomes infinite. This infinite slope is not biologically realistic and is difficult to tie down accurately to these data. This makes the estimates of the ED_{01} unstable and, worse, makes their lower confidence bounds very unstable. The net effect of this restriction is a possible bias towards higher-than-expected ED_{01} value and a truncation in the distribution of observed shapes. The first effect cannot be avoided, but the second should not be a problem because unrestricted estimates of $n<1$ will yield restricted estimates of $n=1$ and the shape will be classified into a grouping of risk approximately proportional to dose.

The second model used here is the power function:

$$R(d) = b + sd^n \quad (5)$$

where *b* and *n* have similar descriptions and *s*, referred to as the scale parameter, describes the magnitude of the effect per unit of dose. Unlike the Hill model, this model has no fixed maximum and is used in this chapter for data with either no experimentally evident maximal response or with few dose groups. This poses a considerable problem in defining effective doses, and caution should be used in applying effective doses derived from the power function model. Quantal data were modeled using the Weibull model given by

$$R(d) = c + (1 - c)[1 - exp(-ad^k)] \quad (6)$$

where $R(d)$ is the probability of response at dose d, c is the expected response in untreated animals ($0 \leq c \leq 1$), a is the magnitude of response per unit dose raised to the k^{th} power ($a \geq 0$), and k is the shape parameter ($k \geq 1$). The Weibull model as used in this analysis estimates threshold-like behavior when k is large. In addition, k was not allowed to be less than 1 to avoid instability in the analysis. The ED_{01} values from quantal data satisfy the excess risk relationship described in equation (1) in Section 8.2.2 where $R(\infty)$ is equal to 1 for quantal endpoints.

The data sets examined in this exercise are found in the published literature. The studies analyzed provided dose-response information on TCDD using at least three dose levels of TCDD and a control. In addition, the mean and an estimate of the variance of the data had to be presented in tabular form in the manuscript. Attempts to estimate the means and variances of data presented in graphical forms proved unreliable, thus publications where the data were presented only in graphs were not included in the analysis. Model fits, calculation of the ED_{01} and ED_{10} and the 95% lower bound on the estimated ED_{01} were carried out using the U.S. Environmental Protection Agency (EPA) Benchmark Dose Software (BMDS) version 1.1b (U.S. EPA, 1999). In some cases, the BMDS software failed to locate a lower confidence bound on the ED_{01}.

The model fits were evaluated with regard to the observed data. The goodness of the model fit was determined as "good" if the model curve included nearly all of the data point means, "marginal" if the model curve was within one standard deviation of the data point means, or "poor" if model fit was not within one standard deviation of the means. There were 242 endpoints for which dose-response analyses could be made (approximately 200 continuous endpoints and approximately 30 quantal effects), obtained from more than 36 published manuscripts (see Appendices). The number of data sets, categorized by species, gender and study type, is shown in Table 8-4. Poor fits were not evaluated further and were not included in the overall assessment of the ED_{01} values.

For the Hill model fits, the Vmax estimates from "good and "marginal" model fits were subjectively evaluated for stability and biological plausibility with regard to the observed data. This evaluation identified some potential problems with some of the Vmax estimates. In some cases the error associated with the Vmax could not be calculated by the BMDS software. In these cases if the Vmax model estimate was similar to the "observed Vmax" (i.e. the difference between the highest dose response level and the control response level) then the Vmax estimate was considered biologically plausible and was used for the calculation of an ED_{01}. Otherwise the "observed Vmax" was used for calculation of the ED_{01}.

In other cases the error associated with the model Vmax estimate was high, indicating a potentially unstable estimate that may not be biologically plausible. The Vmax estimate was considered unstable if the error associated with the Vmax estimate was greater than the Vmax itself.

In these cases an "alternate Vmax" was calculated as 3 standard deviations of the observed control response. If the maximum response associated with the use of this "alternate Vmax" was within 3 standard deviations of the observed highest dose response then this new Vmax value was considered to be biologically plausible and was used for subsequent ED_{01} calculations.

However, if the maximum response was not within 3 standard deviations of the observed highest dose response then this "alternate Vmax" value was considered to not be biologically plausible and was not used. In these cases an "observed Vmax" was determined for use in the ED_{01} calculations. The "observed Vmax" was calculated as the difference between the observed highest dose response and the observed control response.

In all cases where the model estimated Vmax was replaced with a new surrogate Vmax a new ED_{01} was recalculated using this new Vmax. In all cases, the original shape, and ED_{50} parameters were used for calculation of the ED_{01}, i.e. the data were not remodeled using the new assigned Vmax as fixed parameter in the Hill model. Because the data were not remodeled, estimates of the lower confidence interval are not available for these data sets.

There were 284 dose-response data sets found in the peer-reviewed literature that fit the inclusion criteria described above. The Hill, Power or Weibull models were fit to these 284 data sets. Good or marginal fits were attained for 242 of these data sets. The data sets that had poor fits were not included in the synthesis of the modeling results. All fits to the 284 data sets are presented in Appendices I through III.

The analyses of the data are presented as summaries of the endpoint categories in Figure 8-1, Figure 8-2, Figure 8-3, Table 8-5, and Table 8-6. The data are divided into several categories on the basis of exposure regimen and endpoint. Exposure categories are grouped as either single exposures or multiple exposures. For simplicity, effects were categorized as biochemical, hepatic, immune, endocrine, tissue, or toxicity (Table 8-7). Biochemical changes included alterations in mRNA, protein, or enzyme activities. The category of hepatic changes included responses of hepatotoxicity, such as serum enzymes and histological effects. Immune responses included alterations in lymphocyte phenotypes and functional alterations such as altered responses to antigen challenge. Alterations in tissue and body weights were classified as a tissue response. Developmental, reproductive, and tissue toxicities were classified as toxic responses. Finally, there were limited studies on the effects of TCDD on serum thyroid hormone concentrations and alterations in either serum or tissue retinoid concentrations; these studies were categorized as endocrine effects.

Comparison of the ED_{01} between studies can be problematic for several reasons. The effective dose is dependent upon the sensitivity of the endpoint examined and the dosing regimen employed. For example, in studies examining the effects of TCDD following a single exposure, the time after the initial exposure when the determinations were made varied from days to weeks. For

some effects, the differences in the time after the initial exposure probably influence the effective dose. Similarly, in studies employing multiple doses, investigators used a variety of regimens including daily exposure, weekly exposures, and loading/maintenance regimens. These differences in dosing regimens may influence the dose response relationships. In addition, investigators used a variety of exposure routes including dietary, oral gavage, subcutaneous, and intraperitoneal. The different routes and vehicles (diet vs. oil solution) have different absorption rates and percentage absorbed. These differences may result in different tissue concentrations and may influence the dose-response relationships. In order to compare the multiple-dose studies using different routes of exposure, the average daily dose was estimated for each study by calculating the total dose administered to the animal over the course of the study and dividing by the length of the study in days. In addition, for the multiple-dose studies, average steady-state body burden at the ED_{01} was calculated using the equation in Section 8.2.2 and the percentage of dose adsorbed and the half-lives for TCDD in Table 8-1.

In applying a consistent modeling approach across all endpoints, some uncertainty is introduced for those data sets where this approach provides only a marginally adequate fit. In some cases, no trend was apparent below the highest dose examined, thus reducing the confidence that can be placed in accurately estimating the dose associated with a change as small as 1%. In other cases, it appeared that other models could provide a better fit to the data, with a significantly different ED_{01}. For example, sometimes the Hill model gave a dose-response curve with sharp changes in slope, but a Weibull model could have provided a better fit to the data with a smoother curve and a lower ED_{01}. In addition, the ED_{01} and the 95% lower confidence interval (LED_{01}) were sometimes quite far apart (differing by more than tenfold), suggesting that little confidence can be placed in some ED_{01} values as a precise index of toxicity. In such cases, it is useful to look at the LED_{01} as a bound. Whenever the modeling results were problematic for these or other reasons, we noted it and gave less emphasis to those results in our overall synthesis of the data. In this way, the overall conclusions are based on the strongest results.

8.3.4.2. ***Multiple-Dose Studies***

Of 139 endpoints examined from multiple-dose studies, 108 data sets had fits described as good or marginal. Thirteen of the data sets had statistically significant fits designated as poor and 18 data sets did not have statistically significant fits to the models. Data sets with poor fits or non-statistically significant fits were not included in the following analysis. The estimates of the Vmax were unstable in 43 of the 108 data sets with good and marginal fits. Of the 43 data sets with unstable Vmax estimates, five of the modeled Vmax values were accepted as the estimate. The Vmax was set at three standard deviations from controls for 25 of the data sets. For 13 data sets, the Vmax was set as the difference between the response of the highest dose tested with those of the controls.

In the studies examining the effects of TCDD following multiple exposures, the range of the ED_{01} values is highly variable within and across response categories (Figure 8-1). For the multiple dose exposure studies, the ED_{01} values were modeled using the average daily dose from each study. When examined by category, the median values for the ED_{01} for biochemical responses are lower than the median ED_{01} for other types of response by almost an order of magnitude. Biochemical responses have a median ED_{01} of approximately 1 ng/kg/d and hepatic, immune and tissue responses have median ED_{01} values of 10 ng/kg/d or greater. Of the 108 endpoints examined from studies using multiple exposures, ten have ED_{01} values less than 0.1 ng/kg/day. Six of the ten endpoints with an ED_{01} below 0.1 ng/kg/day are markers of immune response. However, the ED_{01} for markers of immune function range over six orders of magnitude, decreasing the confidence of any particular ED_{01} value for this response. In general these ED_{01} values represent dose-response information from female rats and mice, with few studies examining male rats and mice or other species. These knowledge gaps decrease our confidence in making extrapolations between species and gender.

ED_{01}s for single dose exposures were also estimated using body burden as the dose metric (Figure 8-2). Biochemical responses had a median ED_{01} of approximately 13 ng/kg. Hepatic, immune and tissue response had median ED_{01}s greater than 200 ng/kg. Background human exposure to dioxin-like chemicals is approximately 5 ng TEQ/kg. The margin of exposure between the median ED_{01}s for humans is approximately 3 for biochemical effects and approximately 40 for hepatic, immune and tissue responses. Of the 108 data sets for which ED_{01}s were estimated, 42 are less than 50 ng/kg and include responses in the biochemical, endocrine, immune and hepatic categories. There are 11 responses with ED_{01}s of 5 ng/kg or lower. Six of these responses are immune, three are biochemical and one each is endocrine and tissue. These data indicate that a number of the ED_{01}s are at or below present background exposures.

The ED_{10} was also estimated for these data sets and similar trends were observed compared to the ED_{01}. The median ED_{10} for biochemical responses was approximately 200 ng/kg and the

other response categories were 5-10 times higher (Figure 8-3). There were 14 data sets with ED_{10}s less than 50 ng/kg and 9 of these data sets had ED_{10}s that were less than 5 ng/kg. This data suggests that the ED_{10} for a number of endpoints is within an order of magnitude of background exposures. In 44 of the multiple-dose data sets analyzed the ED_{10} was less than 2 times the ED01. In 28 of the data sets the ED_{10} was between 10 and 2 times greater than the ED_{01}.

One measure of the degree of confidence of the ED_{01} estimate is the ratio of the ED_{01} to the lowest dose used in the study from which it was derived (Table 8-5). A ratio of 1 or greater indicates that the ED_{01} is above the lowest dose examined. Ratios between 1 and 0.1 are within one order of magnitude of the lowest dose tested and indicate that the ED_{01} may provide a realistic value. Ratios less than 0.1 indicate that the estimate was more than an order of magnitude below the lowest dose used in the study and should be viewed with caution. Forty-seven of the 108 values had ratios of the ED_{01}/lowest-dose less than 1. However, of these 47 only 37 were less than one order of magnitude below the lowest dose used in the study. Another measure of the stability of the ED_{01} is the ratio of the LED_{01} to the ED_{01} of the 59 data sets for which the LED_{01} was estimated, the median ratio of the LED_{01}/ED_{01} is 0.39. Only 17 of the 59 data sets had an LED_{01} that was an order of magnitude or more less than the ED_{01}.

In general, an estimated shape parameter that is less than 1.5 indicates that the shape of the dose-response curve tends to be linear at low doses, and those with shape parameters greater than 1.5 tend to be threshold-like. Of the 108 endpoints for which an estimate was obtained, 48 had shape parameters less than 1.5, indicating linear dose-response relationships (Table 8-6). Approximately half of the biochemical and half of the tissue responses indicated a linear dose-response relationship. In contrast, only 19% of the immune function responses were linear.

Although there is some consistency of shape within certain categories of these endpoints, in general about half of the responses could be classed as either linear or nonlinear. These observations do not strongly support linearity for TCDD dose-response, nor do they strongly support the existence of thresholds within the observable range.

8.3.4.3. *Single-Dose Studies: Adult Animals*

There were 98 data sets examining the effects of dioxin in adult rats and mice following a single exposure. Good or marginal fits were assigned to 75 of these data sets. The Hill model was used in 58 of these data sets and the Weibull model was applied to 17 of these data sets. The Vmax was considered unstable in 17 out of the 58 data sets with good or marginal fits to the Hill model. The Vmax was set at three standard deviations from control for 5 data sets and the response at the high dose minus the control response was used as the Vmax for 10 data sets. For two of the data sets with unstable Vmax estimates, the modeled estimate was considered acceptable based on the criteria outlined above.

The median ED_{01} is above 1 ng/kg for all endpoints examined (Figure 8-2). Biochemical and immune responses had the lowest median ED_{01} estimates, 207 and 133 ng/kg, respectively. Hepatic and toxic responses gave median ED_{01}s greater than 10,000 ng/kg. Once again there was large variability in the ED_{01}s for a given category. In general the ED_{01}s varied over three orders of magnitude within each category. There were 14 data sets from the immune, tissue and biochemical response categories with ED_{01} values less than 50 ng/kg. The ED_{01} estimates were below the lowest dose tested for 21 of the 74 endpoints for which an estimate was obtained. Of these 21 estimates, the ED_{01} was less than one order of magnitude lower than the lowest dose tested for 13 of the values (Table 8-5). An LED_{01} was estimated for 70 data sets. The median ratio of the LED_{01}/ED_{01} was 0.36 with only 11 out of 70 data sets with ratios less than 0.1.

Estimates of the ED_{10} produced similar trends (figure 8-3). The immune and biochemical response categories had the lowest median ED_{10} values. There are four data sets with ED_{10} values less than 50 ng/kg and all are in the immune response category. There are four response categories that overlap between the single acute studies and the multiple dose studies. The ED_{10}s for the biochemical, hepatic and tissue response categories are higher in the single dose studies than in the multiple dose studies. In contrast, the ED_{10}s in the immune response categories are approximately 4 times less in the single dose studies compared to the multiple dose studies.

In studies examining the effects of dioxin in adult rats and mice following a single exposure, the median ED_{01} is above 1 ng/kg for all endpoints examined Figure 8-2. Biochemical and immune responses had the lowest median ED_{01} estimates, 207 and 133 ng/kg, respectively. Hepatic and toxic responses gave median ED_{01}s greater than 10,000 ng/kg. Once again there was large variability in the ED_{01}s for a given category; in general they varied over three orders of magnitude within each category. The ED_{01} estimates were below the lowest dose tested for 21 of the 74 endpoints for which an estimate was obtained. Of these 21 estimates, the ED_{01} was less than one order of magnitude lower than the lowest dose tested for 13 of the values (Table 8-5).

Following a single exposure to TCDD, 30 of the 74 endpoints examined (40%) had shape parameters less than 1.5, indicating linear dose-response relationships (Table 8-6). There was no consistent pattern in the shape of the dose-response relationships for the biochemical, immune, and tissue response categories. In these categories both linear and threshold-like dose-response relationships were observed. In contrast, all endpoints in the toxicity category exhibited threshold-like dose-response relationships.

8.3.4.4. *Single-Dose Studies: Developmental Studies*

There were 90 data sets classified as developmental studies following a single exposure. The model fits were described as good or marginal for 60 data sets. The Hill model was fit to 55 data sets. Thirty data sets were not included in this analysis because the model fits were either not

statistically significant or were described as poor. The model estimates of the Vmax was considered unstable in 18 out of the 55 data sets with good or marginal fits to the Hill model. In 7 of these data sets, the model estimate of the Vmax was considered acceptable based on the criteria describe above. Vmax was set at 3 standard deviations from controls in 11 of the data sets and for 5 of the data sets Vmax was set as the response at the high dose minus the response in the controls.

Following a single exposure, a number of developmental effects have been examined. These effects have been categorized as biochemical, tissue, or toxic. The majority of the effects examined were considered tissue responses. The range of ED_{01} values was more than five orders of magnitude, and the median values for all response categories were greater than 10 ng/kg, with an overall median of 139 ng/kg (Figure 8-2). The median ED_{01} values for the response categories were lower for developmental effects following a single dose compared to ED_{01} estimates for effects observed in adults after a single dose. The tissue response category was the only category with sufficient studies to compare between the developmental studies and the multiple dose studies in adults. In this case the median ED_{01} for the developmental effects was approximately an order of magnitude less than the median ED_{01} for the multiple dose studies. There were 18 out of the 60 data sets that had ED_{01} values of less than 50 ng/kg and 8 of these were less than 5 ng/kg. ED_{10}s were also estimated for the developmental effects and similar trends were observed. The ED_{10} values for 12 of the developmental data sets was below 50 ng/kg and only one of the data sets had an ED10 less than 5 ng/kg. Decreases in epididymal sperm counts on PND 49 had and ED_{01} of 1.7 ng/kg based on data from Gray et al. (1997).

The ED_{01} values for developmental effects were below the lowest dose tested in 38 out of 60 endpoints for which an estimate was obtained. Of the 28 estimates that were below the experimental range, approximately half (18) were less than an order of magnitude below the lowest dose tested. There were 37 endpoints for which the LED_{01} was estimated. The median ratio of the LED_{01}/ED_{01} was 0.19 and there were 8 endpoints with ratios less than 0.1. The shape parameter for the developmental effects was less than 1.5 (i.e. linear) for only 18 of 60 endpoints analyzed.

The results of the analysis of the single exposure studies had similarities to those of the multiple dose studies. There was a large range of ED_{01} values within response categories which in some instances reached over five orders of magnitude. Similar to the analysis of multiple-dose studies, the biochemical and immune response categories had the lowest ED_{01}s. The median values for all response categories were greater than 10 ng/kg, with an overall median of 139 ng/kg (Figure 8-2).

8.3.4.5. *Summary of the Dose-Response Modeling for Noncancer Endpoints*

The activation of the AhR by TCDD initiates a cascade of events resulting in alterations in growth factors and their receptors, hormones and their receptors, and proteins involved in numerous

cellular functions such as cell cycle regulation and intermediary metabolism (see Chapter 2 for a more detailed discussion of these processes). Many of these biochemical changes, particularly the alterations in growth factors and their receptors, may mediate the toxic effects of TCDD. The role of other biochemical changes, e.g., induction of aldehyde dehydrogenase, is less certain. One can consider the biochemical and toxicological effects of dioxins as a continuum, starting with biochemical changes leading to toxicological events. Hence, understanding the shape of the dose-response relationship for the biochemical effects may provide insight into the shape of the dose-response relationship for toxic responses, particularly in the low-dose region.

Consistent with the hypothesis that the biochemical effects are precursors of the toxic effects is that, in general, the biochemical responses tend to have lower ED_{01} estimates than other types of endpoints examined. However, few of the biochemical changes examined have been directly linked to toxic responses. For example, the induction of CYP1A proteins is perhaps the best-characterized response to TCDD and related chemicals. Despite their known role as modulators of intermediary metabolism for a number of classes of environmental chemicals in both activation and elimination pathways, the direct relevance of these proteins to the toxic effects of TCDD remains uncertain. Induction of CYP1A proteins has been proposed as a dose surrogate for the carcinogenic effects of TCDD (Portier and Kohn, 1996). One of the best examples of biochemical changes leading to toxicities is the TCDD-induced decreases in circulating thyroid hormones. This is likely a result of TCDD-mediated induction in hepatic glucuronosyltransferases (UGTs), which metabolize these hormones and increase their elimination. van Birgelen et al. (1995a) determined total and free plasma thyroxine concentrations and hepatic thyroxine glucuronidation (T_4UGT) in rats exposed to TCDD for 90 days in the diet. The ED_{01} values for total plasma thyroxine, free plasma thyroxine, and T_4UGT are 33, 4.9, and 1.6 ng/kg/day. The increased sensitivity of T_4UGT is consistent with the mechanism by which the plasma concentrations of these hormones are decreased. In female Sprague-Dawley rats exposed biweekly to TCDD for 30 weeks, Sewall et al. (1995) examined the effects of TCDD on UGT mRNA, serum total thyroxine, and serum TSH. All three responses had shape parameters greater than 1.5 and the ED_{01} values were 0.37, 1.3, and 26 ng/kg/day for UGT mRNA, total serum thyroxine, and serum TSH, respectively. Similar to the data of van Birgelen, the induction of UGT is more sensitive than changes in total serum thyroxine, which in turn is more sensitive than are changes in serum TSH. These data indicate that simple biochemical responses have lower ED_{01} values than more complex phenomena such as decreases in thyroxine and alterations in the homeostasis of thyroid hormones.

One concern in the interpretation of the data is whether the study design can affect the ED_{01} or the shape parameters. One example of this is the studies by Diliberto and co-workers. Diliberto et al. (1995) examined both dose-response and time course for CYP1A1-associated hepatic ethoxyresorufin deethylase (EROD) activity at 7, 14, 21, and 35 days after a single exposure to

TCDD. In these studies, the ED_{01} values and the shape parameters increased with time after dosing. The increase most likely stems from the decreasing tissue concentrations of TCDD and the subsequent decreases in enzyme induction from day 7 to day 35. The shape parameter ranged from 1 at 7 days after dosing to 6.5 at the 35-day time point. The ED_{01} increased from 27 ng/kg at 7 days after dosing to 740 ng/kg at the 35-day time point. These data indicate that both the shape parameter and the ED_{01} are sensitive to the study design. Comparisons of studies that determined EROD activity within 7 days of administration of TCDD demonstrate considerable consistency. Four studies examined EROD induction in rats or mice within 7 days of dosing and the ED_{01} values ranged from 16 to 84 ng/kg. The estimated shape parameter is 1 for the Diliberto et al. (1995), Abraham et al. (1988), and Narasimhan et al. (1994) studies and 1.8 for the van Birgelen et al. (1995a) study. It should be noted that two of these studies are in mice and two are in rats, suggesting similar dose-response relationships for enzyme induction between these species.

Another variation in study design that may affect dose-response modeling is dose selection. The dose-response relationship for induction of hepatic EROD activity was modeled for six studies (van Birgelen et al., 1995a,b; DeVito et al., 1994; Johnson et al., 1997; Schrenk et al., 1994; Vogel et al., 1997). Only the data from DeVito et al. (1994) and Johnson et al. (1997) had shape parameters greater than 1.5. While most of the ED_{01} values were approx 1 ng/kg/day, the data of Vogel et al. (1997), resulted in an ED_{01} more than 100-fold lower. Vogel et al. (1997) used a loading/maintenance dosing regimen, and the low dose used was 100 times lower than those of the other studies. The highest dose in the Vogel study was approximately 50-100 times lower than the highest dose used in the other studies. The much lower ED_{01} from this study may be a consequence of the dose pattern and dose selection in this study compared to the other studies.

Another factor to consider is species and strain selection in the studies. The developmental effects of TCDD have generated concern, particularly the developmental reproductive toxicities observed in rats and hamsters (Mably et al., 1992a,c; Gray et al., 1997). These studies demonstrated decreases in epididymal sperm counts on postnatal day 63. However, the shape parameters vary between 1 and 11 and the ED_{01} values vary between 0.65 and 140 ng/kg. The studies used different strains of rats, and perhaps this may account for some of the differences between the data sets. The decreases in the epididymal sperm counts were greater in the Holtzman rat used by Mably et al. (1992a) when compared to the Long Evans rat used by Gray et al. (1997) Overall, the study by Gray et al. (1997) demonstrated smaller effects than the study by Mably et al. (1992a). Also, the data from Gray et al. (1997) demonstrate highly nonlinear responses (shape parameters greater than 2 for all but 3 out of 32 responses examined). In contrast, the effects observed in Mably et al. (1992a) were larger, the shape parameters indicate a more linear dose-response, and the ED_{01} is almost two orders of magnitude lower than those estimated from the data of Gray et al. (1997).

One of the apparent observations of this exercise is the limited number of studies examined compared to the vast literature on the health effects of 2,3,7,8-TCDD. There are thousands of research articles examining health effects of TCDD. Of these articles, less than 50 were analyzed. There are a variety of reasons why only a limited number of articles could be included in this analysis. First, only studies in experimental animals were included, omitting many articles on in vitro studies. Second, only studies providing dose-response data that included a minimum of three dose levels and a control were included. Third, the data had to be presented in tabular form with means, standard deviations or standard error, and the number of samples for which the mean was calculated. It is likely that given the vast number of data sets available, some were inadvertently excluded. However, most of the studies found in the literature did not fit these criteria, either because of inadequate dose-response information or graphical presentation. For some studies that provided adequate dose-response information but presented the data in graphical format, the authors were asked to provide means and standard deviations and kindly did so. One of the conclusions of this exercise is that when preparing data for publication, authors conducting dose-response studies should consider the use of their data and present it in such a way that it is usable in future independent analyses.

Care should be taken in interpreting these analyses. There tends to be a large variation in both the shape parameter and the ED_{01} values for a given endpoint. Most of the studies examined were designed to determine a no-observed-effect-level (NOEL) or lowest-observed-effect-level (LOEL) and, as such, these data contain limited dose-response information. The limited information contributes to the observed variation in the estimates of both the shape parameters and the ED_{01} values. This should not be taken as a critique on the quality of the study designs. In almost all instances, the authors of the studies used analysis of variance as a statistical tool and the studies were designed for such an analysis. In contrast, the present exercise attempts to examine the dose-response relationships using nonlinear regression analysis as a statistical tool. Because of the limited dose-response data available, particular caution should be used when extrapolating to dose levels outside the experimental design. If this situation is to be improved and uncertainties in data interpretation reduced, studies will need to be designed and data produced that are more suitable for nonlinear regression analysis. Second, and perhaps more disappointing, was the frequency of inadequate reporting of the data. Many studies would present a mean and some measure of variance without describing whether the variance was presented as a standard deviation, a standard error of the mean, or some confidence interval. These variables can be adjusted for use in modeling if the proper number of animals/group is provided. However, often the number of animals/group was presented as a range.

Although ED_{01} values are intended as a common measure across studies and endpoints, they must be interpreted in relation to their respective maximal responses. For example, if enzyme

induction varies over a considerably greater range in one strain than another (for example, hepatic EROD induction in the studies by DeVito et al. [1994] compared to that observed in the study of Vogel et al. [1997]), then their respective ED_{01} values will represent different levels of induction. The biological significance of these responses may not be commensurate with their respective ED_{01} values. In addition, comparisons across endpoints must proceed cautiously. A 1% increase in response for decreased body weight may not necessarily be comparable to a 1% excess effect on immune function or enzyme induction.

Several studies have demonstrated that control rats and mice have detectable amounts of TCDD and related chemicals (Vanden Heuvel et al., 1994a; DeVito et al., 1998). The concentrations of these chemicals are at or near the quantification limits. In the present analysis, the background exposures of the control animals were not considered. The inclusion of background exposure levels or tissue concentrations in the dose-response analysis may alter the shape of the dose-response curves and is some cases may possibly increase the ED_{01} estimate and/or the model estimate of the shape parameter. However, it is unlikely that any effect of the estimates would substantially change the observed trends in the estimates or the main conclusions of this dose-response chapter.

An important finding in this analysis is that the biochemical effects tend to have lower ED_{01} values compared to more complex effects such as immunotoxicity or tissue weight loss. This finding is consistent with the hypothesis that the biochemical responses are precursors to the toxic responses of these chemicals. Another difference between the biochemical and toxicological responses is that the biochemical responses tend to have lower shape parameters. Thus, the dose-response relationships for the biochemical responses tend to be linear more often than the toxicological responses. Because of the limited dose-response data available for many of these analyses, caution must be taken when making some of these generalizations. For example, the decrease in thymus weight tends to have estimated shape parameters of 1.

Finally, the present analysis focused on the ED_{01}. This effect level was chosen because it would allow the comparison between the human epidemiological data and the animal data. Typically, the ED_{01}, ED_{05} or ED_{10} is used as the point of departure in risk assessments. Use of either of these alternative risk estimates would result in some differences. Obviously choosing higher effect levels will result in higher dose levels compared to the ED_{01}. Also the estimates of the ED_{10} would most likely be more stable than the estimates of the ED_{01}. However, in the present analysis several data sets had ED_{01}s and ED_{10}s that were less than 50 ng TCDD/kg. Based on a background human exposure at 5 ng TEQ/kg, using either the ED_{01} or ED_{10}s would result in a number of effects with margin of exposures less than an order of magnitude from background human exposures.

8.4. MODE-OF-ACTION-BASED DOSE-RESPONSE MODELING

8.4.1. Introduction

Mode-of-action-based modeling for TCDD encompasses PBPK models for estimating tissue dose and biochemical/tissue response models that describe the consequences of tissue dose. The distinction between tissue dose and response is often maintained in developing mechanism- or mode-of-action-based models. A number of PBPK models for TCDD have been developed. These models have provided insights into key determinants of TCDD disposition in TCDD-treated animals, such as diffusion-limited movement of TCDD between blood and tissue and induction of hepatic binding. PBPK models may be extended to generate predictions for biochemical consequences of the tissue dosimetry of TCDD. The molecular steps leading to observed responses form a causal sequence that describes the mode of action by which pathology is produced. Examples of carcinogenic modes of action include enhanced mutation by direct DNA reactivity, increased cell proliferation related to toxicity or mitogenic stimulation, or diminished apoptosis in a population of altered cells. The predictions of a PBPK model can be used to describe parameters in the mathematical representation of this mode of action. The goal of mode-of-action-based modeling is to express quantitatively the relationships between TCDD exposure, TCDD tissue kinetics, and the biochemical alterations leading to effects on these integrated responses. This section discusses models for dosimetry, biochemical, and tissue responses, and how they ultimately lead to adverse effects of TCDD.

Risk assessments where mechanistic dosimetry models have been used without any attempt to describe the mechanism of tissue response are a viable intermediate stage in the development of mechanism-based risk assessments. This approach to risk assessment also reflects the paucity of mechanistic models of tissue response, relative to models of tissue dosimetry. The more ambitious modeling of the entire exposure-tissue response continuum (Section 8.4.2) carries with it the greater requirement for mechanistic understanding of tissue response. When our understanding of mechanisms of tissue dosimetry and response are different, careful consideration should be given to the sources of uncertainty in the overall modeling effort. The realization that dosimetry and response submodels can contribute unequally to overall model uncertainty can help to guide the choices made in developing the final risk model and the allocation of resources for additional research.

8.4.2. Model Structures and Model Development

8.4.2.1. *PBPK Models*

8.4.2.1.1. ***Issues pertaining to PBPK models***. Tissue dosimetry encompasses the absorption of an administered chemical and its distribution among tissues, metabolism, and elimination from the body (ADME). TCDD dosimetry depends on physicochemical properties of TCDD (e.g., tissue

permeation constants, partition coefficients, kinetic constants, and biochemical parameters) and physiological parameters (e.g., organ volumes and blood flow rates). The mathematical structure that describes the relationship between these factors and ADME constitutes a model for the tissue dosimetry of dioxin. These models describe the pharmacokinetics of TCDD by a series of mass-balance differential equations in which the state variables represent the concentration of TCDD in anatomically distinct regions of the body. These tissue "compartments" are linked by a physiologically realistic pattern of blood perfusion, called a PBPK model. Several research documents discuss the development of PBPK models for general use (Gerlowski and Jain, 1998), and use in risk assessment (Clewell and Anderson, 1985).

PBPK models have been validated in the observable response range for numerous compounds in both animals and humans, making them useful for risk assessment, especially for cross-species extrapolation. In addition, they aid in extrapolation from one chemical to other structurally related chemicals because many of the components of the model are the same or can be deduced for related compounds. The tissue concentrations of several cellular proteins are known to be modified by TCDD, making them useful as dose metrics. A model can be used to predict the concentrations of these proteins as well. If one of these proteins is mechanistically linked to a toxic endpoint, the protein could also serve as a dose metric of toxic effects.

The time course of behavior in each compartment of a PBPK model is defined by an equation containing terms for input and loss of chemical. The specific structure of a PBPK model and the assumptions used to develop the model are encoded in the equations. A careful evaluation of any PBPK model must involve the adequacy of its fit to the data, the relationship of its structure to the underlying biology, and the mathematical details linking the two. Several PBPK models have been developed for TCDD and related chemicals (see Part II. Chapter 1 for a brief overview). Models have also been developed for polychlorinated biphenyls (Lutz et al., 1984; Matthews and Dedrick, 1984; Parham et al., 1997, 1998) and polychlorinated dibenzofurans in several species (King et al., 1983), including humans.

There are four levels of complexity in PBPK models for the effects of TCDD. First is the traditional PBPK model by Leung et al. (1988) with the added complexity of protein binding to CYP1A2 in the liver. The next level of complexity are the models by Andersen et al. (1993) and Wang et al. (1997) using diffusion-limited modeling and protein induction by interaction of DNA binding sites. The third level is represented by the model of Kohn et al. (1993) with extensive hepatic biochemistry and the model for zonal induction of cytochromes P-450 (Andersen et al., 1997b). Finally, there are the models that include coordination of responses in multiple organs (Kohn et al., 1996) for hormonal interactions, and Roth et al. (1994) with its detailed description of gastrointestinal uptake, lipoprotein transport, and mobilization of fat (Figure 8-4).

8.4.2.1.2. ***Initial attempts to include protein induction***. Leung et al. (1988) developed a PBPK model for TCDD disposition in mice, for Sprague-Dawley rats (Leung et al., 1990a) and for 2-iodo-3,7,8-trichlorodi-benzo-*p*-dioxin in mice (Leung et al., 1990b). These initial models considered tissue partitioning, protein binding in blood, specific binding of TCDD to inducible hepatic proteins, binding of TCDD to the AhR, and activation of gene transcription by the Ah-TCDD complex. Subsequent PBPK models have refined the representations of these processes as more biological information became available.

This early PBPK model (Leung et al., 1990a) contained five flow-limited tissue compartments, including blood, liver, fat, and slowly perfused and richly perfused tissues. TCDD binding in blood was described by an effective equilibrium between the bound and free TCDD given by a constant ratio. TCDD also binds to two liver proteins: one corresponding to the high-affinity, low-capacity AhR and the other to a lower affinity, higher capacity microsomal protein inducible by TCDD, now known to be CYP1A2. The predictions from this modeling exercise prompted a series of experiments to examine the nature of these binding proteins in mice (Poland et al., 1989a,b). In the PBPK model (Leung et al., 1990a), the concentration of the AhR is held constant and the concentration of CYP1A2 is calculated using a Michaelis–Menten equation for the instantaneous extent of induction as a function of hepatic TCDD concentration.

In various studies, TCDD has been administered by intravenous, intraperitoneal, or subcutaneous injection; feeding; or by oral intubation (gavage). In the PBPK modeling framework, intravenous injection can be represented by setting the initial amount in the blood compartment equal to the injected dose. Oral intubation and subcutaneous injection were modeled as first-order uptake from the site of administration, with TCDD appearing in the liver blood after oral administration and in the mixed venous blood after subcutaneous injection. Feeding was modeled (Leung et al., 1988, 1990a) as a constant input rate on days that TCDD was included in the diet. With 2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin, the estimated rate constant for oral absorption was considerably larger in TCDD-induced than in naive animals. The physiological basis of this change is unknown, but it may be a consequence of increased hepatic lipid synthesis and elevated plasma lipid following TCDD treatment (Gorski and Rozman, 1987).

The descriptions of the routes of uptake are clearly not defined in specific physiological terms, and this lack of detail represents a common limitation in all of the PBPK models for TCDD. These descriptions of the oral, subcutaneous, and skin routes are simply empirical attempts to estimate an overall rate of uptake of TCDD into the PBPK model. This is one area in which additional research could improve dose-response modeling for TCDD.

Partition coefficients for TCDD were estimated from measurements of tissue and blood concentrations in exposed animals. Leung et al. (1990a) also modeled metabolic clearance as a first-order process with the rate constant scaled inversely with $(\text{body weight})^{0.3}$. In the mouse with

the iodo-derivative, TCDD pretreatment at maximally inducible levels caused a threefold increase in the rate of metabolism, probably through loss of iodine. However, Olson et al. (1994) found that pretreatment of rats with 5 µg TCDD/kg body weight increased metabolism in isolated hepatocytes only when at least 1 mM TCDD was present in the medium. Induction of its own metabolism by TCDD appears to be a minor high-dose effect.

Leung et al. (1990a) kept all physiological parameters (e.g., organ perfusion rates and tissue volumes) constant over the lifetime of the animal. Subsequent PBPK models have included growth of the animals over time and changes in organ size due to growth and toxicity. TCDD and TCDD analogues have dose- and time-dependent kinetics in both rodents (Kociba et al., 1976, 1978; Rose et al., 1976; Abraham et al., 1978; Poland et al., 1989b; Tritscher et al., 1992) and humans (Carrier and Brodeur, 1991; Pirkle et al., 1989). As the exposure level increases in single and short-duration exposures, the proportion of total dose found in the liver increases. This initial model served as the basis of later models as new data were published on dose and time dependence of TCDD tissue concentrations (Abraham et al., 1988 Tritscher et al. 1992).

In discussing the components that form the basis for a mechanistic model for TCDD, we focus on aspects of the model that could lead to nonproportional response for low environmental doses (nonlinear behavior). The model of Leung et al. (1990a) predicted slight nonlinearity between administered dose and tissue concentration in the experimental dose range. In the low-dose range, the model predicts a linear relationship between dose and concentration. The authors argue, however, that tissue dose alone should not be used for risk assessment for TCDD because of the large species specificity in the ability of TCDD to elicit some toxic responses. They suggest instead that use of time-weighted receptor occupancy linked with a two-stage model of carcinogenesis is a better approach to risk estimation. The time-weighted receptor occupancy predictions derived from the Leung et al. (1990a) model are linear in the low-dose region, reaching saturation in the range of high doses used to assess the toxicity of TCDD. This discussion represented one of the early attempts to define a dose metric for the carcinogenic action of TCDD.

8.4.2.1.3. ***Refinements with DNA binding of Ah-TCDD complexes***. Andersen et al. (1993a) modified the model of Leung et al. (1990a) to include Hill kinetics in the induction of CYP1A1 and CYP1A2 and to treat tissue uptake of TCDD as diffusion limited instead of blood flow limited as done by Leung et al. (1990a). Diffusion limitation was incorporated by replacing the blood flow term in the expression for tissue uptake of TCDD by a permeability factor equal to the diffusion coefficient times the cell membrane surface area accessible to the chemical. Andersen et al. (1993a) assumed this quantity to be proportional to the tissue perfusion rate, with a constant of proportionality less than 1. In the model used by Andersen et al. (1993a) each tissue has two subcompartments, the tissue blood compartment and the tissue itself.

This revised model eliminated allometric scaling of the metabolic rate constant used in the model of Leung et al. Instead, it treats TCDD as inducing its own metabolism, with a maximal increase of 100%. The increase is a hyperbolic function similar to that for binding of TCDD to the AhR. This induction led to an improved fit to observed liver and fat TCDD concentrations. Subsequent research (Olson et al., 1994; McKinley et al., 1993) revealed no induction of metabolism of TCDD suggesting that this is likely to be a minor high-dose effect.

Most of the physiological constants and many of the pharmacological and biochemical constants used by Leung et al. (1990a) were modified for the Andersen et al. (1993a) model because Wistar rats instead of Sprague-Dawley rats were used in the experiments they simulated. The parameters in the model were optimized to reproduce tissue distribution and CYP1A1-dependent enzyme activity in a study by Abraham et al. (1988) and liver and fat concentrations in a study by Krowke et al. (1998). For the longer exposure regimens and observation periods, changes in total body weight and the proportion of weight as fat compartment volume were included via piecewise constant values (changes occurred at 840 hours and 1,340 hours).

Induction of CYP1A1 proteins in the model was modeled by including interaction between the Ah-TCDD complex and presumed DNA binding sites. The concentrations of CYP1A2 and CYP 1A1 were modeled as a function of hepatic AhR–TCDD concentration. Although the revised model represented the kinetics with a Hill equation, the Hill exponent was 1, similar to the Michaelis–Menten model used by Portier et al. (1993) for the independent induction of CYP1A2. The Hill exponent for CYP1A2 (2.3) introduced marked sigmoidicity in the computed dose-response of this protein.

Andersen et al. (1993a) noted that the liver/fat concentration ratio changes with dose because of an increase in the amount of microsomal TCDD-binding protein (CYP1A2) in the liver. For high doses in chronic exposure studies, this introduces a nonlinearity into the concentration of TCDD in the liver. In the low-dose region, because the Hill coefficients for CYP1A2 concentration and for TCDD binding to the AhR are equal to 1, the liver TCDD concentration as a function of dose is still effectively linear. In the observable response range, there is a slight nonlinearity in the concentration of TCDD in the liver as a function of dose under chronic exposure (Andersen et al., 1993a). The dose-dependent changes in liver/fat ratio are consistent with animal data and limited human data (Carrier and Brodeur, 1991), and are a necessary part of the modeling for TCDD.

Andersen et al. (1993b) provided a simple comparison of the induction of CYP1A1 and CYP1A2, the concentration of free TCDD in the liver, and the total concentration of TCDD in the liver to tumor incidence (Kociba et al., 1976) and to the volume of altered hepatic foci (Pitot et al., 1987). The computed cumulative hepatic concentrations of TCDD and induced proteins were used as summary metrics of internal exposure. Tumor promotion correlated more closely with predicted induction of CYP1A1 than with the other dose metrics. The choice of an independent induction

model for CYP1A1 and a Hill coefficient greater than 1 leads to nonlinear low-dose behavior. These correlations were not based on any mechanistic considerations of the role of induction of CYP1A1 in hepatocarcinogenesis.

8.4.2.1.4. ***Improving the physiological characteristics of the TCDD models***. Kohn et al. (1993) modeled the binding of TCDD to the AhR using explicit rate constants for association and dissociation of ligand instead of dissociation equilibrium constants. However, large unidirectional specific rates were used, leading to a predicted TCDD–AhR complex concentration similar to that computed by Leung et al. (1990a) and Andersen et al. (1993a). Other binding reactions in the model were handled similarly (e.g., TCDD binding to CYP1A2 and TCDD binding to blood protein). This approach avoids having to solve for the concentration of TCDD in the liver using the mass conservation relationship described in Leung et al. (1990a) as mass balance is automatically achieved. The physiology described in the Kohn et al. (1993) model is dependent on the body weight of the animal. Body weighs as a function of dose and age were recorded by Tritscher et al. (1992) and directly incorporated into the model by cubic spline interpolation among the measured values. Tissue volumes and flows were calculated by allometric formulas based on work by Delp et al. (1991). To allow the model to fit data at both low and high doses (Tritscher et al., 1992), this model includes loss of TCDD from the liver by lysis of dead cells, where the rate of cell death was assumed to increase as a hyperbolic function of the cumulative amount of unbound hepatic TCDD. This assumption is based on the observation of a dose-response for cytotoxicity in livers of TCDD-treated rats (Maronpot et al., 1993) and is consistent with observed tissue burdens of TCDD. No information regarding the rate of TCDD release from lysed cells is available; therefore, this feature of the Kohn et al. (1993) model predicts a net contribution of TCDD clearance by TCDD-induced cell death.

A further extension of this model, incorporating effects on thyroid hormones (Kohn et al., 1996), included tissue blood compartments similar to those used by Andersen et al. (1993a). Blood was distributed among these compartments and a compartment for the major blood vessels, instead of supplementing a generalized blood compartment with the tissue blood. The GI tract was separated from the rapidly perfused tissues compartment to permit a more realistic representation of uptake of TCDD and perfusion of the liver. The allometrically scaled metabolic rate constant used in the Kohn et al. (1993) model was replaced by a Hill rate law, and parameters were estimated to reproduce the kinetic data of Abraham et al. (1988) and the dose-response data of Tritscher et al. (1992).

Transthyretin (also known as prealbumin) can bind hydroxylated PCDDs, (McKinney et al., 1985) and single doses of TCDD can cause prolonged decrease in this protein (Albro et al., 1978). A dose-dependent decrease was included in the model and the algebraic equation for blood binding

was replaced by a differential equation. The revised model, incorporating blood binding, correctly predicted blood TCDD data not used in constructing the model. Ignoring production of binding protein led to serious underestimation of the low-dose data, and ignoring inhibition led to overestimation of the high-dose data. This revised model also differed from the earlier version in its treatment of loss of TCDD from the liver consequent to cytotoxicity. Instead of simply disappearing from the model, TCDD from lysed cells was assumed to pass via the bile into the gut, where it was reabsorbed and redistributed to tissues. This model also explicitly accounted for background exposures of TCDD equivalents in the feed, as observed by Vanden Heuvel et al. (1994a).

The above models have been applied in developing dose metrics for biochemical and tissue-response models. They do not necessarily include every aspect of the distribution of TCDD within the mammalian organism. The following two efforts expand on issues related to TCDD distribution. However, at this time they have not been included in the dose-response models and are unlikely to dramatically change estimates of dose metrics.

8.4.2.1.5. ***Lipid metabolism and sequestration in blood***. The above PBPK models empirically represent sequestration of TCDD in blood without reference to the nature of the pools of TCDD in the blood compartment. Animals exposed to high doses of TCDD and related compounds exhibit alterations in lipid metabolism characterized by mobilization of fat stores and resulting in wasting, hyperlipidemia, and fatty liver. Roth et al. (1993, 1994) constructed a PBPK model of the distribution of TCDD in the rat over a 16-day period following an oral dose. The model did not include tissue blood compartments but did consider diffusion limitation in uptake by multiplying tissue perfusion rates by a fractional extraction, mathematically identical to the formulations of Andersen et al. (1993a) and Kohn et al. (1996). A unique feature of this model was the division of the GI tract into five subcompartments—stomach, duodenum, jejunum, cecum, and colon—with sequential passage of ingested material. The model also separates the rapidly perfused tissues compartment into its constitutive organs and separates white and brown adipose tissue because of their different perfusion rates and differences in ability to mobilize lipid stores. The model included an earlier submodel of fatty acid metabolism in liver and adipose tissues, triglyceride transport via lipoprotein particles in blood plasma, and uptake of lipoprotein by liver and fat (Roth et al., 1994). Regulation of food consumption and lipolysis in white adipose tissue were assumed to be regulated by a cytosolic receptor that binds TCDD.

The model included the possibility for loss of body weight, muscle mass, and fat weight and hypertrophy of the liver subsequent to TCDD administration. It matched data for the initial increases and subsequent declines of TCDD in liver and brown and white fat. Fecal and urinary excretion data also were reproduced. The model included induction of CYP1A2 binding sites for

TCDD. The measured concentration of TCDD in white adipose tissue shows a paradoxical increase at 16 days postdosing despite the fact that TCDD was being cleared from the body. The model of Roth et al. (1994) failed to reproduce this effect, but the concentration in the lipid portion of the tissue did increase because the mass of lipid was decreasing in highly exposed animals. Roth et al. suggested that barriers to uptake and efflux of TCDD may not be symmetrical.

Roth et al. (1994) cited evidence that TCDD is absorbed from the gut, dissolved in dietary fat, carried into the bloodstream by chylomicrons, and secreted into the gut lumen from the intestinal mucosa. There does not appear to be a significant first-pass extraction of these unprocessed lipoprotein particles by the liver. Several tissues (e.g., heart, spleen, and fat) have high levels of receptors for such very-low-density lipoprotein vesicles. So TCDD transport may be regulated by endocytosis of these particles and not be under equilibrium control, as has been assumed in all other pharmacokinetic models. Such a process may reflect the mechanistic origin of diffusion-limitation in TCDD tissue uptake. Further research may be required to resolve this point. Another feature of the Roth et al. (1994) model that suggests additional research is the assumption that white adipose tissue contains a cytosolic TCDD receptor (adipose tissue does express the AhR) which mediates effects on lipid metabolism.

8.4.2.1.6. ***Diffusion limitations in multiple tissues***. Assessment of diffusion limitation in tissue uptake has been hampered by a lack of data at short times after dosing with TCDD. Wang et al. (1997) obtained time-course data for TCDD in blood, several tissues, and the remaining carcass following a single oral dose. They fit an eight-compartment (blood, lung, liver, kidney, spleen, fat, skin, carcass) PBPK model to these data, estimating the values of gut absorption rate, tissue permeability, partition coefficients, AhR concentrations, and CYP1A2 induction parameters by an ad hoc method (no formal optimization). The terminal TCDD half-lives in liver and kidney were assumed to reflect metabolism and were used to calculate an effective first-order rate constant. Time courses in highly vascularized tissues (lung, spleen) could be fit with flow-limited kinetics, but diffusion restriction was required for other tissues, especially kidney. The model by Wang et al. was also used to predict induction of CYP1A1 and CYP1A2 protein in liver and CYP1A1 and CYP1A2 enzyme activity in liver, kidney, lung, and skin (Santostefano et al., 1998). This model has recently been shown to predict the TCDD tissue concentrations from a study by Krowke and coworkers using a loading dose/maintenance dose exposure regimen (Wang et al., 2000). However, it was not demonstrated that the model could reproduce responses to chronic exposure to TCDD.

8.4.2.1.7. ***Modeling of dose-dependent tissue disposition in humans***. Carrier et al. developed a simple empirical model to account for dose-dependent hepatic sequestration of dibenzofurans and other TCDD-like compounds (Carrier et al., 1995a,b). This description had two primary

parameters: a maximum proportion sequestration of body burden in the liver (F_{max}) and a half-saturation constant (K_d)(in units of µg TEQ/kg) for enhanced sequestration with increasing dose. These two parameters were estimated by fitting the model to data on the dose-dependent sequestration in the liver presumed to occur in the livers from human poisoning incidents in Japan and China. The model was also used to derive similar empirical constants from the rat data (Abraham et al., 1988). These two fitting parameters do not contain specific information about the biology of TCDD and related compounds. A PBPK model for TCDD was used recently to infer the relationship between specific biological factors and these two empirical parameters (Evans and Anderson, 2000). With sensitivity analyses, the half saturation constant (K_d) was found to be related to characteristics of the binding of TCDD to the AhR and the AhR-TCDD complex binding to dioxin response elements on DNA. In contrast, the maximum proportion in liver is determined by fat:blood partition coefficients and binding parameters for the interaction of CYP1A2 with TCDD. The composite parameters of Carrier's models (1995a,b) have no obvious relationship to specific biological processes.

In principle, it is possible to convert a PBPK model of disposition of TCDD in a laboratory rodent into one for a human by substituting human parameter values for rodent values. (Andersen et al., 1997c). Although values for anatomical and physiological parameters are available for humans, the biochemical parameters (e.g., TCDD metabolism, binding to the AhR and CYP1A2, and induction of the various proteins cited above) are generally not available for humans. Parameters for protein binding (K_d and basal B_{max}) could be determined in vitro from samples of human tissues obtained either postmortem or from surgical patients, but estimating parameters for induction of proteins would require tissue samples from living individuals exposed to dioxin. Alternatives to measuring human parameter values include allometric scaling of rodent values by the 2/3 or 3/4 power of body weight. This tactic is suspect, as species differences in expression of proteins do not follow a simple pattern for all proteins.

8.4.2.2. *Biochemical, Tissue, and Endocrine Response Models*

The next step after the modeling of the disposition of TCDD within the body is the modeling of effects of TCDD on biological responses that are plausibly linked with activation of the AhR.

8.4.2.2.1. ***Generic receptor-mediated response models***. Looking at one aspect of modeling of TCDD's effects, Portier et al. (1993) examined the relationship between tissue concentration and the response of three liver proteins by TCDD in intact female Sprague-Dawley rats. The effects studied included the induction of two hepatic cytochrome P-450 isozymes, CYP1A1 and CYP1A2, and the reduction in maximal binding of EGF to its receptor in the hepatic plasma membrane.

Portier et al. (1993) modeled the rate-limiting step in the induction of CYP1A1 and CYP1A2 following exposure to TCDD using a Hill equation. Hill equations are commonly used for modeling ligand-receptor binding and enzymatic kinetics data. Consequently, these models could be applied to other receptor-mediated effects and are not specific to TCDD and the AhR. The Hill equation allows for both linear and nonlinear response below the maximal induction range. A complete discussion of Hill kinetics and other models for ligand-receptor binding is given by Boeynaems and Dumont (1980). Examples of the use of Hill kinetics for ligand-receptor binding include the muscarinic acetylcholine receptors (Hulme et al., 1981), nicotinic acetylcholine receptors, opiate receptors (Blume, 1981), the AhR (Gasiewicz and Rucci, 1984), estrogen receptors (Notides et al., 1985), and glucocorticoid receptors (Sunahara et al., 1989). The Hill model can be thought of as a very general kinetic model that reduces to hyperbolic kinetics when the Hill exponent is 1. Portier et al. (1993) also modeled the reduction in maximal binding to the EGF receptor with Hill kinetics, assuming that TCDD reduces expression of the receptor protein from the rate observed in control animals. For all EGFR, CYP1A1, and CYP1A2, proteolysis was assumed to follow Michaelis–Menten kinetics. The proposed models fit the data in the observable response range. The major purpose of this paper by Portier et al. was to emphasize the importance of the mechanism of basal (i.e., uninduced) expression on the curve shape of tissue concentration of protein vs. dose of TCDD. For each protein, they considered two separate models of steady-state protein production.

In the first model, the additional expression of protein induced by TCDD is independent of the basal-level expression. In their second model, basal expression of these proteins is mediated by a ligand of endogenous or dietary origin that competes with TCDD for binding sites on the AhR. Using these simple models, Portier et al. (1993) see virtually no difference in predicted protein concentrations between the independent and additive models in the observable response range, even estimating almost equal Hill coefficients in the two models for all three proteins. In the low-dose range where risk extrapolation would occur, the models differed depending on the value of the Hill coefficient. An estimated Hill exponent exceeding 1 yielded a concave upwards dose-response curve, especially for the independent model. This behavior implies diminished increases in responses at very low doses followed by an accelerated response as the dose increases. For CYP1A2, the Hill exponent was estimated to be about 0.5. When the estimated Hill exponent is less than 1, the dose-response curve was convex upwards, indicating greater than linear increases in response at low doses. Finally, for the EGF receptor, the Hill exponent was approximately 1, in which case the two models are identical.

The additive model is expected to exhibit low-dose linearity because each additional molecule of TCDD adds more ligand to the pool available for binding and, under subsaturating conditions, proportionally increases the concentration of protein. Similar observations have been

made with regard to statistical (Hoel, 1980) and mechanistic (Portier, 1987) models for tumor incidence. Thus, even though these two basic models show almost identical response in the observable response region, their low-dose behavior is remarkably different. If either CYP1A1 or CYP1A2 levels had been used as dose surrogates for low-dose risk estimation, the choice of the independent or additive model would yield differences of several orders of magnitude in the risk estimates for humans. Using CYP1A1 as a dose surrogate, the independent model would predict much lower risk estimates than the additive model. For CYP1A2, the opposite occurs. For EGF receptor, there would be no difference.

8.4.2.2.2. ***Specific biochemical responses to TCDD***. Kohn et al. (1993) have provided an extensive model of the biochemistry of TCDD in the liver to explain TCDD-mediated alterations in hepatic proteins in the rat, specifically considering CYP1A1, CYP1A2, and the Ah, EGF, and estrogen receptors over a wide dose range. The model describes the distribution of TCDD to the various tissues, accounting for both time and dose effects observed by other researchers. A description of the PBPK portion of this model is described above. Earlier PBPK models (Andersen et al., 1993a, Leung et al., 1990a) relied on several single-dose data sets (Rose et al., 1976; Abraham et al., 1988) and were validated against dosimetry results from longer term subchronic and chronic dosing regimens (Kociba et al., 1976; Krowke et al., 1989). These and other studies (Tritscher et al., 1992; Sewall et al., 1993) were used to model the pharmacokinetics and induction of gene products in female Sprague-Dawley rats (Kohn et al., 1993). Among the data reported were concentrations of TCDD in blood and liver, concentrations of hepatic CYP1A1 and CYP1A2, and EGF receptor binding capacity in the hepatocyte plasma membrane. The tissue dosimetry for the model (Kohn et al., 1993) was validated against single-dose and chronic dosing regimen experimental data not used in estimation of model parameters.

In the biochemical effects portion of the model the AhR-TCDD complex upregulates four proteins: CYP1A1, CYP1A2, the AhR, and an EGF-like peptide (treated nominally as transforming growth factor-alpha, TGF-alpha). The induction of an EGF-like peptide is deduced from observations on human keratinocytes (Choi et al., 1991; Gaido et al., 1992) and is quantified on the basis of a presumed interaction with the EGF receptor, resulting in a downregulation and internalization of the EGF receptor. However, TCDD-mediated induction of TGF-alpha or of other EGF-like peptides has not been demonstrated in liver. For all four proteins, synthesis is defined explicitly as a function of occupied AhR concentration. Constitutive rates of expression for CYP1A2, AhR, and EGF receptor are substantial and were assumed independent of the induced expression. The Hill coefficients for the induction of these proteins were estimated to be 1.0, indicating low dose linearity in this response irrespective of the mechanism of basal expression.

Estimated ED_{01} values for TCDD-regulated responses predicted from the dose-response model is shown in Table 8-8.

The model included a background of dioxin-like AhR agonists, which compete with TCDD for binding to the receptor. Induction of CYP1A1 was assumed to be based on additive induction because this enzyme is poorly expressed in the absence of an inducer and expression in control animals is likely due to the background exposure. Again, the Hill exponent was estimated to be 1, leading to low-dose linearity under either additive or independent assumptions. This model predicts that the induction of all gene products appears to be a hyperbolic function of dose without any apparent cooperativity. The discrepancy in the estimates of the Hill exponents between this model and the other models discussed (Andersen et al., 1993a,b; Portier et al., 1993; Kedderis et al., 1993) is probably related to the inclusion only in the Kohn et al. (1993) model of induction of the AhR. The effects of TCDD on the AhR concentration are uncertain. In acute studies, the AhR is decreased following TCDD exposure (Pollenz et al., 1998), whereas in subchronic studies, there is some evidence that the AhR is increased (Sloop and Lucier, 1987). Further studies are required to better understand the regulation of the AhR following TCDD exposure.

The AhR-TCDD complex is assumed to downregulate the EGF receptor in the Kohn et al. (1993) model. It was assumed that the estrogen receptor-estrogen complex synergistically reacts with the AhR-TCDD complex to transcriptionally activate gene(s) that regulate synthesis of an EGF-like peptide. This term was introduced to partially account for the observation of reduced TCDD tumor-promoting potency in ovariectomized females as compared to intact female rats (Lucier et al., 1991). This mechanism of TCDD regulation of these proteins, although supported by some data (Sunahara et al., 1989; Clark et al., 1991), is speculative.

Vanden Heuvel et al. (1994b) provided data on the production of CYP1A1 mRNA and protein following a single oral dose of TCDD. These observations were used to extend the Kohn et al. model and resulted in a model that predicted two critical DNA binding sites for the liganded AhR with different affinities (Vanden Heuvel et al., 1994; Kohn et al., 1994). Both sites had to be occupied in order to activate transcription. This rate equation led to a sigmoidal dose-response curve for the message. Protein synthesis on the mRNA template was modeled by a Hill equation. The optimal Hill exponent was less than 1 and the computed overall dose-response was hyperbolic, as in the Kohn et al. model. This result suggests that the supralinear response of protein to mRNA production compensates for the sublinear response of the message to AhR-TCDD complex formation. It is possible that this reflects the greater sensitivity of the RT-PCR method to detect CYP1A1 mRNA than measurement of CYP1A1 protein. Within this context it is of note that there are more than two DREs within the human CYP1A1 promoter region that may be occupied (Kress et al., 1998).

8.4.2.2.3. ***Tissue response models: zonal induction model***. The mechanistic model of Kohn et al. treats the TCDD-treated liver as a single homogeneous unit. With regard to the induction of cytochromes P-450 in the liver, Tritscher et al. (1992) used antibody staining techniques, showing that the induction of CYP1A1 and CYP1A2 by TCDD in the liver exhibits a regiospecific pattern of induction characterized by increased areas of staining around the central vein of the liver lobule. The size of the induced region in the centrilobular region increased with increasing dose of TCDD. This sharp demarcation in observed induction within hepatocytes could be due to an insensitivity in detection of low levels of CYP proteins in the cell using immunohistochemical techniques: alternatively, it may indicate differences in the sensitivity of hepatocytes to TCDD across the liver. In an attempt to model this regiospecific pattern of induction, Andersen et al. assumed that the observed sharp demarcation in CYP1A expression between induced and noninduced regions indicated that individual hepatocytes were either fully induced or noninduced (Anderson et al., 1997a,b). In this model the liver lobular structure was divided the into five concentric zones with a threefold difference between adjacent zones, in the affinity of DREs for the liganded AhR. The model also further used Hill kinetics for induction, with a Hill exponent of 4. The model reproduced the qualitative features of expanding zonal induction and, with parameters selected to yield a fit to time-course data (Abraham et al., 1988) and CYP1A1 mRNA data (Vanden Heuvel et al., 1994), produced a fit to P-450 data comparable to that obtained with the homogeneous liver model of Kohn et al. (1993). The mRNA data were fitted without proposing multiple DRE binding sites for transcriptional control of message. However, the low-dose extrapolated responses predicted by the regional induction model exhibited greater low-dose sublinearity than a comparable homogeneous liver model. The model predicted an 81-fold difference in AhR-TCDD binding between periportal and centrilobular zones and utilized steep Hill kinetics; these two issues drive the low-dose nonlinearity of this model and are important areas for further research.

8.4.2.2.4. ***Endocrine models: thyroid hormones.*** In addition to models of whole-tissue responses such as that seen in the liver, attempts have also been made to model endocrine effects that encompass changes that may occur in multiple tissues. This is demonstrated in the thyroid hormone model of Kohn et al. (1994). TCDD induces thyroid tumors in male rats and female mice at lower doses than those that induce liver tumors in female rats (NTP, 1982a). Sewall et al. (1995) found increased circulating thyrotropin (TSH) and thyroid hypertrophy and hyperplasia in TCDD-treated rats, suggesting that thyroid tumors may be a consequence of chronically elevated serum TSH (Hill et al., 1989). Because this may be a sensitive endpoint for TCDD carcinogenesis, the Kohn et al. (1993) model was extended (Kohn et al., 1996) to include effects of TCDD on thyroid hormones.

The extended model added compartments for tissues involved in the production (pituitary and thyroid glands) and storage (e.g., kidney, brown fat) of thyroid hormones as well as equations

for secretion and metabolism of the hormones. It reproduced the data used in the original model, blood levels of thyroid hormones and TSH (Sewall et al., 1995), and mRNA (vanden Heuvel et al., 1994b) for the thyroxine metabolizing enzyme UDP-glucuronosyl-transferase-1*6 (UDPGT). It also reproduced experimental data for induction of this enzyme that were not used in the construction of the extended model. In the model, induction of UDPGT by TCDD and subsequent endocrine changes in thyroid hormone homeostasis can lead to chronically elevated serum TSH. This may be related to increased thyroid cancer risk. The estimated dose-response relationships were hyperbolic in the experimental range, supporting a linear dose-response at lower doses.

8.4.2.2.5. ***Dose-response behavior of biochemical/tissue dose-response models*****.** The models of Kohn et al. (1993, 1996) are based on the concept that tissue-level responses are emergent properties that arise from the accumulated molecular effects of exposure to TCDD. Thus, the models were constructed in a bottom-up fashion starting from these more elementary steps, e.g., binding to the AhR, transcriptional activation, translation of mRNA, and the enzymatic functions of the induced proteins. The calculated responses that can serve as dose metrics include altered expression of CYP1A1, CYP1A2, and UDPGT. Because TCDD induces expression of the AhR, lower computed doses are required to obtain the same responses as estimated by models that ignore this effect. The critical steps are binding of the liganded AhR to DREs and translation of the mRNA into protein. The most important lesson of this modeling exercise is that lack of significant sigmoidicity in the dose-response curves calculated for these proteins arises from saturation of protein synthesis at low concentrations of mRNA, compensating for possible sublinearity in transcription. Similar compensatory effects led to low-dose linearity in the more complex responses of EGF receptor internalization and elevation of plasma TSH.

Any of the above responses can serve as indices of toxicity or pathology, and which is selected for such use depends on the hypothesized origin of the endpoint. Use of CYP1A2 as a marker for indirect DNA damage is based on the hypothesis that the catalytic properties of this enzyme lead to the generation of free radicals or DNA-reactive quinones (Yager and Liehr, 1996). Use of the internalized EGF receptor as a marker for promotional effects in the liver is based on the hypothesis that TCDD induces growth factors that are ligands of this receptor. Use of TSH as a marker for promotional effects in the thyroid is based on the goitrogenic properties of this hormone. Further experiments are required to determine if these postulated events are causally related to the pathological responses. Nevertheless, if the computed responses are used as dose metrics, the model indicates that linear extrapolation from the experimental dose range can be used to estimate low-dose effects.

The main hepatic response motivating the regional induction model was the pattern of staining within hepatic lobules in TCDD-treated rats (Tritscher et al., 1992). On the basis of

geometric considerations, hepatic lobular structure was described as a series of concentric lobular regions with differing affinities of DNA binding sites for the Ah-TCDD complex (Andersen et al., 1997a). A main underlying assumption was a linear correspondence between mRNA concentrations and protein levels, modeled by an inducible rate of synthesis and a first-order degradation. The rate of message production was modeled with Hill kinetics with respect to receptor complex concentration. The successful parameterization required differences in binding affinity between adjacent zones and very steep dependence on TCDD and Ah-receptor complex concentration (i.e., the estimated Hill coefficients were large) in order to reproduce experimental data. A single-compartment liver model was also examined. It could reproduce all data except the heterogeneous distribution and low-dose mRNA levels. The major inference drawn from this analysis was that induction should be considered on the level of the cell, not the gene. The effects appear to be coordinate, cooperative expression of a battery of gene products and emergence of new cellular characteristics. This behavior, if true, might be regarded as a reversible differentiation of TCDD-transformed phenotype, rather than induction of single genes in isolation. Overall linear behavior in the entire liver arises from composite responses of individual cells with differing thresholds for induction. The sensitivity of cells in the centrilobular region of the liver would determine the low-dose behaviors.

In the present model the low-dose behavior of this small group of cells would be distinctly nonlinear. The ED_{01} with this regional induction model was about 1.4 ng/kg/day (Table 8-8). This value is close to the estimate of 0.34 for the induction of CYP1A2 estimated by Kohn et al. More significant than the differences in ED_{01} values are the inferences drawn with regard to the shape of the curve in the low-dose region by the two models. Specific studies on regional induction and cellular level responses should be vigorously pursued to discriminate between these two model structures. Regional induction of mRNA needs to be studied on a more quantitative level and methods need to be developed for studying induction in primary hepatocytes. Recent data in rats exposed to TCDD demonstrate that the hepatocytes in the centrilobular region accumulate TCDD to a greater extent in the low-dose region and are more responsive to TCDD than are the periportal hepatocytes (Santostefano et al., 1999).

8.4.3. Application of Models

The goal of biochemical response models is to link TCDD-regulated responses to adverse effects associated with TCDD exposures. In principle, these models could be applied to a variety of adverse responses. The focus of the application of these models has been to carcinogenic endpoints. Much less attention has been given to the application of mathematical models to the development of noncancer pathologies.

TCDD is a potent carcinogen in all animal species tested (see Part II, Chapter 6). TCDD is an operational promoter, as defined in assay systems of skin and/or liver in mice and rats (Schrenk et al., 1994; Maronpot et al., 1993; Clark et al., 1991; Pitot et al., 1980; van Birgelen et al., 1999; Buchman et al., 1994) (see Chapter 6). Mathematical modeling can be a powerful tool for understanding and combining information on complex biological phenomena such as carcinogenesis. For the analysis of tumor promotion by TCDD, much of the focus on the use of mathematical and mechanistic models has been on understanding the mechanism of hepatocarcinogenesis induced by TCDD. Specifically, the focus has been on modeling the development of putatively preneoplastic altered hepatocellular foci (AHF) that exhibit altered expression of marker enzymes such as placental glutathione-s-transferase (PGST), or gamma-glutamyl transpeptidase (GGT). Mechanism-based modeling of carcinogenicity can be accomplished by incorporating linkages between cell growth and mutation and the biochemical/tissue responses of TCDD, within the context of the quantitative dose-response models described above. In addition, analysis of changes in hepatocyte replication has been used to estimate of parameter values for in some models.

8.4.3.1. *Modeling Preneoplastic Lesions*

Within the framework of a two-stage model of carcinogenesis, these models treat AHFs as an initiated phenotype produced by conversion of a normal cell by a mutational event. Models for the numbers of normal and initiated cells also incorporate parameters related to the relative birth rates and death rates of the respective cell populations. These growth and mutational parameters may or may not be directly related to biological processes altered by TCDD. Three research groups have evaluated growth and development of AHFs, using different mathematical approaches, different assumptions of the phenotypic distribution of the AHFs, and different linkages of biological processes to the model parameters.

8.4.3.1.1. ***Models with a single initiated phenotype.*** Portier et al. (1996) estimated the parameters in the first half of a two-stage mathematical model of carcinogenesis from the initiation-promotion data (Maronpot et al., 1993) using previously developed methods (Dewanji et al., 1989). This analysis used daily average dose as the dose metric for examining dose dependent effects of TCDD on model parameters. Maronpot et al. (1993) quantified the number and size of liver AHF lesions expressing the placental form of glutathione-S-transferase (PGST). The modeling results indicate that TCDD stimulates the production of PGST-positive AHF (which could indicate a mutational effect) and promotes the growth of PGST AHF (as a result of either increases in birthrate or decreases in the death rate). Data on cell replication indices and liver weight could not explain the mutational effect of TCDD. Following upon the work of Kohn et al. (1993), Portier et al. (1996)

suggested this finding could be due to an increase in the metabolism of estrogens to catechol estrogens, leading to subsequent increase in free oxygen radicals and eventually to mutations. The analysis also indicated an interaction between DEN and TCDD that results in dose-related formation of initiated cells throughout the study period. Portier et al. (1996) also found that best-fitting curves (using maximum likelihood methods) for the effect of TCDD on the mutation and birth rates reached saturation levels at doses below 3.5 ng/kg/day.

As a validation exercise, Portier et al. (1987) used the same methods to analyze focal lesion data from Pitot et al. The two studies utilized different initiation protocols. In the Maronpot experiments, a necrogenic DEN dose (175 mg/kg) was used, whereas in the Pitot experiments a non-necrogenic dose of DEN (30 mg/kg) was given 24 hours after partial hepatectomy. These two initiation protocols lead to differences in background tumor rates and differences in time course for tumor development following TCDD exposure.

In the Pitot experiment, three types of enzyme-altered AHF were quantified using the marker enzymes gamma-glutamyltranspeptidase (GGT), canalicular adenosine triphosphatase (ATP) and glucose-6-phosphatase (G6P). Portier et al. (1996) found that all four types of AHF from the two different studies produced similar qualitative results; TCDD had effects on both mutation and birth rates. The effect of dose on the birth rates for both data sets produced similar patterns, with an almost identical unexposed birthrate for all of the four lesion types, a maximal increase over the background rate between 33% and 300%, saturation of the increased birthrate at low doses, and a small increase in birthrate because of DEN initiation. The pattern of dose-related changes in the mutation rate is slightly different in the ATP, GGT, and G6P AHF than for the PGST AHF, tending more toward linearity than the hyperbolic response seen for the PGST AHF. However, for all four lesions, the maximal induction rate tended to be the same.

Moolgavkar et al. (1996) analyzed data from Buchmann et al. (1994) on ATP AHF in female Wistar rats exposed to 2,3,7,8-TCDD as well as 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HCDD). The initiation protocol was a non-necrogenic dose (10 mg/kg) for 5 consecutive days. In addition to the mathematical analysis developed by Dewanji et al. (1989), Moolgavkar et al. (1996) used a modification that allowed for cellular replication focused on the edge of the AHF. Although Moolgavkar et al. (1996) did not have information on multiple dose groups, the results of their analysis for TCDD concur qualitatively with those of Portier et al. (1996). In essence, they observed no effect on the birthrate of initiated cells, a significant (sevenfold in noninitiated and twofold in initiated) effect of TCDD on the mutation, and a prolonged effect of DEN following initiation (similar to the interaction effect observed by Portier et al. [1996]). The observed lack of change in birthrates is similar to that of the nonsignificant increase observed by Portier et al. (1996) for PGST+, GGT, and G6P foci, but smaller than that for ATP foci in the Pitot et al. (1980) study. In the DEN-initiated groups, the associated increases in the mutation rates were quantitatively

similar to those observed for PGST lesions in the Portier et al. (1996) study (2.2-fold at 100 ng/kg/day in Moolgavkar et al. (1996), 2.5-fold at 125 ng/kg/day for PGST), but much smaller than those observed for the ATP, GGT, and G6P lesions from the Pitot et al. (1980) study (9.9-fold for ATP, 4.5-fold for GGT and 5.8-fold for G6P). The observed increase in the mutation rate in noninitiated animals was much larger in the Moolgavkar et al. (1996) analysis than that for the Portier et al. (1996) analysis. This study was conducted at a single dose and the comparison is simply treated versus control.

8.4.3.1.2. ***Models with two initiated phenotypes*.** Conolly and Andersen (1997) developed a model for focal lesion growth based upon two types of initiated cells, applying the negative selection mechanism for hepatic tumor promotion proposed by Jirtle et al. (1991a,b). In this model, even though the two types of initiated cells express the same biochemical marker, they respond differently to promotional stimulation in the liver. The model presumes that a promotional stimulus to the liver is countered by mitoinhibitory signals generated by the liver to constrain proliferation. One set of mutated cells is sensitive to this mitoinhibition whereas the other set of mutated cells is insensitive and responds only to the promotional stimulus. The result is that, under increasing doses of the promoter, one group of focal lesions is decreasing in size, and hence number of cells, while the other group is increasing in size.

Conolly and Andersen's model is different from those of Portier et al. (1996) and Moolgavkar et al. (1996) in that it can result in U-shaped dose-response curves for the total number and mean size of observable focal lesions without using U-shaped parametric forms for the mutation rates or the birthrates. Number and size of focal lesions were estimated using the stochastic resampling methods outlined in Conolly and Kimbell (1994), with deterministic growth replacing stochastic growth when colonies exceeded 1,000 cells. Twenty-five replicates for each model output were compared to the data for the combination of all three focal lesion types from the study by Pitot et al. (1980) to obtain parameter estimates for the birth and death rates of the two types of mutated cells. This analysis used administered dose as the tissue dose metric.

The two-cell model adequately fit the data with biologically reasonable parameter values. An alternative model including an effect of TCDD on mutation rates was not considered. Similarly, the earlier analyses of Portier and Moolgavkar did not consider two types of initiated cells, so comparisons between models with one type of initiated cell versus two types of initiated cells relating to the issue of the effect of TCDD on mutation rates cannot be made. This is an area that could use additional research. The birthrates (combined for the two mutated clones in the Conolly and Andersen model) for all three sets of models (Portier et al., 1996; Moolgavkar et al., 1996; Conolly and Andersen, 1997) are comparable in the control groups but differ substantially for the higher dose groups, with the two clone models having much larger rates. This difference is

partially due to the assumption in the Conolly and Andersen model that there is no increase in mutation rate following initiation and partially due to the use of an increasing death rate with exposure to TCDD. Portier et al. (1996) used a fixed death rate in their final model and Moolgavkar et al. (1996) varied the death rate with the birth rate. Results from a study of Stinchcombe et al. (1995) indicate a lack of significant effects of TCDD on cell replication in PGST foci, but remarkable suppression of apoptosis within PGST-positive AHF. This study, however does not supply information on dose dependency of these parameters. Given the lack of sufficient data, it is not possible to simultaneously estimate both the birthrates and death rates for the initiated cell phenotypes.

8.4.3.1.3. ***Alternative dose metrics in promotion studies.*** In the above models, oral dose of TCDD was essentially used as the dose metric. In contrast, Conolly and Andersen used the fraction of the maximum possible induction of CYP1A1 and CYP1A2 calculated from the zonal induction model (Andersen et al., 1997a) as a dose-surrogate for the effect of TCDD on the clonal expansion of both mutated cell types within the framework of a two-cell multistage model. Andersen et al. (1997a) fit their multicompartment geometric model of hepatic zonation (Andersen et al., 1997b) to data derived from several studies on the expression of CYP1A2 in rats (Abraham et al., 1988; Tritscher et al., 1992; van den Heuvel et al., 1994b). The zonal induction model is described previously in this review. The model was linked to the previous PBPK model (Andersen et al., 1993a) with modifications (Andersen et al., 1997b) to account for the regional induction of CYP1A2, rather than to the original model which was based upon uniform expression throughout the liver. Formal optimization methods were not used to obtain model parameters; however, graphical comparisons of the model predictions to these data did not appear to be obviously different from previous descriptions and provided adequate fits. The dissociation constants for binding of the TCDD-AhR complex to dioxin-responsive elements for CYP1A1 (0.6 to 2 nM for compartment 3) and CYP1A2 (0.08 to 1.0 nM for compartment 3) were fit separately for each data set and varied by a factor of 3 from compartment to compartment. This produced a model that fit the fraction of liver volume occupied by focal cells, but failed to fit the number of foci per volume of liver as well as the original analysis. These analyses used percent of liver expressing CYP1A2 as an indicator of the dose metric.

8.4.3.2. *Estimation of Cancer Risks*

Portier and Kohn (1996) combined the biochemical response model of Kohn et al. (1993) with a single initiated phenotype two-stage model of carcinogenesis to estimate liver tumor incidence in female Sprague-Dawley rats from the 2-year cancer bioassay of Kociba et al. (1978). In the simplest of several models tested, the initial mutation rate to the initiated phenotype was

proportional to the instantaneous concentration of CYP1A2 as predicted by the biochemical model of Kohn et al. The birthrate of mutated cells was a linear function of loss of EGFR. All death rates were held constant, as was the second mutation rate from the initiated to the malignant phenotype. This model adequately fit the tumor data, although it overestimated the observed tumor response at the lowest dose in the Kociba et al. (1978) study. The shape of the dose-response curve was approximately linear and the estimated ED_{01} value for this model (0.15 ng/kg/day) is presented in Table 8-8. The corresponding body burden giving a 1% increased effect was 2.7 ng/kg. The use of CYP1A2 as a dose metric for the first mutation rate is consistent with its role as the major TCDD-inducible estradiol hydroxylase in the liver (Hayes et al., 1996; Dannan et al., 1986) and with the hypothesized role of estrogen metabolites leading to increased oxidative DNA damage and increased mutation (Yager and Liehr, 1996; Roy et al., 1992; Cavalieri et al., 1997) .

Even though the thyroid hormone model of Kohn et al. (1996) has not been strictly used for modeling of thyroid neoplasia induced by TCDD, it is important to note that the hypothesis for induction of thyroid neoplasia consequent to growth stimulation by chronically elevated serum TSH is highly plausible. In contrast there is weaker evidence in the liver that alteration in CYP1A2 and EGFR are causally linked to carcinogenesis. Given that the alteration in thyroid hormone homeostasis as a consequence of TCDD induction of UDPGT can be effectively modeled provides an excellent opportunity to mechanistically link activation of gene expression by TCDD with thyroid cancer risk.

8.4.4. Knowledge/Data Gaps

Knowledge gaps still exist with each of the models. All the PBPK models have biological structure and encode hypotheses about the modulation of protein concentrations by TCDD. However, each of them falls between curve fitting and mathematical representations of known biology. Parameters in empirical equations representing overall production of the protein gene products, for example, were estimated using dose-response data for protein concentrations and enzyme activity. Although protein level is a direct consequence of gene expression, this empirical approach constitutes curve fitting. In the cases of CYP1A1 and UDGPT induction, information about both mRNA and protein levels was available, permitting a more realistic, although still empirical, representation of the mechanism of induction. Similarly, equations for metabolism of TCDD and thyroid hormones in the model of Kohn et al. (1996) and of lipids in the model of Roth et al. (1994) are not based on detailed studies of the enzymatic kinetics but are greatly simplified representations. Nonetheless, the structure of the physiological models was specified by information on anatomy, physiology, and qualitative effects of TCDD. These PBPK models reproduce protein concentrations in data sets that were not included in the construction of the model

and that were obtained from experimental designs different from those used to define the model. This constitutes at least a partial mechanistic validation of these models.

Models for tissue response including lipid metabolism and hepatic lobular effects also have aspects that need confirmation. The Roth et al. (1994) model has not been validated for chronic exposures or low doses. Even though the Wang et al. (1997) model has examined CYP1A1 and CYP1A2 induction, it has not been validated for chronic exposures. The regional induction model (Andersen et al., 1997a,b) creates a hypothesis concerning regional induction that should be further studied. An alternative to altering the affinity of DREs to the liganded AhR is a gradient in the receptor concentration across the liver acinus. The concentration of the receptor in centrilobular hepatocytes was found to be more than 40 times that in periportal hepatocytes (Lindros et al., 1997). The use of Hill kinetics to describe at least some of the binding (or metabolic) reactions is a convenience to allow flexibility in estimating dose-response relationships.

The models for estimating values of the dose metrics for exposure or effects differ in their mathematical representations of the same physiological processes while providing comparable fits to the observed responses. The endocrine response model includes TCDD induction of the AhR, binding to multiple DREs, and saturation kinetics for protein synthesis on the mRNA template. This sequence of steps can potentially lead to nonlinear kinetics for the overall responses, but the nonlinearities in the individual steps appeared to compensate for each other, leading to approximately linear low-dose responses. The regional induction model (Andersen et al., 1997a) collapses this sequence into a single overall process and uses Hill kinetics to represent the potential overall nonlinearity. A high Hill exponent was required to reproduce the sharp edge detected for the induced region of the liver, leading to sublinear predicted responses below the experimentally accessible range of doses. Thus, emphasizing different aspects of the underlying biology leads to different mathematical structures with different predicted low-dose behavior. Which of these processes are most important in producing the overall responses cannot be resolved by existing data.

The biochemical and tissue response models were linked to a two-stage cancer model (Portier and Kohn, 1996). Although TCDD is not a mutagen in in vitro systems commonly used to detect mutation through DNA damage, inferences drawn from biochemical data and mechanistic modeling supported a secondary mechanism for TCDD-induced mutations (Portier et al., 1996; Moolgavkar et al., 1996). Another approach, with secondary pathways leading to mutations and two cellular phenotypes, also fit these data but does not require this secondary effect on mutation rate (Andersen et al., 1997a,b; Conolly and Andersen, 1997). Even though this secondary mechanism of mutation is still speculative, these studies present challenges to the application of general models for cancer risk assessment based on direct chemical mutagenesis as a fundamental mechanism for chemically induced or radiation-induced cancer and the notion of a single cellular phenotype as a precursor for cancer.

8.4.5. Summary

The development of PBPK models describing the disposition of TCDD within experimental animals has proceeded through multiple levels of refinement, with newer models incorporating ever-increasing levels of biological complexity. The two most complete PBPK models give similar predictions about TCDD tissue dose metrics. It is unlikely that additional refinement of the current models will have a major impact on the model predictions within the observable dose range. However, further work could better characterize the biological processes involved in disposition.

Despite their availability, these PBPK models have been highly underutilized in aiding empirical dose-response analyses for the effects of TCDD observed in laboratory studies. Differences in dosing regimens in experimental animals, such as exposure duration, route of exposure, time after dosing to necropsy, use of maintenance-loading dose regimen, etc., complicate the use of a simple metric based on administered dose for comparative analyses between studies (Section 8.3). The use of the current PBPK models could provide a more scientifically credible description of a body burden dose metric and may reduce some of the uncertainties introduced when converting a daily averaged dose ED_{01} to a body burden dose metric.

Similarly, the application of these models to human dose-response data, while possible has also not been pursued. The current level of detail in rodent PBPK modes for TCDD has not been included in any current human PBPK model for TCDD. Human exposure assessment for use in dose-response modeling utilizes either back-extrapolation based on a single measurement of a tissue (plasma/serum) concentration or a dose metric based on an estimated external exposure. Although extrapolation of the current generation of rodent PBPK models to humans would have uncertainties, it is unlikely that predictions from such a model would be any less uncertain that current methodologies used for estimating human body burdens.

With regard to the extension of PBPK models to biochemical response, tissue response, and toxicological responses, the differences in interpretation of the mechanism of action of a TCDD-dependent response lead to varying estimates of the dose-dependent behavior for similar responses. In addition, the hypotheses and assumptions used in different models may restrict the shape of the dose-response curves that are calculated and lead to differences in their low-dose behaviors.

The use of specific biochemical/tissue responses as dose metrics for the evaluation of the dose-response for toxicity are based upon hypotheses regarding specific linkages between these responses and toxicity. A greater understanding of the mechanism of linkage of these dose metrics to the toxicological endpoint of concern is required before an interpretation of the shape of the dose-response curve or estimation of low-dose risk is credible.

In summary, the state of the science for mechanism-based modeling has been greatly improved by these newer PBPK models and incorporation of knowledge of the mode of action of

TCDD. These models may allow qualitative assessment of modes of action, i.e., low-dose behavior; however, differences exist in the low-dose expectations of current models. Expanded use of current PBPK models could reduce uncertainty in quantifying actual internal dose following different dosing regimens.

8.5. DATA GAPS

This chapter identified several important data and knowledge gaps. Information to fill these gaps would substantially improve dose-response analysis and risk assessment. The most substantial gaps are summarized below.

There are similarities and differences, both qualitative and quantitative, in responses to TCDD between laboratory animals and humans. These are due to a variety of factors, including disposition of TCDD, AhR properties and regulation, and tissue- and species-specific biochemical responses and specific factors regulating these responses. A better understanding of these factors could substantially improve dose-response analysis and risk assessment.

There are differences between AhR binding curves and dose-response curves for specific toxic endpoints. This suggests that factors in addition to the AhR contribute to these toxic endpoints. For complex endpoints, including frank toxicities, there are likely to be earlier biochemical events, initiated by receptor binding, that lead ultimately to the toxic responses. Detailed quantitative knowledge of this sequence of events would increase reliability in response and species extrapolation, mechanistic modeling, and extrapolation to lower doses.

Also, tissue disposition of TCDD plays a critical role in the approach to risk assessment for this chemical. Knowledge about the disposition of TCDD at or near the background exposures experienced by the general population is limited. PBPK models can make predictions about tissue disposition at these low levels of exposure, though these predictions tend to be below the dose ranges for which the models have been validated. Lack of knowledge of disposition of low doses is especially applicable to human exposures and exposures that may occur in the embryo at critical time points. Furthermore, there is uncertainty about half-life in humans and about the heterogeneity in this half-life among individuals. These factors add to the difficulty in determining the proper dose metric for different endpoints and across different species. PBPK modeling could help to address this problem if the existing models developed for laboratory rodents were extrapolated to humans. Although there would be uncertainty associated with this extrapolation, it would not necessarily be greater than, nor even as great as, the uncertainty associated with the current approach.

In animals, more information is needed about background levels of exposure and how they may affect dose-response analyses. This is especially true because greater emphasis is being placed

on low levels of exposure in animal experiments. Including background exposure data may alter the shape of the dose-response curve and affect the estimate of the ED_{01}.

Quantitative mechanism-of-action-based models can provide insights into the complex interrelationships of the molecular and biochemical events that comprise a mechanism or mode of action. However, the level of confidence in the models and their predictions should not be greater than the level of confidence in the quality of the database and degree of scientific consensus about the mechanism or mode of action that the model describes. This is particularly true when the model is to be used for risk assessment. It is possible to use alterations in the concentrations of proteins known to be altered by TCDD as potential dose metrics. However, more information is needed about the mechanistic linkages of these proteins to toxic endpoints to improve estimations of shapes of dose-response curves and estimates of low-dose risks.

8.6. SUMMARY

Data available for several biochemical and toxicological effects of TCDD, and on the mechanism of action of this chemical, indicate that there is good qualitative concordance between responses in laboratory animals and humans. For example, human data on exposure and cancer response appear to be qualitatively consistent with animal-based risk estimates derived from carcinogenicity bioassays. These data would suggest that animal models are generally an appropriate basis for estimating human responses. Nevertheless, there are clearly differences in responses between animals and humans, and recognition of these is essential when using animal data to estimate human risk. The level of confidence in any prediction of human risk depends on the degree to which the prediction is based on an accurate description of these interspecies extrapolation factors.

Almost all data are consistent with the hypothesis that the binding of the TCDD to the AhR is the first step in a series of biochemical, cellular, and tissue changes that ultimately lead to toxic responses observed in both experimental animals and humans. As such, an analysis of dose-response data and models should use, whenever possible, information on the quantitative relationships between ligand (i.e., TCDD) concentration, receptor occupancy, and biological response. However, it is clear that multiple dose-response relationships are possible when considering ligand-receptor mediated events. For example, dose-response relationships for relatively simple responses, such as enzyme induction, may not accurately predict dose-response relationships for complex responses such as developmental effects and cancer. Cell-specific factors may determine the quantitative relationship between receptor occupancy and the ultimate response. Indeed, for TCDD there is much experimental data from studies using animal and human tissues to indicate that this is the case.

One of the most difficult issues in risk assessment is the dose metric to use for animal-to-human extrapolations. The most appropriate dose metric should reflect both the magnitude and frequency of exposure, and should be clearly related to the toxic endpoint of concern by a well-defined mechanism. However, considering the variety of endpoints in different species, it is unlikely that a single dose metric will be adequate for interspecies extrapolation for all of these endpoints. Furthermore, the use of different dose metrics with respect to the same endpoint may lead to widely diverse conclusions. Nevertheless, it is possible to express dose in a form that allows for comparison of responses for selected endpoints and species. This can be done by either choosing a given exposure and comparing responses or choosing a particular response level and comparing the associated exposures. For particular endpoints, and considering the large differences in half-lives for TCDD across multiple species, it is best to compare the dose metric as body burden rather than daily intake. A useful and common metric for comparison is the 1% effective dose or ED_{01}, which is the exposure dose resulting in 1% change in a particular endpoint. The possibility that existing PBPK models could be used to a greater extent to compare tissue doses across experimental designs and between species deserves further study.

TCDD has been classified as a known human carcinogen, and is a carcinogen in all species and strains of laboratory animals tested. However, it is generally difficult to find human data with sufficient information to model dose-response relationships. For those data that are available, the uncertainties involved in the modeling of these data are considerable, and notably include extrapolation of occupational exposure many years after it took place, and the type and shape of the curve for the dose-response model used in the extrapolation. A linear model is often used because the number of exposure groups for analysis is too small to support more complex models. On the other hand, analysis of animal data suggests that many complex responses to TCDD are nonlinear (Figures 8-1, 8-2, 8-3). Nevertheless, with these qualifications, it is possible to apply simple empirical models to studies in which exposure data for TCDD are available in human populations. An analysis of epidemiological studies of two studies of occupationally-exposed individuals suggests an effect of TCDD on all cancers at body burden ED_{01}s for total cancers ranging from 1.4 ng/kg to 40 ng/kg. This was slightly smaller than the estimates from empirical modeling from the animal studies which ranged from 14 ng/kg to 1190 ng/kg (most estimates were in the range from 14 to 500 ng/kg), and in most cases slightly above the 2.7 ng/kg estimate from the single mechanism-based model. The two lowest human ED_{01} values (1.4 and 1.8 ng/kg) were associated with the power model used by Steenland et al. (2001) which predicts an unrealistic risk for the background exposure; the next lowest value was 6 ng/kg.

At this point, sufficient data are not available to model noncancer endpoints in humans. Many studies are available to estimate ED_{01} values for noncancer endpoints in animals. However, there are a number of difficulties and uncertainties that should be considered when comparing

endpoints across species. Some of these include differences in sensitivity of endpoints, times of exposure, exposure routes, species and strains, use of multiple or single doses, and variability between studies even for the same response. The estimated ED_{01} values may be influenced by experimental design, suggesting that caution should be used in comparing values from different designs. In addition, caution should be used when comparing studies that give ED_{01} estimates outside the experimental range. Furthermore, comparing values between different categories of inducible responses may result in misleading estimates of a potential health risk. For example, the human health risk for a 1% change of body weight may not be comparable to a 1% change in enzyme activity. Finally, background exposures are not often considered in these calculations simply because they were not known. The latter consideration is particularly important as the inclusion of these may alter the shape of the dose-response curve, possibly increasing the shape parameter so that the responses would demonstrate more threshold-like effects. Nevertheless, given these considerations several general trends were observed. The lowest ED_{01} values tended to be for biochemical effects, followed by hepatic responses, immune responses, and responses in tissue weight. An analysis of shape parameters implies that many dose-response curves, for a variety of responses, were consistent with linearity over the range of doses tested. This does not imply that the curves would be linear outside this range of doses. The lower shape parameters, suggesting linearity, were for biochemical responses, whereas the higher values for shape parameters, suggesting nonlinearity, were for tissue responses. Overall, these data suggest that biochemical responses to TCDD are more likely to be linear within the experimental dose range, while the more complex responses including frank toxicity are more likely to assume a nonlinear shape. For cancer, the shapes were split between linear (eight analyses) and nonlinear (five analyses).

The tissue weight changes seen for animals (using only data sets with good or moderate empirical fits to the model) yielded a median ED_{01} of 510 ng/kg in the multidose studies (range 11 to 28,000 ng/kg) and a median ED_{01} of 160 ng/kg (range 0.0001 to 9,700 ng/kg) in the single- dose studies. Toxicity endpoints from the single-dose studies resulted in a median value of 4,300 ng/kg (range 1.3 to 1,000,000 ng/kg). For tissue weight changes, 43% of the dose-response curves exhibited linear response. In contrast, the toxicity endpoints from the single-dose studies exhibited predominantly nonlinear responses (80%). All multidose studies demonstrated a greater degree of linear response (41%) than did single-dose studies (37%), especially for tissue weight changes and toxicity endpoints (50% linear for multidose versus 34% for single dose). In general it is not possible to specify the differences between cancer and noncancer dose-response as being due to differences in endpoint response or to differences in the length of dosing and exposure. Also, a greater percentage of the noncancer ED_{01} values were below the experimental dose range (42%) than was the case for the cancer endpoints (8% in animals and no extrapolations in humans). However, many more noncancer data sets were examined compared to the cancer endpoints.

Empirical models have advantages and disadvantages relative to mechanism-based models. Empirical models provide a simple mathematical model that adequately describes the pattern of response for a particular data set and can also provide the means for hypothesis testing and interpolation between data points. In addition, empirical models can provide qualitative insights into underlying mechanisms. However, the major disadvantage is their inability to quantitatively link data sets in a mechanistically meaningful manner. On the other hand, comprehensive mechanism-based models can be powerful tools for understanding and combining information on complex biological systems. Use of a truly mechanism-based approach can in theory enable reliable and scientifically sound extrapolations to lower doses and between species. However, any scientific uncertainty about the mechanisms that the models describe is inevitably reflected in uncertainty about the predictions of the models.

PBPK models have been validated in the observable response range for numerous compounds in both animals and humans. The development of PBPK models for disposition of TCDD in animals has proceeded through multiple levels of refinement, with newer models showing increasing levels of complexity by incorporating data for disposition of TCDD and its molecular actions with the AhR and other proteins, as well as numerous physiological parameters. These have provided insights into key determinants of TCDD disposition in treated animals. The most complete PBPK models give similar predictions about TCDD tissue dose metrics. The PBPK models have been extended to generate predictions for early biochemical consequences of tissue dosimetry of TCDD such as induction of CYP1A1. Nevertheless, extension of these models to more complex responses is more uncertain at this time. Differences in interpretation of the mechanism of action lead to varying estimates of dose-dependent behavior for similar responses. The shape of the dose-response curves governing extrapolation to low doses is determined by these hypotheses and assumptions. In the observable range around 1% excess response, the quantitative differences are relatively small. Below this response, the different mechanisms can diverge rapidly. The use of predicted biochemical responses as a dose metric for toxic responses is considered a potentially useful application of these models. However, greater understanding of the linkages between these biochemical effects and toxic responses is needed to reduce the potentially large uncertainty associated with these predictions.

8.7. CONCLUSIONS

Once an environmental agent has been deemed a health hazard, the two main questions to be addressed in any dose-response assessment are: (1) What can be said about the shape of the dose-response function in the observable range, and what does this imply about dose-response in the range of environmental exposures? and (2) What is a reasonable limit (critical dose or point of departure) at the edge of the observable range, and what risk is associated with this exposure? For

the dose-response assessment of TCDD, these questions are complicated by the multiplicity of responses observed and the complexity of the mechanisms known to impact upon those responses. In the dose-response evaluation conducted for this chapter, we have attempted to use the best available analytic procedures to provide insight into the answers to these questions. This includes both the critical assessment of formal empirical dose-response analyses of the available data and, where appropriate, predictions of dose-response behavior using mechanism-based models of TCDD.

Many different shapes of dose-response curves were seen in the observable range. Although human data were available, the data were not adequate for addressing curvature of the dose-response relationship. Consequently, the main conclusions on the shape of the dose-response for TCDD are based on animal models.

Under simple empirical dose-response models, about half of the cancer endpoints observed in animals were linear in the observable range and about half were not. Noncancer endpoints had a greater degree of nonlinearity, with only 40% of the observed responses being linear. Biochemical endpoints (more closely coupled to activation of the AhR) tended to exhibit linear dose-response curves, whereas TCDD-inducible responses, which are likely more complex and involve multigene interactions, exhibited more nonlinear behavior. Mechanism-based modeling provided two different answers depending upon the approach used in the analysis and the assumptions used in the approaches. The variability in the available data for mechanism-based modeling did not allow us to clearly decide upon any one given model in favor of another. For intermediate biochemical endpoints and preneoplastic lesions in the rat liver, we saw model fits that strongly supported nonlinear dose-response shapes in the observable range. This was based upon the assumptions of a nonlinear expression of proteins in the liver and upon multiple types of focal lesions responding differently to the effects of TCDD. In contrast, using an alternative model resulted in effectively linear dose-response (defined as response proportional to dose in the low-exposure region, not necessarily the higher experimental doses) for both endpoints and the proposition of a secondary effect of TCDD on increasing mutations through changes in estrogen metabolism.

All humans tested contain detectable body burdens of TCDD and other dioxin-like compounds that are likely to act through the same mode of action. This consideration, together with the high percentage of observed linear responses, suggests that a proportional model should be used when extrapolating beyond the range of the experimental data rather than using a margin-of-exposure analysis. However, this decision would have to be based upon a policy choice because this analysis dose not strongly support either choice.

Because we had human data for dose-response analysis and a strong desire to stay within the range of responses estimated by these data, the risk chosen for determining a point of departure was the 1% excess risk. Doses and exposures associated with this risk (the ED_{01}s) were estimated from

the available data using both mechanistic and empirical models. Comparisons were made on the basis of body burdens (either averaged, steady-state or administered dose) to account for differences in half-life across the numerous species studied. In humans, restricting the analysis to dose-response models from the literature for two occupational cohorts resulted in body burden ED_{01}s for total cancers ranging from 1.4 ng/kg to 40 ng/kg. This was slightly smaller than the estimates, from empirical modeling from the animal studies which ranged from 14 ng/kg to 1190 ng/kg (most estimates were in the range from 14 to 500 ng/kg), and in most cases slightly above the 2.7 ng/kg estimate from the single mechanism-based model. The two lowest human ED_{01} values (1.4 and 1.8 ng/kg) were associated with the power model used by Steenland et al. (2001) which predicts an unrealistic risk for the background exposure; the next lowest value was 6 ng/kg. Estimates for non-cancer endpoints showed much greater variability. In general, the noncancer endpoints displayed lower body burdens at the ED_{01} for longer term exposures versus short-term exposures, and for simple biochemical endpoints versus more complex endpoints such as tissue weight changes or toxicity. In addition, the noncancer endpoints generally displayed higher estimated body burdens at the ED_{01} than the cancer endpoints, with most estimates ranging from 100 ng/kg to 100,000 ng/kg. For some endpoints, however, the body burdens at the ED_{01} were below the range of the cancer endpoints. The mechanism-based models for noncancer endpoints gave a lower range of body burdens at the ED_{01} (0.17 to 105 ng/kg). While most of these estimates were based upon a single model, the estimate from the hepatic zonal induction model gave a body burden for the ED_{01} for CYP1A2 induction of 51 ng/kg and hence was within the same range.

These estimates, although highly variable, suggest that any choice of body burden, as a point-of-departure, above 100 ng/kg would likely yield greater than 1% excess risk for some endpoints in humans. Also, choosing a point-of-departure below 1 ng/kg would in general only be supported by analyses that gave estimates that were below the range of these data, and would likely represent a risk of less than 1%. Any choice in the middle range of 1ng/kg to 100 ng/kg, would be supported although the data provide the greatest support in the range of 10 ng/kg to 20 ng/kg.

This Chapter has produced an extensive summary of dose-response relationships as is feasible at this time. The analyses and discussions synthesize a considerable breadth of data and model types, drawing upon this information to highlight strengths and weaknesses in the information base, gaps in our qualitative and quantitative understanding and the uncertainties inherent in making a decision concerning a point-of-departure for risk characterization. While such an extensive evaluation may not be necessary for most environmental contaminants, the concepts envisioned here can serve as a framework for evaluation in other settings. This unique document hopefully marks the beginning of more objective, quantitative reviews of information pertaining to risk decisions for environmental agents.

Table 8-1. Estimated half-lives for species considered in the analyses to follow and used for converting between daily exposures and steady-state body burdens

Species	Half-life (days)
C57BL/6N mice	10
All other mouse strains	11
Golden Syrian hamster	12
Wistar rats	22
All other rat strains	25
Human	2,593

TABLE 8-2: Total cancer risk in humans through age 75 (units are constant body burden in ng/kg not adjusted for lipid). Upper and lower 95% confidence limits (where available) are in parentheses after ED values

Study	Model and Sex	ED_{10}	ED_{05}	ED_{01}	Unit excess risk for 1 ppt body burden above background
Steenland et. al, (2001)	power male	500 (46.4, 2.91 x 10^7)	33.9 (8.23, 1.59 x 10^4)	1.38 (0.71, 8.95)	0.0079 (0.0027, 0.0132)
	power female[1]	1315 (84.4, 4.5 x 10^8)	64.5 (12.6, 2.50 x 10^4)	1.84 (0.92, 14.9)	0.0064 (0.0022, 0.0107)
	piecewise linear male	• (92.9, •[3])	83.6 (51.8, •[3])	18.6 (11.5, 48.3)	0.00052 (0.00020, 0.00084)
	piecewise linear female[2]	•[8] (108.9, •[3])	100.7 (62.39, •[3])	23.1 (14.3, 59.8)	0.00042 (0.00016, 0.00067)
Becher et al., (1998)	power-male	120.3	41.17	5.971	0.0018
	power-female[4]	170.9	55.44	7.580	0.0014
	additive-male	192.8	93.35	18.22	0.00055
	additive-female[5]	239.1	116.2	22.75	0.00044
	multiplicative-male	258.9	144.4	32.16	0.00030
	multiplicative-female[6]	304.4	173.8	39.82	0.00024
Ott and Zober (1996)	multiplicative-male	411.7 (201.9, □)	229.0 (112.3, □)	50.9 (25.0, □)	0.00019 (0, 0.00039)
	multiplicative-female[7]	478.0 (234.4, □)	272.1 (133.4, □)	62.1 (30.5, □)	0.00015 (0, 0.00032)

[1] Relative risk RR proportional to $(AUC)^{0.097}$, with 15 year lag
[2] Relative RR proportional to exp (0.000015 AUC). This is based on the linear function in the lower range of the piecewise linear model.
[3] When body burden exceeds 133 ng/kg, the AUC years exceeds 40,000 ppt years and the model cannot achieve the prescribed risk level
[4] Relative risk RR proportional to $(0.00017\ AUC + 1)^{0.326}$
[5] Relative risk RR proportional to (1+0.000016 AUC)
[6] Relative RR proportional to exp (0.00000869 AUC).
[7] Relative RR proportional to exp (0.0003522 x lipid concentration).

Table 8-3. Doses yielding 1% excess risk (95% lower confidence bound) based upon 2-year animal carcinogenicity studies using simple multistage models

		ED_{01}	
Tumor	**Shape**	**Intake for 1% excess risk (ng/kg/day)**	**Steady-state body burden (ng/kg) at ED_{01}**
Liver cancer in female rats (Kociba)	Linear	0.77 (0.57)	14 (10)
Squamous cell carcinoma of the tongue in male rats (Kociba)	Linear	14.1 (5.9)	254 (106)
Squamous cell carcinoma of the nasal turbinates or hard palate in male rats (Kociba)	Cubic	41.4 (1.2)	746 (22)
Squamous cell carcinoma of the lung in female rats (Kociba)	Cubic	40.4 (2.7)	730 (48)
Squamous cell carcinoma of the nasal turbinates or hard palate in female rats (Kociba)	Linear	5.0 (2.0)	90 (36)
Thyroid follicular cell adenoma in male rats (NTP)	Linear	4.0 (2.1)	144 (76)
Thyroid follicular cell adenoma in female rats (NTP)	Cubic	33.0 (3.1)	1,190 (112)
Liver adenomas and carcinomas in female rats (NTP)	Quadratic	13.0 (1.7)	469 (61)
Liver adenomas and carcinomas in male mice (NTP)	Linear	1.3 (0.86)	20.6 (13.6)
Liver adenomas and carcinomas in female mice (NTP)	Linear	15.1 (7.8)	239 (124)
Thyroid follicular cell adenomas and carcinomas in female mice (NTP)	Linear	30.1 (14.0)	478 (222)
Subcutaneous tissue sarcomas in female mice (NTP)	Lin-Cubic	43.2 (14.1)	686 (224)
Leukemias and lymphomas in female mice (NTP)	Linear	10.0 (5.4)	159 (86)

Table 8-4. Noncancer endpoints used for comparing ED_{01} values

Species	Gender	Multi-dose	Single-dose		Total
			Adult	Developmental	
Mouse	Female	25	23	5	53
	Male	0	35	20	55
	Unknown	—	—	3	3
Rat	Female	62	10	0	72
	Male	21	4	32	57
Hamster	Female	0	0	0	0
	Male	0	2	0	2
Total		**108**	**74**	**60**	**242**

Table 8-5. Ratio of ED_{01}/lowest dose, categorized by study type and endpoint type*

	Multi-dose		Single-Adult		Single-Developmental	
Category	**Out of range**	**In-range**	**Out of range**	**In-range**	**Out of range**	**In-range**
Biochemical	21 (16)	7	1 (1)	15	1(1)	0
Hepatic	4 (4)	9	0	13	—	—
Immune	8 (6)	8	13 (8)	3	—	—
Endocrine	6 (4)	3	—	—	—	—
Tissue	8 (6)	34	7 (4)	9	31 (17)	21
Toxicity	—	—	0	13	6 (0)	1
Subtotals	**47 (37)**	**61**	**21 (13)**	**53**	**38 (18)**	**22**
TOTALS	**108**		**74**		**60**	

* These data do not include analyses where a poor fit of the model to the data was obtained. "Out of range" indicates studies where the ED_{01} estimate was lower than the lowest dose used in the study. "In-range" indicates the estimate was within the experimental dose range used in the study from which the estimate was derived. Number of endpoints where the estimate was less than 1 order of magnitude lower than the lowest dose used are shown in parentheses.

Table 8-6. Estimated shape parameters, categorized by study type and endpoint type

	Multi-dose		Single-Adult		Single-Development	
Category	**Linear***	**Non-linear**	**Linear**	**Non-linear**	**Linear**	**Non-linear**
Biochemical	15	13	6	10	0	1
Hepatic	3	10	4	9	—	—
Immune	3	13	10	6	—	—
Endocrine	5	4	—	—	—	—
Tissue	21	21	10	6	14	38
Toxicity	—	—	0	13	4	3
Subtotals	**47**	**61**	**30**	**44**	**18**	**42**
TOTALS	**108**		**74**		**60**	

* "Linear" shape parameters are those where the Hill model coefficient n<1.5
These data do not include analyses where a poor fit of the model to the data was obtained.

Table 8-7. Categorization of specific endpoints

Category	Endpoint	
Biochemical	CYP 1A1 mRNA	Liver benzopyrene hydroxylase (CYP1A1 activity)
	CYP1A1 (Protein)	Liver cytochrome P-450 (Total)
	CYP1A1 EROD in liver, lung, and skin	Renal retinol concentration
	CYP1A2 (Protein)	Renal RPH activity
	CYP1A2 ACOH	Serum testosterone
	CYP1A2 mRNA	Superoxide anion production by PLC
	CYP1A2 MROD	T4UGT
	CYP1B1 mRNA	Total Ah Receptor binding
	EGF dissociation (Kd)	UGT mRNA
	EGFR autophosphorylation	UGT1A1
	EGFR maximum binding	
Hepatic	Serum 5'-nucleotidase	Serum Not Esterified chloesterol
	Serum alkaline phosphatase	Serum S. Dehydrogenase
	Serum ALT	Serum SGPT
	Serum BUN	Serum TBA
	Serum bilirubin (total, indirect, direct)	Serum total cholesterol
	Serum esterified cholesterol	Serum triglycerides
	Serum glucose	
Immune	CD4+/CD8+	Immune footpad swelling (following SRBC)
	CD8+/CD4-	Immune increment in ear thickness (following oxazalone)
	CD8-/CD4-	PFC/106 splenocytes
	CD4+/CD8-	PFC/spleen(x10-4)
	Cells/spleen(x10-6)	Total thymic cells/mouse
	Immune titer	

Table 8-7. Categorization of specific endpoints (continued)

Endocrine	Hepatic retinol	Plasma retinol	Hepatic retinyl-palmitate
	Thyroid-stimulating hormone	Thyroxine Free T4	
	Thyroxine	Thyroxine Total T4	
Tissue	Age at puberty	Epididymal sperm count	Relative kidney weight
	Body weight	Epididymidis weight	Relative liver weight
	Brain weight	Eye opening	Relative spleen weight
	Caput/corpus epid. sperm numbers	Eye opening in F/M	Relative thymus weight
	Cauda epid. sperm numbers	Glans penis weight	Seminal vesicle weight
	Cauda epididymal weight	Heart weight	Spleen atrophy
	Coagulating glands	Incisor eruption	Spleen cellularity
	Daily sperm production	Kidney weight	Testes weight
	Dorsal prostate weight	Liver weight	Thymus atrophy
	DSP/g D day 120	Ovarian weight	Thymus weight
	Endometrial lesion diameter	Ovulation (ova/rat)	Uterine horn weight
	Endometrial lesion weight	Paired epididymal weight	Uterus weight
		Pituitary gland weight	Ventral prostate weight
Toxicity	Cleft palate	Liver BDH	Sperm morphology
	Fertility index	Liver fatty change	Stomach edema
	Gestation period	Liver HCC	Testes MNGC
	Hydronephrosis	Liver HCK	Testes SFEN
	Litter size	Number of copulatory plugs	Testis descent
	Live birth index (%)	Pinna detachment	Total testis sperm numbers

Table 8-8. Steady-state ED_{01} values calculated using mechanism-based dose-response models of dioxin-regulated responses

	Response value			
Response	**Control (0 μg/kg/day)**	**Maximum (10 μg/kg/day)**	**ED_{01} (ng/kg/day)**	**Body burden $_{01}$ (ng/kg)[a]**
CYP1A1 (nmol/g)[b]	0.0216	6.09	0.0047	0.17
CYP1A2 (nmol/g)[b]	0.558	7.17	0.34	12.3
CYP1A2 (% liver induced)[c]			1.4	50.5
Internalized-EGFR (pmol/g)[b]	0	2.09	0.28	10.1
T_4 (nM)[b]	29.0	3.96	0.27	9.7
UGT RNA pmol/g	1.13	14.1	0.85	30.7
UDPGT (nmol/g)[b]	0.118	0.416	2.9	104.6
TSH pM[b]	77.8	179	1.3	46.9
Liver cancer[d]	0.35	1.00	0.15	2.7

[a]Steady-state body burdens were calculated from the formula in Section 8.2.3. assuming 100% absorption, except for the liver cancer model, which used 50% absorption.
[b]Values obtained using the extended thyroid hormone model.
[c]Values from the zonal induction model.
[d]Mechanism-based cancer model.

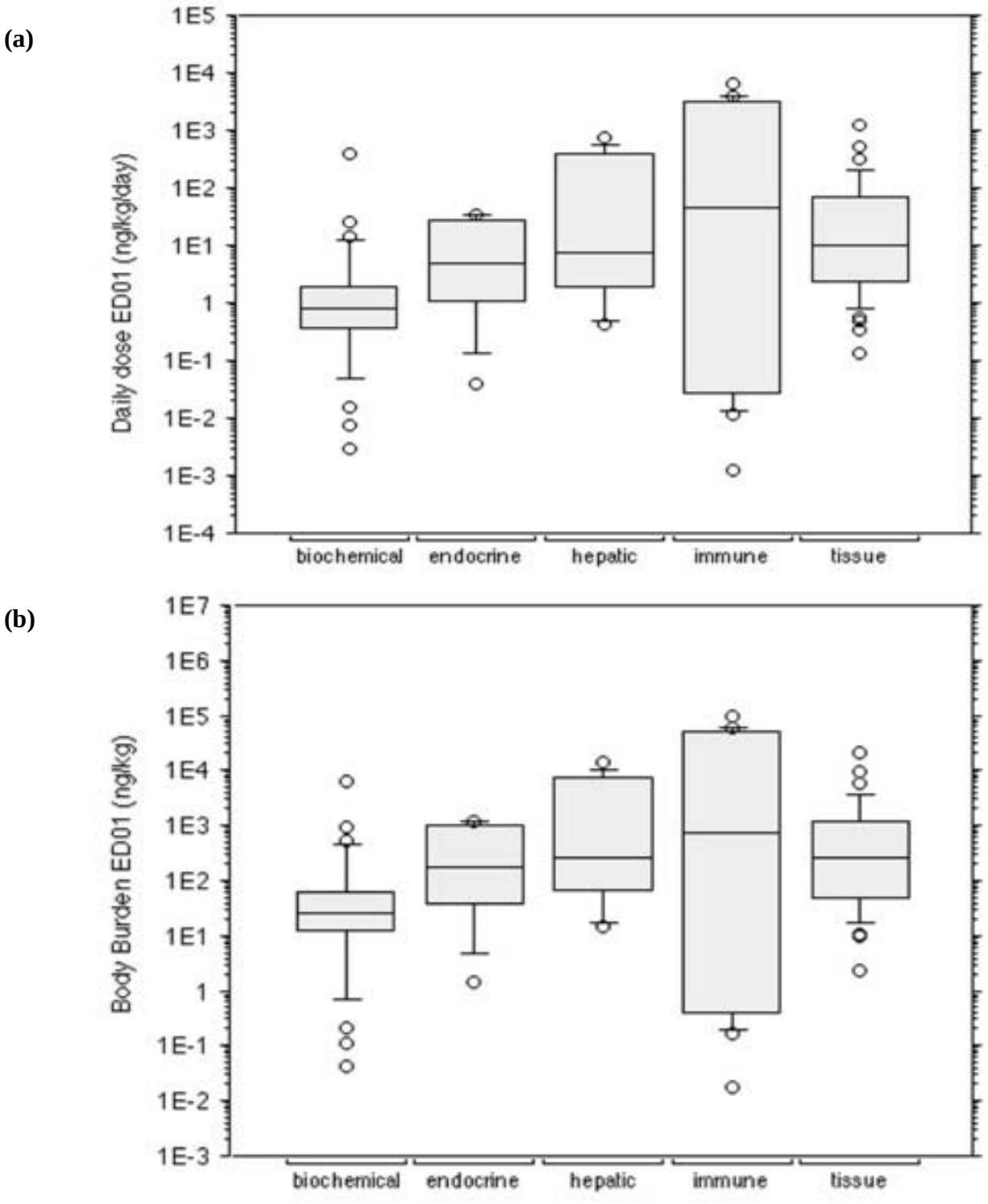
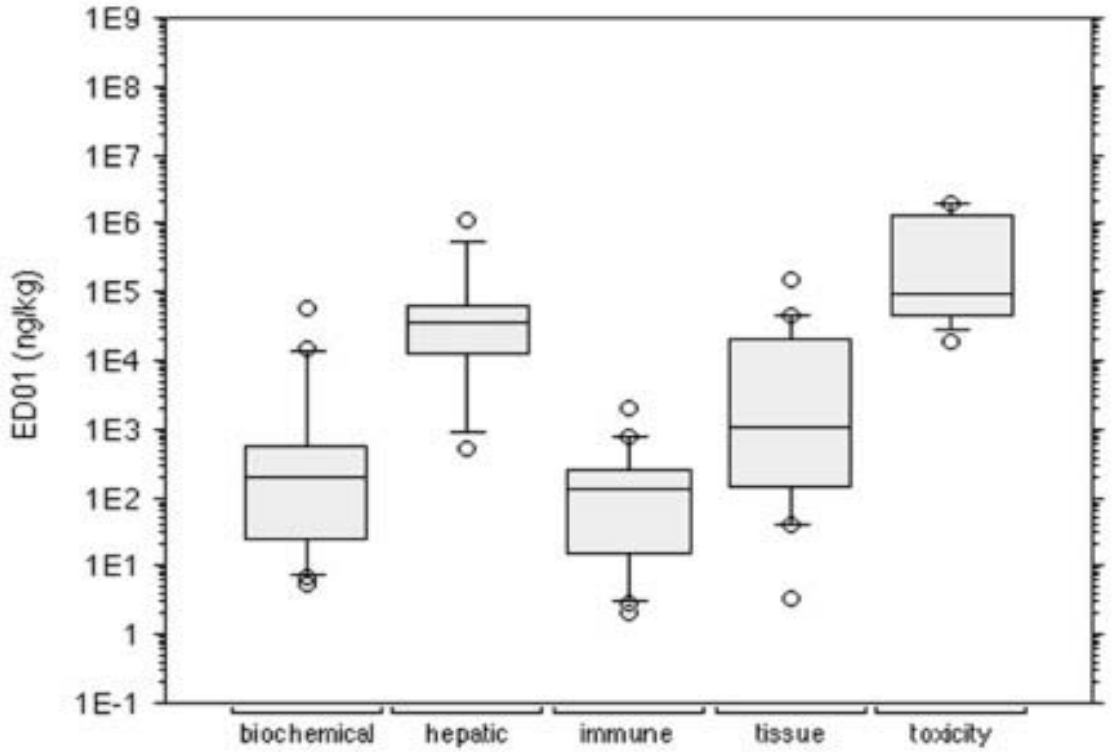


Figure 8-1. Distribution of ED_{01} and BB_{01} values in multidose studies by endpoint.
(a) ED_{01} values. **(b)** Body burden values at the ED_{01}. The distribution of individual values is presented as box plots. The boxed region contains values within the 25th to the 75th percentiles of the sample distribution, with the median value (50th percentile) shown as a line within the boxed region. The error bars represent values within the 10th to the 90th percentiles. Values above the 90th percentile and below the 10th percentile are shown as individual data points. Values are categorized according to Table 8-7.

(a)



(b)

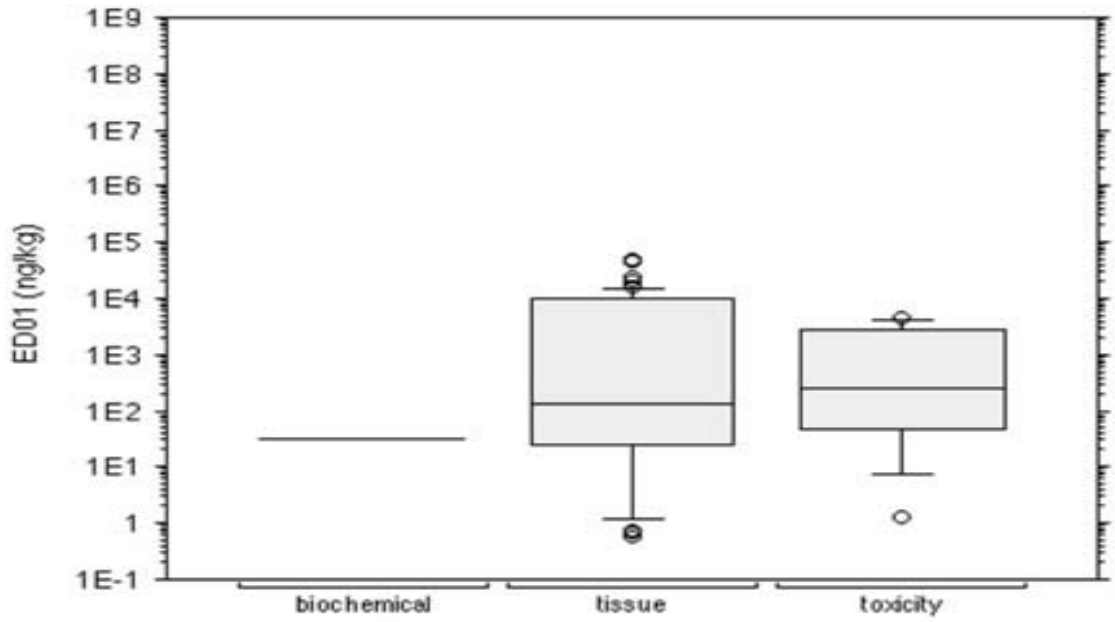


Figure 8-2. Distribution of ED_{01} values in single-dose studies by endpoint.
(a) Adult endpoints. **(b)** Developmental endpoints. The distribution of individual values is presented as box plots. The boxed region contains values within the 25th to the 75th percentiles of the sample distribution, with the median value (50th percentile) shown as a line within the boxed region. The error bars represent values within the 10th to the 90th percentiles. Values above the 90th percentile and below the 10th percentile are shown as individual data points. Values are categorized according to Table 8-7.

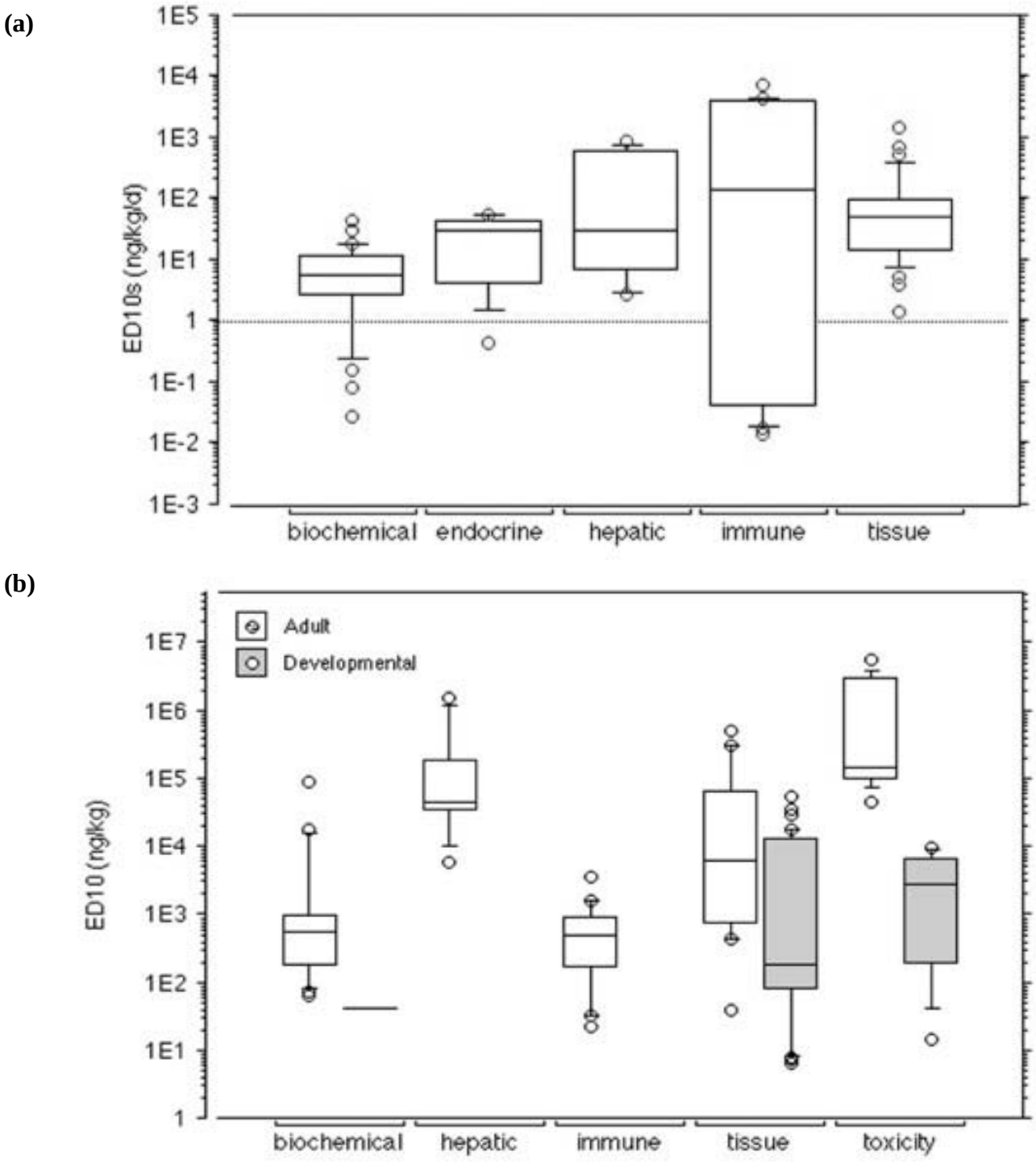


Figure 8-3. Distribution of ED_{10}s in multi-dose studies and single-dose studies by endpoint.
(a) Multi-dose studies. **(b)** Single-dose studies. The distribution of individual values is presented as box plots. The boxed region contains values within the 25th to the 75th percentiles of the sample distribution, with the median value (50th percentile) shown as a line within the boxed region. The error bars represent values within the 10th to the 90th percentiles. Values above the 90th percentile and below the 10th percentile are shown as individual data points. Values are categorized according to Table 8-7.

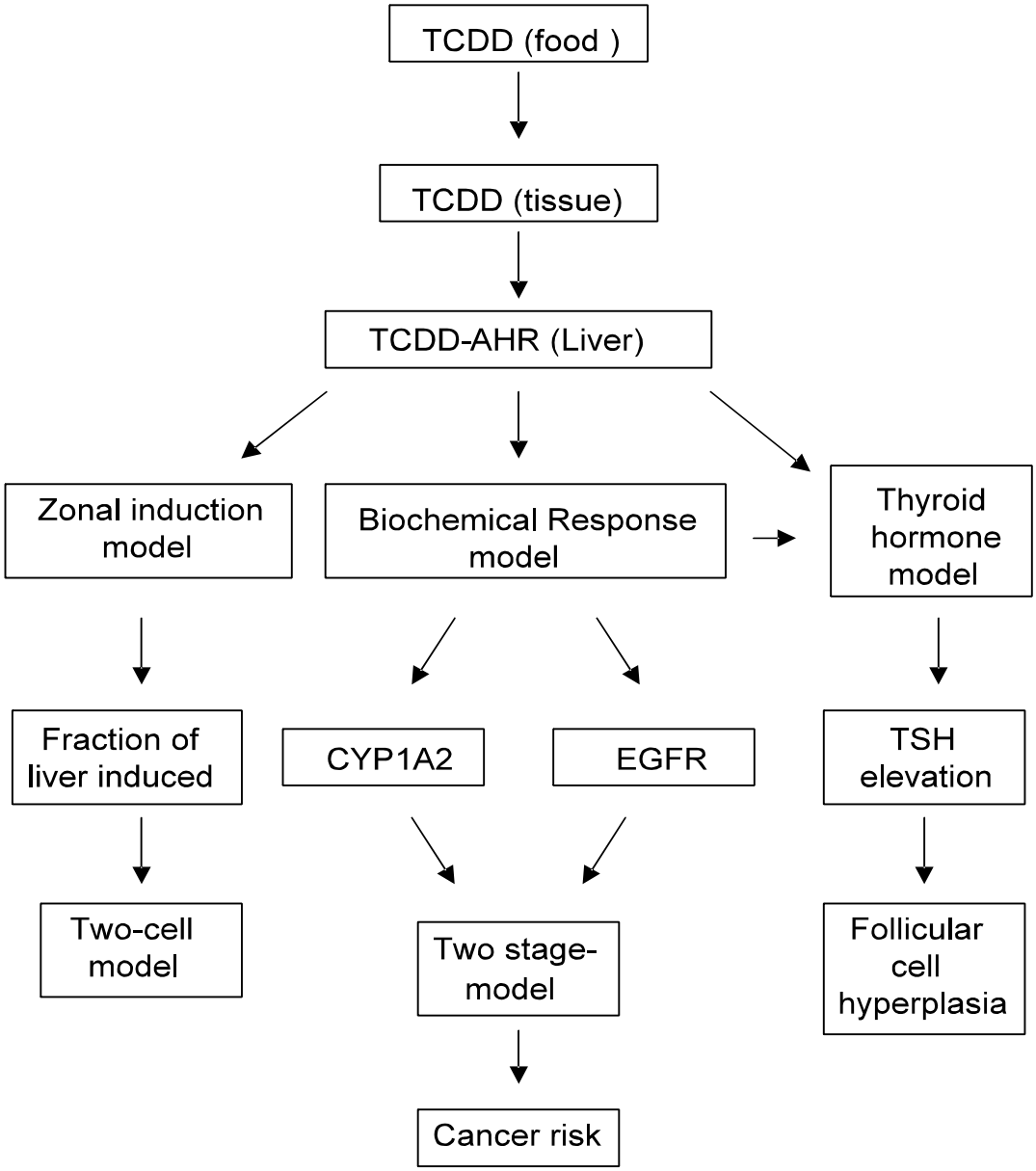


Figure 8-4. Schematic representation of the linkage of current PBPK models and biochemical/tissue response models for TCDD action.

Appendix I: Multiple-dose studies

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Body burden ED_{01}(ng/kg)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Body burden ED_{10} (ng/kg)	Relative ED_{10}[c]	Quality of fit[d]
Kociba et al. (1976), male Sprague-Dawley rats	13 weeks, 5x/wk, 1 ng/kg	Body weight	18.0	9.1E+01	1.6E+03	9.1E+01	1.0E+02	1.9E+03	1.0E+02	M
		Brain weight	5.7	5.4E+01	9.8E+02	5.4E+01	8.3E+01	1.5E+03	8.3E+01	M
		Rel brain weight	4.9	3.2E+02	5.9E+03	3.2E+02	5.2E+02	9.4E+03	5.2E+02	M
		Heart weight	6.4	6.8E+01	1.2E+03	6.8E+01	9.9E+01	1.8E+03	9.9E+01	M
		Kidney weight	7.4	6.2E+01	1.1E+03	6.2E+01	8.5E+01	1.5E+03	8.5E+01	M
		Liver weight	1.0	1.2E-01	2.3E+04	1.2E-01	1.4E+00	2.6E+01	1.4E+00	G
		Rel liver weight	1.0	1.1E+00	1.9E+01	1.1E+00	1.2E+01	2.1E+02	1.2E+01	G
		Serum alkaline phosphatase	6.2	3.8E+02	6.8E+03	3.8E+02	5.5E+02	9.9E+03	5.5E+02	M
		Serum BUN	6.9	5.1E+02	9.2	5.1E+02	7.1E+02	1.3E+04	7.1E+02	M
		Serum direct bilirubin	NA[i]	NA	NA	NA	NA	NA	NA	NF[j]
		Serum indirect bilirubin	NA	NA	NA	NA	NA	NA	NA	NF
		Serum total bilirubin	7.0	4.8E+02	8.7E+03	4.8E+02	6.7E+02	1.2E+04	6.7E+02	G
		Spleen weight	6.4	5.4E+01	9.8E+02	5.4E+01	7.9E+01	1.4E+03	7.9E+01	M
		Rel spleen weight	8.6	5.3E+02	9.5E+03	5.3E+02	6.9E+02	1.2E+04	6.9E+02	M

Appendix I: Multiple-dose studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Body burden ED_{01}(ng/kg)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Body burden ED_{10} (ng/kg)	Relative ED_{10}[c]	Quality of fit[d]
		Rel testes weight	7.1	7.3E+01	1.3E+03	7.3E+01	1.0E+02	1.9E+03	1.0E+02	M
		Thymus weight	1.0	3.7E+00	6.7E+01	3.7E+00	4.0E+01	7.3E+02	4.0E+01	M
		Rel thymus weight	1.0	2.6E+00	4.8E+01	2.6E+00	3.0E+01	5.4E+02	3.0E+01	M
Kociba et al. (1976), female Sprague-Dawley rats	13 weeks, 5x/wk, 1 ng/kg	Body weight	1.0	4.8E+00	8.6E+01	4.8E+00	4.7E+01	8.5E+02	4.7E+01	G
		Brain weight	1.0	5.8E+00	1.1E+02	5.8E+00	6.5E+01	1.2E+03	6.5E+01	M
		Rel brain weight	5.8	6.8E+01	1.2E+03	6.8E+01	1.1E+02	1.9E+03	1.1E+02	M
		Heart weight	5.5	5.2E+01	9.4E+02	5.2E+01	8.1E+01	1.5E+03	8.1E+01	M
		Kidney weight	7.3	5.1E+02	9.8E+03	5.1E+02	7.0E+02	1.3E+04	7.0E+02	M
		Liver weight	7.1	6.0E+00	1.1E+02	6.0E+00	8.4E+00	1.5E+02	8.4E+00	M
		Rel liver weight	1.1	5.4E-01	9.8E+00	5.4E-01	5.2E+00	9.4E+01	5.2E+00	G
		Serum alkaline phosphatase	7.7	7.3E+00	1.3E+02	7.3E+00	9.9E+00	1.8E+02	9.9E+00	M
		Serum direct bilirubin	1.0	6.8E+00	1.2E+02	6.8E+00	7.5E+01	1.3E+03	7.5E+01	M
		Serum indirect bilirubin	NA	NA	NA	NA	NA	NA	NA	NF
		Serum total bilirubin	18.0	7.7E+02	1.4E+04	7.7E+02	8.8E+02	1.6E+04	8.8E+02	M

Appendix I: Multiple-dose studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Body burden ED_{01}(ng/kg)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Body burden ED_{10} (ng/kg)	Relative ED_{10}[c]	Quality of fit[d]
		Serum SGPT	14.1	2.3E+02	4.2E+03	2.3E+02	2.8E+02	5.0E+03	2.8E+02	P
		Thymus weight	1.0	1.3E+00	2.3E+01	1.3E+00	1.4E+01	2.5E+02	1.4E+01	G
		Rel thymus weight	1.0	1.0E+00	1.9E+01	1.0E+00	1.1E+01	2.0E+02	1.1E+01	G
Clark et al. (1981), male C57Bl/6 mice	4 weeks, 1x/wk, 1 week after last dose, 400 ng/kg	Immune footpad swelling (following SRBC)	7.0	2.6E+03	3.8E+04	5.7E+01	2.8E+04	4.1E+05	6.1E+02	P
		Immune increment in ear thickness (following oxazalone)	18.0	1.6E+02	2.3E+03	3.4E+00	1.6E+03	2.3E+04	3.4E+01	P
Tritscher et al. (1992), female Sprague-Dawley rats	31 weeks, 1x/2 weeks, 3.5 ng/kg/day	CYP1A1 (Protein) (DEN)	1.2	4.1E-01	1.5E+01	1.2E-01	3.0E+00	1.1E+02	8.6E-01	G
		CYP1A1 (Protein) (saline)	1.0	3.5E-01	1.3E+01	10.0E-02	3.8E+00	1.4E+02	1.1E+00	G
		CYP1A2 (Protein) (DEN)	1.0	5.1E-01	1.9E+01	1.5E-01	5.6E+00	2.0E+02	1.6E+00	G
		CYP1A2 (Protein) (saline)	1.0	3.6E-01	1.3E+01	1.0E-01	3.9E+00	1.4E+02	1.1E+00	G

Appendix I: Multiple-dose studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Body burden ED_{01}(ng/kg)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Body burden ED_{10} (ng/kg)	Relative ED_{10}[c]	Quality of fit[d]
Fox et al. (1993), female Sprague-Dawley rats	7 days, 5 ng/kg (initial dose), 0.9 ng/kg/4 days until 0.03 ng/g steady-state achieved	Body weight	15.2	1.2E+03	2.2E+04	1.4E+03	1.4E+03	2.5E+04	1.7E+03	M
		Body weight change	2.5	7.9E+01	1.4E+03	9.4E+01	2.0E+02	3.6E+03	2.4E+02	M
		Liver weight	11.2	3.3E+01	6.0E+02	3.9E+01	4.1E+01	7.3E+02	4.8E+01	M
		Liver weight:body weight ratio	1.0	9.6E-01	1.7E+01	1.1E+00	9.8E+00	1.8E+02	1.2E+01	G
Fox et al. (1993), female Sprague-Dawley rats	14 days, 5 ng/kg (initial dose), 0.9 ng/kg/4 days until 0.03 ng/g steady-state achieved	Body weight	1.0	2.0E+00	3.6E+01	3.7E+00	2.1E+01	3.8E+02	3.9E+01	G
		Body weight change	2.7	5.5E+01	1.0E+03	1.0E+02	1.4E+02	2.4E+03	2.5E+02	G
		Liver weight	1.0	1.2E+00	2.2E+01	2.2E+00	1.3E+01	2.4E+02	2.4E+01	G
		Liver weight:body weight ratio	1.0	1.9E+01	3.4E+02	3.5E+01	1.9E+02	3.4E+03	3.5E+02	M

Appendix I: Multiple-dose studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Body burden ED_{01}(ng/kg)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Body burden ED_{10} (ng/kg)	Relative ED_{10}[c]	Quality of fit[d]
Fox et al. (1993), male Sprague-Dawley rats	7 days, 5 ng/kg (initial dose), 0.9 ng/kg/4 days until 0.03 ng/g steady-state achieved	Body weight	5.3	9.2E-06	1.7E-04	1.1E-05	1.5E-05	2.6E-04	1.7E-05	P
		Body weight change	2.4	1.3E+02	2.3E+03	1.5E+02	3.4E+02	6.2E+03	4.1E+02	M
		Liver weight	1.0	2.8E+00	5.0E+01	3.3E+00	3.1E+01	5.5E+02	3.6E+01	G
		Liver weight:body weight ratio	3.1	7.7E+01	1.4E+03	9.1E+01	1.7E+02	3.0E+03	2.0E+02	G
Fox et al. (1993), male Sprague-Dawley rats	14 days, 5 ng/kg (initial dose), 0.9 ng/kg/4 days until 0.03 ng/g steady-state achieved	Body weight	18	1.2E-05	2.2E-04	2.3E-05	1.4E-05	2.6E-04	2.6E-05	P
		Body weight change	18	1.1E+03	2.0E+04	2.0E+03	1.3E+03	2.3E+04	2.3E+03	P
		Liver weight	6.2	6.3E+00	1.1E+02	1.1E+01	9.2E+00	1.7E+02	1.7E+01	M
		Liver weight:body weight ratio	2.5	3.4E+01	6.1E+02	6.2E+01	8.9E+01	1.6E+03	1.6E+02	G
Maronpot et al. (1993), female Sprague-Dawley rats	31 weeks, 1x/2weeks, 3.5 ng/kg/day (DEN-initiated)	Serum 5'-nucleotidase	1.9	8.3E-01	3.0E+01	2.4E-01	3.0E+00	1.1E+02	8.5E-01	G

Appendix I: Multiple-dose studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Body burden ED_{01}(ng/kg)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Body burden ED_{10} (ng/kg)	Relative ED_{10}[c]	Quality of fit[d]
		Serum alkaline phosphatase	2.4	7.6E+00	2.7E+02	2.2E+00	2.0E+01	7.4E+02	5.8E+00	M
		Serum s. dehydrogenase	1.0	5.1E-01	1.8E+01	1.5E-01	5.6E+00	2.0E+02	1.6E+00	G
		Serum total cholesterol	1.3	4.2E-01	1.5E+01	1.2E-01	2.6E+00	9.3E+01	7.4E-01	G
		Serum triglycerides	18.0	2.8E+01	1.0E+03	8.0E+00	3.2E+01	1.2E+03	9.2E+00	M
Maronpot et al. (1993), female Sprague-Dawley rats	31 weeks, 1x/2 weeks, 3.5 ng/kg/day (SALINE)	Serum 5'-nucleotidase	18.0	2.6E+01	9.2E+02	7.3E+00	2.9E+01	1.1E+03	8.3E+00	G
		Serum total cholesterol	2.0	2.3E+00	8.3E+01	6.6E-01	7.4E+00	2.7E+02	2.1E+00	G
		Serum triglycerides	18.0	9.1E+01	3.3E+03	8.0E+00	1.1E+02	3.8E+03	3.0E+01	P
Sewall et al. (1993), female Sprague-Dawley rats	31 weeks, 1x/2 weeks, 3.5 ng/kg/day (DEN-initiated and saline-treated)	EGF dissociation (K_d) (DEN)	1.0	8.1E-01	2.9E+01	2.3E-01	8.9E+00	3.2E+02	2.6E+00	M
		EGF dissociation (K_d) (saline)	18.0	1.4E+01	5.0E+02	4.0E+00	1.6E+01	5.9E+02	4.7E+00	M

Appendix I: Multiple-dose studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Body burden ED_{01}(ng/kg)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Body burden ED_{10} (ng/kg)	Relative ED_{10}[c]	Quality of fit[d]
		EGFR autophospho-rylation	1.0	4.9E-01	1.8E+01	1.4E-01	5.1E+00	1.8E+02	1.5E+00	G
		EGFR Maximum binding (DEN)	1.6	1.7E+00	6.1E+01	4.8E-01	7.7E+00	2.8E+02	2.2E+00	G
		EGFR Maximum binding (saline	1.5	3.8E-01	1.4E+01	1.1E-01	1.9E+00	6.8E+01	5.4E-01	G
DeVito et al. (1994), female B6C3F1 mice	13 weeks, 5x/week, 1.5 ng/kg/day	CYP1A1 EROD	1.6	3.2E+00	5.1E+01	2.1E+00	1.5E+01	2.3E+02	9.8E+00	G
		CYP1A1 EROD lung	1.3	6.1E-01	9.7E+00	4.1E-01	3.7E+00	5.8E+01	2.5E+00	G
		CYP1A1 EROD skin	NA	NA	NA	NA	NA	NA	NA	NF
		CYP1A2 ACOH	1.0	1.2E-01	1.9E+00	8.2E-02	1.3E+00	2.1E+01	9.0E-01	G
Schrenck et al. (1994), female Wistar rat	13 weeks, 1x/2 weeks, 2 ng/kg	Body weight	10.7	1.3E+01	4.2E+02	6.6E+00	1.7E+01	5.3E+02	8.3E+00	G
		CYP1A1 EROD	1.2	8.2E-01	2.6E+01	4.1E-01	5.6E+00	1.8E+02	2.8E+00	G
		Relative liver weight	1.0	3.5E-01	1.1E+01	1.8E-01	3.9E+00	1.2E+02	1.9E+00	G

Appendix I: Multiple-dose studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Body burden ED_{01}(ng/kg)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Body burden ED_{10} (ng/kg)	Relative ED_{10}[c]	Quality of fit[d]
Sewall et al. (1995), female Sprague-Dawley rats	31 weeks, 1x/2 weeks, 3.5 ng/kg/day, (DEN-initiated)	CYP 1A1 mRNA	18.0	2.6E+01	9.4E+02	7.4E+00	3.0E+01	1.1E+03	8.5E+00	M
		Thyroid-stimu-lating hormone	12.1	2.6E+01	9.3E+02	7.4E+00	3.1E+01	1.1E+03	9.0E+00	M
		Thyroxine	1.9	1.3E+00	4.8E+01	3.8E-01	4.7E+00	1.7E+02	1.3E+00	G
		UGT mRNA	16.0	3.7E-01	1.3E+01	1.1E-01	4.3E-01	1.6E+01	1.2E-01	M
VanBirgelen et al. (1995), female Sprague-Dawley rats	13 weeks, 1x/day, 14 ng/kg/d	CYP1A1 EROD	1.3	1.0E+00	3.8E+01	7.5E-02	6.9E+00	2.5E+02	4.9E-01	G
		T4UGT	1.0	1.6E+00	5.8E+01	1.1E-01	1.8E+01	6.3E+02	1.3E+00	G
		Thyroxine ft4	1.0	4.9E+00	1.8E+02	3.5E-01	5.4E+01	1.9E+03	3.8E+00	M
		Thyroxine tt4	16.6	3.3E+01	1.2E+03	2.4E+00	3.8E+01	1.4E+03	2.7E+00	M
		UGT1A1	1.7	1.5E+00	5.3E+01	1.0E-01	6.0E+00	2.2E+02	4.3E-01	M
VanBirgelen et al. (1995b), female Sprague-Dawleyrats	13 weeks, 1x/day, 14ng/kg/d	Body weight	1.0	4.3E+00	1.6E+02	3.1E-01	4.7E+01	1.7E+03	3.4E+00	G
		CYP1A1 EROD	1.0	6.1E-01	2.2E+01	4.3E-02	6.7E+00	2.4E+02	4.8E-01	G
		CYP1A2 ACOH	2.1	2.1E+00	7.4E+01	1.5E-01	6.5E+00	2.3E+02	4.6E-01	M

Appendix I: Multiple-dose studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Body burden ED_{01}(ng/kg)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Body burden ED_{10} (ng/kg)	Relative ED_{10}[c]	Quality of fit[d]
		Hepatic retinol	1.0	2.8E-01	1.0E+01	2.0E-02	3.1E+00	1.1E+02	2.2E-01	G
		Hepatic retinyl-palmitate	1.0	4.0E-02	1.5E+00	2.9E-03	4.4E-01	1.6E+01	3.2E-02	G
		Liver weight	18.0	2.2E+02	8.0E+03	1.6E+01	2.5E+02	9.1E+03	1.8E+01	P
		Liver weight:body weight ratio	1.0	4.9E+00	1.8E+02	3.5E-01	5.4E+01	2.0E+03	3.9E+00	G
		Plasma retinol	1.2	2.3E+00	8.2E+01	1.6E-01	1.7E+01	6.0E+02	1.2E+00	G
		Relative kidney weight	1.0	4.8E-01	1.7E+01	3.4E-02	5.3E+00	1.9E+02	3.8E-01	G
		Relative spleen weight	0.9[f]	4.9E+00	1.8E+02	3.5E-01	7.4E+01	2.7E+03	5.3E+00	G
		Relative thymus weight	1.0	3.0E+00	1.1E+02	2.1E-01	3.3E+01	1.2E+03	2.3E+00	M
		Thymus weight	1.0	2.5E+00	8.9E+01	1.8E-01	2.7E+01	9.7E+02	1.9E+00	M
		Thyroxine ft4	1.0	4.9E+00	1.8E+02	3.5E-01	5.4E+01	1.9E+03	3.8E+00	G
		Thyroxine tt4	16.6	3.3E+01	1.2E+03	2.4E+00	3.8E+01	1.4E+03	2.7E+00	M
Rhile et al. (1996), female DBA/2 mice	11 days, 1x/day, 100 ng/kg	Total thymic cells/mouse	8.5	6.5E+02	1.0E+04	6.5E+00	8.6E+02	1.4E+04	8.6E+00	M
		CD8+ cells	NA	NA	NA	NA	NA	NA	NA	NF
		CD8+/CD4+	18.0	6.4E+03	1.5E+05	6.4E+01	7.2E+03	1.1E+05	7.2E+01	M
		CD8-/CD4-	NA	NA	NA	NA	NA	NA	NA	NF

Appendix I: Multiple-dose studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Body burden ED_{01}(ng/kg)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Body burden ED_{10} (ng/kg)	Relative ED_{10}[c]	Quality of fit[d]
		CD4+	17.5	1.7E+02	2.7E+03	1.7E+00	1.9E+02	3.0E+03	1.9E+00	M
Rhile et al. (1996), female C57 BL/6 mice	11 days, 1x/day, 100 ng/kg	Total thymic cells/mouse	15.0	7.5E+01	1.2E+03	7.5E-01	8.9E+01	1.4E+03	8.9E-01	M
		CD8+ cells	13.5	3.4E+03	5.4E+04	3.4E+01	4.1E+03	6.5E+04	4.1E+01	M
		CD8+/CD4+	11.2	3.2E+03	4.9E+04	3.1E+01	3.8E+03	6.1E+04	3.8E+01	G
		CD8-/CD4-	1.0	9.9E-01	1.6E+01	9.9E-03	1.1E+01	1.7E+02	1.1E-01	G
Rhile et al. (1996), female C57BL/6 lpr/lpr mice	11 days, 1x/day, 100 ng/kg	Total thymic cells/mouse	1.0	1.6E+01	2.5E+02	1.6E-01	1.8E+02	2.8E+03	1.8E+00	G
		CD8+ cells	18.0	3.8E+03	6.1E+04	3.8E+01	4.3E+03	6.9E+04	4.3E+01	M
		CD8+/CD4+	18.0	2.8E+04	4.5E+05	2.9E+02	3.3E+04	5.2E+05	3.3E+02	P
		CD8-/CD4-	15.3	1.2E+04	1.9E+05	1.2E+02	1.4E+04	2.3E+05	1.4E+02	P
		CD4+	18.0	3.6E+04	5.7E+05	3.6E+01	4.1E+03	6.5E+04	4.1E+01	M
Vogel et al. (1997), female C57BL/6 mice	23 days, 1 ng/kg (initial dose), 0.2 ng/kg/week (3x total)	Immune CD4+/CD8- (23 d)	6.1	2.9E-02	4.2E-01	4.2E-01	4.3E-02	6.3E-01	6.2E-01	G
		Immune CD4-/CD8- (23 d)	1.0	1.3E-03	1.8E-02	1.8E-02	1.4E-02	2.0E-01	2.0E-01	M
		Immune CD4-/CD8+ (23 d)	6.1	2.5E-02	3.7E-01	3.6E-01	3.8E-02	5.5E-01	5.4E-01	G

Appendix I: Multiple-dose studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Body burden ED_{01}(ng/kg)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Body burden ED_{10} (ng/kg)	Relative ED_{10}[c]	Quality of fit[d]
		ImmuneCD4+/CD8+ (23 d)	5.5	2.7E-02	3.9E-01	3.8E-01	4.2E-02	6.0E-01	5.9E-01	G
Vogel et al. (1997), female C57BL/6 mice	79 days, 1 ng/kg (initial dose), 0.2 ng/kg/week, (7x total)	Immune CD4+/CD8- (79 d)	13.4	6.2E-02	8.9E-01	2.1E+00	7.4E-02	1.1E+00	2.5E+00	P
		Immune CD4-/CD8- (79 d)	18.0	7.9E-02	1.1E+00	2.6E+00	9.0E-02	1.3E+00	3.0E+00	G
		ImmuneCD4-/CD8+ (79 d)	6.6	1.2E-02	1.7E-01	4.0E-01	1.7E-02	2.5E-01	5.7E-01	M
Vogel et al. (1997), female C57BL/6 mice	135 days, 1 ng/kg (initial dose), 0.2 ng/kg/week until 0.034 ng/kg steady-state reached	CYP1A1 EROD (135 d)	1.0	7.4E-03	1.1E-01	2.2E-01	8.0E-02	1.2E+00	2.4E+00	G
		CYP1A1 mRNA (135 d)	8.1	1.7E+00	2.5E+01	5.0E+01	2.3E+00	3.3E+01	6.7E+01	G
		CYP1A2 mRNA (135 d)	1.1	3.0E-03	4.3E-02	8.7E-02	2.7E-02	3.9E-01	7.9E-01	G
		CYP1A2 MROD (135 d)	1.0	1.5E-02	2.2E-01	4.6E-01	1.6E-01	2.4E+00	4.8E+00	G

Appendix I: Multiple-dose studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Body burden ED_{01}(ng/kg)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Body burden ED_{10} (ng/kg)	Relative ED_{10}[c]	Quality of fit[d]
Johnson et al. (1997), female B6C3F1 mice	18 weeks, 1x/3 wks (5x total), 3 weeks after last, 1,000 ng/kg	CYP1A1 EROD	2.8	1.9E+01	6.2E-03	8.2E+00	4.2E+01	6.6E+02	8.8E-01	G
		Endometrial lesion diameter	NA	NA	NA	NA	NA	NA	NA	NF
		Endometrial lesion weight	NA	NA	NA	NA	NA	NA	NA	NF
		Liver weight	1.1	7.7E+00	1.2E+02	1.6E-01	6.2E+01	9.8E+02	1.3E+00	G
		Thymus weight	NA	NA	NA	7.3E+00	NA	NA	NA	NF
		Ovarian weight	15.2	3.5E+02	5.5E+03	NA	4.1E+02	6.4E+03	8.5E+00	P
Walker et al. (1999), female Sprague-Dawley Rats	31 weeks, 1x/2 weeks, 3.5 ng/kg/day, (DEN-initiated)	CYP1A1 mRNA	2.0	1.6E+00	5.9E+01	4.7E-01	5.6E+00	2.0E+02	1.6E+00	G
		CYP1A2 mRNA	3.0	7.6E+00	2.7E+02	2.2E+00	1.7E+01	6.1E+02	4.8E+00	G
		CYP1B1 mRNA	3.1	7.0E+00	2.5E+02	2.0E+00	1.5E+01	5.4E+02	4.3E+00	G

[a]Dose regimen is described by study duration, exposure frequency, and lowest dose used in the study.
[b]Unless noted otherwise, the Hill model was used to fit these data.
[c]Relative ED_X effect is the ratio of daily ED_X to the lowest daily doselevel used in the study from the study.
[d]Qualitative assessment of fit: G=good (model curve goes through/near all data point mean); M=marginal (model within one std. deviation of mean); P=poor (model not within one std. deviation of means).
[e]NR- In some cases, BMDS (U.S. EPA, 1999) fails to locate a lower confidence bound on the 1% effective dose.
[f]Power model was used for these data.
[h]NR- Quality of fit was not assessed for this endpoint.

Appendix I: Multiple-dose studies (continued)

[i]NA-Models in BMDS (U.S. EPA, 1999) not applicable to these data.
[j]NF - Quality of fit not assessed for this endpoint.

Appendix II: Single-dose adult studies

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Relative ED_{10}[c]	Quality of fit[d]
Kitchin & Woods (1979), female Sprague-Dawley rats	3 days, 0.6 ng/kg	Liver cytochrome P-450 (total)	1.0	1.5E+01	2.6E+01	1.7E+02	2.8E+02	G
		Liver benzopyrene hydroxylase (CYP1A1 activity)	17.7	1.4E+03	2.4E+03	1.6E+03	2.7E+03	P
Olson et al. (1980), male Golden Syrian hamsters	50 days, 5,000 ng/kg	Thymus weight	1.1	3.7E+03	7.3E-01	3.5E+04	6.9E+00	G
		Spleen weight	3.5	1.5E+05	3.1E+01	3.0E+05	6.1E+01	M
Vecchi et al. (1983), female B6 mice	12 days, 1,200 ng/kg	Body weight	12.0	2.0E+04	1.6E+01	2.4E+04	2.0E+01	G
		Thymus weight	1.4	1.5E+02	1.3E-02	8.3E+02	6.9E-01	G
		PFC/1E+06 splenocytes	1.0	2.7E+00	2.3E-04	1.3E+02	1.1E-01	G
		PFC/spleen	1.0	3.9E+00	3.3E-04	2.1E+02	1.7E-01	G
Vecchi et al. (1983), female C3 mice	12 days, 1,200 ng/kg	Body weight	11.1	4.4E+03	3.6E-01	5.4E+03	4.5E+00	P
		Thymus weight	1.0	3.9E+01	3.3E-03	4.3E+02	3.6E-01	G
Vecchi et al. (1983), female D2 mice	12 days, 1,200 ng/kg	Body weight	17.8	3.8E+05	3.1E+02	4.3E+05	3.6E+02	P
		Thymus weight	1.0	3.5E+00	2.9E-03	3.8E+01	3.2E-02	M
		PFC/1E+06 splenocytes	1.0	5.2E+01	4.3E-02	5.7E+02	4.7E-01	G
		PFC/spleen	1.3	1.3E+02	1.1E-01	8.5E+02	7.1E-01	G

Appendix II: Single-dose adult studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Relative ED_{10}[c]	Quality of fit[d]
Vecchi et al. (1983), female B6D2F1 mice	12 days, 1,200 ng/kg	Thymus weight	1.0	6.1E+01	5.1E-02	6.6E+02	5.5E-01	G
		PFC/1E+06 splenocytes	1.0	1.4E+01	1.2E-03	1.6E+03	1.3E+00	G
		PFC/spleen	1.0	1.4E+01	1.2E-03	1.5E+03	1.2E+00	G
Abraham et al, (1988), female Wistar rats	7 days, 1 ng/kg	Liver EROD (CYP1A1 activity)	1.1	1.6E+01	1.6E+01	7.3E+01	7.3E+01	G
		Liver cytochrome P450 (total)	1.0	6.7E+00	6.7E+00	1.4E+02	1.4E+02	G
Davis and Safe (1988), male 657BL/6J mice	9 days, 1 nmol/kg	Spleen cellularity	18.0	4.5E+02	1.4E+00	5.2E+02	1.6E+00	M
		PFCs/spleen	4.2	2.0E+02	6.3E-01	3.6E+02	1.1E+00	G
		PFCs/1E+06 viable cells	4.0	2.1E+02	6.5E-01	3.8E+02	1.2E+00	G
Birnbaum et al. (1990), male C57BL/6J (Ahb/b) mice	35 days, 50 ng	Serum TBA	18.0	4.6E+04	9.1E+02	5.2E+04	1.0E+03	M
		Serum SDH	2.8	1.7E+04	3.4E+02	3.9E+04	7.8E+02	M
		Serum ALT	2.4	1.6E+04	3.2E+02	4.3E+04	8.6E+02	M
		Serum 5'-NUC	18.0	8.8E+04	1.8E+03	1.0E+05	2.0E+03	M
		Serum glucose	18.0	5.3E+04	1.1E+03	6.0E+04	1.2E+03	M
Birnbaum et al. (1990), male C57BL/6J (Ahb/b) mice	35 days, 50 ng	Serum total cholesterol	18.0	3.5E+04	6.9E+02	4.0E+04	7.9E+02	M
		Serum NEChol	4.7	7.6E-04	1.5E-05	1.3E-03	2.5E-05	P

Appendix II: Single-dose adult studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Relative ED_{10}[c]	Quality of fit[d]
		Serum Echol	18.0	3.5E+04	7.1E+02	4.0E+04	8.1E+02	M
		Liver Hepatocellular cytomegaly	7.2[g]	8.5E+04	1.7E+03	1.2E+05	2.3E+03	G
		Liver Hepatocellular karyomegaly	5.8[g]	3.0E+04	6.0E+02	4.5E+04	8.9E+02	G
		Fatty liver change	7.9[g]	5.8E+04	1.2E+03	7.8E+04	1.6E+03	G
		Liver bile duct hyperplasia	2.6[g]	4.8E+04	9.6E+02	1.2E+05	2.4E+03	G
		Thymic atrophy	2.0[g]	2.3E+04	4.6E+02	7.6E+04	1.5E+03	G
		Splenic atrophy	1.9[g]	1.6E+04	3.3E+02	5.5E+04	1.1E+03	G
		Testes: multinucleated spermatid giant cells	2.3[g]	3.7E+04	7.4E+02	1.0E+05	2.1E+03	G
		Testes: seminiferous tubule epithelium necrosis	6.9[g]	1.0E+05	2.0E+03	1.4E+05	2.9E+03	G
		Gland. stomach edema	1.5[g]	1.8E+04	3.7E+02	8.6E+04	1.7E+03	G
Birnbaum et al. (1990), male C57BL/6J (Ahd/d) mice	35 days, 400 ng	Serum TBA	2.3	4.0E+05	9.9E+02	1.1E+06	2.7E+03	M
		Serum SDH	7.1	1.1E+06	2.1E+04	1.5E+06	3.8E+03	M
		Serum ALT	1.0	4.2E+04	1.0E+02	4.2E+05	1.0E+03	M
		Serum 5'-NUC	18.0	3.2E+05	8.1E+02	3.7E+05	9.2E+02	P
		Serum glucose	18.0	6.1E+05	1.5E+03	6.9E+05	1.7E+03	P

Appendix II: Single-dose adult studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Relative ED_{10}[c]	Quality of fit[d]
		Serum triglycerides	18.0	1.8E+06	4.6E+03	2.1E+06	5.3E+03	P
		Serum total cholesterol	1.0	5.1E+02	1.3E+00	5.6E+03	1.4E+01	G
		Serum NEChol	1.0	1.0E+03	2.5E+00	1.1E+04	2.8E+01	G
		Serum Echol	1.0	1.7E+03	4.2E+00	1.8E+04	4.6E+01	G
		Liver Hepatocellular cytomegaly	4.2[g]	1.5E+06	3.8E+03	2.7E+06	6.7E+03	M
		Liver Hepatocellular karyomegaly	3.1[g]	9.2E+04	2.3E+02	1.9E+05	4.9E+02	M
		Fatty Liver change	2.6[g]	6.9E+05	1.7E+03	1.7E+06	4.3E+03	M
		Liver BDH	1.6[g]	1.3E+06	3.2E+03	5.4E+06	1.3E+04	M
		Thymic atrophy	1.0[g]	4.7E+04	1.2E+02	4.9E+05	1.2E+03	M
		Splenic atrophy	1.0[g]	2.3E+04	5.8E+01	2.4E+05	6.1E+02	M
		Testes: seminiferous tubule epithelium necrosis	4.2[g]	1.9E+06	4.9E+03	3.4E+06	8.5E+03	G
		Gland. stomach edema	4.2[g]	1.9E+06	4.9E+03	3.4E+06	8.5E+03	G
Jurek et al. (1990), male Sprague-Dawley rats	12 days, 1 nmol/kg	Body weight	1.0	9.2E+02	2.9E+00	1.0E+04	3.1E+01	M
		Liver weight:body weight ratio	8.2	1.1E+06	3.5E+03	1.4E+03	4.2E+00	P
		Kidney weight:body weight ratio	2.7	3.4E-03	1.1E-05	8.3E-03	2.6E-05	P

Appendix II: Single-dose adult studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Relative ED_{10}[c]	Quality of fit[d]
		Renal retinol concentration	12.3	2.0E+03	6.3E+00	2.5E+03	7.6E+00	M
		Renal RPH activity	18.0	1.5E+04	4.5E+01	1.7E+04	5.2E+01	M
Alsharif et al. (1994), female Sprague-Dawley rats	1 day, 5 ng/kg	Superoxide anion production by PLC	5.4	5.7E+04	1.1E+04	8.9E+04	1.8E+04	G
Narasimhan et al. (1994), female B6C3F1 mice	24 hrs., 5 ng/kg	Liver EROD (CYP1A1 activity)	1.1	8.4E+01	1.7E+01	7.2E+02	1.4E+02	G
		Liver CYP1A1 (mRNA)	1	5.6E+00	1.1E+00	6.2E+01	1.2E+01	G
		Liver CYP1A2 (mRNA)	3.2	1.7E+02	3.4E+01	3.7E+02	7.3E+01	G
		Spleen PFC/1E+06cells	1.0	2.0E+00	4.1E-01	2.2E+01	4.5E+00	G
	4 days, 5 ng/kg	Total AhR binding	3.8	3.5E+02	7.0E+01	6.5E+02	1.3E+02	G
Harper et al. (1994), male C57BL/6 mice	8 days, 0.6 mg/kg	Immune titer	4.8	3.0E+02	5.0E-01	5.0E+02	8.3E-01	G
		PFC/1E+06 cells	6.1	3.3E+02	5.5E-01	4.9E+02	8.1E-01	M
Smialowicz et al. (1994), male F344 rats	1x followed by immunization with SRBC 7 days later, 100 ng/kg	PFC/1E+06 cells	18.0	1.6E+04	1.6E+02	1.8E+04	1.8E+02	P
		PFC/spleen($\times 10^{-4}$)	18.0	2.3E+04	2.3E+02	2.6E+04	2.6E+02	P
		Cells/spleen($\times 10^{-6}$)	18.0	7.3E+03	7.3E+01	8.3E+03	8.3E+01	P
		Titer(log2)	1.4	1.2E+02	1.2E+00	6.9E+02	6.9E+00	G

Appendix II: Single-dose adult studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Relative ED_{10}[c]	Quality of fit[d]
Smialowicz et al. (1994), female F344 rats	1x followed by immunization with SRBC 7 days later, 100 ng/kg	PFC/1E+06 cells	1.0	3.4E+02	3.4E+00	3.4E+03	3.4E+01	P
		PFC/spleen($\times 10^{-4}$)	1.0	3.6E+02	3.6E+00	3.6E+03	3.6E+01	P
Smialowicz et al. (1994), female B6C3F1 mice	1x followed by immunization with SRBC 7 days later, 300 ng/kg	PFC/1E+06 cells	1.0	2.9E+00	9.6E-03	3.2E+01	1.1E-01	M
		PFC/spleen($\times 10^{-4}$)	1.1	4.4E+00	1.5E-02	4.0E+01	1.3E-01	G
Vanden Heuvel et al. (1994a), female Sprague-Dawley rats	4 days, 0.1 ng/kg	CYP1A1 mRNA	3.6	3.9E+02	3.9E+03	7.7E+02	7.7E+03	G
		UGT mRNA	1.4	3.5E+01	3.5E+02	1.9E+02	1.9E+03	G
Diliberto et al. (1995), female B6C3F1 mice	S, 7, 14, 21, 35 days, 100 ng/kg	Liver EROD (CYP1A1): 7 days	1.0	2.7E+01	2.7E-01	3.0E+02	3.0E+00	P
		Liver EROD (CYP1A1): 14 days	3.5	2.8E+02	2.8E+00	5.5E+02	5.5E+00	G
		Liver EROD (CYP1A1): 21 days	2.8	2.4E+02	2.4E+00	5.7E+02	5.7E+00	G
		Liver EROD (CYP1A1): 35 days	6.5	7.4E+02	7.4E+00	1.1E+03	1.1E+01	M
Li et al. (1995), female Sprague-Dawley rats	4 days, 300 ng/kg	Body weight	3.7	1.2E+03	3.9E+00	2.2E+03	7.4E+00	G
		Ovarian weight	1.0	1.7E+02	5.7E-01	1.9E+03	6.2E+00	G

Appendix II: Single-dose adult studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	Daily ED_{01} (ng/kg/day)	Relative ED_{01}[c]	Daily ED_{10} (ng/kg/day)	Relative ED_{10}[c]	Quality of fit[d]
		Ovulation (ova/rat)	1.4	1.5E+02	4.9E-01	8.7E+02	2.9E+00	G
VanBirgelen et al. (1996), female B6C3F1 mice	S, 7 days, 100 ng/kg	CYP1A1 EROD	1.8	7.1E+01	7.1E-01	2.7E+02	2.7E+00	G

[a]Dose regimen is described by study duration (total days after single administration) and lowest dose used in the study.
[b]Unless noted otherwise, the Hill model was used to fit these data.
[c]Relative ED_X is the ratio of the ED_X to the lowest dose tested in the study.
[d]Qualitative assessment of fit: G=good (model curve goes through/near all data point means); M=marginal (model within one std. deviation of means); P=poor (model not within one std. deviation of means).
[e]NR- In some cases, BMDS (U.S. EPA, 1999) fails to locate a lower confidence bound on the 1% effective dose.
[f]Power model used to fit these data.
[g]Weibull model used to fit these data.
[h]NA - Models in BMDS (U.S. EPA, 1999) not applicable to these data.
[i]NF- Quality of fit not assessed for this endpoint.

Appendix III: Single-dose developmental studies

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	ED_{01} (ng/kg/day)	Relative ED_{01}c	ED_{10} (ng/kg/day)	Relative ED_{10}[C]	Quality of fit[d]
Birnbaum et al. (1989), C57BL/6N mice	GD 10 or 12, 8 or 6 days (sacrificed on GD 18), 6,000 ng/kg	Cleft palate GD-10[e]	3.5	3.3E+03	3.3E+00	6.4E+03	1.1E+00	G
		Cleft palate GD-12[e]	6.4	4.4E+03	4.4E+00	6.3E+03	1.1E+00	G
		Hydronephrosis GD-10[e]	1.0	3.2E+01	3.2E-02	3.3E+02	5.5E-02	M
		Hydronephrosis GD-12[e]	2.3	2.1E+02	2.1E-01	5.7E+02	9.5E-02	P
Mably et al. (1992b,c), pregnant female, male offspring, Holtzman Sprague-Dawley rats	GD 15, postnatal day (PND) 49, 63, or 120, 64 ng/kg	Sperm morph. – day 120	4.4	8.7E+01	1.4E+00	1.5E+02	2.3E+00	G
		Fertility index	NA[g]	NA	NA	NA	NA	NF[h]
		Cauda sperm count day 63	1.0	6.6E-01	1.0E-02	7.2E+00	1.1E-01	G
		Cauda sperm count - day 120	1.0	7.6E-01	1.2E-02	8.3E+00	1.3E-01	G
		Cauda sperm count/g - day 120	1.7	3.7E+00	5.8E-02	1.5E+01	2.3E-01	G
		DSP/g - day 63	1.0	5.6E-01	8.8E-03	6.2E+00	9.7E-02	G
		DSP/g - day 120	1.4	1.4E+00	2.2E-02	7.9E+00	1.2E-01	G
		DSP/g – day 49	1.7	6.6E+00	1.0E-01	2.8E+01	4.4E-01	G
		Reproductive outcomes of females:						
		Litter size	18.0	7.9E+01	1.2E+00	9.1E+01	1.4E+00	P
		Live birth index (%)	NA	NA	NA	NA	NA	NF

Appendix III. Single-dose developmental studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	ED_{01} (ng/kg/day)	Relative ED_{01}c	ED_{10} (ng/kg/day)	Relative ED_{10}[C]	Quality of fit[d]
		Age of indices of dev. in pups:						
		Pinna detachment	17.0	7.7E+02	1.2E+01	8.8E+02	1.4E+01	P
		Incisor eruption	1.0	1.3E+01	2.0E-01	1.3E+02	2.1E+00	G
		Eye opening	1.0	7.0E+00	1.1E-01	7.4E+01	1.2E+00	G
		Testis descent	1.0	1.3E+00	2.1E-02	1.4E+01	2.3E-01	G
Theobald et al. (1997), pregnant female, male and female offspring ICR mice	GD 14, PND 44, 15,000 ng/kg	Testes weight	1.0	7.4E+02	6.5E+00	6.9E+03	4.6E-01	M
		Epididymidis wt.	18.0	4.8E+04	3.2E+00	5.4E+04	3.6E+00	M
		Dorsal prostate wt.	1.0	3.0E+02	2.0E-02	3.3E+03	2.2E-01	P
		Ventral prostate wt.	2.9	1.2E-04	8.0E-09	2.8E-04	1.9E-08	M
		Coagulating glands	1.7	3.3E+03	2.2E-01	1.3E+04	8.7E-01	G
		Seminal vesicles	18.0	4.5E+04	3.0E+00	5.2E+04	3.4E+00	M
		Ovary weight	18.0	2.4E+04	1.6E+00	2.8E+04	1.8E+00	M
		Uterus weight	4.5	9.8E+03	6.5E-01	1.7E+04	1.1E+00	G
Theobald et al. (1997), pregnant female, male and female offspring ICR mice	GD 14, PND 65, 15,000 ng/kg	Testes weight	18.0	1.1E+04	7.5E-01	1.3E+04	8.5E-01	M
		Epididymidis wt.	3.1	1.4E-04	9.5E-09	9.4E-04	6.2E-08	P
		Ventral prostate wt.	18.0	1.1E+04	NR	1.3E+04	8.6E-01	M

Appendix III. Single-dose developmental studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	ED_{01} (ng/kg/day)	Relative ED_{01}c	ED_{10} (ng/kg/day)	Relative ED_{10}[C]	Quality of fit[d]
		Coagulating glands	18.0	1.1E+04	7.5E-01	1.3E+04	8.6E-01	M
		Seminal vesicles	1.0	1.2E+03	7.5E-01	1.2E+04	7.8E-01	M
		Sperm production: ESN	13.4	1.0E+04	7.8E-02	1.2E+04	8.2E-01	M
		Sperm production: DSP	18.0	1.5E+04	6.8E-01	1.7E+04	1.1E+00	M
		Pituitary gland wt. (males) (PND 65)	11.5	3.0E+05	9.8E-01	3.7E+05	2.5E+01	P
Theobald et al. (1997), pregnant female, male and female offspring ICR mice	GD 14,PND 114/128, 15,000 ng/kg	Epididymidis wt.	NA	NA	2.0E+01	NA	NA	NF
		Dorsal prostate wt.	1.0	5.0E+02	NA	5.3E+03	3.6E-01	P
		Ventral prostate wt.	18.0	1.1E+04	3.4E-02	1.3E+04	8.4E-01	M
		Coagulating glands	18.0	1.1E+04	7.3E-01	1.3E+04	8.6E-01	M
		Seminal vesicles	NA	NA	7.6E-01	NA	NA	NF
		Sperm production: ESN (PND 114/128)	NA	NA	NA	NA	NA	NF
		Female rep: ovary wt. (PND 114)	18.0	1.6E+04	NA	1.8E+04	1.2E+00	M
		Female rep: uterus wt. (PND 114)	4.5	2.1E+04	1.0E+00	3.5E+04	2.4E+00	G
Theobald et al. (1997), pregnant female, male and female offspring ICR mice	GD 14,PND 114/128, 15,000 ng/kg	Pituitary gland wt. (males) (PND 128)	NA	NA	1.4E+00	NA	NA	NF

Appendix III. Single-dose developmental studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	ED_{01} (ng/kg/day)	Relative ED_{01}c	ED_{10} (ng/kg/day)	Relative ED_{10}[C]	Quality of fit[d]
		Pituitary wt. (females) (PND 128)	18.0	1.1E+04	NA	1.2E+04	8.2E-01	M
		Hydronephrosis (females)	1.1[e]	1.2E+03	7.2E-01	9.4E+03	6.3E-01	M
		Eye opening (females)	1.0	3.8E+01	8.0E-02	4.2E+02	2.8E-02	M
		Thymus weight (females)	1.0	3.2E+02	2.5E-03	3.5E+03	2.3E-01	M
		Hydronephrosis (males)	1.0[e]	2.6E+02	2.1E-02	2.7E+03	1.8E-01	M
		Eye opening (males)	1.0	7.6E+01	1.7E-02	8.4E+02	5.6E-02	G
		Thymus weight (males)	3.4	1.6E-04	5.1E-03	3.2E-04	2.1E-08	P
Gray et al. (1997), Long Evans Hooded rat male offspring	GD 15, PND 49,50 ng/kg	Body weight (day 49)	9.6	1.4E+02	1.0E-08	1.8E+02	3.5E+00	G
		Testes weight (49)	1.1	1.0E+01	2.7E+00	8.4E+01	1.7E+00	G
		Paired epididymal weight (49)	13.9	1.4E+02	2.1E-01	1.7E+02	3.4E+00	M
		Cauda epididymus (49)	18.0	7.9E+01	2.9E+00	9.0E+01	1.8E+00	G
		Epididymal sperm count (49)	1.0	1.5E-01	1.6E+00	1.7E+00	3.4E-02	P
		Ventral prostate weight (49)	12.4	1.4E+02	3.0E-03	1.6E+02	3.3E+00	G
		Seminal vesicle weight (49)	17.9	1.5E+02	2.7E+00	1.7E+02	3.5E+00	M

Appendix III. Single-dose developmental studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	ED_{01} (ng/kg/day)	Relative ED_{01}c	ED_{10} (ng/kg/day)	Relative ED_{10}[C]	Quality of fit[d]
		Daily sperm production (49)	14.1	5.9E+02	3.0E+00	6.9E+02	1.4E+01	M
		Age at puberty (49)	2.8	4.0E+01	1.2E+01	9.4E+01	1.9E+00	P
		Body weight at puberty (49)	13.6	1.4E+02	1.3E+03	1.6E+02	3.2E+00	M
		Pituitary (49)	8.9	9.6E+01	7.9E-01	1.3E+02	2.5E+00	M
Gray et al. (1997), Long Evans Hooded rat male offspring	GD 15, PND 63,50 ng/kg	Body weight (63)	17.5	1.6E+02	2.7E+00	1.8E+02	3.6E+00	P
		Testes weight (63)	10.8	1.3E+02	1.9E+00	1.6E+02	3.2E+00	G
		Paired epididymal weight (63)	14.2	1.4E+02	3.2E+00	1.6E+02	3.3E+00	P
		Cauda epididymus (63)	12.1	1.3E+02	2.6E+00	1.6E+02	3.1E+00	G
		Epididymal sperm count (63)	11.2	1.4E+02	2.8E+00	1.7E+02	3.5E+00	G
		Ventral prostate weight (63)	14.0	1.4E+02	2.6E+00	1.7E+02	3.4E+00	P
		Seminal vesicle weight (63)	11.3	1.6E+02	2.8E+00	2.0E+02	4.0E+00	G
		Daily sperm production (63)	13.6	5.4E+02	2.8E+00	6.4E+02	1.3E+01	M
		Serum testosterone (63)	10.3	3.3E+01	3.2E+00	4.1E+01	8.2E-01	M
		Pituitary (63)	8.7	3.7E+01	1.1E+01	4.9E+01	9.7E-01	M

Appendix III. Single-dose developmental studies (continued)

Study description	Dose regimen[a]	Endpoint[b]	Shape parameter	ED_{01} (ng/kg/day)	Relative ED_{01}c	ED_{10} (ng/kg/day)	Relative ED_{10}[C]	Quality of fit[d]
Gray et al. (1997), Long Evans Hooded rat male offspring	GD 15, offspring examined 15 months, 50 ng/kg	Body weight	13.0	1.6E+02	6.5E-01	1.9E+02	3.8E+00	M
		Seminal vesicle weight	18.0	7.8E+01	7.4E-01	8.9E+01	1.8E+00	G
		Glans penis weight	1.4	3.8E+00	3.1E+00	2.2E+01	4.5E-01	G
		Paired epididymal weight	18.0	7.3E+01	5.5E+02	8.4E+01	1.7E+00	P
		Cauda epididymal weight	10.7	3.3E+01	1.6E+00	4.1E+01	8.2E-01	P
		Epididymal sperm numbers	4.3	3.8E+01	7.6E-02	6.6E+01	1.3E+00	G
		Caput/corpus epid. sperm numbers	15.5	1.2E+02	1.5E+00	1.4E+02	2.9E+00	P
		Cauda epid. sperm numbers	2.9	1.4E+01	6.5E-01	3.1E+01	6.3E-01	G
		Number of copulatory plugs	2.4	1.1E-06	7.5E-01	3.2E-06	6.3E-08	P
		Total testis sperm numbers	12.3	1.6E+02	2.5E+00	2.0E+02	4.0E+00	P
		Pituitary weight	18.0	7.7E+01	2.7E-01	8.8E+01	1.8E+00	P

[a] Dose regimen is described by specific time of single administration, duration or offspring examination day, and lowest dose used in the study.
[b] Unless noted otherwise, the Hill model was used to fit these data.
[c] Relative ED_X is the ratio of the ED_X to the lowest dose tested in the study
[d] Qualitative assessment of fit: G=good (model curve goes through/near all data point mean); M=marginal (model within one std. deviation of means); P= poor (model not within one std. deviation of means).
[e] The Weibull model was fit to these data.

Appendix III. Single-dose developmental studies (continued)

[f] NR-In some cases, the BMDS (U.S. EPA, 1999) fails to locate a lower confidence bound on the 1% effective dose.
[g] NA-Models in BMDS (U.S. EPA, 1999) not applicable to these data.
[h] NF-Quality of fit was not assessed for this endpoint.

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