8. DOSE-RESPONSE MODELING

Preface

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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 aromatic 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 over 5,000 publications and abstracts since the identification of TCDD as a chloracnogen [1]. Some data sets on members of this family of compounds other than TCDD are clearly amenable to dose-response modeling. However, this chapter has focused 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 the Introduction is found in more extensive detail later in this chapter or in the other parts of this reassessment. The 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. It also introduces the reader to the type 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 our knowledge relating to the evaluation of TCDD dose-response are identified. Understanding these gaps and

their impact on the certainty of 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?

It is critical when performing dose-response analyses 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 a comparison across species and endpoints are discussed in Section 8.2 of this chapter.

Most, if not all, of the effects elicited by TCDD are mediated by the ability of this chemical to bind to the Ah receptor. The activation of this protein leads to a series of molecular and biochemical events which ultimately contribute to particular biological responses (see 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 when exposure occurs, and whether the exposure is acute or chronic. These responses vary from biochemical alterations, such as enzyme induction, which may require only acute exposures, 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 based on several assumptions and observations made at only 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 like biochemical alterations, that are closely coupled to activation of the AhR, to more complicated responses like 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 each endpoint 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.[2-5] 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 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 Chapter

2, Mechanism(s) of Action. It is accepted by most researchers that most, if not all, cellular responses to TCDD require the initial interaction between TCDD and the Ah receptor.

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 has been reviewed in several sections of this document as well as in the peer-reviewed literature.[6-8] 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 analogs to the AhR (see 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 P450 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.

The AhR has been identified in numerous mammalian species including humans [9][10-15], several non mammalian vertebrates including chicken embryo[16] and newts,[17] and several aquatic species from whales to teleosts and elasmobranchs.[18] The broad phylogenetic distribution in vertebrate evolution [18], and the phylogenetic conservation of this receptor also suggests 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 analogs in animals also occur in humans. Data on effects of TCDD and its analogs 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.[19] 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 analogs.[20] Consistent with this contention, the *in vitro* EC_{50} for TCDD-mediated induction of CYP1A1-dependent enzyme activities is ~1.5 nM when using either rodent or human lymphocytes.[21] The human Ah receptor appears to have greater than a 20-fold range in TCDD affinity.[9] This range of affinity 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 Ah receptor[15], 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 analogs 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 transformng growth factor-α (TGF-α).[22, 23] Placentas from humans exposed to rice oil contaminated with polychlorinated dibenzofurans also exhibited markedly reduced EGF-stimulated autophosphorylation of the EGF receptor, and this effect occurred with similar sensitivity as observed in rats.[20, 24] 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 non-human 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 in addition to the Ah receptor. For example, mice congenic at the Hr locus demonstrate altered sensitivity to the chloracnegenic and tumor promoting effects of TCDD.[25] These data suggests that there may be multiple factors (e.g. genetics) that my 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. These studies are evaluated in Chapter 7a, Epidemiology/Human Data, 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.[26] 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 [27] with similar results seen in Air Force personnel following exposure to Agent Orange.[28, 29] Another interesting finding of these studies was a positive relationship between TCDD exposure and diabetes (see Chapter 7b).

There are also differences between human and animal effects associated with TCDD. For example, chloracne has been observed in exposed humans but only in some animal species. Similarly, increases in humans of certain cancers such as soft tissue sarcoma have not been observed in animals (see Chapters 6 and 7). Also, immunotoxic endpoints consistently seen in animals have rarely been demonstrated, or looked for, in humans (see 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; (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 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 models (e.g. a mental image of how something works), biological models (e.g. transgenic mice as a surrogate for a human system), physical models (e.g. a three-dimensional model of the human heart) and mathematical models (e.g. a physiologically-based pharmacokinetic model (PBPK)). Any model is defined by a set of parameters which are 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 are the major components 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 between ligand concentration, receptor occupancy, and biological response. For example, Roth and Grunfeld[30] 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 this 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 proportional 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 non-linear 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 which 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; (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 which 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 provide 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. [5] 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 which 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 (US EPA Guidelines for Carcinogen Risk Assessment, EPA600Z96001). 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 than is meant by mode of action. 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 [31] "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 Ah receptor, are generally response-independent. The response-dependent events are species-, sex-, organ-, tissue-, cell- and developmental stage specific. If the 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 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 than 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 end points 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 Ah receptor to accessory proteins, have not been modeled extensively due to lack of data. Because of this deficiency, several alternate mechanistic hypothesis 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 at which 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 the animal-to-human dose extrapolation. To provide significant insight into differences in sensitivity among species, the 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) while 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 which 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 [32] and the BASF chemical plant in Ludwigshafen, Germany, in 1953 [33]. Increased daily exposures over background to dioxins have occurred in occupational exposed populations using some herbicides, for example, during the Vietnam War [34] and in agricultural workers [35]. 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[a] (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 [36, 37].

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. [38]

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 [26] 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 Ah receptor [39], induced CYP1A2 [40, 41] and reduced EGFR [42].

Different dose metrics can lead to widely diverse conclusions. For example, the lowest dose with an increased tumorigenic response (thyroid tumors) in a rat [43] 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 this 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%[b]. The current body burden 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

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[a] 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).

[b] 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.

intake for humans is likely lower than historical levels and is biased downward due to unknown sources leading to a discrepancy between body burdens and daily intake. For example, the predicted steady state body burden for humans given a daily intake of TCDD of 0.2 pg/kg/day, a 7.1 year half-life and 50% bioavailability is 0.4ng/kg. (For a discussion, see Part I).

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, the 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 in the developing organism are on the orders of hours to days. One of the difficulties is that the time span of the window of susceptibility is often too short to clearly discriminate between dose metrics such as peak concentration, steady-state body burden, or average body burden. When attempting these types of comparisons for TCDD, 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. 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 the developmental effects since 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 the mechanism-based cancer modeling, instantaneous values of a does-metric are used since these can be used as surrogates for mutational rates and growth rates within a two–stage cancer model. For the 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 which integrate dose over a specific time period. In this case 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 Calculation of Effective Doses

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, comparison of responses are made using estimated exposures associated with a given level of excess risk. To avoid large extrapolations, this common level of excess risk was chosen such that for most studies, the estimated exposure is in or near the range of the exposures seen in the studies being compared [44-49] 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. While effective dose reporting for the 2%, the 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. Based upon 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 that dose satisfying the excess risk relationship

$$\frac{R(ED_{01}) - R(0)}{R(\infty) - R(0)} = 0.01$$

where $R(d)$ represents the response (either risk or other measure) at the given exposure or dose level, d, and $R(\infty)$ is the maximum response possible (e.g. $R(\infty)$=1 for risk endpoints).

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 [26]. Under steady-state conditions, it is possible to calculate total body burdens (ng/kg) for TCDD as

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

where f is the fraction of dose absorbed and is assumed to be 50% for absorption from food [50] and 100% for other routes. Half-lives for converting between daily exposures and steady-state body burden are presented in Table 8.2.

Table 8.2: 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 mice strains	11
Golden Syrian Hamster	12
Wistar Rats	22
All other rat strains	25
Human	2593

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 the U.S. 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 [51]. Epidemiological data have suggested increases in soft-tissue sarcomas, respiratory system tumors and all cancers combined (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 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 [43, 52-54].

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 applies simple empirical models to the few studies for which exposure-response data for TCDD are available in human populations.

Modeling cancer in humans uses slightly different approaches than those used for the animal studies that will be presented later in this chapter. The modeling approach used in the analysis of the human epidemiology data for all cancers combined and lung cancer involves applying

estimated human body burden to cancer response, and estimating parameters in a linear risk model for each data set. A linear risk model is the simplest form that can be applied to these data. In all three cohorts studied there are three exposure groups and one reference group; this is sufficient information to consider more complicated dose-response models. However considering the complexity of the epidemiological data the potential impacts of bias and confounding and the erudity of the exposure measures used, this simple model is warranted. Evaluation of the shape of the dose-response data for the human studies was not done. Access to the raw data may make it possible to use more complicated mathematical forms which allow for the evaluation of shape [55]. In the one case in which this has been done [55], the estimated shape of the dose-response curve was supralinear (dose raised to a power <1).

8.3.2.1 All Cancers Combined and Lung Cancer

There exist three studies of human occupational exposure which provide enough information to perform a quantitative dose-response analysis. These are the NIOSH Study [36], the Hamburg Cohort Study [56], and the BASF Cohort Study [33].

NIOSH Study. Aylward *et al.*[57] presented a dose-response analysis using data from a cohort study of 5172 male workers at 12 plants in the U. S. that produced TCDD-contaminated chemicals [36] considering only cancers occurring after 20 years of exposure. Workers were classified into groups by length of exposure with each group assigned a TCDD exposure value calculated using a linear first-order elimination model for concentration of TCDD in serum lipid. The model assumed a constant concentration of 5 ppt in serum lipid for all years including those before and after first exposure, constant input of TCDD over the period of exposure, and an exponential decay during the years following industrial exposure. Persons with serum lipid levels below 10 ppt, at time of measurement , were assumed to have had no excess occupational exposure. The elimination half-life was assumed to be 7.5 years for all subjects. Three dose metrics were derived from the reconstructed TCDD concentration profile over time: area under the time-concentration curve (AUC; units of ppt-years), peak serum lipid concentration (ppt), and mean serum lipid concentration (AUC/age at time of observation). The serum measurements for 253 workers from one plant were used to estimate the doses of four exposure groups consisting of workers from all twelve plants. Each of the dose metrics was found to increase with

duration of exposure. Excess risk for lung cancer death was calculated from the standard mortality ratios from the original study, see Table 8.3.1.[36] Excess risk for respiratory cancer increased with each of the dose metrics given.

To provide ED_{01} estimates for comparison in this chapter, Poisson regression[58] was used to fit a linear model to these data. Table 8.3.1 presents the estimates for the steady-state body burden predicted to yield a 1% additional effect over background. Also presented in Table 8.3.1 are the observed and predicted relative risks (based upon the linear Poisson regression model), and the mean exposures used in each category of exposure. Other analyses of exposure and response exist for this occupational cohort, [37, 59-61]. None of these studies presented estimates of the EDs_{01} so it is not possible to obtain a direct quantitative comparison; however, the results are similar to those presented in this chapter.

Hamburg Cohort Study. Another cohort studied was based on 1189 men who worked at a herbicide plant in Hamburg, Germany [55, 56, 62, 63]. Flesch-Janys *et al.*[62] 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 [58]. The SMRs for the tenth decile of TCDD concentration were significantly elevated while 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 3 of the 5 categories.

Flesch-Janys *et al.*[63] 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% C. I. 121-240) and for all workers combined (SMR 141, 95% C. I. 117-168). The overall cancer SMR is increased over the results of Manz *et al.*[56] which included mortality only up to 1989. For all workers combined, lung cancer mortality was significantly increased (SMR 151, 95% C. I. 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. For this chapter, these data were modeled using the Poisson regression method applied earlier to the NIOSH data used by Aylward *et al.*[57] The results are presented in Table 8.3.1.

Another recent article [55] 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 as calculated by Flesch-Janys *et al.*[63]. 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 results obtained are discussed later in this chapter.

BASF Cohort Study. Zober *et al.*[33] studied a cohort of 247 workers from a 1953 accident at a BASF factory in Germany which 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% C.I. 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% C. I. 122-305).

Another study of the BASF cohort [64] 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% C. I. 100- 500). The incidence was not significantly increased for either 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 (relative risk 1.22; 95% C. I. 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 non-smokers or ex-smokers. These data were also modeled in this analysis using the Poisson regression described earlier, with the results presented in Table 8.3.1.

Other Studies. Hooiveld *et al.* [65] 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. 1031 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% C. I. 1.2-2.8) and high (RR 1.9, 95% C. I. 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, [66] a single episode of exposure to TCDD occurred following an explosion at a local chemical plant. Men, woman 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.*[67] 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. Since this chapter has focused primarily on the effects of TCDD, this cohort will not be included in the modeling effort here. In addition, Collins *et al.*[68] 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. Since that chemical plant is included in the NIOSH study cohort [36], it is discussed in chapter 7.

8.3.2.2 Average Body Burden

As described above, the data used in the analyses presented in Table 8.3.1 are from the analysis by Aylward *et al.*[57] of the NIOSH study, Flesch-Janys *et al.*[63] for the Hamburg cohort, and Ott and Zober[64, 69] for the BASF cohort. The limited information available from these studies is in the form of SMRs and/ or risk ratios categorized by exposure subgroups with some estimate of cumulative subgroup exposures. Exposure subgroups were defined either by number of years of exposure to dioxin-yielding processes[57] or by extrapolated TCDD levels.[63, 69] No study sampled TCDD blood serum levels for more than a fraction of their cohort and these samples were generally taken decades after last known exposure. In each study, serum fat or body fat levels of TCDD were back calculated using a first-order kinetic model. The assumed

half-life of TCDD used in the model varied from study to study. Aylward *et al.*[57] used the average TCDD levels of those sampled in an exposure subgroup to represent the entire subgroup. Flesch-Janys *et al.*[63] and Ott and Zober[69] performed additional calculations, using regression procedures with data on time spent at various occupational tasks to estimate TCDD levels for all members of their respective cohorts. They then divided the cohorts into exposure groups based on the estimated TCDD levels. The information presented in the literature cited above was used to calculate estimated average TCDD dose levels.

As discussed in Section 8.2, a useful dose metric for risk estimation is the time average body burden. The body burdens obtained from Flesch-Janys *et al.*[63] were presented with background exposure of the general population subtracted; the calculations from the other studies do not. However, the calculated concentrations in the study cohorts are much larger than background levels. The analysis here takes this into account and assumes a background level of 5 ng/kg in blood lipid. The data from the NIOSH cohort [36, 57] are taken from deaths with a 20-year latency; data from the other two cohorts used here do not take latency into account.

Using body burden as the dose metric allows one to estimate either effective dose or lifetime risk based on an assumption of equivalence of acute exposure to continuous exposure. However, this may not be realistic if the effect of TCDD is related to the timing of exposure, or if it is related to body levels attained above a threshold level which would never be reached with constant exposure. Considering the periodic nature of the occupational exposure and given the limited amount of information available, this is felt to be the most workable approach.

The estimates derived in this analysis and presented in Table 8.3.1 can be compared to those of Becher *et al.*[55] who used several models to estimate dose-response for total cancer mortality using data from the Hamburg cohort. If we assume an average lifetime daily intake above background of 1pgTCDD/kg/day and 100% absorption, the estimated steady-state body burden would be 0.4 ngTCDD/kg Using the risk calculated from the Hamburg cohort data, this gives a total excess cancer mortality risk per pg/kg/day of 57 per 10000 exposed. The risk estimates of Becher *et al.*, derived from data for male workers with a ten-year latency and taking greater

caution over other factors affecting risk, range from 13 per 10000 to 56 per 10000 per pg/kg/day intake.

Table 8.3.1. Maximum likelihood (95% lower bound) estimates for average body burden yielding 1% added risks for lung cancer and total cancer response from three epidemiological studies.

Study	Exposure Groups	Exposures (ng/kg whole body) [a]	# subjects in exposure group	All cancer deaths			Lung cancer deaths		
				Relative risk: observed	Relative risk: predicted	ED_{01}(95% lower bound) (ng/kg)	Relative risk: observed	Relative risk: predicted	ED_{01}(95% lower bound) (ng/kg)
NIOSH Cohort [57][b]	<1 year	27.8	Not given in article	1.02	1.03	39.9 (23.0)	0.96	1.04	116.2 (58.2)
	1-5 years	103.3		1.65	1.11		1.26	1.14	
	5-15 years	184.5		1.38	1.20		1.46	1.26	
	>15 years	554.5		1.15	1.61		1.56	1.78	
Hamburg Cohort [63][c]	1st quartile	1.4	297[d]	1.24	1.00	5.7 (3.5)	1.56	1.00	23.6 (10.5)
	2nd quartile	2.5	297	1.34	1.01		1.63	1.01	
	3rd quartile	6.5	297	1.34	1.04		1.22	1.04	
	4th quartile	101.2	297	1.73	1.77		1.66	1.69	
BASF Cohort [69]	<0.1 [e]	4.6	108	0.80	1.00	80.2 (37.5)	1.00	1.00	161.1 (77.4)
	0.1-0.99	51.9	66	1.20	1.03		0.50	1.05	
	1.0-1.99	200.1	47	1.40	1.11		n/a	n/a	
	2.0+	2012.0	22	2.00	2.10		n/a	n/a	
	1.0+	1352.0	69	n/a	n/a		2.40	2.37	

[a] Group mean, including background; where necessary, lipid-adjusted numbers were converted to body-burden by assuming 25% of body mass is lipid

[b] Based upon the cohort of Fingerhut *et al.*[36]

[c] based upon the cohort of Manz *et al.* [56]

[d] Study had 1189 subjects divided into quartiles

[e] Groups are by TCDD body burden at first exposure, in µg/kg

8.3.2.3 Non-Cancer endpoints

Cardiovascular Disease.

A pattern of increased risk of cardiovascular and ischemic heart disease mortality was observed by Flesch-Janys *et al.*[62] across 6 exposure categories. There was a statistically significant trend (p=0.04) in relative risk for mortality for all cardiovascular diseases when using gas workers 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 (344.7-3890.2 ppt) showed a significant relative risk of 1.99 (CI; 1.05-3.75). When using national rates 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.* [63, 70]. Using 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 [61] or for the BASF cohort [33, 71].

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 [72-74] 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, while based on actual body measurements, may not be fully representative or precise. Although 253 subjects were sampled in the Fingerhut *et al.*[36] study, the blood samples were all taken decades after last exposure and were from two plants from a total of twelve plants. Subjects from the larger of these two plants had the higher TCDD levels but a lung cancer SMR=72 based on seven deaths, while 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.*[36] 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 and when necessary in this analysis, average body burden was calculated on the basis of a one-compartment model with first order elimination. This analysis assumed a half-life of 7.1 years; half-life assumptions in the literature varied but were close to that. Some data, however, suggest a shorter half-life of as little as 5.8 years [69]while others suggest a longer half-life of 11.3 years [75]. A recent study [76] suggests a half-life of 9.5 years. A longer half-life than 7.1 years would result in higher calculated body burdens and hence lead to a reduced 1% excess risk estimate. Conversely a shorter half-life would increase the risk estimate. However, the assumption of a single half-life is uncertain since 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[77]. If this were the case, the actual initial exposure may have been higher than predicted using a single half-life. This would also lead to a reduced 1% excess risk estimate. 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, that is inducible via the AhR. In humans, while the dose–response for induction of CYP1A2 by TCDD is not known, or the effect this may have on disposition of TCDD, it is likely that the half lives for dioxin-like compounds are not independent.

Another uncertainty is that of possible interaction or of 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 [78]. 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.*[33] cohort; for the 37 cancer cases, only 2 were stated as being nonsmokers. Of the eleven men with lung cancer, only one reported never smoking. The Ott and Zober [69] analysis, which includes smoking as a covariate, did appear to show an effect of smoking on TCDD dose-response. While similar SMRs from other smoking-related diseases in the two subcohorts in Fingerhut *et al.*[36] suggest similar smoking prevalence across this multi-factory cohort, the effects with higher levels of TCDD could be synergistic for cancer.

Other potential confounders in all three studies include exposures concomitant with TCDD exposures; other chlorinated hydrocarbons in the case of Zober *et al.*[33] and Manz *et al.*[56] and miscellaneous chemicals including 4-aminobiphenyl, a known human bladder carcinogen, in the case of Fingerhut *et al.*[36]. These confounders raise the question of whether the increased SMR's 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 a linear model for analysis. Table 8.3.1 shows a strict pattern of increasing relative risk with increasing dose for total cancer mortality in the Hamburg and the BASF cohorts, and for lung cancer in the NIOSH cohort, but for none of the data sets in the table is the increase in risk simply linear with dose. The Becher *et al.* [55] 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.

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 total TCDD eqiavalents (TEQ) basis (Chapter 9), 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. This 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 the extent of an initial exposure to dioxin-like compounds with shorter half-lives. For example, a current lipid level of 1ppt 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 1024 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 sub-population used for the bio-monitoring of TCDD levels in human blood is not representative for 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 three occupational studies were sufficient to calculate risk estimates. Estimates derived from the human data (Table 8.3.1) suggest an ED_{01} based on body burden in the range of 6-80 ng/kg for all cancers combined and in the range of 24-161 ng/kg for lung cancer.

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.

Back-calculation of present TCDD body burden, used assumptions to derive an individual body burden over time, which was then converted to the dose metric of time-averaged body burden used in this analysis. Assumptions varied from study to study. Half-lives used in the calculations varied, and not all calculations took into account variation in weight and in percentage body fat. Some of the calculations assumed that there was no non-background exposure to TCDD except from the primary occupational source. The Poisson regression used for risk calculations assumed that the dose-response is linear and proportional to background response. Since little is known about the validity of these assumptions, no modulation of the models used above were possible to account for them. Sensitivity of the results reported so far on the presence of extreme measured TCDD concentration values of persons from the population used for the back-calculation and of predicted TCDD concentration of persons from the complete cohort has to be considered in future analyses. The low correlation in the range of 0.5 between measured and predicted concentration levels add to the uncertainty.

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 strength and the weaknesses of different metrics under different scenarios.

This dose-response analysis was restricted to a grouping of the exposed population into a few categories of increasing TCDD levels. Analysis of individual data -making use of statistical resampling methods- may be very useful to estimate population heterogeneity.

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 co-exposure. 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 co-exposure 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_{s01} presented in Table 8.3.1 are based on a simple dose-response model. The analysis uses the crude endpoint of all cancers combined, or the most frequent cancer in men, lung cancer. 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 sub-cohort sampling as a possible source of information.

Risk estimates could not be calculated for infant or non-adult exposure. This is to some extent due to insufficiencies in study design for use in risk estimation for the total population and due to 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 non-cancer 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 models [54] and can be improved by taking the results of an existing mechanism-basedmodel on receptor-based effects of TCDD within the context of a physiologically based pharmacokinetic (PBPK) model [41] and using these results in a detailed multistage model of carcinogenesis [42]. Both approaches have been attempted. For a mechanism-based approach see section 8.4.3.2.

Portier *et al.* [54] used a simple multistage model of carcinogenesis with up to two mutation stages affected by exposure to model the five tumor types observed to be increased in the 2 year

feed study of Kociba *et al.* [52] (Sprague-Dawley rats) and the eight tumor types observed to be increased in the 2 year gavage cancer study conducted by the National Toxicology Program. [43] (Osborne-Mendel rats and B6C3F$_1$ mice). The findings from this analysis are presented in Table 8.3.2. All but one of the estimated EDs$_{01}$ are above the lowest dose used in the experiment (approximately 1ng/kg/day in both studies) and are thus interpolations. 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.*[52] study (feed experiment) and 100%[79] for the NTP study [43] (gavage experiment). Also presented in Table 8.3.2 are the shapes of the dose-response curves as determined by Portier *et al.*[54]

Table 8.3.2: Doses yielding 1% excess risk (95% lower confidence bound) based upon 2-year animal carcinogenicity studies using simple multistage models[54].

Tumor	Shape	ED_{01}	
		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)	1190 (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)

The predominant shape of the dose-response curve in the experimental region is linear; this does not imply that a non-linear 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 for these cases [80]. 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 EDs_{01} range from a low

value of 14ng/kg based upon the linear model associated with liver tumors in female rats, to as high as 1190 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 1190 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 (See 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 [42].

8.3.3.3 Knowledge Gaps in Animal Cancer Dose-Response Modeling

The dose-response data for cancer in animals following TCDD exposure is limited to only three exposure groups. Although non-linear models could be applied to these data [54] 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 is obtained from the same experiment.

Hepatocellular carcinoma 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 e.g. seen in the NTP study. 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 end-point specific mathematical models. Instead they have relied on safety assessment involving determination of a dose which is likely to be without risk taking both data and model uncertainties into account. While 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 used and the statistical details follow similar analyses done by McGrath *et al.* [49] and Murrell *et al.*[44] In brief, two different models were applied to the 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

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

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. Since 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 as the shape parameter.

In the present exercise, n was not allowed to vary below 1 and subsequently the model as used does not predict sublinearity. 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 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 would be expected EDs_{01} and a truncation in the distribution of observed shapes. The first effect cannot be avoided, but the second should not be a problem since unrestricted estimates of n<1 will yield restricted estimates of n=1 and the shape will be classified into the grouping of risk approximately proportional to dose.

The second model used here is the power function:

$$R(d) = b + sd^n$$

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 and/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.

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 1% effective doses (ED_{01}) and 95% lower bound on the estimated ED_{01} , were carried out using of the U.S. Environmental Protection Agency (EPA) Benchmark Dose Software (BMDS) version 1.1b [81]. In some cases, the BMDS software failed to locate a lower confidence bound on the ED_{01}. Qualitative assessment of the goodness of the model fit was determined as either; Good, model curve included nearly all of the data point means; Marginal, model curve was within one standard deviation of the data point means; Poor, model fit was not within one standard deviation of the means. There were 234 endpoints for which dose-response analyses could be made,

obtained from over 45 published manuscripts (See Appendix A). The number of data sets, categorized by species, gender and study type, is shown in Table 8.3.3.

The analyses of the data are presented as summaries of the endpoint categories in Figure 8.3.1, Figure 8.3.2, and in Table 8.3.5, and Table 8.3.6 at the end of this section. The data are divided into several categories based on exposure regimen and endpoint. Exposure categories are grouped as either single exposures or multiple exposures. For simplicity, effects were categorized as either *biochemical*, *hepatic, immune, toxicity, tissue, retinol, or thyroid* (Table 8.3.4). 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 weight were classified as a *tissue* response. Body weight changes, 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 and these studies were categorized as either *thyroid* or *retinol*.

Table 8.3.3 Non cancer endpoints used for comparing ED_{01} values.

Species	Gender	Multi-dose	Single-dose		Total
			Adult	Developmental	
Mouse	Female	26	24	5	55
	Male	0	36	18	54
	Unknown	--	---	3	3
Rat	Female	59	10	0	69
	Male	16	4	32	52
Hamster	Female	0	0	0	0
	Male	0	1	0	1
Total		101	75	58	**234**

Table 8.3.4 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 NEChol	
	serum Alkaline Phosphatase	serum S. Dehydrogenase	
	serum ALT	serum SGPT	
	serum BUN	serum TBA	
	serum bilirubin (total, indirect, direct)	serum Total cholesterol	
	serum Echol	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		
Retinol	Hepatic retinol	Plasma retinol	Hepatic retinyl-palmitate
Thyroid	Thyroid-stimulating hormone	Thyroxine Free T4	
	Thyroxine	thyroxine Total T4	
Tissue	Age at puberty	Epididymal sperm count	Relative kidney weight
	Body weight	Epididymides 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 wt.	liver weight	Thymus atrophy
	DSP/g _ 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 morph.ology
	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

Comparison of the 1% effective dose (ED_{01}) between studies is 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 dosing when the determinations were made varied from days to weeks. For some effects, the differences in the time after the initial exposure probably influences the effective dose. Similarly, in studies employing multiple doses, investigators used a variety of regimens including daily exposure, weekly exposures and loading/maintenance regimens. 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. 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} based on was calculated using the equation in Section 8.2.2, the percentage of dose, adsorbed and the half-lives for TCDD in Table 8.2.

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 percent. 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, while 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 10-fold), suggesting that little confidence can be placed in some ED_{01} 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

In the studies examining the effects of TCDD following multiple exposures, the range of the EDs_{01} is highly variable within and across response categories (Figure 8.3.1). When examined by category, the median values for the ED_{01} for biochemical and retinol responses are lower than the median ED_{01} for other types of response. Of the 106 endpoints examined from studies using multiple exposures, 11 have ED_{01} values less than 0.1 ng/kg/day. Seven of the 11 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 6 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 decreases our confidence in making extrapolations between species and gender.

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 8.3.5). A ratio of 1 or greater indicates that the ED_{01} is within the doses 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 of ED_{01}/lowest dose tested less than 0.1 indicate that the estimate was greater than an order of magnitude below the lowest dose used in the study and should be viewed with caution. Forty five of the 101 values had a ratios of the ED_{01}/lowest-dose less than 1. However, of these 45 only 36 were less than one order of magnitude below the lowest dose used in the study.

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 106 endpoints for which an estimate was obtained, 43 had shape parameters less than 1.5, indicating linear dose-response relationships (Table 8.3.6). Approximately half of the biochemical and half of the tissue responses indicated a linear dose-response relationship. The median shape parameter for the tissue responses is heavily influenced by the consistently linear shapes for alterations in thymic weight (10 of 11 dose-response curves

for thymic changes had shape parameters less than 1.5). In contrast, only 18% of the immune function responses were linear.

While 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 non-linear. These observation 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: Adults Animals

In studies examining the effects of dioxin in adult rats and mice following a single exposure, the median ED_{01} is above 10 ng/kg for all endpoints examined. Biochemical and immune responses had the lowest median ED_{01} estimates, 180 and 65 ng/kg, respectively. Hepatic and toxic responses gave median EDs_{01} greater than 10,000 ng/kg. Once again there was large variability in the EDs_{01} for a given category and in general, varied approximately three orders of magnitude, within each category. The ED_{01} estimates were below the lowest dose tested for 23 of the 75 endpoints examined. Of these 23 estimates, the ED_{01} was less than one order of magnitude lower than the lowest dose tested for approximately half (10) of the values (Table 8.3.5).

Following a single exposure to TCDD, 33 of the 77 (43%) endpoints examined had shape parameters less than 1.5, indicating linear dose-response relationships (Table 8.3.6). 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. All endpoints in the toxicity category exhibited threshold-like dose-response relationships.

8.3.4.4 Single Dose Studies: Developmental Studies

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 EDs_{01} was more than five orders of magnitude and the median values for all response categories were greater than 100 ng/kg, with an overall median of 140 ng/kg (Figure 8.3.2). One of the more recent findings on the effects of TCDD is its developmental reproductive effects in rats, hamsters and mice [82-86]. One striking difference is that the EDs_{01} for the reproductive developmental effects in mice are 10 to 1,000 times higher than those in the rats. The EDs_{01} for the developmental effects were within the dose range tested in 26 out of 58 endpoints for which an estimate was obtained. Of the 32 estimates that were below the experimental range, approximately half (17) were less than an order of magnitude below the lowest dose tested (Table 8.3.5). The shape parameter for the developmental effects was less than 1.5 for only 18 of the 60 endpoints analyzed (Table 8.3.6).

8.3.4.5 Summary of the Dose-Response Modeling for Non-Cancer 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 relationships 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 dose surrogates for the carcinogenic effects of TCDD [42]. 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.*[87] 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} 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.* examined the effects of TCDD on UGT mRNA, serum total thyroxine, and serum TSH[88]. All three responses had shape parameters greater than 1.5 and the EDs_{01} 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 is more sensitive than are changes in serum TSH. These data indicate that simple biochemical responses have lower EDs_{01} 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 impact the ED_{01} or the shape parameters. One example of this is the studies by Diliberto and coworkers. Diliberto *et al.*[89] 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 EDs_{01} and the shape parameters increased with time after dosing. The increase in these parameters 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 seven days after dosing to 6.5 at the 35 day time point. The ED_{01} increased from 27 ng/kg at seven 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 seven days of administration of TCDD demonstrate considerable consistency. Four studies examined EROD induction in rats or mice within seven days of dosing and the EDs_{01} range from 16 to 84 ng/kg. The estimated shape

parameter is 1, for the Diliberto *et al.*[89], Abraham *et al.*[90], Narasimhan *et al.*[91]studies and 1.8 for the Van Birgelen *et al.*[87] study. It should be noted that that two of these studies are in mice and two are from rats, suggesting similar dose-response relationships for enzyme induction between these species.

Another variation in study design that may affect the dose-response modeling is dose selection. The dose-response relationship for induction of hepatic EROD activity was modeled for six studies[87, 92-96]. Only the data from DeVito *et al.*[92] and Johnson *et al.*[93] had shape parameters greater than 1.5. The EDs_{01} ranged from 0.4-3.2 ng/kg/day except for the data of Vogel *et al.*[96] which resulted in an ED_{01} over 100 fold lower. Vogel *et al.*[96] used a loading /maintenance dosing regimen and the doses used were 100 times lower than those of 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 that are observed in rats and hamsters.[82, 84, 85] These studies demonstrated decreases in epididymal sperm counts on post- natal day 63. However, the shape parameters are vary between 1 and 11 and the EDs_{01} 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.[82] when compared to the Long Evans rat used by Gray *et al.*[85] Overall, the study by Gray *et al.*[85] demonstrated smaller effects than the study by Mably *et al.*[82] Also, the data from Gray *et al.*,[85] demonstrate highly non-linear responses (shape parameters greater than 2 for all but 3 out of 32 responses examined). In contrast, the effects observed in Mably *et al.*[82] 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.*[85]

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 fifty 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 due to 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 is in a format 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 EDs_{01} 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 dose-response information available contributes to the observed variation in the estimates of both the shape parameters and the EDs_{01}. 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 non-linear 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 which are more suitable for non-linear regression. Second, and perhaps more disappointing was the frequency of inadequate reporting of the data. Many studies would a 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.*[92] compared to that observed in the study of Vogel *et al.*[96]), then their respective EDs_{01} will represent different levels of induction. The biological significance of these responses may not be commensurate with their respective EDs_{01}. 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.

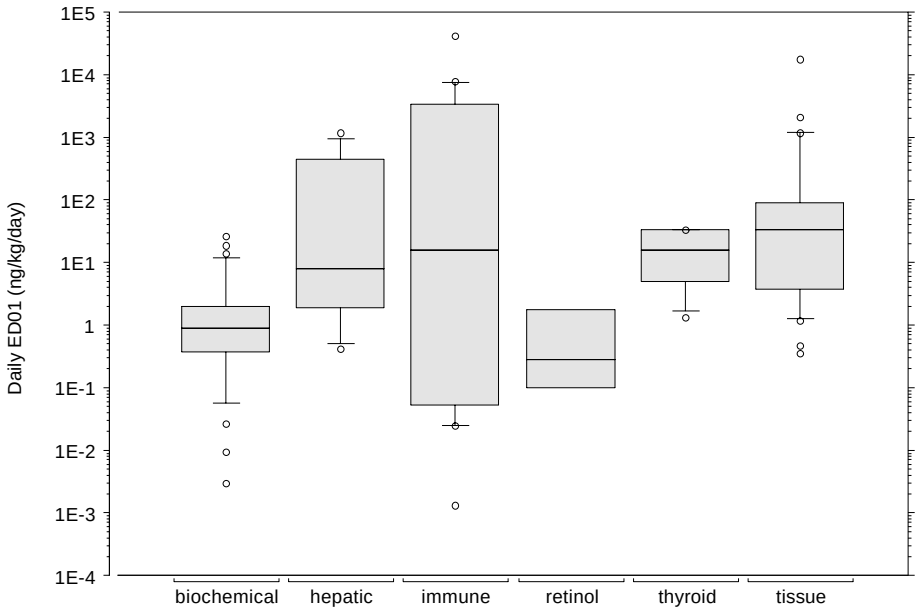
Several studies have demonstrated that control rats and mice, have detectable amounts of TCDD and related chemicals[97, 98]. The concentrations of these chemicals in the control animals are at or near the quantification limits. In the present analysis, the background exposures of the control animals were not considered. The inclusion of background exposure levels or tissue concentrations in the control animals in the dose-response analysis may alter the shape of the dose-response curves and is some cases may possibly increase both the ED_{01} estimate and/or increase 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 these 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. Due to the limited dose-response data available for many of

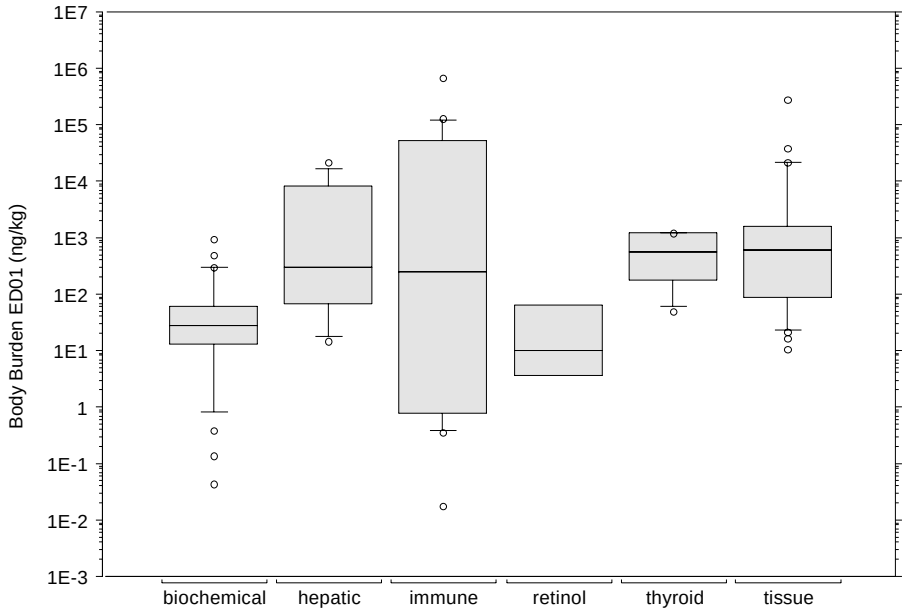
these analysis, caution must be taken when making some of these generalities. For example, the decrease in thymus weight tends to have estimated shape parameters of one.

Figure 8.3.1- Distribution of ED_{01} and BB_{01} values in multi-dose studies by endpoint.

I. ED_{01} values



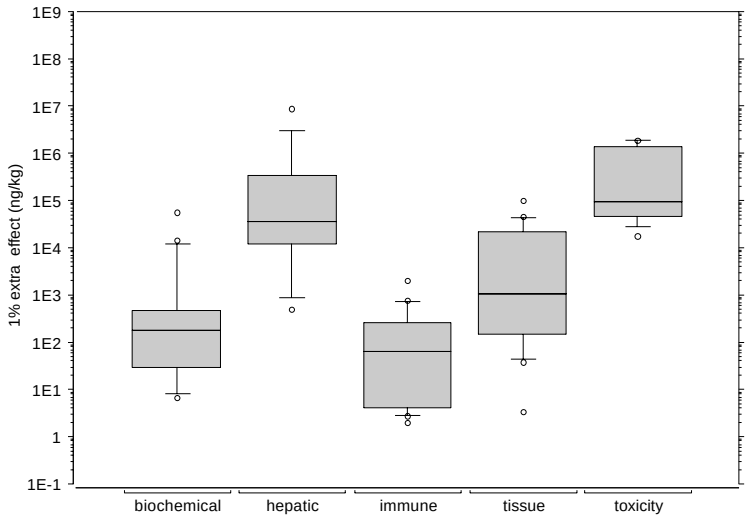
II. Body Burden values at the ED_{01}



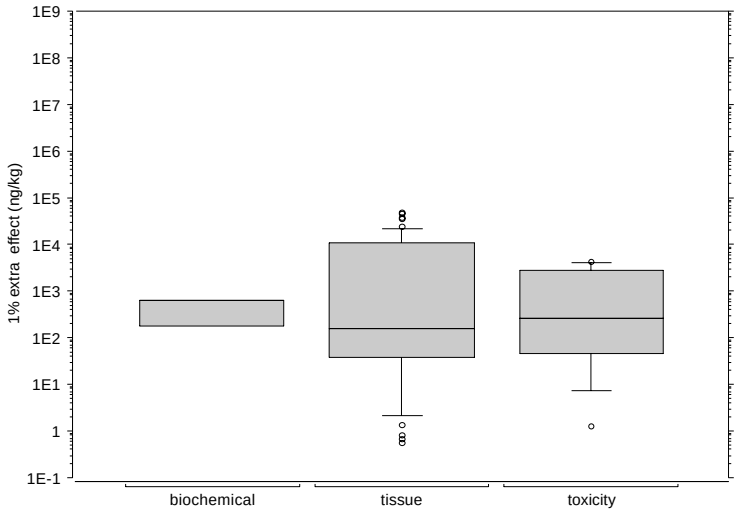
The distribution of individual values is presented as box plots. The boxed region contains values within the 25th to the 75th percentiles of the sample distribution, with the median value (50th percentile) shown as a line within the boxed region. The error bars represent values within the 10th to the 90th percentiles. Values above the 90th percentile and below the 10th percentile are shown as individual data points. Values are categorized according to Table 8.3.4

Figure 8.3.2- Distribution of ED_{01} values in single-dose studies by endpoint.

I. Adult endpoints



II. 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 8.3.4

Table 8.3.5 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	22 (18)	6	3 (2)	14	0	3
Hepatic	4 (4)	9	0	13	--	--
Immune	7(5)	10	13 (5)	3	--	--
Retinol	3 (1)	0	--	--	--	--
Thyroid	3 (3)	3	--	--	--	--
Tissue	6 (5)	28	7 (3)	9	26 (15)	22
Toxicity	--	--	0	13	6 (2)	1
Sub-Totals	45 (36)	56	23 (10)	52	32 (17)	26
Totals	101		75		58	

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 (value was between 0.1 and 1) are shown in parentheses.

Table 8.3.6 Estimated shape parameters, categorized by study type and endpoint type

	Multi-dose		Single-Adult		Single-Developmental	
Category	Linear	Non-linear	Linear	Non-linear	Linear	Non-linear
Biochemical	15	13	6	11	0	3
Hepatic	3	10	4	9	--	--
Immune	3	14	11	6	--	--
Retinol	3	0	--	--	--	--
Thyroid	2	4	--	--	--	--
Tissue	17	17	12	5	14	36
Toxicity	--	--	0	13	4	3
Sub-Totals	43	58	33	44	18	42
Totals	106		77		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.
Note, that in some cases an empirical model could fit the data but no ED_{01} value could be calculated and therefore total groups counts for the shape parameters are not the same as those for the ED_{01} values.

8.4 Mode of Action-based dose-response modeling

8.4.1 Introduction

Mode of action based modeling for TCDD encompasses physiologically based pharmacokinetic (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 the levels of 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 Physiologically-based pharmacokinetic (PBPK) models

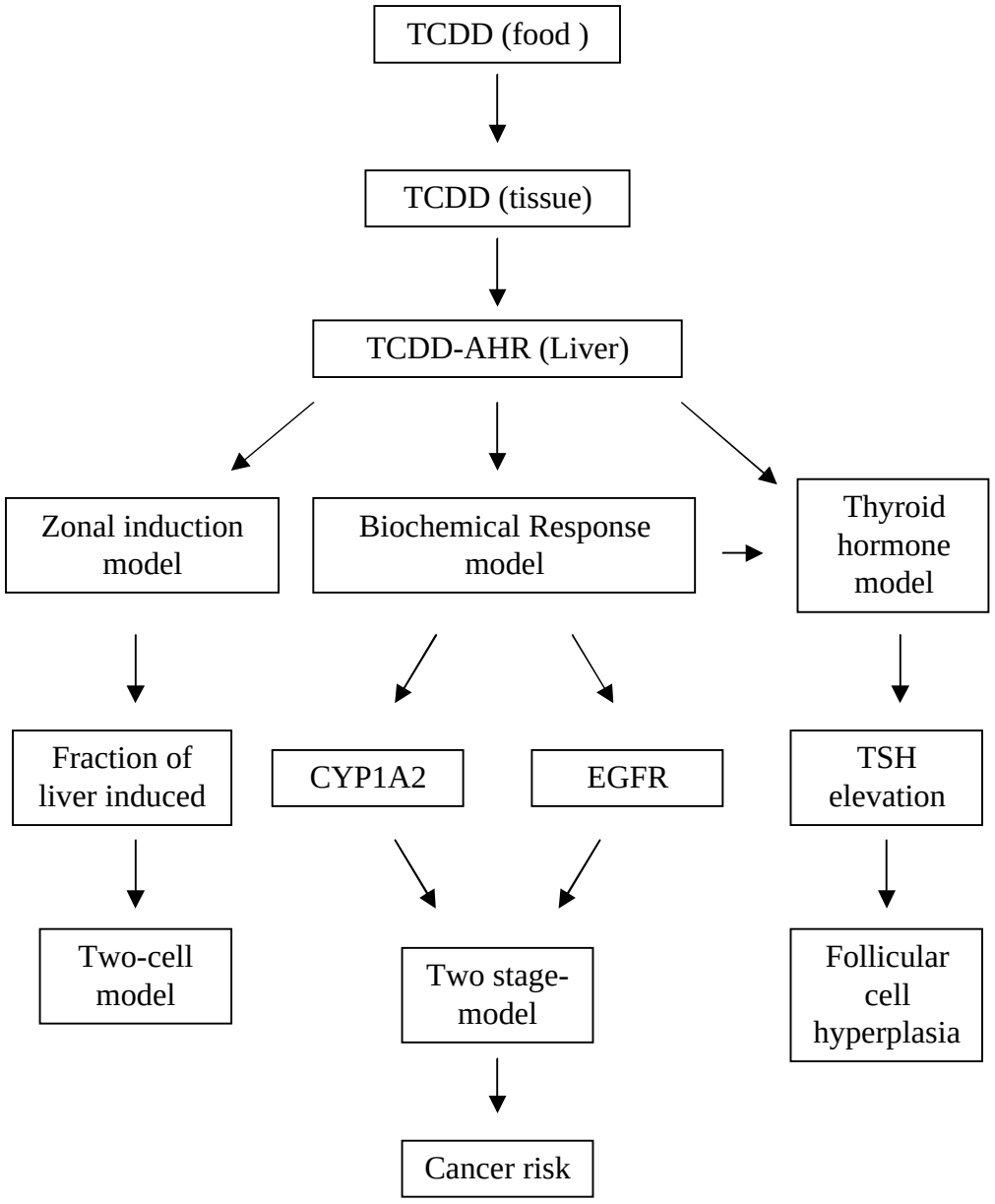
Issues Pertaining to PBPK Models . Tissue dosimetry encompasses the absorption of an administered chemical and its distribution among tissues, metabolism, and elimination from the body (ADME). TCDD dosimetry depends on physicochemical properties of TCDD (e.g., tissue permeation constants, partition coefficients, 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, and such a model is called a PBPK model. Several research documents discuss the development of PBPK models for general use[99] and for use in risk assessment.[100]

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 end point, 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 these two. Several PBPK models have been developed for TCDD and related chemicals (see Chapter 1, Disposition and Pharmacokinetics, for a brief overview). Models have also been developed for polychlorinated biphenyls [101-104] and polychlorinated dibenzofurans in several species [105] including humans [106, 107].

There are four levels of complexity in PBPK models for the effects of TCDD. First is the traditional PBPK model by Leung *et al.*[108] with the added complexity of protein binding to CYP1A2 in the liver. The next level of complexity are the models by Andersen *et al.*[109], and Wang *et al.*[110] 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.*[41] with extensive hepatic biochemistry and the model for zonal induction of cytochromes P450 [111]. Finally, there are the models which include coordination of responses in multiple organs [112] for hormonal interactions and Roth *et al.*[113] with its detailed description of gastrointestinal uptake, lipoprotein transport, and mobilization of fat (Figure 8.4).

Figure 8.4 Schematic representation of the linkage of current PBPK models and biochemical/tissue response models for TCDD action.



Initial Attempts to Include Protein Induction: Leung *et al.*[108] developed a PBPK model for TCDD disposition in mice, for Sprague–Dawley rats[114] and for 2-iodo-3,7,8-trichlorodibenzo-p-dioxin in mice.[115] These initial models considered tissue partitioning, protein binding in blood, specific binding of TCDD to inducible hepatic proteins, binding of TCDD to the Ah receptor, 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 [114] contained five flow-limited tissue compartments including blood, liver, fat, 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 Ah receptor and the other to a lower affinity, higher capacity microsomal protein which is 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 [116, 117]. In the PBPK model [114], the concentration of the Ah receptor 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[108, 114] 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. [118]

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.*[114] also modeled metabolic clearance as a first-order process with the rate constant scaled inversely with (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 due to loss of iodine. However, Olson *et al.*[119] 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.*[114] 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 analogs have dose- and time-dependent kinetics in both rodents[50, 52, 79, 90, 117, 120] and humans.[107, 121] 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. [90, 120]

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 non-proportional response for low environmental doses (non-linear behavior). The model of Leung *et al.*[114] 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 due to 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.*[114] 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.

Refinements with DNA Binding of Ah-TCDD Complexes: Andersen et al[109] modified the model of Leung *et al.*[114] 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.*[114] 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.*[109] 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.*[109] each tissue has two sub-compartments, 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 Ah receptor. This induction led to an improved fit to observed liver and fat TCDD concentrations. Subsequent research [119, 122] 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.*[114] were modified for the Andersen *et al.*[109] model because Wistar rats instead of Sprague-Dawley 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.*[90] and liver and fat concentrations in a study by Krowke *et al.*[123] 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 Ah receptor–TCDD concentration. Although they represented the kinetics with a Hill equation, the Hill exponent was 1, similar to the Michaelis–Menten model used by Portier *et al.*[124] for the independent induction of CYP1A2. Their Hill exponent estimated 2.3 introduced marked sigmoidicity in the computed dose–response of this protein.

Andersen *et al.*[109] noted that the liver/fat concentration ratio changes as dose changes due to 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 Ah receptor 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.[109] The dose-dependent changes in liver/fat ratio are consistent with animal data and limited human data,[107] and are a necessary part of the modeling for TCDD.

Andersen *et al.*[125] 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[50] and to the volume of altered hepatic foci.[126] 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.

Improving the Physiological Characteristics of the TCDD Models: Kohn *et al.*[41] modeled the binding of TCDD to the Ah receptor 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–Ah receptor complex concentration similar to that computed by Leung *et al.*[114] and Andersen *et al.*[109]. 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.*[114] as mass balance is automatically achieved. The physiology described in the Kohn *et al.*[41] model is dependent on the body weight of the animal. Body weight as a function of dose and age were recorded by Tritscher *et al.*[120] 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.*[127] To allow the model to fit data at both low and high doses[120], 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 [128] 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.* [41] model predicts a net contribution of TCDD clearance by TCDD-induced cell death.

A further extension of this model, incorporating effects on thyroid hormones [112], included tissue blood compartments similar to those used by Andersen *et al.*[109]. 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.*[41] model was replaced by a Hill rate law, and parameters were estimated to reproduce the kinetic data of Abraham *et al.*[90] and the dose–response data of Tritscher *et al.*[120]

Transthyretin (also known as prealbumin) can bind hydroxylated PCDDs [129], and single doses of TCDD can cause prolonged decrease in this protein [130]. 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.*[97]

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.

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.*[113, 131] 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.*[109] and Kohn *et al.*[112]. A unique feature of this model was the division of the GI tract into five sub-compartments—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[113]. 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 post-dosing despite the fact that TCDD was being cleared from the body. The model of Roth *et al.*[113] 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. They suggested that barriers to uptake and efflux of TCDD may not be symmetrical.

Roth *et al.*[113] 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.*[113] model that suggests additional research is the assumption that white adipose tissue contains a cytosolic TCDD receptor (adipose tissue does express the Ah receptor) which mediates effects on lipid metabolism.

Diffusion-Limitations in Multiple Tissues: Assessment of diffusion limitation in tissue uptake had been hampered by in the lack of data at short times after dosing with TCDD. Wang *et al.*[110] 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, Ah receptor 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 [132]. 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[133]. However, it was not demonstrated that the model could reproduce responses to chronic exposure to TCDD.

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.[134, 135]. 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 mgTEQ/kg) for enhanced sequestration with increasing dose. These two parameters were estimated by fitting the model to date 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 [90]. 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 used to infer the relationship between specific biological factors and these two empirical parameters [136]. Using sensitivity analyses, the half saturation constant (K_d) was found to be related to characteristics of the binding of TCDD to the Ah receptor and the Ah receptor-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 [134, 135] have no obvious relationship to 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 [137] Although values for anatomical and physiological parameters are available for humans, the biochemical parameters (e.g. TCDD metabolism, binding to the Ah receptor 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 post mortem 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 Ah receptor.

Generic Receptor-Mediated Response Models: Looking at one aspect of modeling of TCDD's effects, Portier *et al.*[124] 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 P450 isozymes, CYP1A1 and CYP1A2, and the reduction in maximal binding of EGF to its receptor in the hepatic plasma membrane.

Portier *et al.*[124] 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 Ah receptor. 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.[138] Examples of the use of Hill kinetics for ligand-

receptor binding include the muscarinic acetylcholine receptors,[139] nicotinic acetylcholine receptors, opiate receptors,[140] the Ah receptor,[141] estrogen receptors,[142] and glucocorticoid receptor.[24] 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.*[124] modeled the reduction in maximal binding to the EGF receptor with Hill kinetics also, 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.*[124] 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 Ah receptor. Using these simple models, Portier *et al.*[124] 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 yields 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 is 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 since each additional molecule of TCDD adds more ligand to the pool available for binding and, under sub-saturating conditions, proportionally increases the concentration of protein. Similar observations have been made with regard to statistical[143] and mechanistic[144] 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.

Specific Biochemical Responses to TCDD: Kohn *et al.*[41] 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[109, 114] relied on several single-dose data sets[79, 90] and were validated against dosimetry results from longer term subchronic and chronic dosing regimens.[50, 123] These and other studies [120, 145] were used to model the pharmacokinetics and induction of gene products in female Sprague-Dawley rats[41] Among the data reported[120, 145] 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[41] 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 Ah receptor–TCDD complex up-regulates four proteins; CYP1A1, CYP1A2, the Ah receptor, 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[22, 146] and is quantified based on a presumed interaction with the EGF receptor, resulting in a down-regulation and internalization of the EGF receptor (EGFR). 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 Ah receptor concentration. Constitutive rates of expression for

CYP1A2, Ah receptor, 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 8.4

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 hyperbolic functions of dose without any apparent cooperativity. The discrepancy in the estimates of the Hill exponents between this model and the other models discussed[109, 124, 125, 147] is probably related to the inclusion in only the Kohn *et al.*[41] model of induction of the Ah receptor. The effects of TCDD on the Ah receptor concentration is uncertain. In acute studies, the Ah receptor is decreased following TCDD exposure[148], while in subchronic studies, there is some evidence that the Ah receptor is increased [149]. Further studies are required to better understand the regulation of the Ah receptor following TCDD exposure.

The Ah receptor–TCDD complex is assumed to down-regulate the EGF receptor in the Kohn *et al.*[41] model. It was assumed that the estrogen receptor–estrogen complex synergistically reacts with the Ah receptor–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[150]. This mechanism of TCDD regulation of these proteins, although supported by some data[24, 151], is speculative.

Vanden Heuvel *et al.*[152] 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 Ah

receptor with different affinities[152, 153]. 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 supra-linear response of protein to mRNA production compensates for the sub-linear response of the message to Ah receptor–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 [154].

Tissue Response Models : Zonal Induction Model: The mechanistic model of Kohn *et al.* treat the TCDD-treated liver as a single homogeneous unit. With regard to the induction of cytochromes P450 in the liver, Tritscher *et al.*[120] used antibody staining techniques, showed that the induction of CYP1A1 and CYP1A2 by TCDD in the liver exhibits a regio-specific 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 level 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 regio-specific pattern of induction, Andersen *et al.* assumed that the observed sharp demarcation in CYP1A expression between induced and non-induced regions indicated that individual hepatocytes were either fully induced or non-induced,[40, 111]. In this model the liver lobular structure was divided the into five concentric zones with a 3-fold difference between adjacent zones in the affinity of DREs for the liganded Ah receptor. The model also further used Hill kinetics for induction using 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 [90] and CYP1A1 mRNA data [152],and produced a fit to P450 data comparable to that obtained with homogeneous liver model of Kohn *et al.* The mRNA data were fit 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 sub-linearity than a comparable homogeneous liver model. The model predicted an 81-fold difference in AhR-TCDD binding between periportal and centrilobular zones and utilizes steep Hill kinetics; these two issues drive the low-dose nonlinearity of this model and are important areas for further research.

Endocrine Models: Thyroid Hormones: In addition to 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.*[41]. TCDD induces thyroid tumors in male rats and female mice at lower doses than those which induce liver tumors in female rats.[43] Sewall *et al.*[88] 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 [155]. Because this may be a sensitive end point for TCDD carcinogenesis, the Kohn *et al.*[41] model was extended[112] 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 and equations for secretion and metabolism of the hormones. It reproduced the data used in the original model, blood levels of thyroid hormones and TSH [88], and mRNA [152] for the thyroxine metabolizing enzyme UDP-glucuronosyltransferase-1*6 (UDPGT). It also reproduced experimental data for induction of this enzyme that was 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.

Dose-response behavior of biochemical/tissue dose-response models. The models of Kohn *et al.* [41, 112] 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 Ah receptor, 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 Ah receptor, 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 Ah receptor 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 let 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 end point. 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[156]. 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 which 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[120]. Based on 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 [40]. 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 kinetic 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 due to 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 non-linear. The ED_{01} with this regional induction model was about 1.4 ng/kg/day (Table 8.4). 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 EDs_{01} 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 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 [157].

Table 8.4 Steady state ED_{01} values calculated using mechanism-based dose–response models of dioxin regulated responses.

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

[a] Values obtained using the extended thryoid hormone model[112]
[b] Values from the zonal induction model[40, 111]
[c] Mechanism based cancer-model[42]
[d] 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.

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 non-cancer pathologies.

TCDD is a potent carcinogen in all animal species tested (See Chapter 6). TCDD is an operational promoter, as defined in assay systems of skin and/or liver in mice and rats.[94, 128, 151, 158-160] (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 the analysis of changes in hepatocyte replication has been used for the estimation of parameter values for use in some models of these 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.

Models with A Single Initiated Phenotype: Portier *et al.*[161] estimated the parameters in the first half of a two-stage mathematical model of carcinogenesis from the initiation-promotion data[128] using previously developed methods.[162] This analysis used daily average dose as the dose metric for examining dose dependent effects of TCDD on model parameters. Maronpot *et al.*[128] 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 birth rate or decrease 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.*,[41] Portier *et al.*[161] 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 which results in dose-related formation of initiated cells throughout the study period. Portier *et al.*[161] 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, they used the same methods to analyze focal lesion data from Pitot *et al.*[126] 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 hr 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.*[161] 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 were shown to produce similar patterns with an almost identical unexposed birth rate for all of the four lesion types, a maximal increase over the background rate between 33% to 300%, saturation of the increased birth rate at low doses and a small increase in birth rate due to 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.*[163] analyzed data from Buchmann *et al.* [160] 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.* [162], Moolgavkar *et al.*[163] used a modification which allowed for cellular replication focused on the edge of the AHF. While Moolgavkar *et al.*[163] do not have information on multiple dose groups, the results of their analysis for TCDD concur qualitatively with those of Portier *et al.*[161] In essence, they observed no effect on the birth rate of initiated cells, a significant (seven-fold in non-initiated and two-fold 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.*[161]). The observed lack of change in birth rates is similar to that of the non-significant increase observed by Portier *et al.*[161] for PGST+, GGT and G6P foci but smaller than that for ATP foci in the Pitot *et al.*[158] 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.*[161] study (2.2-fold at 100 ng/kg/day in Moolgavkar *et al.*,[163] 2.5 fold at 125 ng/kg/day for PGST), but much smaller than that observed for the ATP, GGT and G6P lesions from the Pitot *et al.*[158] 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 non-initiated animals was much larger in the Moolgavkar *et al.*[163] analysis than that for the Portier *et al.*[161] analysis. This study was conducted at a single dose and the comparison is simply treated versus control.

***Models with Two Initiated Phenotypes*:** Conolly and Andersen[164] 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.*[165, 166] 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 mito-inhibitory signals generated by the liver to constrain proliferation. One set of mutated cells is sensitive to this mito-inhibition while 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.

Their model is different from those of Portier *et al.*[161] and Moolgavkar *et al.*[163] 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 birth rates. Number and size of focal lesions were estimated using the stochastic resampling methods outlined in Conolly and Kimbell[167] with deterministic growth replacing stochastic growth when colonies exceeded 1000 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.*[158] 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 birth rates (combined for the two mutated clones in the Conolly and Andersen model) for all three sets of models[161, 163, 164] are comparable in the control groups, but differ substantially for the higher dose groups with the two clone model having much larger rates. This difference is partially due to their assumption 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.*[161] used a fixed death rate in their final model and Moolgavkar *et al.*[163] varied the death rate with the birth rate. Results from a study of Stinchcombe *et al.* [168] 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 birth rates and death rates for the initiated cell phenotypes.

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 [40] 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.*[40] fit their multi-compartment geometric model of hepatic zonation[111] to data on the expression of CYP1A2 in rats derived from several studies.[90, 120, 152] The zonal induction model is described previously in this review. The model was linked to the previous PBPK model[109] with modifications[111] to account for the regional induction of CYP1A2 rather than 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 2nM for compartment 3) and CYP1A2 (0.08 to 1.0nM 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 which 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[42] combined the biochemical response model of Kohn *et al.*[41] with a single initiated phenotype two stage model of carcinogenesis to estimate liver tumor incidence in female Sprague-Dawley rats from the two-year cancer bioassay of Kociba *et al.*[52] 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 birth rate of mutated cells was a linear function of loss of EGFR. All death rates were held constant, as was the second mutation rate from 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.*[52] study. The shape of the dose-response curve was approximately linear and the estimated ED_{01} value for this model (1.3ng/kg/day) is presented in Table 8.4. 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 [169, 170] and the hypothesized

role of estrogen metabolites leading to increased oxidative DNA damage and increased mutation [156, 171, 172].

While the thyroid hormone model of Kohn *et al.*[112] 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 fall 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.*[112] and of lipids in the model of Roth *et al.*[113] 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.*[113] model has not been validated for chronic exposures or low doses. While the Wang *et al.*[110] model has examined CYP1A1 and CYP1A2 induction it has not been validated for chronic exposures. The regional induction model [40, 111] creates a hypothesis concerning regional induction which should be further studied. An alternative to altering the affinity of DREs to the liganded Ah receptor is a gradient in the receptor concentration across the liver acinus. The concentration of the receptor in centrilobular he*patocyt*es was found to be more than 40 times that in periportal hepatocytes.[173] 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 Ah receptor, 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 [40]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 [42]. While 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.[161, 163] 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.[40, 111, 164] 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 have 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. While 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 curve 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 hypothesis 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, Ah receptor properties and regulation, and tissue- and species-specific biochemical responses and specific factors regulating these responses. A better understanding these factors could substantially improve dose-response analysis and risk assessment.

- There are differences between Ah receptor binding curves and dose-response curves for specific toxic endpoints. This suggests that factors in addition to the Ah receptor 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.

- 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 for exposures that may occur in the embryo at critical timepoints. 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 when different

species are compared. PBPK modeling could help to address this problem if the existing models developed for laboratory rodents were extrapolated up to humans. While 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 the dose-response analyses. This is especially true since 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 Ah receptor 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, there uncertainties involved in the modeling of these data are considerable and notably include; extrapolation of the exposure observed many years after the critical occupational exposure being modeled, and the type and shape of the curve for the dose-response model used in the extrapolation. A linear model is often used since 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 8.3.1 and 8.3.2). 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 occupationally exposed individuals, suggest an effect of TCDD on all cancers, and lung cancers in the adult human male. The EDs_{01} based upon average excess body burden of TCDD ranged from 6 ng/kg to 161 ng/kg in humans. This compared well with the steady state body burdens estimated in animals that ranged from 3 ng/kg to 1190ng/kg. For the effect of TCDD on lung cancers, the only tumor site increased in both rodents and humans, the human EDs_{01} ranged from 24ng/kg to 161 ng/kg, compared with the single estimate of 730 ng/kg in the rat.

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 the same or different 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 EDs_{01} 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 gave 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 since 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 EDs_{01} 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 category 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, while the higher value 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 (8 analyses) and non-linear shapes (5 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 28000 ng/kg) and a median ED_{01} of 160 ng/kg (range 0.0001 to 9700 ng/kg) in the single dose studies. Toxicity endpoints from the single dose studies resulted in a median value of 4300

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 non-linear responses (80%). All multi dose 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 dissociate the differences between cancer and non-cancer dose-response as being due to differences in endpoint response or simply due to differences in the length of dosing and exposure. Also, a greater percentage of the non-cancer ED_{01}s 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 non-cancer 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, they 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 a 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.

Physiologically-based pharmacokinetic (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, its molecular actions with the Ah receptor 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 are 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 are 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 metrics for toxic responses is considered as 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? 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 types 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. While 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 is based on animal models.

Using 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 Ah receptor) tended to exhibit linear dose-response curves whereas TCDD-inducible responses that are likely more complex and involve multi-gene interactions exhibited more non-linear behavior. Mechanism-based modeling provided two different answers depending upon the approach used in the analysis and the assumptions used in the different 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 a more traditional 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 since 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 linear models resulted in cancer ED_{01}s ranging from 6 ng/kg to 161 ng/kg. This was similar to 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 2.7 ng/kg for the single mechanism-based model.

Estimates for non-cancer endpoints showed much greater variability, ranging over 10 orders of magnitude. 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. However for some endpoints 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 by the analyses, although the data provide the greatest support in the range of 10ng/kg to 50 ng/kg.

This Chapter has produced an extensive a 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.

Appendix I: Multiple-dose studies

Study Description	*Dose Regimen*[a]	*Endpoint*[b]	*Shape Parameter*	*Daily ED01 (ng/kg/day)*	*Lower conf. Bound (ng/kg/day)*	*Body Burden ED01 (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative ED01*[c]	*Quality of Fit*[d]
Kociba *et al.*, 1976 [50] Male Sprague-Dawley rats	13 weeks, 5x/wk, 1 ng/kg	Body weight	18.0	9.1E+01	8.8E+00	1.6E+03	1.6E+02	9.1E+01	M
		Brain weight	5.7	5.4E+01	NR[e]	9.8E+02	NR	5.4E+01	M
		Heart weight	6.4	6.8E+01	3.5E+00	1.2E+03	6.3E+01	6.8E+01	M
		Kidney weight	7.4	6.2E+01	1.3E+00	1.1E+03	2.3E+01	6.2E+01	M
		liver weight	1.0	5.6E+02	3.9E+00	1.0E+04	7.1E+01	5.6E+02	G
		serum alkaline phosphatase	6.2	4.2E+02	4.9E+00	7.7E+03	8.8E+01	4.2E+02	M
		serum BUN[f]	9.7	NC[g]	NC	NC	NC	NC	NF[h]
		serum Direct bilirubin	NA[i]	NA	NA	NA	NA	NA	NF
		serum Indirect bilirubin	NA	NA	NA	NA	NA	NA	NF
		serum SGPT	8.0	1.2E+03	NR	2.2E+04	NR	1.2E+03	P
		serum Total bilirubin	7.0	5.5E+02	NR	9.9E+03	NR	5.5E+02	G
		Spleen weight	6.4	5.4E+01	1.0E+01	9.8E+02	1.8E+02	5.4E+01	M
		Testes weight	NS[j]	NS	NS	NS	NS	NS	NF
		Thymus weight	1.0	4.2E+00	1.4E+00	7.6E+01	2.4E+01	4.2E+00	M
Kociba *et al.*, 1978 [50] Female Sprague-Dawley rats	13 weeks, 5x/wk, 1 ng/kg	Body weight	1.0	4.8E+00	1.1E+00	8.6E+01	2.0E+01	4.8E+00	G
		Brain weight	1.0	5.3E+00	NR	9.5E+01	NR	5.3E+00	M
		Heart wt	5.5	5.2E+01	NR	9.4E+02	NR	5.2E+01	M
		Kidney weight	7.3	5.7E+02	5.6E+00	1.0E+04	1.0E+02	5.7E+02	M
		Liver weight	7.1	6.0E+00	NR	1.1E+02	NR	6.0E+00	M

[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_{01} is the ratio of daily ED_{01} to lowest daily dose estimated from 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, the BMDS[81] fails to locate a lower confidence bound on the 1% effective dose.
[f] Power model was used for these data.
[g] NC – BMDS [81] does not calculate excess risk for model type selected.
[h] NR – Quality of fit was not assessed for this endpoint.
[i] NA – Models in BMDS[81] not applicable to these data.
[j] NS – No dose-response for this endpoint.

Study Description	*Dose Regimen*[a]	*Endpoint*[b]	*Shape Parameter*	*Daily ED01 (ng/kg/day)*	*Lower conf. Bound (ng/kg/day)*	*Body Burden ED01 (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative ED01*[c]	*Quality of Fit*[d]
		serum alkaline phosphatase	7.7	7.3E+00	1.8E-01	1.3E+02	3.3E+00	7.3E+00	M
		Serum Direct bilirubin	1.0	6.8E+00	1.1E+00	1.2E+02	2.0E+01	6.8E+00	M
		serum Indirect bilirubin	NA	NA	NA	NA	NA	NA	NF
		serum Total bilirubin	18.0	8.8E+02	9.3E-01	1.6E+04	1.7E+01	8.8E+02	M
		serumBUN	NS	NS	NS	NS	NS	NS	NF
		serumSGPT	14.1	2.3E+02	2.5E-04	4.2E+03	4.5E-03	2.3E+02	P
		Spleen weight	NS	NS	NS	NS	NS	NS	NF
		Thymus weight	1.0	1.3E+00	8.4E-01	2.3E+01	1.5E+01	1.3E+00	G
Clark *et al.*, 1981 [174] 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	NR	3.8E+04	NR	5.7E+01	P
		immune Increment in Ear Thickness (following oxazalone)	18.0	1.6E+02	NR	2.3E+03	NR	3.4E+00	P
Tritscher *et al.*, 1992 [120] Female Sprague-Dawley rats	31 weeks, 1x/2weeks, 3.5 ng/kg/day	CYP1A1 (Protein) (DEN)	1.2	4.1E-01	1.9E-01	1.5E+01	7.0E+00	1.2E-01	G
		CYP1A1 (Protein) (Saline)	1.0	3.5E-01	2.7E-01	1.3E+01	9.9E+00	10.0E-02	G
		CYP1A2 (Protein) (DEN)	1.0	5.1E-01	3.3E-01	1.9E+01	1.2E+01	1.5E-01	G
		CYP1A2 (Protein) (Saline)	1.0	3.6E-01	1.5E-01	1.3E+01	5.3E+00	1.0E-01	G
Fox *et al.*, 1993 [175] 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	7.0E+01	2.2E+04	1.3E+03	1.4E+03	M
		body weight change	2.5	9.7E+01	6.8E+00	1.7E+03	1.2E+02	1.2E+02	M
		Liver weight	11.2	3.3E+01	1.2E+01	6.0E+02	2.2E+02	3.9E+01	M
		Liver weight:Body weight ratio	1.0	3.1E+00	1.9E+00	5.6E+01	3.4E+01	3.7E+00	G
Fox *et al.*, 1993 [175] 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	3.7E+00	1.1E+00	*6.8E+01*	*2.1E+01*	6.8E+00	G
		body weight change	2.7	5.6E+01	2.6E+00	*1.0E+03*	*4.7E+01*	1.0E+02	G
		Liver weight	1.0	1.2E+00	8.8E-03	*2.2E+01*	*1.6E-01*	2.2E+00	G
		Liver weight:Body weight ratio	1.0	2.1E+03	6.4E-03	*3.8E+04*	*1.2E-01*	3.9E+03	M

Study Description	*Dose Regimen*[a]	*Endpoint*[b]	*Shape Parameter*	*Daily ED01 (ng/kg/day)*	*Lower conf. Bound (ng/kg/day)*	*Body Burden ED01 (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative ED01*[c]	*Quality of Fit*[d]
Fox *et al.*, 1993 [175] 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	9.2E-06	1.7E-04	1.7E-04	1.1E-05	P
		Body weight change	2.4	1.2E+02	5.4E+00	2.2E+03	9.7E+01	1.4E+02	M
		Liver weight	1.0	2.9E+00	1.4E+00	5.2E+01	2.5E+01	3.4E+00	G
		Liver weight:Body weight ratio	3.1	7.7E+01	NR	1.4E+03	NR	9.1E+01	G
Fox *et al.*, 1993 [175] 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	NR	*2.2E-04*	*NR*	2.3E-05	P
		Body weight change	18	1.2E+03	NR	*2.2E+04*	*NR*	2.2E+03	P
		Liver weight	6.2	6.3E+00	1.4E-01	*1.1E+02*	*2.4E+00*	1.1E+01	M
		Liver weight:Body weight ratio	2.5	3.4E+01	2.6E-01	*6.1E+02*	*4.7E+00*	6.2E+01	G
Maronpot *et al.*, 1993 [128] Female Sprague-Dawley Rats	31 weeks, 1x/2weeks, 3.5 ng/kg/day (DEN-initiated)	serum 5'-Nucleotidase	1.9	8.3E-01	2.4E-02	3.0E+01	8.8E-01	2.4E-01	G
		serum Alkaline Phosphatase	2.4	7.9E+00	6.5E-01	2.9E+02	2.3E+01	2.3E+00	M
		serum S. Dehydrogenase	1.0	5.1E-01	5.3E-02	1.8E+01	1.9E+00	1.5E-01	G
		serum Total cholesterol	1.3	4.2E-01	5.5E-02	1.5E+01	2.0E+00	1.2E-01	G
		serum Triglycerides	18.0	2.8E+01	9.0E-02	1.0E+03	3.2E+00	8.0E+00	M
Maronpot *et al.*, 1993 [128] Female Sprague-Dawley Rats	31 weeks, 1x/2weeks, 3.5 ng/kg/day (SALINE)	serum 5'-Nucleotidase	18.0	2.6E+01	NR	9.2E+02	NR	7.3E+00	G
		serum Alkaline Phosphatase	NS	NS	NS	NS	NS	NS	NF
		serum S. Dehydrogenase	NS	NS	NS	NS	NS	NS	NF
		serum Total cholesterol	2.0	2.3E+00	1.5E-01	8.3E+01	5.4E+00	6.6E-01	G
		serum Triglycerides	18.0	8.6E+01	NR	3.1E+03	NR	2.5E+01	P
Sewall *et al.*, 1993 [145] Female Sprague-Dawley Rats	31 weeks, 1x/2weeks, 3.5 ng/kg/day (DEN-initiated and saline-treated)	EGF Dissociation (K_d) (DEN)	1.0	8.1E-01	1.4E-02	2.9E+01	5.0E-01	2.3E-01	M
		EGF Dissociation (K_d) (saline)	18.0	1.4E+01	5.2E-01	5.0E+02	1.9E+01	4.0E+00	M
		EGFR Autophosphorylation	1.0	1.4E+00	2.7E-01	4.9E+01	9.7E+00	3.9E-01	G
		EGFR Maximum Binding (DEN)	1.6	1.7E+00	4.1E-01	6.1E+01	1.5E+01	4.8E-01	G
		EGFR Maximum Binding (saline	1.5	3.8E-01	6.0E-02	1.4E+01	2.2E+00	1.1E-01	G
DeVito *et al.*, 1994 [92] Female B6C3F1 mice	13 weeks, 5x/week, 1.5 ng/kg/day	cyp1a1 erod	1.6	3.2E+00	2.7E+00	5.1E+01	4.2E+01	2.1E+00	G
		cyp1a1 erod lung	1.3	6.1E-01	5.2E-01	9.7E+00	8.2E+00	4.1E-01	G
		cyp1a1 erod skin	NA	NA	NA	NA	NA	NA	NF
		cyp1a2 acoh	1.0	1.2E-01	8.4E-02	1.9E+00	1.3E+00	8.2E-02	G

Study Description	*Dose Regimen*[a]	*Endpoint*[b]	*Shape Parameter*	*Daily ED01 (ng/kg/day)*	*Lower conf. Bound (ng/kg/day)*	*Body Burden ED01 (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative ED01*[c]	*Quality of Fit*[d]
Schrenck *et al.*, 1994 [94] Female Wistar Rat	13 weeks, 1x/2 weeks, 2 ng/kg	Body weight	10.7	1.3E+01	3.3E-02	4.2E+02	1.0E+00	6.6E+00	G
		cyp1a1 erod	1.2	8.2E-01	4.0E-01	2.6E+01	1.3E+01	4.1E-01	G
		Relative liver weight	1.0	3.5E-01	1.1E-01	1.1E+01	3.5E+00	1.8E-01	G
Sewall *et al.*, 1995 [88] Female Sprague-Dawley Rats	31 weeks, 1x/2 weeks, 3.5 ng/kg/day, (DEN-initiated)	CYP 1A1 mRNA	18.0	2.6E+01	5.7E+00	9.4E+02	2.0E+02	7.4E+00	M
		Thyroid-stimulating hormone	12.1	2.6E+01	7.3E-01	9.3E+02	2.6E+01	7.4E+00	M
		Thyroxine	1.9	1.3E+00	1.0E-01	4.8E+01	3.6E+00	3.8E-01	G
		UGT mRNA	16.0	3.7E-01	NR	1.3E+01	NR	1.1E-01	M
VanBirgelen *et al.,* 1995 [87] Female Sprague-Dawley rats	13 weeks, 1x/day, 14 ng/kg/d	cyp1a1 erod	1.3	1.0E+00	5.0E-01	3.8E+01	1.8E+01	7.5E-02	G
		T4UGT	1.0	1.6E+00	1.2E+00	5.8E+01	4.2E+01	1.1E-01	G
		thyroxine ft4	1.0	4.9E+00	2.3E+00	1.8E+02	8.3E+01	3.5E-01	M
		thyroxine tt4	16.6	3.3E+01	1.2E+01	1.2E+03	4.5E+02	2.4E+00	M
		UGT1A1	1.7	1.5E+00	3.0E-01	5.3E+01	1.1E+01	1.0E-01	M
VanBirgelen *et al.,* 1995 [95] Female Sprague-Dawley Rats	13 weeks, 1x/day, 14 ng/kg/d	Body weight	1.0	4.3E+00	1.7E+00	1.6E+02	6.0E+01	3.1E-01	G
		cyp1a1 erod	1.0	6.1E-01	5.5E-01	2.2E+01	2.0E+01	4.3E-02	G
		cyp1a2 acoh	2.1	2.1E+00	1.0E+00	7.4E+01	3.6E+01	1.5E-01	M
		Hepatic retinol	1.0	2.8E-01	2.1E-01	1.0E+01	7.6E+00	2.0E-02	G
		Hepatic retinyl-palmitate	1.0	4.0E-02	2.9E-02	1.5E+00	1.1E+00	2.9E-03	G
		liver weight	18.0	2.2E+02	4.2E+01	8.0E+03	1.5E+03	1.6E+01	P
		Liver weight:Body weight ratio	1.0	4.9E+00	3.0E+00	1.8E+02	1.1E+02	3.5E-01	G
		Plasma retinol	1.2	2.3E+00	1.1E+00	8.2E+01	4.1E+01	1.6E-01	G
		Relative kidney weight	1.0	4.8E-01	3.1E-01	1.7E+01	1.1E+01	3.4E-02	G
		Relative spleen wt.[f]	0.9	NC	NC	NC	NC	NC	NF
		Relative thymus weight	1.0	3.0E+00	2.3E+00	1.1E+02	8.2E+01	2.1E-01	M
		Thymus weight	1.0	2.5E+00	2.0E+00	8.9E+01	7.1E+01	1.8E-01	M
		thyroxine ft4	1.0	4.9E+00	2.3E+00	1.8E+02	8.3E+01	3.5E-01	G
		thyroxine tt4	16.6	3.3E+01	3.0E+01	1.2E+03	1.1E+03	2.4E+00	M

Study Description	*Dose Regimen*[a]	*Endpoint*[b]	*Shape Parameter*	*Daily ED01 (ng/kg/day)*	*Lower conf. Bound (ng/kg/day)*	*Body Burden ED01 (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative ED01*[c]	*Quality of Fit*[d]
Rhile *et al.*, 1996 [176] Female DBA/2 mice	11 days, 1x/day, 100 ng/kg	Total thymic cells/mouse	8.5	6.5E+02	3.2E+01	1.0E+04	5.0E+02	6.5E+00	M
		CD8+ cells	NA	NA	NA	NA	NA	NA	NF
		CD8+/CD4-	18.0	8.1E+03	NR	1.3E+05	NR	8.1E+01	M
		CD8+/CD4+	NA	NA	NA	NA	NA	NA	NF
		CD4+	17.5	1.7E+02	NR	2.7E+03	NR	1.7E+00	M
Rhile *et al.*, 1996 [176] Female C57 BL/6 mice	11 days, 1x/day, 100 ng/kg	Total thymic cells/mouse	15.0	7.5E+01	NR	1.2E+03	NR	7.5E-01	M
		CD8+ cells	13.5	3.5E+03	2.2E+02	5.6E+04	3.5E+03	3.5E+01	M
		CD8+/CD4-	11.2	3.3E+03	1.8E+01	5.2E+04	2.9E+02	3.3E+01	G
		CD8-/CD4-	1.0	9.9E-01	1.2E-02	1.6E+01	1.9E-01	9.9E-03	G
		CD4+	NS	NS	NS	NS	NS	NS	NF
Rhile *et al.*, 1996 [176] Female C57Bl/6 lpr/lpr mice	11 days, 1x/day, 100 ng/kg	Total thymic cells/mouse	1.0	1.6E+01	1.2E+01	2.5E+02	1.9E+02	1.6E-01	G
		CD8+ cells	18.0	4.9E+03	NR	7.8E+04	NR	4.9E+01	M
		CD8+/CD4-	18.0	2.9E+04	2.1E-02	4.6E+05	3.3E-01	2.9E+02	P
		CD8-/CD4-	15.3	1.4E+04	2.0E-02	2.2E+05	3.2E-01	1.4E+02	P
		CD4+	18.0	4.3E+04	4.5E+02	6.8E+05	7.1E+03	4.3E+02	M
Vogel *et al.*, 1997 [96] 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	1.1E-07	4.2E-01	1.7E-06	4.2E-01	G
		immune CD4-/CD8- (23 d)	1.0	1.3E-03	4.9E-05	1.8E-02	7.0E-04	1.8E-02	M
		immune CD4-/CD8+ (23 d)	6.1	2.5E-02	6.2E-05	3.7E-01	9.0E-04	3.6E-01	G
		ImmuneCD4+/CD8+ (23 d)	5.5	2.7E-02	6.5E-05	3.9E-01	9.4E-04	3.8E-01	G
Vogel *et al.*, 1997 [96] 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	4.3E-02	8.9E-01	6.2E-01	2.1E+00	P
		immune CD4-/CD8- (79 d)	18.0	7.9E-02	NR	1.1E+00	NR	2.6E+00	G
		ImmuneCD4-/CD8+ (79 d)	6.6	1.2E-02	3.8E-04	1.7E-01	5.4E-03	4.0E-01	M
		immune CD4+/CD8- (79 d)	3.7	5.5E+00	NR	7.9E+01	NR	1.8E+02	M
Vogel *et al.*, 1997 [96] 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	9.4E-03	4.0E-03	1.4E-01	5.8E-02	2.8E-01	G
		CYP1A1 mRNA (135 d)	8.1	1.8E+00	2.7E-01	2.6E+01	4.0E+00	5.3E+01	G
		CYP1A2 mRNA (135 d)	1.1	3.0E-03	9.1E-04	4.3E-02	1.3E-02	8.7E-02	G
		cyp1a2 MROD (135 d)	1.0	2.7E-02	1.0E-02	3.9E-01	1.5E-01	7.9E-01	G

Study Description	*Dose Regimen*[a]	*Endpoint*[b]	*Shape Parameter*	*Daily ED01 (ng/kg/day)*	*Lower conf. Bound (ng/kg/day)*	*Body Burden ED01 (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative ED01*[c]	*Quality of Fit*[d]
Johnson *et al.*, 1997 [93] Female B6C3F1 mice	18 weeks, 1x/3 wks (5x total), 3 weeks after last, 1000 ng/kg	cyp1a1 EROD	2.8	1.9E+01	2.4E+00	3.0E+02	3.8E+01	4.0E-01	G
		Endometrial lesion diameter	NA	NA	NA	NA	NA	NA	NF
		Endometrial lesion weight	NA	NA	NA	NA	NA	NA	NF
		Liver weight	1.1	1.8E+04	NR	2.8E+05	NR	3.8E+02	G
		Ovarian weight	15.2	3.8E+02	1.5E+01	6.0E+03	2.3E+02	8.0E+00	P
		Thymus weight	NA	NA	NA	NA	NA	NA	NF
		Uterine horn weight	NS	NS	NS	NS	NS	NS	NF
Walker *et al.*, 1999 [177] Female Sprague-Dawley Rats	31 weeks, 1x/2weeks, 3.5 ng/kg/day, (DEN-initiated)	CYP1A1 mRNA	2.0	1.6E+00	1.1E+00	5.9E+01	3.8E+01	4.7E-01	G
		CYP1A2 mRNA	3.0	7.6E+00	5.5E+00	2.7E+02	2.0E+02	2.2E+00	G
		CYP1B1 mRNA	3.1	7.0E+00	6.2E+00	2.5E+02	2.2E+02	2.0E+00	G

Appendix II: Single–dose adult studies

Study Description	*Dose Regimen*[a]	*Endpoint*[b]	*Shape Parameter*	*1% extra effect (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative 1% extra effect*[c]	*Quality of Fit*[d]
Kitchin & Woods, 1979 [178] Female Sprague-Dawley rats	3 days, 0.6 ng/kg	Liver Cytochrome P-450 (Total)	1.0	1.5E+01	9.4E+00	2.6E+01	G
		Liver Benzopyrene Hydroxylase (CYP1A1 Activity)	17.7	1.4E+03	NR[e]	2.4E+03	P
Olson *et al.*, 1980 [179] male golden syrian hamsters	50 days, 5000 ng/kg	Thymus weight	1.1	3.7E+03	1.6E+03	7.3E-01	G
		Spleen weight[f]	1.0	NC[g]	NC	NC	NF[h]
Vecchi *et al.*, 1983 [180] Female B6 mice	12 days, 1200 ng/kg	Body Weight	12.0	2.1E+04	1.4E+03	1.8E+00	G
		Thymus weight	1.4	1.5E+02	4.8E+01	1.3E-02	G
		PFC/1E+06 splenocytes	1.0	2.7E+00	1.6E+00	2.3E-04	G
		PFC/spleen	1.0	3.9E+00	3.5E+00	3.3E-04	G
Vecchi *et al.*, 1983 [180] Female C3 mice	12 days, 1200 ng/kg	Body Weight	11.1	4.4E+03	3.6E+02	3.6E-01	P
		Thymus weight	1.0	3.9E+01	2.4E+01	3.3E-03	G
Vecchi *et al.*, 1983 [180] Female D2 mice	12 days, 1200 ng/kg	Body Weight	17.8	4.2E+05	NR	3.5E+01	P
		Thymus weight	1.0	3.3E+00	NR	2.7E-04	M
		PFC/1E+06 splenocytes	1.0	3.0E+01	1.5E+01	2.5E-03	G
		PFC/spleen	1.3	1.0E+02	4.2E+01	8.3E-03	G
Vecchi *et al.*, 1983 [180] Female B6D2F1 mice	12 days, 1200 ng/kg	Body Weight	5.5	6.5E+05	4.8E-02	5.4E+01	P
		Thymus weight	1.0	7.6E+01	4.6E+01	6.4E-03	G
		PFC/1E+06 splenocytes	1.0	1.4E+01	1.3E+01	1.2E-03	G
		PFC/spleen	1.0	1.4E+01	1.3E+01	1.2E-03	G
Abraham *et al.*, 1988 [90] female Wistar rats	7 days, 1 ng/kg	Liver EROD (CYP1A1 Activity)	1.1	1.6E+01	1.3E+01	1.6E+01	G
		Liver Cytochrome P450 (Total)	1.0	6.7E+00	4.7E+00	6.7E+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 1% extra effect is the ratio of 1% extra effect 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] NR – In some cases, BMDS [81] fails to locate a lower confidence bound on the 1% effective dose.
[f] Power model used to fit these data.
[g] NC – BMDS [81] does not calculate excess risk for model type selected.
[h] NF – Quality of fit not assessed for this endpoint.

Study Description	*Dose Regimen*	*Endpoint*	*Shape Parameter*	*1% extra effect (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative 1% extra effect*	*Quality of Fit*
Davis and Safe, 1988 [181] Male 657BL/6J mice	9 days, 1 nmol/kg	Spleen cellularity	18.0	4.5E+02	2.7E+00	1.4E+00	M
		PFCs/spleen	4.2	2.0E+02	1.1E+02	6.3E-01	G
		PFCs/1E+06 viable cells	4.0	2.1E+02	1.1E+02	6.5E-01	G
Birnbaum *et al.*, 1990 [182]Male C57BL/6J (Ahb/b) mice	35 days, 50ng	Serum TBA	18.0	4.6E+04	2.4E+04	9.1E+02	M
		Serum SDH	2.8	1.7E+04	8.2E+03	3.4E+02	M
		Serum ALT	2.4	1.6E+04	5.0E+03	3.2E+02	M
		Serum 5'-NUC	18.0	8.8E+04	4.3E+04	1.8E+03	M
		Serum Glucose	18.0	5.3E+04	2.8E-01	1.1E+03	M
		Serum Triglycerides	18.0	3.4E+05	2.8E-02	6.9E+03	P
Birnbaum *et al.*, 1990 [182]Male C57BL/6J (Ahb/b) mice	35 days, 50ng	Serum Total cholesterol	18.0	3.5E+04	4.6E-02	6.9E+02	M
		Serum NEChol	4.7	7.7E-04	NR	1.5E-05	P
		Serum Echol	18.0	3.5E+04	8.5E+02	7.1E+02	M
		Liver HCC	7.2	8.5E+04	5.8E+04	1.7E+03	G
		Liver HCK	5.8	3.0E+04	1.3E+04	6.0E+02	G
		Fatty Liver Change	7.9	5.8E+04	2.0E+04	1.2E+03	G
		Liver BDH	2.6	4.8E+04	1.7E+04	9.6E+02	G
		Thymic Atrophy	2.0	2.3E+04	4.4E+03	4.6E+02	G
		Splenic Atrophy	1.9	1.6E+04	5.1E+03	3.3E+02	G
		Testes: MNGC	2.3	3.7E+04	1.2E+04	7.4E+02	G
		Testes: SFEN	6.9	1.0E+05	4.9E+04	2.0E+03	G
		Gland. Stomach Edema	1.5	1.8E+04	3.6E+03	3.7E+02	G
Birnbaum *et al.*, 1990 [182] Male C57BL/6J (Ahb/b) mice	35 days, 400ng	Serum TBA	2.3	1.5E+06	1.2E+05	3.6E+03	M
		Serum SDH	7.1	1.1E+06	2.7E+04	2.1E+04	M
		Serum ALT	1.0	8.6E+06	3.3E+04	2.1E+04	M
		Serum 5'-NUC	18.0	3.2E+05	1.1E+05	8.1E+02	P
		Serum Glucose	18.0	6.1E+05	8.3E+04	1.5E+03	P
		Serum Triglycerides	18.0	1.8E+06	8.3E+05	4.6E+03	P
		Serum Total cholesterol	1.0	5.1E+02	1.3E-01	1.3E+00	G
		Serum NEChol	1.0	1.0E+03	3.2E-02	2.5E+00	G
		Serum Echol	1.0	1.7E+03	3.9E+02	4.2E+00	G
		Liver HCC	4.2	1.5E+06	1.9E+05	3.8E+03	M
		LiverHCK	3.1	9.2E+04	3.3E+04	2.3E+02	M
		Fatty LiverChange	2.6	6.9E+05	1.1E+05	1.7E+03	M
		Liver BDH	1.6	1.3E+06	2.5E+05	3.2E+03	M

Study Description	*Dose Regimen*	*Endpoint*	*Shape Parameter*	*1% extra effect (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative 1% extra effect*	*Quality of Fit*
Birnbaum *et al.*, 1990 [182] Male C57BL/6J (Ahb/b) mice	35 days, 400ng	Thymic Atrophy	1.0	4.7E+04	2.5E+04	1.2E+02	M
		Splenic Atrophy	1.0	2.3E+04	1.7E+04	5.8E+01	M
		Testes: MNGC	NS[a]	NS	NS	NS	NF
		Testes: SFEN	4.2	1.9E+06	3.2E+05	4.9E+03	G
		Gland. Stomach Edema	4.2	1.9E+06	3.2E+05	4.9E+03	G
Jurek *et al.,* 1990 [183] Male Sprague-Dawley rats	12 days, 1 nmol/kg	Body weight	1.0	9.2E+02	3.8E+02	2.9E+00	M
		Liver weight:Body weight ratio	8.2	1.1E+06	5.2E-01	3.5E+03	P
		Kidney weight:Body weight ratio	2.7	3.5E-03	NR	1.1E-05	P
		Renal Retinol concentration	12.3	2.0E+03	9.2E+02	6.3E+00	M
		Renal RPH activity	18.0	1.5E+04	8.6E+02	4.5E+01	M
Alsharif *et al.*, 1994 [184]Female Sprague-Dawley rats	1 day, 5 ng/kg	Superoxide anion production by PLC	5.4	5.7E+04	2.1E+04	1.1E+04	G
Narasimhan *et al.*, 1994 [91]Female B6C3F1 mice	24 hrs., 5 ng/kg	Liver EROD (CYP1A1 Activity)	1.1	8.4E+01	5.9E+01	1.7E+01	G
		Liver CYP1A1 (mRNA)	1	5.0 E-03	3.2E-03	9.9E-04	G
		Liver CYP1A2 (mRNA)	3.2	1.8E+02	7.1E+01	3.5E+01	G
		Total Ah receptor binding	3.8	3.5E+02	2.8E+02	7.0E+01	G
	4 days, 5 ng/kg	Spleen PFC/1E+06cells	1.0	2.0E+00	NR	4.1E-01	G
Harper *et al.*, 1994 [185] Male C57BL/6 mice	8 days, 0.6 mg/kg	Immune Titer	4.8	3.0E+02	1.8E+02	5.0E-01	G
		PFC/1E+06 cells	6.1	3.3E+02	2.1E+02	5.5E-01	M
Smialowicz *et al.*, 1994 [186] Male F344 rats	1x followed by immunization with SRBC 7 days later, 100 ng/kg	PFC/1E+06 cells	18.0	1.6E+04	5.5E+03	1.6E+02	P
		PFC/spleen(x10-4)	18.0	2.4E+04	4.3E+03	2.4E+02	P
		Cells/spleen(x10-6)	18.0	7.3E+03	1.9E+01	7.3E+01	P
		Titer(log2)	1.4	1.2E+02	1.2E+01	1.2E+00	G
Smialowicz *et al.*, 1994 [186] Female F344 rats	1x followed by immunization with SRBC 7 days later, 100 ng/kg	PFC/1E+06 cells	1.0	4.1E+03	3.4E+03	4.1E+01	P
		PFC/spleen(x10-4)	1.0	1.7E+04	4.3E+02	1.7E+02	P
		Cells/spleen(x10-6)	18.0	7.9E+02	NR	7.9E+00	M
		Titer(log2)	1.0	NR	NR	NR	P

[a] NS – No dose-response for this endpoint.

Study Description	*Dose Regimen*	*Endpoint*	*Shape Parameter*	*1% extra effect (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative 1% extra effect*	*Quality of Fit*
Smialowicz *et al.*, 1994 [186] Female Long-Evans rats	1x followed by immunization with S RBC 7 days later, 100 ng/kg	PFC/1E+06 cells	18.0	2.4E+04	2.7E+03	2.4E+02	P
		PFC/spleen(x10-4)	14.8	2.5E+04	3.4E+03	2.5E+02	P
		Cells/spleen(x10-6)	4.3	2.0E+03	9.7E+01	2.0E+01	G
		Titer(log2)	18.0	3.4E+05	NR	3.4E+03	P
Vanden Heuvel *et al.*, 1994 [152] Female Sprague-Dawley Rats	4 days, 0.1 ng/kg	CYP1A1 mRNA	3.6	3.9E+02	NR	3.9E+03	G
		UGT mRNA	1.4	3.5E+01	1.4E+01	3.5E+02	G
Diliberto *et al.*, 1995 [89] Female B6C3F1 mice	S, 7, 14, 21, 35 days, 100 ng/kg	Liver EROD (CYP1A1): 7 days	1.0	2.7E+01	2.3E+01	2.7E-01	P
		Liver EROD (CYP1A1): 14 days	3.5	2.8E+02	6.8E+01	2.8E+00	G
		Liver EROD (CYP1A1): 21 days	2.8	2.4E+02	7.4E+01	2.4E+00	G
		Liver EROD (CYP1A1): 35 days	6.5	7.4E+02	4.1E+02	7.4E+00	M
Li *et al.,* 1995 [187] Female Sprague-Dawley rats	4 days, 300 ng/kg	Body weight	3.7	1.2E+03	6.7E+02	3.9E+00	G
		Ovarian weight	1.0	1.7E+02	1.1E+02	5.7E-01	G
		Ovulation (ova/rat)	1.4	1.5E+02	3.5E+01	4.9E-01	G
Smialowicz *et al.*, 1994 [186] Female B6C3F1 mice	1x followed by immunization with SRBC 7 days later, 300 ng/kg	PFC/1E+06 cells	1.0	2.9E+00	1.7E+00	9.6E-03	M
		PFC/spleen(x10-4)	1.1	4.4E+00	1.3E+00	1.5E-02	G
VanBirgelen *et al.*, 1996 [188] Female B6C3F1 mice	S, 7 days, 100 ng/kg	Cyp1A1 EROD	1.8	7.1E+01	2.1E+01	7.1E-01	G

Appendix III: Single-dose developmental studies

Study Description	*Dose Regimen*[a]	*Endpoint*[b]	*Shape Parameter*	*1% extra effect (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative 1% extra effect*[c]	*Quality of Fit*[d]
Birnbaum *et al.*, 1989 [189]C57BL/6N mice	GD 10 or12, 8 or 6 days (sacrificed on GD 18), 6000 ng/kg	Cleft palate GD-10[e]	3.5	3.3E+03	1.4E+03	3.3E+00	G
		Cleft palate GD-12[e]	6.4	4.4E+03	2.7E+03	4.4E+00	G
		Hydronephrosis GD-10[e]	1.0	3.2E+01	1.9E+01	3.2E-02	M
		Hydronephrosis GD-12[b]	2.3	2.1E+02	NR[f]	2.1E-01	P
Mably *et al.*, 1992[82, 84] Preg. 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	5.5E+00	1.4E+00	G
		Fertility index	NA[g]	NA	NA	NA	NF[h]
		Cauda sperm count day 63	1.0	6.6E-01	5.1E-01	1.0E-02	G
		Cauda sperm count - day 120	1.0	8.1E-01	7.3E-01	1.3E-02	G
		Cauda sperm count/g - day 120	1.7	3.7E+00	1.2E+00	5.8E-02	G
		DSP/g - day 49	1.0	5.7E-01	4.5E-01	9.0E-03	G
		DSP/g - day 63	1.4	1.4E+00	2.1E-01	2.2E-02	G
		DSP/g – day 120	1.7	6.6E+00	2.8E+00	1.0E-01	G
		Reproductive Outcomes of Females:					
		Gestation period	18.0	2.0E+03	NR	3.2E+01	P
		Litter size	18.0	7.9E+01	NR	1.2E+00	P
		Live birth index(%)	NA	NA	NA	NA	NF
		Age of Indices of Dev. In Pups:					
		Pinna detachment	17.0	8.6E+02	2.3E+02	1.3E+01	P
		Incisor eruption	1.0	3.5E+01	4.3E+00	5.5E-01	G
		Eye opening	1.0	3.3E+01	7.0E+00	5.2E-01	G
		Testis descent	1.0	1.3E+00	8.2E-01	2.1E-02	G

[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 1% extra effect is the ratio of 1% extra effect 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.
[f] NR – In some cases, the BMDS [81] fails to locate a lower confidence bound on the 1% effective dose.
[g] NA – Models in BMDS [81] not applicable to these data.
[h] NF-Quality of fit was not assessed for this endpoint.

Study Description	*Dose Regimen*	*Endpoint*	*Shape Parameter*	*1% extra effect (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative 1% extra effect*	*Quality of Fit*
Theobald *et al.,* 1997 [86] Pregnant female, male and female offspring ICR mice	GD 14, PND 44, 15000 ng/kg	Testes weight	1.0	9.7E+04	NR	6.5E+00	M
		Epididymides wt.	18.0	4.7E+04	5.2E+03	3.1E+00	M
		Dorsal prostate wt.	1.0	3.0E+02	6.0E-04	2.0E-02	P
		Ventral prostate wt.	2.9	1.4E-04	NR	9.5E-09	M
		Coagulating glands	1.7	7.7E+03	2.6E+03	5.1E-01	G
		Seminal vesicles	18.0	4.8E+04	4.8E-01	3.2E+00	M
		Ovary weight	18.0	2.4E+04	2.3E+03	1.6E+00	M
		Uterus weight	4.5	9.8E+03	3.4E+03	6.5E-01	G
Theobald *et al.,* 1997 [86]Pregnant female, male and female offspring ICR mice	GD 14, PND 65, 15000 ng/kg	Testes weight	18.0	1.1E+04	2.3E+03	7.5E-01	M
		Epididymides wt.	3.1	1.4E-04	NR	9.5E-09	P
		Dorsal prostate wt.	1.0	NR	NR	NR	P
		Ventral prostate wt.	18.0	1.1E+04	4.2E+03	7.5E-01	M
		Coagulating glands	18.0	1.1E+04	2.4E+03	7.5E-01	M
		Seminal vesicles	1.0	3.9E+04	1.1E-01	2.6E+00	M
		Sperm production: ESN	13.4	1.0E+04	2.3E+02	6.8E-01	M
		Sperm production: DSP	18.0	1.5E+04	5.4E+03	9.8E-01	M
		Pituitary gland wt (Males) (PND 65)	11.5	4.5E+05	4.1E-01	3.0E+01	P
Theobald *et al.,* 1997 [86] Pregnant female, male and female offspring ICR mice	GD 14, PND 114/128, 15000 ng/kg	Testes weight	NS	NS	NS	NS	NF
		Epididymides wt.	NA	NA	NA	NA	NF
		Dorsal prostate wt.	1.0	8.3E+02	1.2E+02	5.5E-02	P
		Ventral prostate wt.	18.0	1.1E+04	1.3E+03	7.3E-01	M
		Coagulating glands	18.0	1.1E+04	3.2E+03	7.6E-01	M
		Seminal vesicles	NA	NA	NA	NA	NF
		Sperm production: ESN (PND 114/128)	NA	NA	NA	NA	NF
		Sperm production: DSP (PND 114/128)	NA	NA	NA	NA	NF
		Female Rep: Ovary wt. (PND 114)	18.0	1.6E+04	9.0E+03	1.0E+00	M
		Female Rep: Uterus wt. (PND 114)	4.5	3.7E+04	1.4E+04	2.5E+00	G

Study Description	*Dose Regimen*	*Endpoint*	*Shape Parameter*	*1% extra effect (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative 1% extra effect*	*Quality of Fit*
Theobald *et al.,* 1997 [86] Pregnant female, male and female offspring ICR mice	GD 14, PND 114/128, 15000 ng/kg	Pituitary gland wt (Males) (PND 128)	NA	NA	NA	NA	NF
		Pituitary wt (females) (PND 128)	18.0	1.1E+04	2.5E+03	7.2E-01	M
		Hydronephrosis (females)	1.1	1.2E+03	4.3E+02	8.0E-02	M
		Eye opening (females)	1.0	3.8E+01	6.4E-01	2.5E-03	M
		Thymus weight (females)	1.0	3.2E+02	7.6E+01	2.1E-02	M
		Hydronephrosis (males)	1.0	2.6E+02	1.8E+02	1.7E-02	M
		Eye opening (males)	1.0	7.6E+01	1.9E+01	5.1E-03	G
		Thymus weight (males)	3.4	1.4E-04	NR	9.5E-09	P
Gray *et al.,* 1997 [85]Long Evans Hooded Rat Male Offspring	GD 15, PND 49, 50 ng/kg	Body weight (day 49)	9.6	1.4E+02	8.8E+00	2.7E+00	G
		Testes weight (49)	1.1	4.5E+02	NR	9.0E+00	G
		Paired epididymal weight (49)	13.9	1.4E+02	NR	2.9E+00	M
		Cauda epididymus (49)	18.0	7.9E+01	2.3E+01	1.6E+00	G
		Epididymal sperm count (49)	1.0	1.2E-01	1.7E-04	2.3E-03	P
		Ventral prostate weight (49)	12.4	1.4E+02	2.2E+01	2.7E+00	G
		Seminal vesicle weight (49)	17.9	1.5E+02	2.3E+01	3.0E+00	M
		Daily sperm production (49)	14.1	6.1E+02	4.5E+01	1.2E+01	M
		Serum testosterone (49)	13.5	6.4E+02	2.1E+01	1.3E+03	M
		Age at puberty (49)	2.8	4.0E+01	1.1E+01	7.9E-01	P
		Body weight at puberty (49)	13.6	1.4E+02	1.1E+01	2.7E+00	M
		Pituitary (49)	8.9	9.6E+01	1.1E+01	1.9E+00	M

Study Description	*Dose Regimen*	*Endpoint*	*Shape Parameter*	*1% extra effect (ng/kg)*	*Lower conf. Bound (ng/kg)*	*Relative 1% extra effect*	*Quality of Fit*
Gray *et al.,* 1997 [85] Long Evans Hooded Rat Male Offspring	GD 15, PND 63, 50 ng/kg	Body weight (63)	17.5	1.6E+02	1.0E+01	3.2E+00	P
		Testes weight (63)	10.8	1.3E+02	5.3E+00	2.6E+00	G
		Paired epididymal weight (63)	14.2	1.4E+02	2.5E+01	2.8E+00	P
		Cauda epididymus (63)	12.1	1.3E+02	1.2E+01	2.6E+00	G
		Epididymal sperm count (63)	11.2	1.4E+02	1.8E+01	2.8E+00	G
		Ventral prostate weight (63)	14.0	1.4E+02	2.0E+01	2.8E+00	P
		Seminal vesicle weight (63)	11.3	1.6E+02	2.0E+01	3.2E+00	G
		Daily sperm production (63)	13.6	5.7E+02	7.3E+01	1.1E+01	M
		Serum testosterone (63)	10.3	3.3E+01	NR	6.5E-01	M
		Pituitary (63)	8.7	3.7E+01	8.5E+00	7.4E-01	M
Gray *et al.,* 1997 [85] Long Evans Hooded Rat Male Offspring	GD 15, offspring examined 15 months, 50 ng/kg	Body Weight	13.0	1.6E+02	8.0E+00	3.1E+00	M
		Testes Weight	5.7	2.7E+04	NR	5.5E+02	P
		Ventral prostate weight	6.7	4.5E+03	2.6E-03	9.0E+01	P
		Seminal vesicle weight	18.0	7.8E+01	3.3E+01	1.6E+00	G
		Glans penis weight	1.4	3.8E+00	1.1E+00	7.6E-02	G
		Paired epididymal weight	18.0	7.3E+01	2.7E+01	1.5E+00	P
		Cauda epididymal weight	10.7	3.3E+01	4.5E+00	6.5E-01	P
		Epididymal sperm numbers	4.3	3.8E+01	1.3E+01	7.5E-01	G
		Caput/corpus epid. sperm numbers	15.5	1.2E+02	2.5E+01	2.5E+00	P
Gray *et al.,* 1997 [85] Long Evans Hooded Rat Male Offspring	GD 15, offspring examined 15 months, 50 ng/kg	Cauda epid. Sperm numbers	2.9	1.4E+01	2.5E+00	2.7E-01	G
		Number of copulatory plugs	2.4	1.1E-06	NR	2.3E-08	P
		Total testis sperm numbers	12.3	1.6E+02	9.9E+00	3.3E+00	P
		Serum testosterone	10.2	6.2E+02	NR	1.2E+01	M
		Piuitary weight	18.0	7.7E+01	7.0E+00	1.5E+00	P

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