The Application of Internal Dose Measures, Biokinetics, and Biomonitoring Data in the Risk Assessment of Dioxin-Like Compounds

Lesa Lynn Aylward

The Application of Internal Dose Measures, Biokinetics, and Biomonitoring Data in the Risk Assessment of Dioxin-Like Compounds/ Lesa Lynn Aylward

PhD Thesis, Institute of Risk Assessment Sciences (IRAS)
Utrecht University, 2009

ISBN: 978-90-393-5149-9

Printed by: Book1One, Rochester, NY

The Application of Internal Dose Measures, Biokinetics, and Biomonitoring Data in the Risk Assessment of Dioxin-Like Compounds

De toepassing van interne blootstellingsmetingen, biokinetiek en biomonotoring gegevens bij de risicobeoordeling van dioxine-achtige verbindingen

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. J.C. Stoof,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op dinsdag 1 oktober 2009
des ochtends te 10.30 uur

door

Lesa Lynn Aylward

geboren op 16 augustus 1962 te Rockford, Illinois, United States of America

Promotoren: Prof. dr. M. van den Berg

Prof. dr. B.J. Blaauboer

Research included in this thesis was sponsored in part by the Research Foundation for Health and Environmental Effects and by the Chlorine Chemistry Division of the American Chemistry Council.

Table of Contents

Chapter 1: Introduction

The biokinetic properties of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related coplanar polychlorinated dioxin (PCDD), furan (PCDF), and biphenyl (PCB) compounds have played an integral role in the identification, experimental evaluation, and risk assessment of potential health effects of these compounds dating from the earliest occupational evaluations of manufacturing workers engaged in the production of chlorinated phenoxyacetic acid compounds. Because these compounds are highly persistent in human tissues, the detection and quantification of exposure using biomarkers such as adipose or blood lipid concentrations is possible in both populations with elevated exposure potential (such as occupational cohorts) and in persons in the general population exposed to trace levels of these compounds accumulated in the food chain. Availability of these biomarkers has also led to the estimation of half-lives of elimination for TCDD and many related compounds in humans through the analysis of serial biological samples in individuals. In turn, such estimates have resulted in efforts to back-extrapolate estimated internal doses in individuals exposed in specific occupational or accidental incidents years or decades prior to collection of a biological sample.

In parallel, studies of biokinetic properties of TCDD and related compounds in laboratory animals have informed the design and interpretation of toxicological studies. In the risk assessment context, extrapolations across congeners, from high to low dose, and from animals to humans are commonly performed. Because TCDD has historically been the most-studied compound of the class, generalizations based on data on TCDD have been applied, sometimes inaccurately, across the whole class of congeners. In addition, experimental data in animals and data from human studies has revealed concentration-dependent behavior for the elimination of TCDD and other congeners. Finally, physiological differences in humans compared to laboratory rodents can also influence the biokinetics of these compounds. However, these inter-congener, across dose, and inter-species differences have not always been integrated into the risk assessments performed for dioxins.

This dissertation presents research and analysis pertinent to the integration of biokinetic understanding and biomonitoring data into the risk assessment of potential health effects of TCDD and related compounds.

Background – Biological Monitoring of Exposure to TCDD and Related Compounds

The extreme persistence of the skin condition "chloracne" in workers following an acute accidental exposure to a runaway chemical reaction during the production of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was noted by

occupational physicians treating these workers and provided an indication that the underlying toxicant might also be highly persistent (reviewed in Suskind and Hertzberg 1984). When toxicological evaluations of 2,4,5-T began to identify and isolate TCDD as a toxic contaminant (Courtney and Moore 1971), biokinetic evaluations in rats (Piper et al. 1973; Fries and Marrow 1975; Rose et al. 1976) demonstrated the substantial persistence and lipophilicity of TCDD, with estimated half-lives for whole body elimination on the order of 25 days and accumulation of TCDD in the fat and liver observed.

A series of accidental exposure situations led to collection and analysis of human biological samples for TCDD and related compounds. Some of the earliest efforts to use biomarkers to evaluate human exposures to dioxin-like compounds were made by Masuda and coworkers in their studies of victims of yusho poisoning in Japan in 1968 and later, for victims of the similar yucheng episode in Taiwan in 1979 (Masuda et al. 1974 as reviewed in Masuda et al. 1985). The 2,4,5-T reactor accident in Seveso, Italy, in July, 1976, resulted in the release of a substantial quantity of TCDD through the air to the surrounding residential population (Bertazzi et al. 1998). Blood samples were collected and stored beginning within two weeks after the accident for hundreds of residents and periodically continuing for many years for a substantial number of these individuals (Sweeney and Mocarelli 2000) and were later analyzed to quantify TCDD when the analytical capabilities became available. Evaluations of United States Vietnam veterans potentially exposed to Agent Orange led to collection and analysis of adipose tissue samples (see for example CDC 1988) and blood samples, most notably as part of the large Ranch Hand study of members of the United States Air Force unit that handled and sprayed Agent Orange in Vietnam. Finally, distribution of TCDD in residential communities in Missouri occurred when contaminated waste oil was sprayed on rural dirt roads for dust control. Adipose tissue samples from individuals potentially exposed during this incident were collected and analyzed for TCDD (Patterson et al. 1986).

Limited numbers of adipose tissue samples were also collected from occupationally exposed individuals and analyzed, primarily for TCDD, during this same time period (Patterson et al. 1986, 1990). Autopsy studies were also conducted on tissue samples from individuals from the general population (Ryan et al. 1985; Graham et al. 1984) and on samples of opportunity from surgical patients (Patterson et al. 1990).

A breakthrough in the use of biological samples to study and characterize exposure came when Patterson et al. (1988) published their method for sensitive analysis of TCDD and related compounds in blood and demonstrated the high correlation of lipid-based blood concentrations to lipid-based concentrations measured in adipose tissue. The availability of a method for analysis and detection of TCDD and related compounds in blood led to the

feasibility of the use of this method to quantify internal exposures in a larger number of potentially exposed and general population individuals and studies.

Such measurements provided exposure quantification that could not be obtained in any other way for any of these populations. For the occupationally and accident-exposed populations, exposures were generally historical, with no empirical data available to quantify external exposures. For persons in the general environment, the sources of exposure are apparently trace levels of dioxins in food or air, but quantification of such exposures was generally impossible at the analytical sensitivities available during the 1980s. Thus, the measured concentrations reflecting long-term accumulation in humans resulting from the extremely slow elimination provided the most sensitive and accurate method for quantifying exposure to dioxins. In addition, from a toxicological perspective, such measures of internal dose are likely to be more relevant to potential adverse effects than external exposure estimates, and therefore of more interest.

Estimates of Human Rates of Elimination

Estimates of the half-life of elimination of TCDD in humans began to be made in the 1980s. Of note, Poiger and Schlatter (1986) reported a half-life of elimination of 5.8 years from a human self-administration experiment with TCDD, and Pirkle et al. (1989) reported an average half-life of 7.1 years based on analysis of serial blood samples taken 5 years apart from 36 Ranch Hand veterans. Ryan et al. (1993) reported estimated half-lives for various chlorinated furan and biphenyl compounds in individuals from the yusho and yucheng poisoning incidents, and Flesch-Janys et al. (1996) estimated half-lives for a number of dioxin and furan compounds based on data from occupationally exposed individuals from Germany. A full review of studies reporting estimated human half-lives of elimination is presented in Milbrath et al. (2009).

In general, these efforts have assumed simple first-order elimination mechanisms and estimated half-lives for elimination on this basis. Similarly, back-extrapolations used in the estimation of cumulative serum lipid TCDD area under the curve (AUC) for occupational cohorts have relied upon the simple first-order elimination rate assumption (Steenland et al. 2001; Flesch-Janys et al. 1998; Ott and Zober 1996).

However, Ryan et al. (1993) noted extreme differences in the estimated rates of elimination of 2,3,4,7,8-pentachlorodibenzofuran among persons from yucheng and yusho, with far more rapid elimination occurring in persons with the most elevated concentrations (also corresponding to measurements taken

closest to the time of exposure). Ryan et al. (1993) discussed a number of possible explanations for these observations, but pointed to the biokinetic modeling work of Carrier (unpublished at the time) as presenting a physiologically plausible and mathematically elegant model that predicted the apparent concentration-dependence of the elimination rate. The Carrier model (Carrier et al. 1995a, 1995b) posited first-order clearance from the liver, with a concentration-dependent induction of hepatic sequestration, consistent with available animal data on distribution of dioxins. The concentration-dependent elimination mechanism resulted in a greater reservoir of available compound for elimination in the liver at higher body concentrations. The consequence of the concentration-dependent hepatic sequestration behavior, in conjunction with first-order elimination from the liver, is an apparently non-linear elimination rate reflected in the adipose/blood tissue compartments.

Biokinetic Issues Relevant to Risk Assessment for Dioxins

The classical risk assessment paradigm relies upon extrapolation from laboratory animal experiments conducted at relatively high doses (or studies of highly exposed human populations) to estimates of tolerable or risk-specific doses for humans. Dose-response assessments from studies demonstrating adverse effects in animals or humans are used to estimate a point of departure corresponding to no observed effect or to a low effect level useful as the basis for extrapolation to lower doses expected to have no significant toxic effects in a genetically diverse population. Classically, dose-response assessments have been based on external doses administered to animals or estimated for human populations. For TCDD and related compounds, a significant disconnect exists between available exposure information in toxicological studies, which usually (but not always) is limited to external administered dose, and available exposure information in humans, which is dominated by measures of lipid concentrations. Understanding of the biokinetic properties of dioxins in humans and laboratory animals is critical to the meaningful integration of data from laboratory animals and humans.

Risk assessment for TCDD and related compounds generally incorporates an additional step: the use of Toxic Equivalency Factors (TEFs), which are estimates of relative potency of polychlorinated dioxin, furan, and biphenyl congeners compared to TCDD. These TEF values are explicitly based on comparisons of potency among congeners on an external dose basis, and allow the summation of exposure to multiple congeners into a single TCDD-equivalent (TEQ) dose (van den Berg et al. 1998; van den Berg et al. 2006). The TEF system is a practical response to some of the challenges of risk assessment for dioxin. In particular, the TEF system provides a tractable

means for conducting risk assessments for real-world mixtures of dioxins and related compounds. In addition, the TEF system addresses the reality that full toxicological characterizations do not exist for most congeners other than TCDD. Thus, estimates of risks due to other compounds (which typically constitute the vast majority of dioxin-like exposures) are made based on the data available for TCDD in conjunction with the relative potency factors provided by the TEF estimates.

Biokinetic issues that arise in quantitative risk assessment for dioxin-like compounds fall into several categories, including selection of relevant target tissue and dose metric for interspecies extrapolation; accounting for differences among congeners in biokinetic behavior; appropriate application of the TEF approach to internal dose measures provided by biomonitoring or analytical data; and issues relevant to the use of human studies for quantitative risk assessment, including appropriate methods for back-extrapolation of exposure estimates for human populations. These issues are discussed briefly below, and are addressed in more detail in the subsequent chapters.

Identification of relevant dose metrics for interspecies extrapolation

Extrapolations conducted in chemical risk assessment often include both inter- and intra-species extrapolation steps. Expected allometric differences in metabolic rates are often accounted for through application of either explicit scaling of doses between species based on bodyweight to the 2/3rd or ¾ power, or through application of uncertainty factors that include components attributable to default assessments of biokinetic differences among species (Dorne and Renwick 2005; Renwick 1991). In practice, this translates to a default assumption that human elimination half-lives for chemical compounds may be three to approximately six times longer than those in typical laboratory species.

However, estimated half-lives for TCDD in humans are approximately 100 times as long as the corresponding estimates for laboratory rats. Ignoring any differences in distribution, this difference in half-life implies that for a given external dose rate, humans will develop internal concentrations approximately 100 times higher than the corresponding internal concentrations in rodents. Recognition of the implications of these interspecies differences led to the proposal that extrapolation between species be conducted on a basis of total body concentration, or "body burden" (DeVito et al. 1995; WHO 1998; JECFA 2001; ECSCF 2001). Body "burdens" in animal studies have been estimated

through the analysis and summation of concentrations in liver, adipose, and sometimes skin tissues, while estimates for human studies have been based on measured or back-extrapolated lipid-adjusted concentrations and assumptions regarding the typical percentage body fat.

While the use of estimated body burden as a metric for interspecies extrapolation and comparison of dioxin exposures is likely to be superior to the use of administered dose, use of this metric does not address all of the questions relevant to selection of appropriate dose metric for interspecies extrapolation. Specifically, relevant target tissue concentrations may differ substantially between species at the same "body burden." In addition, use of "body burden" does not address the question of the time-dependence of the exposure pattern. While humans exposed environmentally have extremely stable concentrations of dioxins over long periods of time, laboratory animals, by virtue of the shorter elimination half-life, may have substantial swings in tissue concentrations for a given average body burden.

Relevant tissue concentrations for estimating exposure-response relationships are affected not only by elimination rate, but also by distribution patterns within the body. As mentioned above, distribution of TCDD and related compounds is strongly influenced by their lipophilicity. In the absence of other factors, TCDD will distribute proportionally to tissues based solely on the relative fat content of those tissues. However, other factors influence distribution as well.

A second major factor controlling the distribution of TCDD is the presence and induction of the cytochrome P450 1A2 (CYP1A2) protein, primarily in the liver. TCDD and other dioxion-like compounds, through binding to the aryl hydrocarbon receptor (AhR), induces the protein and activity levels of CYP1A1 and 1A2. As demonstrated in studies using CYP1A2 knock-out mice, dioxin-like compounds not only induce, but are also bound by, CYP1A2 protein in the liver (Diliberto et al. 1997, 1999). Although only a few congeners were tested in the knock-out mouse model, the relative avidity of induction and binding to CYP1A2 for different congeners can be inferred from data on liver:adipose tissue concentration ratios (DeVito et al. 1998). Based on data from these studies, different congeners have substantially different sequestration behaviors, with maximum liver:adipose tissue concentration ratios ranging from less than 1 for the tested PCB compounds (indicating distribution to the liver primarily based on relative lipid content) to greater than 40 for 4-PeCDF (DeVito et al. 1998). Also as discussed above, this hepatic sequestration demonstrates significant dose-dependency, with increasing sequestration at increasing dose rates (presumably corresponding to increasing levels of CYP1A2 protein) (see, for example, Diliberto et al. 2001). Thus, at higher experimental doses, a majority of the body burden of retained compound in laboratory animals may be resident in the liver, while in humans at

environmental exposure levels, the vast majority of body burden is distributed in lipid throughout the body.

This hepatic sequestration may distort estimated potency of a compound *if* the CYP1A2-bound compound is relatively inactive, or unavailable, for causing dioxin-like responses through binding to the AhR. Because toxic responses of most interest in human populations are not generally hepatic responses, this potential distortion may be quite significant in a risk assessment context. In addition, because the induction of CYP1A2 protein takes some time, proportional distribution to non-hepatic tissues following a bolus dose of dioxins may be greater than observed under steady-state conditions at the same body concentration of dioxin (Hurst et al. 2000a, 2000b; Bell et al. 2007). If the adverse response of interest is non-hepatic, use of bolus dosing may overestimate potency of a compound compared to a steady-state administration protocol or relative to typical human exposure conditions. Finally, induction of CYP1A2 protein occurs in a dose-dependent manner. In most rodent studies, significant hepatic sequestration of TCDD is apparent at most tested doses (see, for example, DeVito et al. 1998; Diliberto et al. 2001). However, the limited available data on the distribution of dioxin-like compounds among human tissues in persons with typical general population levels suggest limited hepatic sequestration at these exposure levels (Kitamura et al. 2001; Iida et al. 1997), although such sequestration was evident in autopsy data from highly exposed yusho patients (Masuda et al. 1985).

Another factor that bears upon estimation of relevant target tissue concentrations between species is the quantity of body fat. Laboratory rodents, particularly younger animals typically used in experimental toxicology, have relatively low body fat content, on the order of 7 to 10 percent of bodyweight. For the dioxin compounds not bound to hepatic CYP1A2, this translates to a relatively small volume of distribution and correspondingly higher non-hepatic tissue concentrations available to induce toxic responses. Comparatively speaking, humans tend to have much larger adipose tissue reserves, with percentages of body fat generally greater (and often much greater) than 20 percent. The consequence of this difference is that for a given "body burden," humans have a much larger volume of distribution than typical laboratory rodents, resulting in lower tissue concentrations for the same "body burden."

Inter-congener differences in biokinetic behavior

The biokinetic properties of the various dioxin, furan, and PCB compounds included as dioxin-like compounds for risk assessment vary substantially.

While all of the compounds are relatively persistent, the degree of persistence varies, with several specific congeners displaying elimination half-lives in humans and animals that are significantly shorter or longer than that of TCDD (Milbrath et al. 2009; Geyer et al. 2002). In addition, congeners vary in their pattern of distribution in the body. As noted above, while some congeners such as 2,3,4,7,8-pentachlorodibenzofuran display extreme hepatic sequestration at elevated dose levels in rodents, other congeners such as 2,3,7,8-tetrachlorodibenzofuran display little or no binding to CYP1A2 and resulting hepatic sequestration (DeVito et al. 1998). These differences in distribution can influence the relative proportion of compound available to extra-hepatic tissues compared to TCDD. Finally, there is some indication that the higher chlorinated, higher molecular weight, compounds may not distribute as freely through body lipid stores as the lower molecular weight compounds (Wittsiepe et al. 2007) and that molecular size may also influence distribution from the liver to the remaining body tissues (Iida et al. 1999).

Application of the TEF approach to internal dose measures

The TEF approach to aggregating exposures to selected PCDD, PCDF, and PCB compounds has proved undeniably useful as a practical tool. While numerous theoretical and detailed experimental efforts have demonstrated potential uncertainties and limitations, the TEF approach works remarkably well in its ability to provide a rough estimate of the toxicity of dioxin mixtures. However, almost from the beginning, the approach has been applied in ways that are inconsistent with the development of the relative potency factors (TEFs). While the TEF estimates were set based on assessments of relative potency calculated on an external, or intake, dose basis, the TEF system has consistently been used to aggregate the concentrations of dioxin-like compounds measured in human biological samples in to TEQ estimates as well. If all of the congeners included in the process had similar biokinetic properties, this application of the TEF approach would not produce significant distortions. However, because of the disparate elimination rates and distribution patterns among the congeners, application of the TEF approach in this way may overemphasize the toxic contributions of some congeners compared to others.

For example, 4-PeCDF has been assigned a relatively high TEF of 0.3 in the most recent WHO TEF evaluation. This relative potency estimate in the TEF framework reflects, in practice, both intrinsic toxicity and the tendency for 4-PeCDF to accumulate to a greater degree than TCDD. However, when applied to measurements of blood or tissue concentration, the relatively high potency estimate may overestimate actual relative potency on a concentration basis,

because the tendency of 4-PeCDF to accumulate is now being doubly emphasized. As a practical matter, these sorts of distortions may be unimportant if estimates of dose-response based on TEQ estimated on a concentration basis are applied to other internal TEQ concentration estimates based on the same relative mix of congeners. However, if the internal TEQ dose estimates are applied across different congeners or to a mixture with a different congener composition, the potential for misleading results becomes greater.

Issues in the use of human studies for quantitative risk assessment

The availability of numerous studies of populations exposed to elevated levels of TCDD and related compounds and the availability of biological measures of exposure for many of those studies has led to attempts to use these data for quantitative risk assessment, particularly for cancer dose-response (see, for example, Steenland et al. 2001). As discussed above, analytical methods allowing the quantification of dioxins in serum samples became available substantially after the end of most 2,4,5-T manufacturing. Thus, measured levels in occupational cohorts are influenced both by the duration and intensity of past exposure as well as by the elimination that occurred after occupational exposure ceased. That is, a given serum concentration could be the result of a very high exposure a long time ago, or a more moderate exposure that occurred more recently, even if the rate of elimination was constant for two individuals. Thus, when measurements are taken years or decades after last exposure, the assessment of relevant exposure metrics for use in risk assessment must consider the timing and intensity of exposure as well as the biokinetics of accumulation and elimination. In addition, when chronic health conditions are the endpoint of interest, some measure of cumulative internal exposure (such as AUC) has been considered to be of more relevance than the measured concentration at any particular point in time.

As discussed above, the typical approach has been to assume simple first-order elimination of TCDD and related compounds in humans, and to use this assumption to back-extrapolate to peak levels at the date of last exposure. Further assumptions regarding workplace exposures can lead to an estimate of a concentration vs. time profile during employment leading up to that peak concentration. However, as discussed above, substantial datasets now suggest that humans, like laboratory rodents, may display concentration-dependent elimination of TCDD and related compounds. The impact of such elimination rate changes may be substantial when measured serum concentrations are back-extrapolated over decades. In addition, interindividual variation in elimination rates at a given concentration can be

substantial. The resulting uncertainty and variations in estimated cumulative exposures may substantially impact a quantitative risk assessment derived based on such estimated exposures.

These issues are avoided when risk assessments can be conducted by relying on studies in which serum concentrations are at steady state and responses are measured coincident with the measured serum concentrations. A growing body of epidemiologic studies involves examination of endpoints of interest other than cancer, including potential subtle developmental and endocrine system effects (see, for example, Koopman-Esseboom et al. 1994 and related studies; Maervoet et al. 2007; Eskenazi et al. 2003, Bacarelli et al. 2008 and related studies on the Seveso population; Wilhelm et al. 2008). In such studies, measurement of serum concentrations of TCDD and related compounds occurs coincident in time with the occurrence of the endpoint of interest, and therefore, no back-extrapolations are needed. A number of challenges are posed for risk assessors by such studies, including the need to distinguish causal from non-causal associations, potential confounding introduced by the practice of lipid-adjusting measured serum concentrations of dioxins, and difficulty in using results from certain epidemiological study designs in a dose-response framework for application to assessment of general population risks.

Investigations and Applications of Biokinetics in the Risk Assessment of Dioxins

The chapters in this dissertation address many of the topics and issues described above. The first several chapters address topics related to the use of biokinetic data and internal dose evaluations in risk assessments of dioxin-like compounds based on toxicological data.

- Chapter 2 presents an early analysis using cumulative internal dose estimates derived from simple biokinetic models applied to occupational and accidentally exposed cohorts to compare the apparent dose-response for carcinogenesis from occupational cohorts to that from animal bioassay data. This analysis extended the developing concept of "body burden" as a dose metric to estimation of time-varying serum lipid concentrations in occupational cohorts and proposed a variety of considerations for selection of dose metrics and interspecies comparisons of dioxin toxicity.

- Chapter 3 discusses a variety of biokinetic and dose metric issues in the context of risk assessment for potential developmental effects of dioxins. This chapter explores in some detail the impact of inter-

congener differences on the extrapolation of data on the reproductive and developmental toxicity of TCDD to other congeners and to human exposures.

- Chapter 4 uses the tissue concentration data from the recent National Toxicology Program (NTP) long term carcinogenesis bioassays of TCDD, 4-PeCDF, PCB 126, and the TEQ mixture of the three (NTP 2006a, 2006b, 2006c, 2006d) to examine the relative carcinogenic potency of the three compounds on an internal dose basis. The relative potency estimates resulting from this analysis are compared to the Toxic Equivalency Factors (TEFs) used for dioxin risk assessment on an intake dose basis.

- Chapter 5 implements the Carrier et al. concentration-dependent biokinetic model framework for three dioxin-like compounds using the NTP bioassay tissue concentration data. This analysis demonstrates similarities and differences in the distribution and elimination behavior of these compounds and provides modeling tools that can be useful in estimation of internal doses in other laboratory animal studies that do not include a tissue analysis component.

The next section of the dissertation includes chapters that address the incorporation of biokinetics, internal dose concepts, and biomonitoring data in the evaluation and use of epidemiological data for risk assessment of environmental exposures to dioxins.

- Chapter 6 presents the modification and application of the Carrier concentration-dependent biokinetic model for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) based on the rich datasets from Seveso and the Austrian poisoning patients (Abraham et al. 2002; Geusau et al. 2002). In this effort, recent data demonstrating passive elimination mechanisms for dioxins were incorporated into the Carrier et al. (1995) model structure, and the overall TCDD model parameterized based on the detailed serial sampling datasets from highly exposed humans.

- Chapter 7 evaluates the impact of use of the concentration-dependent model compared to a simple first-order model on the cumulative TCDD exposure estimates for the National Institute of Occupational Safety and Health (NIOSH) 2,4,5-T manufacturing cohort.

- Chapter 8 illustrates the potential impact of the alternative biokinetic approach on the assessment of cancer mortality patterns and potency estimates derived from the NIOSH cohort. The full mortality dataset from the NIOSH study was re-analyzed using the exposure

reconstructions resulting from the concentration-dependent model. Cancer potency estimates were compared to previous analyses derived from use of a simple first-order kinetic model.

- Chapter 9 illustrates the use of benchmark dose modeling with selected biomonitoring-based epidemiological data sets to derive a quantitative, margin-of-exposure framework for assessing human health risks of dioxins at environmental exposure levels. Numerous issues inherent in exposure quantification, dose-response assessment, and interpretation of the results in the context of background exposures are addressed.

Finally, Chapter 10 presents a discussion of the major findings of Chapters 2 through 9 in the context of recommendations for scientifically sound methods for risk assessment of dioxins that account for the detailed biokinetic understanding available for these compounds. Remaining data needs in this area are identified.

References

Abraham K, Geusau A, Tosun Y, Helge H, Bauer S, Brockmoller J. 2002. Severe 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) intoxication: insights into the measurement of hepatic cytochrome P450 1A2 induction. Clin Pharmacol Ther 72(2):163-174.

Baccarelli A, Giacomini SM, Corbetta C, Landi MT, Bonzini M, Consonni D, Grillo P, Patterson DG, Pesatori AC, Bertazzi PA. 2008. Neonatal thyroid function in Seveso 25 years after maternal exposure to dioxin. PLoS Med 5(7):e161.

Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S. 2007. Relationships between tissue levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), mRNAs, and toxicity in the developing male Wistar(Han) rat. Toxicol Sci 99(2):591-604.

Bertazzi PA, Bernucci I, Brambilla G, Consonni D, Pesatori AC. 1998. The Seveso studies on early and long-term effects of dioxin exposure: a review. Environ Health Perspect 106 Suppl 2:625-633.

Carrier G, Brunet RC, Brodeur J. 1995. Modeling of the toxicokinetics of polychlorinated dibenzo-p-dioxins and dibenzofurans in mammalians, including humans. I. Nonlinear distribution of PCDD/PCDF body burden between liver and adipose tissues. Toxicol Appl Pharmacol 131(2):253-266.

Carrier G, Brunet RC, Brodeur J. 1995. Modeling of the toxicokinetics of polychlorinated dibenzo-p-dioxins and dibenzofurans in mammalians, including humans. II. Kinetics of absorption and disposition of PCDDs/PCDFs. Toxicol Appl Pharmacol 131(2):267-276.

Centers for Disease Control (CDC). 1988. Serum 2,3,7,8-tetrachlorodibenzo-p-dioxin levels in US Army Vietnam-era veterans. The Centers for Disease Control Veterans Health Studies. J Am Med Assoc 260(9):1249-1254.

Courtney KD, Moore JA. 1971. Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 20(3):396-403.

DeVito MJ, Birnbaum LS, Farland WH, Gasiewicz TA. 1995. Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. Environ Health Perspect 103(9):820-831.

DeVito MJ, Ross DG, Dupuy AE, Jr., Ferrario J, McDaniel D, Birnbaum LS. 1998. Dose-response relationships for disposition and hepatic sequestration of polyhalogenated dibenzo-p-dioxins, dibenzofurans, and biphenyls following subchronic treatment in mice. Toxicol Sci 46(2):223-234.

Diliberto JJ, Burgin D, Birnbaum LS. 1997. Role of CYP1A2 in hepatic sequestration of dioxin: studies using CYP1A2 knock-out mice. Biochem Biophys Res Commun 236(2):431-433.

Diliberto JJ, Burgin DE, Birnbaum LS. 1999. Effects of CYP1A2 on disposition of 2,3,7, 8-tetrachlorodibenzo-p-dioxin, 2,3,4,7,8-pentachlorodibenzofuran, and 2,2',4,4',5,5'-hexachlorobiphenyl in CYP1A2 knockout and parental (C57BL/6N and 129/Sv) strains of mice. Toxicol Appl Pharmacol 159(1):52-64.

Diliberto JJ, DeVito MJ, Ross DG, Birnbaum LS. 2001. Subchronic Exposure of [3H]- 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in female B6C3F1 mice: relationship of steady-state levels to disposition and metabolism. Toxicol Sci 61(2):241-255.

Dorne JL, Renwick AG. 2005. The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. Toxicol Sci 86(1):20-26.

Eskenazi B, Mocarelli P, Warner M, Chee WY, Gerthoux PM, Samuels S, Needham LL, Patterson DG, Jr. 2003. Maternal serum dioxin levels and birth outcomes in women of Seveso, Italy. Environ Health Perspect 111(7):947-953.

European Commission Scientific Committee on Foods (ECSCF). 2001. Opinion of the Scientific Committee on Food on the risk assessment of dioxins and dioxin-like PCBs in food. Update based on new scientific information available since the adoption of the SCF opinion of 22nd November 2000. Rep. CS/CNTM/DIOXIN/20 final. http://europa.eu.int/comm/food/fs/sc/scf/out90_en.pdf CS/CNTM/DIOXIN/20 final. Brussels, Belgium: European Commisssion.

Flesch-Janys D, Becher H, Gurn P, Jung D, Konietzko J, Manz A, Papke O. 1996. Elimination of polychlorinated dibenzo-p-dioxins and dibenzofurans in occupationally exposed persons. J Toxicol Environ Health 47(4):363-378.

Flesch-Janys D, Steindorf K, Gurn P, Becher H. 1998. Estimation of the cumulated exposure to polychlorinated dibenzo-p-dioxins/furans and standardized mortality ratio analysis of cancer mortality by dose in an occupationally exposed cohort. Environ Health Perspect 106 Suppl 2:655-662.

Fries GF, Marrow GS. 1975. Retention and excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin by rats. J Agric Food Chem 23(2):265-269.

Geusau A, Schmaldienst S, Derfler K, Papke O, Abraham K. 2002. Severe 2,3,7,8-tetrachlorodibenzo- p-dioxin (TCDD) intoxication: kinetics and trials to enhance elimination in two patients. Arch Toxicol 76(5-6):316-325.

Geyer HJ, Schramm KW, Feicht EA, Behechti A, Steinberg C, Bruggemann R, Poiger H, Henkelmann B, Kettrup A. 2002. Half-lives of tetra-, penta-, hexa-, hepta-, and octachlorodibenzo-p-dioxin in rats, monkeys, and humans--a critical review. Chemosphere 48(6):631-644.

Graham M, Hileman FD, Orth RG, Wendling JM, Wilson JD. 1986. Chlorocarbons in adipose tissue from a Missouri population. Chemosphere 15:1595-1600.

Hurst CH, DeVito MJ, Birnbaum LS. 2000. Tissue disposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in maternal and developing long-evans rats following subchronic exposure. Toxicol Sci 57(2):275-283.

Hurst CH, DeVito MJ, Setzer RW, Birnbaum LS. 2000. Acute administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in pregnant Long Evans rats: association of measured tissue concentrations with developmental effects. Toxicol Sci 53(2):411-420.

Iida T, Hirakawa H, Matsueda T, Nagayama J, Nagata T. 1999. Polychlorinated dibenzo-p-dioxins and related compounds: correlations of levels in human tissues and in blood. Chemosphere 38(12):2767-2774.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2001. Fifty-seventh meeting Rome, 5-14 June 2001. Summary and conclusions. http://www.who.int/ipcs/food/jecfa/summaries/en/summary_57.pdf

Kitamura K, Nagao M, Yamada T, Sunaga M, Hata J, Watanabe S. 2001. Dioxins in bile in relation to those in the human liver and blood. J Toxicol Sci 26(5):327-336.

Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van der Paauw CG, Tuinstra LG, Brouwer A, Sauer PJ. 1994. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res 36(4):468-473.

Maervoet J, Vermeir G, Covaci A, Van Larebeke N, Koppen G, Schoeters G, Nelen V, Baeyens W, Schepens P, Viaene MK. 2007. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. Environ Health Perspect 115(12):1780-1786.

Masuda Y, Kuroki H, Haraguchi K, Nagayama J. 1985. PCB and PCDF congeners in the blood and tissues of yusho and yu-cheng patients. Environ Health Perspect 59:53-58.

Milbrath MO, Wenger Y, Chang CW, Emond C, Garabrant D, Gillespie BW, Jolliet O. 2009. Apparent half-lives of dioxins, furans, and polychlorinated biphenyls as a function of age, body fat, smoking status, and breast-feeding. Environ Health Perspect 117(3):417-425.

National Toxicology Program (NTP). 2006a. NTP technical report on the toxicology and carcinogenesis studies of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (CAS No. 1746-01-6) in female Harlan Sprague-Dawley rats (Gavage Studies). Natl Toxicol Program Tech Rep Ser(521):4-232.

National Toxicology Program (NTP). 2006b. NTP Toxicology and Carcinogenesis Studies of 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4) in Female Harlan Sprague-Dawley Rats (Gavage Studies). Natl Toxicol Program Tech Rep Ser(525):1-198.

National Toxicology Program (NTP). 2006c. NTP toxicology and carcinogenesis studies of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in female Harlan Sprague-Dawley rats (Gavage Studies). Natl Toxicol Program Tech Rep Ser(520):4-246.

National Toxicology Program (NTP). 2006d. NTP Toxicology and Carcinogenesis Studies of a Mixture of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) (CAS No. 1746-01-6), 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4), and 3,3',4,4',5-Pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in Female Harlan Sprague-Dawley Rats (Gavage Studies). Natl Toxicol Program Tech Rep Ser(526):1-180.

Ott MG, Zober A. 1996. Cause specific mortality and cancer incidence among employees exposed to 2,3,7,8-TCDD after a 1953 reactor accident. Occup Environ Med 53(9):606-612.

Patterson DG, Jr., Hoffman RE, Needham LL, Roberts DW, Bagby JR, Pirkle JL, Falk H, Sampson EJ, Houk VN. 1986. 2,3,7,8-Tetrachlorodibenzo-p-dioxin levels in adipose tissue of exposed and control persons in Missouri. An interim report. Jama 256(19):2683-2686.

Patterson DG, Jr., Needham LL, Pirkle JL, Roberts DW, Bagby J, Garrett WA, Andrews JS, Jr., Falk H, Bernert JT, Sampson EJ, Houk VN. 1988. Correlation between serum and adipose tissue levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in 50 persons from Missouri. Arch Environ Contam Toxicol 17(2):139-143.

Patterson DG, Needham LL, Andrews JS, Jr. 1990. Polychlorinated dibenzo-p-dioxins. In: Clinical Management of Poisoning and Drug Overdose (Haddad LM, Winchester JF, eds). Philadelphia:W. B. Saunders, 528-537.

Piper WN, Rose JQ, Gehring PJ. 1973. Excretion and tissue distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Environ Health Perspect 5:241-244.

Pirkle JL, Wolfe WH, Patterson DG, Needham LL, Michalek JE, Miner JC, Peterson MR, Phillips DL. 1989. Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Vietnam Veterans of Operation Ranch Hand. J Toxicol Environ Health 27(2):165-171.

Poiger H, Schlatter C. 1986. Pharmacokinetics of 2,3,7,8-TCDD in man. Chemosphere 15:1489-1494.

Renwick, A.G. 1991. Safety factors and establishment of acceptable daily intakes. Food Add. Contam. 8:135 -.149.

Ryan JJ, Levesque D, Panopio LG, Sun WF, Masuda Y, Kuroki H. 1993. Elimination of polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) from human blood in the Yusho and Yu-Cheng rice oil poisonings. Arch Environ Contam Toxicol 24(4):504-512.

Ryan JJ, Schecter A, Lizotte R, Sun WF, Miller L. 1985. Tissue distribution of dioxins and furans in humans from the general population. Chemosphere 14(6/7):929-932.

Steenland K, Deddens J, Piacitelli L. 2001. Risk assessment for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) based on an epidemiologic study. Am J Epidemiol 154(5):451-458.

Suskind RR, Hertzberg VS. 1984. Human health effects of 2,4,5-T and its toxic contaminants. JAMA 251(18):2372-2380.

Sweeney MH, Mocarelli P. 2000. Human health effects after exposure to 2,3,7,8-TCDD. Food Addit Contam 17(4):303-316.

van den Berg M, Birnbaum L, Bosveld AT, Brunstrom B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FX, Liem AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 106(12):775-792.

van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Peterson RE. 2006. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 93(2):223-241.

WHO (World Health Organization). 1998. WHO (World Health Organization). 1998. Executive summary. Assessment of the health risks of dioxins: re-evaluation of the Tolerable Daily Intake (TDI). WHO Consultation, May 25-29, 1998, Geneva, Switzerland. Available: http://www.who.int/ipcs/publications/en/exe-sum-final.pdf [accessed May 15, 2008].

Wilhelm M, Wittsiepe J, Lemm F, Ranft U, Kramer U, Furst P, Roseler SC, Greshake M, Imohl M, Eberwein G, Rauchfuss K, Kraft M, Winneke G. 2007. The Duisburg birth cohort study: Influence of the prenatal exposure to PCDD/Fs and dioxin-like PCBs on thyroid hormone status in

newborns and neurodevelopment of infants until the age of 24 months. Mutat Res.
Wittsiepe J, Furst P, Schrey P, Lemm F, Kraft M, Eberwein G, Winneke G, Wilhelm M. 2007. PCDD/F and dioxin-like PCB in human blood and milk from German mothers. Chemosphere 67(9):S286-294.

Chapter 2: Relative Susceptibility of Animals and Humans to the Cancer Hazard Posed by 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Using Internal Measures of Dose

Aylward, L.L., S.M. Hays, N.J. Karch, and D.J. Paustenbach. 1996. Environ. Sci. Technol. 30(12): 3534–3543.

Abstract

An analysis of the cancer dose-response relationship for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in humans and animals was performed based on measured tissue and serum lipid TCDD concentrations and dosimetrics other than administered dose. Basic pharmacologic principles indicate that measures of internal dose such as AUC should be used to describe the dose-response relationship in cancer risk assessments of TCDD and other highly persistent compounds. The TCDD-related liver tumor response in female rats (1, 2) was compared to that in humans (respiratory tract cancer rates in Fingerhut et al.; 3). Three measures of lifetime dose were used: serum lipid TCDD area-under-the-curve (AUC), peak serum lipid concentration (Cpeak), and average serum lipid concentration (Cavg). Serum lipid TCDD concentration vs time profiles for the ratwere constructed assuming first-order elimination and a half-life of 25 days. Concentration vs time profiles for humans were estimated based on measured serum lipid TCDD concentrations and known dates of first and last exposure, assuming a 7.5-year half-life and first-order elimination. A comparison of rat and human responses indicated that, using all three of these dosimetrics, humans are much less sensitive than rats to the carcinogenic effects of TCDD. For example, at the peak concentration measured in rats exposed to 0.1 μg $kg^{-1}d^{-1}$ for 2 years, the human cancer response was more than 9-fold lower than that observed in rats. At comparable average lifetime serum lipid TCDD concentrations, the human cancer response was about 4-fold lower than observed in rats. When AUC was used as the dosimetric, the highest rat dose group (0.1 μg $kg^{-1}d^{-1}$) had a 9-fold greater response at approximately $1/10^{th}$ the AUC of the most highly exposed human group; that is, the rat dose-response was more than 90-fold steeper than the human dose-response. Interestingly, regardless of the dosimetric chosen, the cancer rate in humans in the NIOSH cohort, if due to TCDD, is almost completely insensitive to dose. Our analysis indicates that human exposure to background levels of TCDD (about 5 ppt serum lipid concentration) should not pose an incremental cancer risk.

Introduction

Experimental animals vary widely in sensitivity to the toxic effects of TCDD; for example, the LD50 varies over more than 3 orders of magnitude in rodents (4). A number of scientists have suggested that humans appear to be less sensitive than most animals to the acute and chronic toxic effects of TCDD (5-7). Others believe that humans may not be less sensitive than experimental animals, and the EPA's recent reassessment concluded that "average humans can be reasonably assumed to be of average sensitivity [compared to animals]

for various effects" (8). Until recently, the discussion about relative susceptibility in the regulatory arena has focused primarily on daily dose (μg $kg^{-1}d^{-1}$) rather than on internal dosimetrics like lifetime area-under-the-curve (AUC), lifetime peak or average body burden, peak or average blood concentration, etc.

The evaluation of the relative sensitivity of humans compared to laboratory animals depends upon identifying a biologically relevant dose and a specific response in both humans and in laboratory animals. An increased risk of cancer has been the primary endpoint of concern associated with exposure to TCDD based on data from chronic bioassays. Data from highly exposed human populations now provide at least preliminary data for evaluating the relative sensitivity of humans to TCDD-induced cancer.

The relationship between tissue dose and the likelihood of cancer depends upon the postulated mode of action (9). For example, forDNA-reactive chemicals, the rate of adduct formation or other changes in DNA and, indirectly, the mutation rate probably are a direct function of the concentration of the toxicant in the tissue of interest. Similarly, the likelihood of tumor development is probably a function of the total number of these adducts or mutations formed over time, and therefore a function of the AUC of the blood/tissue concentration of the chemical vs time. Due to the many factors influencing tumor development, this relationship is probably not a simple linear one.

Tissue dose-response relationships can also be postulated for non-DNA-reactive chemicals. For example, for a chemical that produces tumors through repeated cell cytotoxicity, a combination of peak concentration of chemical and time of elevated concentration in blood and tissues, or AUC, is likely to be the relevant dosimetric (9). The appropriateness of AUC for understanding the effects of drugs and chemicals that act through a receptor-mediated mechanism has been noted in pharmacology texts (10-13). In addition, AUC is a fundamental dosimetric used in many current physiologically based pharmacokinetic models for xenobiotics (14-16) and to evaluate the proper dosing regimen for pharmaceuticals (11-13).

The primary mode of action for TCDD-induced carcinogenesis is generally thought to be due to its capacity to promote tumors rather than initiate them (17). TCDD is believed to act through binding to the Ah receptor; the ligand-receptor complex then interacts with DNA, probably with the involvement of other proteins. The exact mechanism of promotion is a matter of investigation and debate, but current theories using the two-stage cancer model paradigm postulate that TCDD promotes tumors through increased cell replication, perhaps with selective enhancement of replication in preneoplastic tissue (9, 17, 18). Increased cell replication may occur through alteration of protein

growth regulatory products, and as for other promotional mechanisms, the eventual development of tumors probably depends upon a sustained tissue dose of sufficient level to continually alter expression of these growth factors. Thus, both tissue concentration (which is directly related to concentration of the ligand-receptor complex) and time at a certain tissue concentration are almost certainly the key factors in predicting the magnitude of the cancer risk due to TCDD. Therefore, AUC dosimetry (perhaps incorporating a threshold to reflect a minimum concentration for gene activation) should be appropriate for predicting the likelihood of a carcinogenic response for TCDD (19).

AUC, with or without a threshold, should be superior to daily dose (mg kg^{-1} day^{-1}) as a measure of the biologically relevant dose of TCDD since daily dose fails to reflect the long period of biologically relevant exposure associated with even short-term, acute dosing episodes due to TCDD's long elimination half-life in humans. Daily dose also fails because it does not account for species differences in elimination half-life. On the other hand, for chemicals with a short biological half-life, it is not surprising that typical dosimetrics like daily dose (mg kg^{-1} day^{-1}) provide an adequate surrogate for tissue concentration(16). However, in the case of TCDD and other highly lipophilic, persistent chemicals, daily dose is too simplistic a dosimetric to capture the key pharmacokinetic elements that are likely to dictate human risk. For example, radioactivity associated with a single labeled dose of 0.0001 µg TCDD was measured and followed in a human volunteer for several years (20), resulting in an estimate of the elimination half life of about 7 years. In contrast, a single 1 mg dose of trichloroethylene can be measured in humans for less than 24 h (21). Thus, dose-response assessments for toxic effects including cancer that rely upon dosimetrics such as lifetime average daily dose (LADD) can be highly misleading.

TCDD is present in the environment and in foods at extremely low levels and has been present in the workplace as a contaminant rather than as a product. In general, estimates of human intakes of TCDD from the diet have not been precise, and because dermal and inhalation exposures have been impractical to measure in the occupational setting, uptake by humans in the occupational setting has not been quantified. However, because TCDD can now be measured in blood at parts per quadrillion (ppq) levels and because of the long half-life of TCDD in humans, exposures that occurred 40 or more years ago can now be estimated with reasonable accuracy. For example, Scheuplein and Bowers (*22*) recently used blood sampling data for workers from various industrial cohorts in conjunction with existing Food and Drug Administration (FDA) cancer slope factors and estimates of low-dose tissue concentrations in rats to examine the question of whether exposures in various human cohorts were sufficient to have been likely to lead to an observable increase in human cancers. An explicit assumption in their analysis was that humans and rats are equally susceptible to tumor development on a tissue dose basis. They

concluded that only the study of the National Institute of Occupational Safety and Health (NIOSH) cohort (*3*) had sufficient statistical power to detect the magnitude of cancer response that was likely to have resulted from the estimated exposures.

The work described here attempts to answer a different question than that addressed by Scheuplein and Bowers. Specifically, we asked "What is the relative susceptibility of humans and rats to the cancer risk posed by exposure to TCDD?". We evaluated this question using several internal dose measures (AUC, C_{peak}, and C_{avg}) for both rats and humans. Our primary goal was to evaluate whether humans are as sensitive as rats to the cancer hazard posed by TCDD.

Approach

Study Selection. A comparison of the relative susceptibility of laboratory animals and humans requires studies in both humans and animals that have data on both dose and response. Cancer was the only response evaluated for this paper. Several published studies were evaluated to identify those that provide data on both dose and response. Those that had data on both administered dose and internal dose (in this case, some measurement of blood lipid or adipose tissue TCDD levels) were given highest priority.

The rat bioassay conducted by Kociba et al. (1) is the most widely used and generally accepted animal study of the cancer hazard posed by TCDD. It provides both administered dose (0.001, 0.01, and 0.1 μg $kg^{-1}day^{-1}$) and data on liver and adipose tissue concentrations of TCDD at the end of a 2-year study period; none of the other animal bioassays provide data on tissue levels. In this bioassay, tumors of the liver (female only), lung, hard palate/nasal turbinates, and tongue were elevated while tumors of the mammary gland, uterus, pituitary, pancreas, and adrenal gland were decreased. The liver tissue pathology has been re-evaluated twice, first by Squire (EPA; 23) and then by Goodman and Sauer (2). The latest re-evaluation was prompted by evolving interpretations and reclassification of rat liver tissue lesions and reflects current histopathologic criteria and classification for evaluating liver tumors in rats. The dose-response information using the most recent tumor classification scheme (2) was used for our analysis (Table 1).

Table 1: Liver Tumor Counts and TCDD Tissue Concentrations in Rats Given TCDD for 2 Years (1)

Dose μg kg^{-1}d^{-1}	Terminal TCDD tissue concn (ppt)		Total liver tumors	
	Adipose	Liver	Original pathology	Goodman and Sauer (2)
0	ND	ND	9/86	2/86
0.001	540	540	3/50	1/50
0.01	1 700	5 100	18/50	9/50
0.1	8 100	24 000	34/48	18/45

ND- Not determined

The potential cancer response to TCDD in humans has been evaluated in studies of herbicide sprayer populations (24), case control studies for various rare tumors (24), Vietnam veterans who had potential exposure to TCDD through exposure to Agent Orange (25), industrial populations exposed through accidents or in herbicide manufacture (3, 26-28), and residential populations exposed through accidental contamination (29, 30). Of these, only a few of the more recent studies contain exposure data in the form of measurements of serum lipid concentration of TCDD for some members of the population (Table 2). The most complete and useful data are available for the Ranch Hand and NIOSH cohorts. Of these two, the NIOSH cohort is larger, and it has greater epidemiologic power to detect any increased incidence of cancer. Exposure levels appear to have been greater for a majority of the NIOSH cohort than for the Ranch Hand cohort. To date, no increased cancer mortality has been observed in the Ranch Hand cohort (25). Finally, the NIOSH database contains additional informationon the dates of first and last exposure as well as the date of serum measurement. These data, which were obtained from NIOSH, allow one to construct an estimated concentration-time profile for each of the 253 persons for whom serum TCDD measurements were obtained.

Dosimetrics Reconstruction. *Rats*. The concentration vs time profile for TCDD in the adipose tissue of the rats in the Kociba et al. bioassay (1) was represented as a simple accumulation whose shape is described

$$C(t) = \frac{D}{Vk}(1 - e^{-kt}) \qquad (1)$$

Where D is the dose (ng/day), V is the volume of distribution in the body (L or kg), and k is the first-order elimination rate constant (day^{-1}). The half-life of TCDD in the Sprague-Dawley rat is approximately 25 days (31). Using the measured adipose tissue concentration at the end of the study as an approximation of the steady-state serum lipid concentration (32), the lifetime concentration vs time profile in rats can be characterized (Figure 1, Table 3).

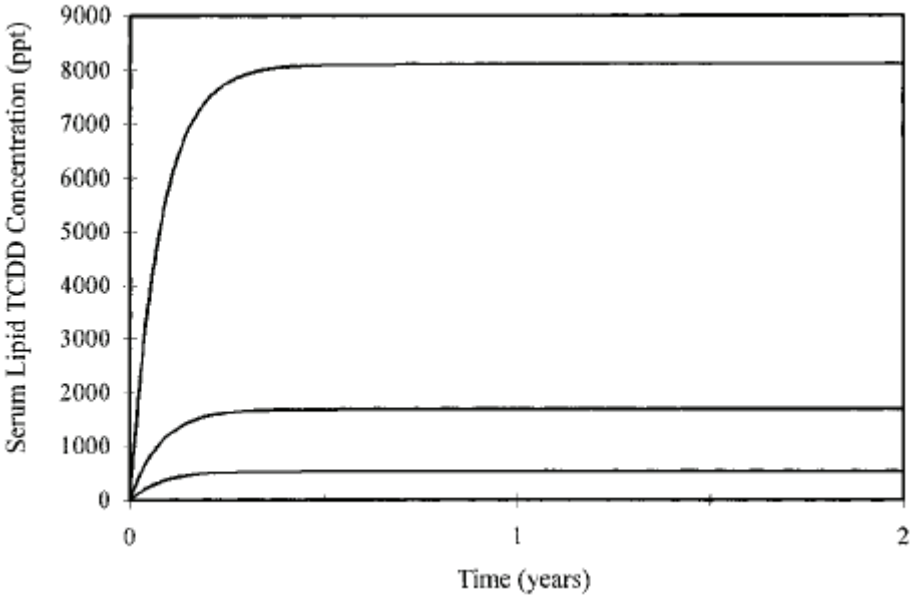


Figure 1: Estimated concentration-time profiles of the serum lipid TCDD concentrations in the three dose groups in the Kociba et al. bioassay (1) (1, 10, and 100 ng kg-1 day-1 in diet) based on reported concentrations in adipose tissue at terminal sacrifice and using a first-order half-life of 25 days (24). Steady state is reached approximately 4 months after dosing begins.

TABLE 2: Human Cancer Studies - TCDD Exposure and Cancer Response Data

Study/subcohort	no. in group	no. sampled	mean TCDD (ppt)	median TCDD (ppt)	range TCDD (ppt)	stat. sig. findings SMR (95% CI)
NIOSH (3)						
full cohort	5172	253	233	76	2-3400	all cancers combined: SMR =115 (102-130); unspecified sites: SMR) 162 (104-241)
<1 yr exposure			69	24		no epidemiologic analysis available
>1 yr exposure			418	231		no epidemiologic analysis available
<1 yr exposure and >20 yr latency	1516	81	78			none
>1 yr exposure and >20 yr latency	1520	95	462			all cancers: SMR) 146 (121-176)
Zober (27)						
C1[a]	69	10		24.5		none
C2	84	7		9.5		other/unspecified site: SMR) 321 (90% CI) 127-675)
C3	94	11		8.4		none
chloracne or erythema	127	16		15		none in full group; all cancers were elevated in subgroup with 20 yr latency SMR) 201 (90% CI 122-315); excess due to borderline nonsig. excess in lung and excess in colorectal
no chloracne	(120?)	12		5.8		no data reported
Manz (26)						
high	459	37	296 (SD=479)	137		group with employment duration >20 yrs (n = 49) all cancers: SMR= 2.54 (1.10-5.00) alternative analysis, subgroup entering workforce before 1954 (n= 96) all cancers: SMR=2.11 (1.25-3.34); excess largely due to lung
medium + low	689	11	83 (SD=73)	60		nonsig. elevation in all cancers in >20 yr subcohort (n=69), SMR = 1.54
Ranch Hand (25)						
full cohort		866		12.8	0-617.8	no elevation in malignant neoplasms of all sites or any site
flying officers		300		7.9	0-42.6	no elevation in malignant neoplasms of all sites or any site
nonflying officers		19		6.7	3.1-24.9	no elevation in malignant neoplasms of all sites or any site
flying enlisted		148		18.1	0-195.5	no elevation in malignant neoplasms of all sites or any site
nonflying enlisted		399		24.0	0-617.8	no elevation in malignant neoplasms of all sites or any site

a This study grouped and analyzed the subjects in two ways, with exposure classification by job title and with exposure classification as determined by presence or absence of chloracne (27).

Table 3: Calculated AUC, C_{peak}, and C_{avg} Serum TCDD for Rats in the Kociba et al. Bioassay (1)

Administered dose ($\mu g\ kg^{-1}d^{-1}$)	Terminal measured adipose TCDD (ppt)	Calculated exposures		
		AUC (ppt*yr)	C_{avg} (ppt)	C_{peak} (ppt)
0	ND[a]	ND	ND	ND
0.001	540	1 038	519	540
0.01	1 700	3 268	1 634	1 700
0.1	8 100	15 572	7 786	8 100

[a] ND- Not determined

Humans. Scheuplein and Bowers (22) constructed approximate concentration vs time profiles for persons in the NIOSH cohort using a three-part curve. In our analysis we used the same approach, dividing the concentration vs time curve into three parts (time before, during, and after occupational exposure; see Figure 2). Construction of the complete curve required an assumption about elimination half-life and five pieces of information: date of birth, measured serum concentration, date of measurement, date of first exposure, and date of last exposure. These data are available from the NIOSH database for each person with a measured serum concentration. The curve was constructed using a three-step process. First, the measured serum lipid level was back-calculated to a peak concentration (C_{peak}) (assumed to occur on the date of last exposure) using the date of serum measurement (which occurred after the last date of occupational exposure):

$$C_{peak} = \frac{C_{meas}}{e^{-k\Delta t}} \qquad (2)$$

where C_{meas} is the measured serum lipid TCDD concentration (ppt), k is the first-order rate constant for elimination ($year^{-1}$), and Δt is the time in years between last exposure and time of serum measurement. Second, the concentration-time curve over the period of occupational exposure was estimated using eq 1 and assuming a constant infusion rate, so that the peak concentration on day of last exposure corresponds to the peak concentration back-calculated in the first step. Finally, a constant 5 ppt serum lipid concentration was assumed for the years prior to first industrial exposure (Figure 2).

This procedure assumes a constant rate of exposure during the period of occupational exposure. Although the actual oral, dermal, and inhalation uptake of TCDD may have varied enormously on a day-to-day or week-to-week basis, the shape of the overall concentration-time curve is virtually insensitive to fluctuations in intake levels (22) due to the extremely slow excretion of TCDD.

During periods of minimum or no intake, the circulating concentration of TCDD remains virtually unchanged. Thus, the assumption of constant intake over the entire period of employment leads to a reasonable approximation of the overall concentration-time relationship. The half-life for TCDD elimination in humans has been determined for the Ranch Hand cohort (33), for industrial workers in Germany (34), in a scientist who voluntarily ingested TCDD (20), and in the Seveso population (35). Mean or median values for biologic half-life in humans from these studies range from 5.2 to 9.7 years. An intermediate value of 7.5 years was used for our analysis.

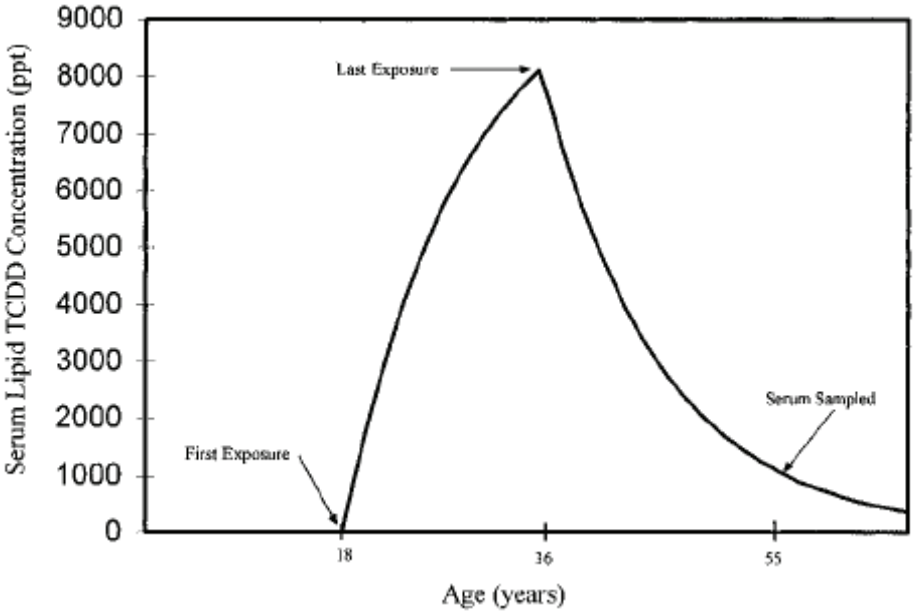


Figure 2: Reconstructed concentration-time profile of the serum lipid TCDD concentration for a highly exposed person from the NIOSH cohort, assuming a first-order elimination half-life of 7.5 years. The time profile shows that steady state is not reached even after many years of exposure. For this individual, exposure to the TCDD contaminated process began at the age of 18 and ceased at the age of 36.5. Serum lipid TCDD concentration was measured at age 55. Serum lipid concentration before industrial exposure was assumed to be 5 ppt. This exposure profile yields an estimated peak serum lipid concentration (Cpeak) of 8269 ppt, an average serum lipid concentration (Cavg) of 2763 ppt, and an integrated serum lipid area under-the-curve (AUC) of 171 076 ppt*yr.

Once the serum lipid TCDD concentration vs time curve was developed for each member of the cohort with measured TCDD levels, measures of lifetime internal dose were calculated using this curve. Dosimetrics chosen for this analysis were all derived from the AUC. Since AUC is heavily influenced by the lifetimes of the two species, rat and human, we also calculated the lifetime average serum lipid concentration (C_{avg}) through the end of the followup period of the Fingerhut et al. (3) study. In order to avoid overestimating exposure levels among those with low measured serum levels, persons with measured serum lipid levels below 10 ppt at the time of measurement (approximately 1987) were not assumed to have experienced any excess occupational exposures during their work years; their serum levels were assumed to have been constant at the measured level throughout their employment period.

Response Measures. We elected to use an "extra risk" formulation for evaluating and comparing the response in the rat and human populations:

$$ER = \frac{P(d) - P(0)}{1 - P(0)} \times 100 \qquad (3)$$

where P(d) and P(0) are the fraction responding at a given dose, d, and zero dose, respectively. This metric is widely used in risk assessment for evaluation of quantal data and allows comparison of responses when the background rates are different (as in this case, for rat liver tumors and human lung and bronchus cancers) (36).

Humans. In their study of herbicide manufacturers, Fingerhut et al. (3) reported standard mortality ratios (SMRs) for individual causes of death. The SMR is the ratio of the observed number of deaths to the expected number of deaths from a given cause based on the age distribution of the population. SMRs were reported for the cohort as a whole and then for the portion of the cohort that had at least 20 years latency (defined as time since first occupational exposure) and, thus, would be more likely to exhibit a cancer response since sufficient time for cancer development would have passed. Fingerhut et al. (3) then divided the 20-year latency cohort into high and low exposure groups (greater than and less than1 year exposure, respectively) for epidemiologic analysis. In addition, for the cancers found to be elevated in the overall mortality analysis, Fingerhut et al. (3) reported SMRs based on additional divisions of exposure time (less than 1 year, 1-5 years, 5-15 years, and greater than 15 years) (Table 4). Assuming that exposure duration is correlated with total exposure, this breakdown of cancer responses is the most useful for developing a dose-response curve for human cancer. For the purposes of our analysis, in order to assign values of AUC, C_{peak}, and C_{avg} to

these exposure duration groups, the 253 members of the NIOSH cohort with serum samples were grouped into corresponding exposure duration groups.

Table 4: Respiratory and All Cancer SMRs by Exposure Duration in NIOSH Subcohort with 20-Year Latency (*3*)

Exposure duration (years)	SMR (20 year latency subcohort) and estimated 95% confidence intervals[a]	
	Trachea, bronchus, and lung cancer	All cancers
<1	96 (56-147)	102 (75-133)
1-<5	126 (73-192)	165 (119-198)
5-<15	146 (79-232)	138 (97-186)
>= 15	156 (71-272)	115 (68-175)

[a] SMRs from Fingerhut et al. (3); confidence intervals estimated by the authors.

The concentration-time profiles developed above were used to calculate AUC, C_{peak}, and C_{avg} for each member. These values were then averaged for each exposure duration group (see Table 5 and Figure 3). Extra risk for each group was calculated based on an assumption that the observed SMRs will hold for the entire lifetime of the cohort. The background lifetime risk of dying from cancer of the lung and bronchus in the male general population, 7.02%, was used as the background response rate (37). SMRs were applied to this value to obtain predicted total mortality rates for respiratory tract cancers in each exposure duration group.

Rats. The extra risk was calculated for each rat dose group from the Kociba et al. (1) bioassay using eq 3. Ninety five percent confidence intervals on the rat extra risk values were estimated using a Monte Carlo simulation assuming a binomial distribution on the observed response in each rat dose group. Ten thousand "bioassays" were simulated, and extra risk calculations were performed for each instance. Upper and lower confidence intervals were identified from the simulations.

Results

The means, medians, and ranges for each exposure duration group from Fingerhut et al. (3) and each dosimetric (AUC, C_{avg}, and C_{peak}) are presented in Table 5. Figure 3 contains box plots illustrating the distribution of the calculated dosimetrics AUC, C_{avg}, and C_{peak} by exposure duration group for the subset of 253 individuals with measured serum TCDD levels from the NIOSH cohort. The values for the different measures of dose for each group showed high interindividual variation, but the central two quartiles (25^{th}-75^{th} percentiles) were relatively narrow in each group regardless of the dosimetric. The calculated dosimetrics were clearly related to the exposure duration.

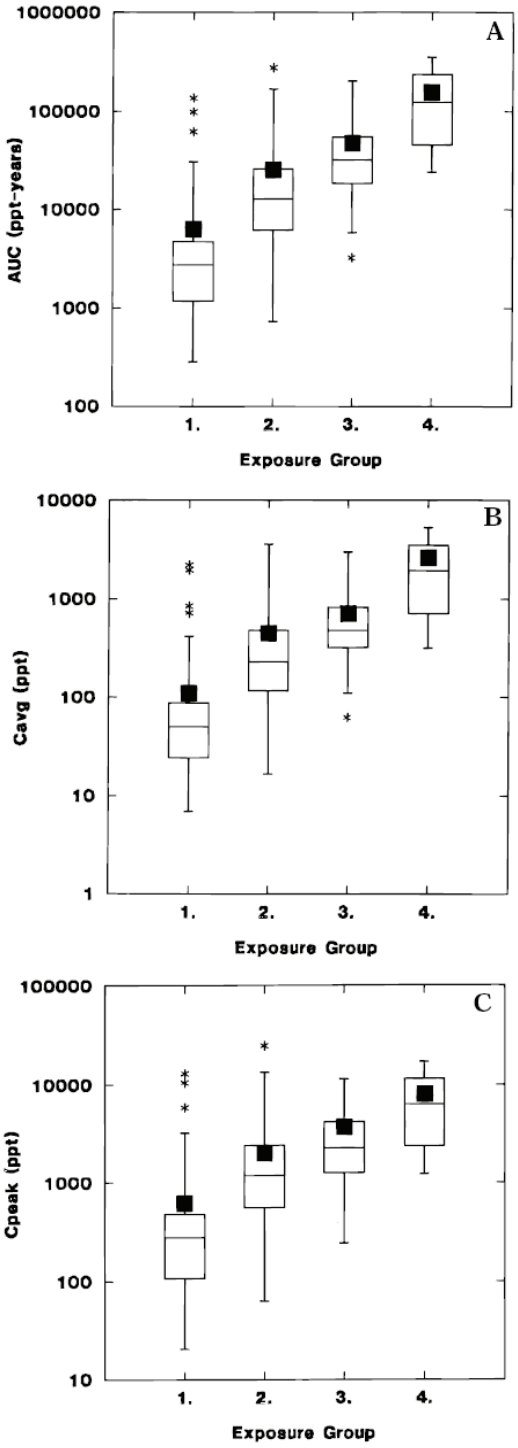
Figures 4-6 show the rat liver and human respiratory tract cancer responses (extra risk) as a function of AUC, C_{peak}, and C_{avg}, respectively. Regardless of which dosimetric is used, the cancer response in rats is much more pronounced than that observed in the NIOSH population. For all three measures, the NIOSH workers experienced internal exposures that were comparable to (or higher than) those experienced by the rats. An interesting observation is that, regardless of which of these three dose measures is used, these data indicate that the respiratory tract cancer response (the most sensitive site in the NIOSH study) in the NIOSH workers is strikingly insensitive to dose.

Rats exhibited a greater tumor response than humans at comparable dose levels, regardless of the dosimetric chosen. At peak serum lipid TCDD levels of approximately 7000 ppt, the tumor response in rats was more than 9-fold greater than that observed in humans. At average concentrations of about 1600 ppt, the tumor response in rats was more than 4-fold greater than that observed in humans. When AUC was used as the dosimetric, the highest dose group (0.1 μg kg^{-1} day^{-1} administered dose) exhibited a 9-fold greater tumor response at approximately 1/10th the AUC of the most highly exposed human group. In short, the slope of the rat AUC dose-response curve was more than 90 times greater than the slope of the human AUC dose-response curve.

Table 5: Calculated AUC, C_{peak}, and C_{avg} Serum TCDD for NIOSH Workers by Exposure Duration

Exposure duration (years)	AUC (ppt*yrs)		C_{peak} (ppt)		C_{avg} (ppt)	
	Mean	Median (Range)	Mean	Median (Range)	Mean	Median (Range)
<1	6 059	2 739 (196-136 823)	597	278 (5-12 977)	111	50 (5-2 176)
1-<5	25 536	12 671 (736-276 359)	2 290	1 189 (63-24 855)	413	227 (17-3 572)
5-<15	47 172	32 135 (3 234-201 843)	3 213	2 250 (243-11 435)	738	477 (62-2 970)
>=15	148 200	130 479 (24 052-353 018)	7 288	6 512 (1 214-17 238)	2 218	2 045 (315-5 252)

Figure 3: Box plots of calculated dose measures by exposure duration group as defined by Fingerhut et al. (3). Group 1, exposed less than 1 year; group 2, exposed 1 to <5 years; group 3, exposed 5 to <15 years; group 4, exposed <=15 years. (A) Area-under-the-curve (AUC) of serum TCDD concentration, ppt*yr. (B) Average serum TCDD concentration (C_{avg}), ppt. (C) Peak serum TCDD concentration (C_{peak}), ppt. Group means indicated by ■.



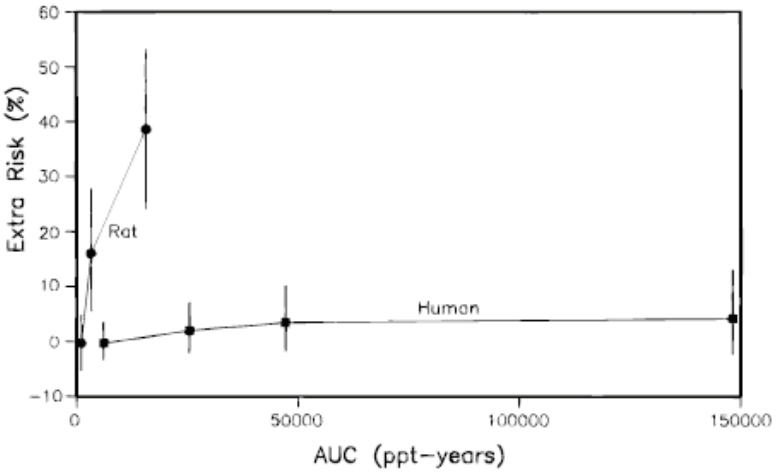


Figure 4: Comparison of the human and rat cancer dose-response curves using AUC (ppt/years) as the dosimetric. Responses plotted are the extra risk of human respiratory tract cancer standard mortality ratios (9) for the various exposure duration subcohorts (with 20 years latency) in Fingerhut et al. (3) (by exposure duration groups; see Figure 3 and text) and the liver tumor extra risk (IRs, b) from the Kociba et al. rat bioassay (1) using the tumor incidences determined by Goodman and Sauer (2). Bars represent 95% confidence intervals.

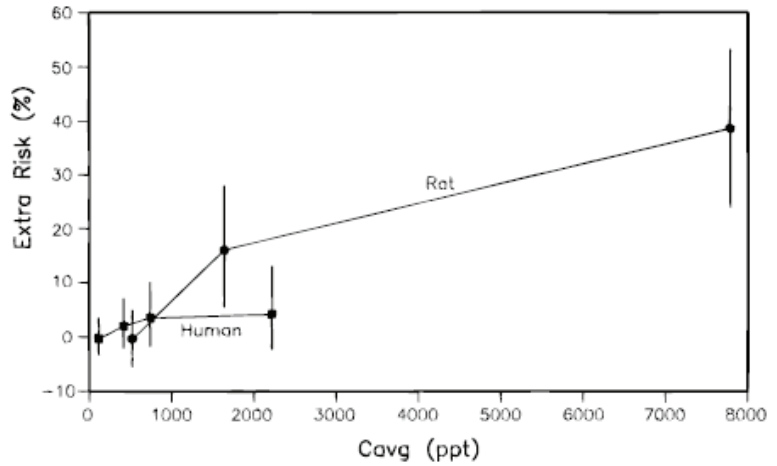


Figure 5: Comparison of the human and rat cancer dose-response curves using average serum lipid TCDD concentration (Cavg, ppt) as the dosimetric. Responses plotted are the extra risk of human respiratory tract cancer (9) for the various exposure duration subcohorts (with 20 years latency) in Fingerhut et al. (3) (by exposure duration groups; see Figure 3 and text) and the liver tumor extra risk (b) from the Kociba et al. rat bioassay (1) using the tumor incidences determined by Goodman and Sauer (2). Bars represent 95% confidence intervals.

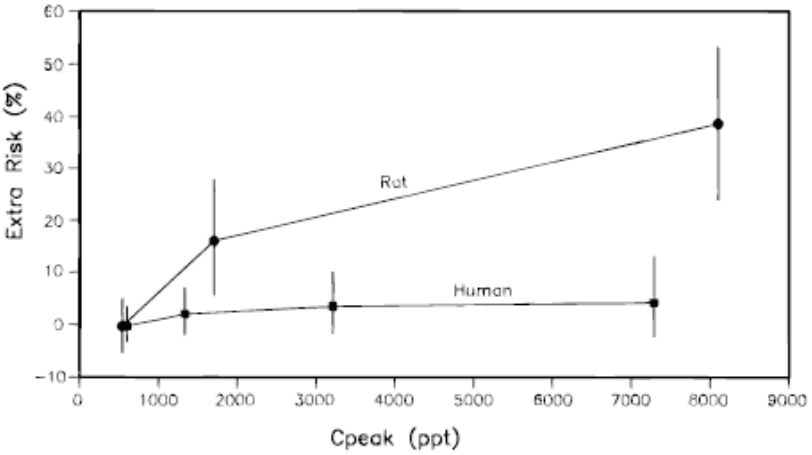


Figure 6: Comparison of the human and rat cancer dose-response curves using peak serum lipid TCDD concentration (C_{peak}, ppt) as the dosimetric. Responses plotted are the extra risk of human respiratory tract cancer (9) for the various exposure duration subcohorts (with 20 years latency) in Fingerhut et al. (3) (by exposure duration groups; see Figure 3 and text) and the rat liver tumor extra risk (b) from the Kociba et al. rat bioassay (1) using the tumor incidences determined by Goodman and Sauer (2). Bars represent 95% confidence intervals.

Discussion

These results indicate that the NIOSH population was exposed to a range of tissue doses of TCDD that are comparable (C_{avg}, C_{peak}) or, assuming that AUC is the proper dosimetric, much higher than those of the rats in the Kociba *et al.* bioassay (*1*). The similarity of internal tissue doses (i.e., delivered doses) indicates that use of a low-dose extrapolation model such as the linearized multistage model is unnecessary to estimate the cancer risks of the NIOSH population based on the rat data. Of course, some extrapolation model is necessary to predict risk due to exposures lower than those of the NIOSH cohort.

Our analysis differs from the Scheuplein and Bowers (*22*) analysis in precisely this area. Specifically, they used estimated rat tissue concentrations and extrapolated cancer slope factors to back-extrapolate to a tissue concentration equivalent to a virtually safe dose (VSD) (the tissue concentration associated with a $1x10^{-6}$ cancer risk) in rats. The risk associated with the VSD tissue level was then re-extrapolated by multiplying by the ratio of the estimated human tissue levels to the rat VSD level. However, the similarity of tissue levels and availability of response data in both the NIOSH cohort and the rat study make

this extrapolation and re-extrapolation unnecessary; since the doses are similar, the responses can be compared directly across species for these two studies. Our analysis does not rely on any assumptions regarding low-dose linearity.

Our analysis has implications for extrapolation of cancer risk to background human exposure levels. For example, the average AUC in the 1 to <5 year exposure group in the NIOSH cohort (in which the SMR for all cancers and for respiratory tract cancers was slightly elevated) was nearly 1000 times greater than average "background" AUC in the general human population. The workers exposed for less than 1 year, which represent an apparent "no-effect level" group in the NIOSH cohort (SMRs less than 100), had average AUCs of approximately 17 times background. Similarly, the members of the Ranch Hand cohort, which has a latency periodof at least 20 years and whose exposures were elevated above background, also have exhibited no increase in cancer risk (*25*). These results indicate that, regardless of the measure of internal dose used, there is no evidence that a cancer risk would be expected at current background levels of exposure to TCDD.

The observation that no cancer risk is observed at the lower doses is not surprising since several experiments in rodents provide evidence that there may be a true threshold for cancer for TCDD. This is consistent with current hypotheses that suggest that TCDD acts as a late-stage carcinogen or promoter. The Kociba *et al.* bioassay (*1*) showed a slight decrease in tumor counts at the low dose level (0.001 μg kg^{-1} day^{-1}). A similar decrease was observed in altered hepatic foci in rats initiated with DEN and exposed to TCDD for 6 months at dose levels of 0.0001 and 0.001 μg kg^{-1} day^{-1} (*38*). Altered hepatic foci were at similar incidences as controls in the 0.01 μg kg^{-1} day^{-1} dose group, while they were elevated at 0.1 μg kg^{-1} day^{-1}. A series of experiments examining various indices of cell proliferation and growth of preneoplastic foci in the two-stage rat liver model also found evidence for a no-effect level on these parameters (39, 40). Lucier et al. (40) concluded that, in contrast to the data regarding enzyme induction, the evidence indicated "there may be a threshold dose for TCDD-mediated increases in cell proliferation and preneoplastic foci". Accordingly, these authors acknowledged that the shape of the TCDD dose-response curve for carcinogenesis may well be different than that for enzyme induction.

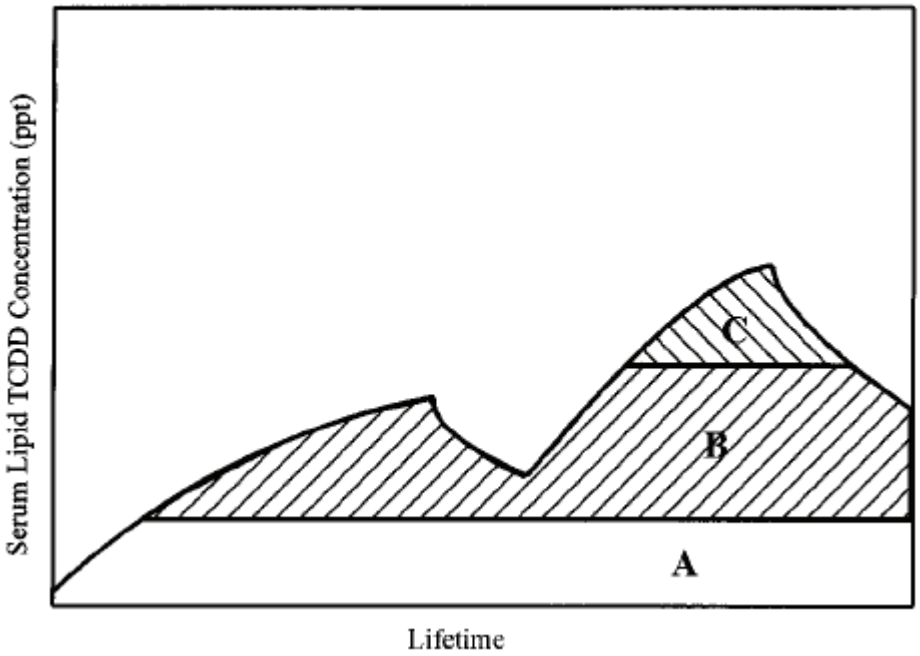


Figure 7: Theoretical concentration vs time curve for TCDD illustrating one possible relationship between AUC and response. This figure illustrates the possible combination ofAUC and thresholds for production of various responses: area A, no effect; area B, enzyme induction; area C, increased cell proliferation.

Given the evidence that TCDD acts as a promoter, given the theoretical and experimental basis for a threshold for the cancer response to TCDD, and using a dosimetric like AUC, it should be possible to identify a practical threshold for TCDD carcinogenesis in humans. Both the NIOSH group exposed less than 1 year and the Ranch Hand cohort suggest that there may be a no-effect level in humans. More sophisticated dosimetry like AUC or AUC above a certain TCDD blood level should also be useful in conducting risk assessments for health effects other than cancer (see Figure 7). Unless an effect is believed to be related to an acute, high-level exposure (for instance, chloracne), an assessment of exposure that accounts for both the magnitude and the duration of tissue levels (although perhaps over shorter periods than a lifetime) is necessary to predict the incidence of an adverse effect for chemicals with a long half-life. In all likelihood, human health data for noncarcinogenic end points such as immunotoxicity should be assessed using AUC and related dosimetrics in order to identify NOAELs and LOAELs and to properly understand potential differences ininterspecies sensitivity (41). This issue deserves further evaluation.

The AUC approach has been considered the dosimetric of choice for nearly 30 years for chemicals that act through a biologic mechanism which depends on maintaining a specific blood or tissue concentration for a specific period of time in order to produce a specific response (10-13). It is especially useful for estimating the efficacy of drugs or the toxicity of chemicals with a moderate to long biologic half-life since daily dose does not capture the fact that a single exposure can produce, at least in the case of TCDD, 40 years of systemic circulation of the toxicant. Since the heavily halogenated chemicals like DDT, PCBs, PBBs, and chlordane all share a number of similar properties (e.g., high lipid solubility, poorly metabolized, long half-life, and lack of genotoxicity), almost certainly the AUC dosimetric
should be a more appropriate predictor of chronic adverse effects than daily dose.

Several uncertainties and limitations are inherent in this analysis:

(1) Effect of Different Half-Life Assumptions. As noted above, estimates of the half-life for TCDD elimination in humans vary. Data from the Ranch Hand population demonstrate that half-life increases slightly with percent body fat (33). If the true half-life is shorter than estimated here (7.5 years), then the estimates of AUC, C_{avg}, and C_{peak}, presented here for the NIOSH cohort underestimate actual values. If the true half-life is longer than 7.5 years, then the values here overestimate actual exposures. The effect of the relatively small variations in estimates of half-life do not significantly alter the results of this analysis. For example, use of a 9-year half-life would reduce estimates of AUC by approximately 25 percent.

(2) Lifetime Scaling Issues. Estimates of AUC for humans will appear much larger than those for animals because, all else being equal, humans have much longer lives (70 years vs 2 years for rats). On a mechanistic basis, AUC may still be an appropriate dosimetric for evaluating the cancer response to promoters and nongenotoxic agents. Scaling on the basis of cell turnover rate in the tissues of interest (rat liver and human respiratory tract) might improve the comparison; however, we were unable to locate appropriate comparable cell turnover rates for the two tissues. Instead, we included a calculation in which we scaled for the difference in lifetimes by dividing the estimated AUC values by the time period of exposure (2 years for rats, birth to end of followup for humans), resulting in an average concentration. We have presented results in this analysis both on the basis of an unadjusted AUC and on the basis of the average concentration. The results are qualitatively similar, although the degree of difference in sensitivity is not as great when evaluated on the basis of C_{avg} rather than AUC.

It is important to note that the half-life for TCDD in humans is longer as a fraction of lifetime than the half-life in rats (7.5 years/70 years vs 25

days/730 days, or 10.7% vs 3.4% of lifetime). This indicates that, for a given administered dose, humans experience higher tissue concentrations for a longer period of time, even when adjusted for lifetime. These species differences are accounted for in our analysis through the evaluation of relative sensitivity using both AUC and C_{avg} as dosimetrics.

(3) Data Limitations. Serum sampling for TCDD was performed on only 253 persons out of a cohort of almost 5200. These 253 were from two of 10 plants involved in the study and did not constitute a random, representative sample. If the exposures of the 253 differed substantially in degree or kind from those of the remaining 4900 workers, estimates of their concentration-time profiles will not be representative of the whole cohort. Another limitation is inherent in the classification of exposure duration using years exposed by Fingerhut et al. (3) for the calculation of group SMRs. Our calculations indicate that while there is a definite trend in internal dosimetrics with exposure duration, there is substantial misclassification as well. This could obscure the true dose-response relationship.

(4) Assumption That NIOSH Cancers Are Due to TCDD. The cause or causes of the observed cancer response in the NIOSH cohort are still a matter of debate (42-44). The dose-response pattern seen here, in which cancer mortality rates were strikingly insensitive to large differences in total exposure, suggests that some other factor might be responsible for the excess cancer seen in this cohort. For example, smoking data were gathered for only about 5% of the cohort, and the 5% was not randomly selected from the cohort. In addition, two cases of mesothelioma were observed, which strongly indicate asbestos exposure. Since respiratory tract cancers (particularly lung) were the only specific cancer site elevated in the study, the possibility of confounding by smoking and asbestos exposure cannot be ignored. Finally, workers in the chemical plants included in the NIOSH study had exposure to numerous other industrial chemicals. None of these other exposures were analyzed or included in a formal way in the Fingerhut et al. analysis (3), although the authors have presented their views about this and other relevant issues (45). Thus, the attribution of the observed respiratory tract cancers response to TCDD exposure is a conservative assumption, that is, is not likely to underestimate (but may have overestimated) any actual effect due to TCDD exposure.

(5) Response Metric. We compared rat liver tumors observed in a bioassay to human respiratory tract cancers observed in a cross-sectional cohort study. Use of different responses in the two species raises several questions. For example, the rat response includes tumors found at a defined time point, i.e., terminal sacrifice, while the human SMR includes only those tumors that produced fatalities. However, the mortality rate from lung cancer is very high, and thus the SMR should be a reasonable estimate of the incidence of respiratory tract cancers. Further, the majority

of liver tumors in the bioassay were found in rats dying before terminal sacrifice. These two responses were the most sensitive responses observed in the two populations.

We also evaluated the absolute increase in cancer and an incidence ratio formulation (observed response at each dose divided by the control response) for the cancer response metric. Use of either of these approaches increases the apparent differences in sensitivity in the two species. For instance, the maximum rat liver tumor response compared to the maximum human respiratory cancer response was 9.2 times greater in the rats than in humans using the extra risk formulation. The maximum rat response was 9.5 and 11 times greater using the absolute increase and incidence ratio formulations, respectively. The analysis presented here is based on followup of 20 years since first exposure. As this cohort is followed in the
coming years, it is plausible that the degree of elevation in respiratory tract cancers as compared to expected levels may increase or decrease or that other sites may show cancer elevations. Rates of diagnosis of lung cancer in the general population peak around age 60 and then decline, so the background rate of lung cancer is likely to fall in this population over time (37). Clearly, more complete data on the smoking habits in this population would be extremely useful.

(6) Effect of More Complex Distribution Patterns. Several physiologically based pharmacokinetic (PB-PK) models have been developed in an attempt to understand tissue distribution patterns of TCDD in rodents and to improve the accuracy of low-dose extrapolations (46-51). As these analyses have noted, the distribution of TCDD in most species is affected by three factors: sequestration in lipids due to the high lipophilicity of the compound, specific binding to the Ah receptor and CYP 1A2 protein, and induction of specific and nonspecific binding sites. Using a model that provided for the induction of CYP 1A2 as a binding protein in the liver for TCDD, Leung et al. (46, 47) were able to model the distribution of TCDD and to reproduce the observed hepatic sequestration that occurs at higher doses in mice and rats. Andersen et al. (51) extended the model a step further by incorporating terms describing the binding of the TCDD-receptor complex to DNA into the rodent model. Most recently, Carrier et al. (49, 50) developed a general toxicokinetic PB-PK model addressing both the distribution and kinetics of PCDDs and PCDFs for several mammalian species, including humans, for the explicit purpose of interspecies extrapolation (49, 50). The key feature of the distribution profile involves the identification of the body burden at which the liver induction of CYP 1A2, which is believed to bind TCDD, begins to change the ratio between liver and adipose concentrations. Experimentally, this ratio is about 1:10 at background levels where CYP 1A2 is uninduced (indicating partitioning based solely on lipid solubility) to more than10:1 at extremely high body burdens. The Carrier et al. (49, 50) model predicts this crossover in distribution ratios at similar body

burdens for several mammalian species, including humans, and would likely become important at adipose tissue concentrations in the range of 300-700 ppt. If this model is correct, then substantial CYP 1A2 induction and resulting hepatic sequestration of TCDD would have occurred in at least some of the NIOSH cohort during periods of higher body burden.

An indirect marker of CYP 1A2 activity was examined in 58 workers from the NIOSH cohort with known current TCDD serum levels ranging from background levels to 1742 ppt (52). Caffeine metabolite ratios (CMR) were examined in this group and in125 controls. No statistically significant relationship between serum TCDD level and CMR were found in the analysis, although the effects of smoking and alcohol consumption were clearly evident. Thus, for this group of workers at current serum levels, no evidence for significant induction of CYP 1A2 (and thus, hepatic sequestration of TCDD) due to TCDD was found. However, such sequestration and binding might have occurred at periods of higher serum TCDD levels in the NIOSH cohort. No evidence for hepatic sequestration was found by Facchetti et al. (53), who analyzed various tissues from a woman that died of pancreatic cancer several months after the Seveso accident. TCDD was present in the liver at 150 ppt; the adipose tissue concentration was 1840 ppt (a ratio of approximately 1:12).

(7) Hormonal Influences. Liver tumors occur in female but not male rats. Liver tumor development is prevented in female rats by removal of the ovaries (40). Thus, the use of liver tumors in female rats to predict the cancer incidence rates in a largely male human population (workers studied
by NIOSH) may not be appropriate. However, the current EPA slope factor is also based largely upon the female rat liver response and is thus the basis for the current regulatory approach to cancer risk assessment for TCDD.

(8) Sensitive Subpopulations. This analysis is limited because the NIOSH cohort consists of male workers exposed during adulthood to TCDD. We cannot evaluate the potential response of potentially sensitive subpopulations such as persons exposed *in utero* or during childhood. Nor can we evaluate the response of subpopulations that may be sensitive due to genetic factors in this genetically heterogeneous population.

None of the possible limitations in this analysis change the conclusion that humans appear to be less sensitive than rats over a wide range of doses to the cancer hazard posed by TCDD or that background exposure levels of TCDD are not likely to pose a significant cancer hazard to humans. To develop a more complete understanding of the relative susceptibility of humans to the cancer hazard posed by TCDD compared to rats, including identification of a possible threshold for carcinogenesis, additional representative serum TCDD data for members of the NIOSH cohort are needed. Further, it would be useful to conduct a similar analysis of the residents of Seveso, Italy, and the Ranch

Hand population. Previous analysis of these groups indicates that some exposures at Seveso were as high or higher than those of the NIOSH cohort (54, 55). These are among the best groups for improving our understanding of the potential human health risks from TCDD at moderate to high doses and to identify an accurate method for estimating any low dose risks.

Literature Cited

(1) Kociba, R. J.; Keyes, D. G.; Beyer, J. E.; Carreon, R. M.; Wade, C. E.; Dittenber, D. A.; Kalnins, R. P.; Frauson, L. E.; Park, C. N.; Barnard, S. D.; Hummel, R. A.; Humiston, C. G. *Toxicol. Appl. Pharmacol.* **1978**, *46*, 279-303.

(2) Goodman, D. G.; Sauer, R. M. *Regul. Toxicol. Pharmacol.* **1992**, *15*, 245-252.

(3) Fingerhut, M. A.; Halperin, W. E.; Marlow, D. A.; Piacitelli, L. A.; Honchar, P.A.; Sweeney,M.H.;Greife,A. L.;Dill, P.A.; Steenland, K.; Suruda, A. J. *N. Engl. J. Med.* **1991**, *324*, 212-218.

(4) Poland, A.; Knutson, J. C. *Annu. Rev. Pharmacol.* **1982**, *22*, 517-554.

(5) Kimbrough, R. D. *J. Toxicol. Environ. Health* **1990**, *30*, 261-271.

(6) Gough, M. *Dioxin, Agent Orange: The Facts*; Plenum Press: New York, 1986.

(7) Leung, H.W.; Wendling, J.M.; Orth, R.; Hileman, F.; Paustenbach, D. J. *Toxicol. Lett.* **1990**, *50*, 275-282.

(8) *Health Assessment Document for 2,3,7,8-tetrachlorodibenzo-pdioxin (TCDD) and Related Compounds, Volumes I-III, Review Draft.* U.S. Environmental Protection Agency, Office of Research and Development: Washington, DC, June 1994; EPA/600/BP-92/001.

(9) Krewski, D.; Withey, J. R.; Ku, L. F.; Andersen, M. E. *Environ. Health Perspect.* **1994**, *102* (11), 37-50.

(10) Gibaldi, M.; Perrier, D. *Pharmacokinetics*; Marcel Dekker: New York, 1975.

(11) Derendorf, H.; Hochhaus, G. *Handbook of Pharmacokinetic/ Pharmacodynamic Correlation*; CRC Press: Boca Raton, FL, 1995.

(12) Goldstein, A.; Aronow, L.; Kalman, S. M. *Principles of Drug Action: The Basis of Pharmacology*, 2nd ed.; John Wiley & Sons: New York, 1984.

(13) Gilman, A. G.; Rall, T. W.; Nies, A. S.; Taylor, P. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th ed.; Pergamon: New York, 1990.

(14) Fisher, J. W.; Allen, B. C. *Risk Anal.* **1993**, *13*, 87-95.

(15) Reitz,R.H.; McCroskey, P. S.; Park,C.N.; Andersen, M.E.;Gargas, M. L. *Toxicol. Appl. Pharmacol.* **1990**, *105*, 37-54.

(16) Andersen, M. E.; MacNaughton, M. G.; Clewell, H. J., III; Paustenbach, D. J. *Am. Ind. Hyg. Assoc. J.* **1987**, *48*, 335-343.

(17) Andersen, M. E.; Mills, J.; Fox, T. R.; Goldsworthy, T. L.; Conolly, R. B. Birnbaum L. S. In *Receptor-Mediated Biological Processes: Implications for Evaluating Carcinogenesis*; Wiley-Liss: New York, 1994; pp 295-310.
(18) Andersen, M. E.;Mills, J. J.; Jirtle, R. L.;Greenlee,W. F. *Toxicology* **1995**, *102*, 223-237.
(19) *Review of the Office of Research and Development's Reassessment of Dioxin and Dioxin-Like Compounds by the Dioxin Reassessment Review Committee. An EPA SAB Report: A Second Look at Dioxin;* Science Advisory Board of the U.S. EPA: Washington, DC, Sep 29, 1995.
(20) Schlatter, C. *Banbury Report 35: Biological Basis for Risk Assessment of Dioxins and Related Compounds*; Cold Spring Harbor Laboratory: Plainview, NY, 1991; pp 215-228.
(21) Piotrowski, J. *The Application of Metabolic and Excretion Kinetics to the Problems of Industrial Toxicology*; National Library of Medicine, U.S. Government Printing Office: Washington, DC, 1971.
(22) Scheuplein, R. J.; Bowers, J. C. *Risk Anal.* **1995**, *15*, 319-333.
(23) *Health Assessment Document for Polychlorinated Dibenzo-p-Dioxins*; Environmental Protection Agency, Office of Health and Environmental Assessment: Washington, DC, Sep 1985; EPA/600/8-84/014F.
(24) Bond, G. G.; Bodner, K. M.; Cook, R. R. *Fund. Appl. Toxicol.* **1989**, *12*, 172-188.
(25) Wolfe, W. H.; Michalek, J. E.; Miner, J. C. *J. Toxicol. Environ. Health* **1994**, *41*, 481-488.
(26) Manz, A.; Berger, J.; Dwyer, J. H.; Flesch-Janys, D.; Nagel, S.; Waltsgott, H. *Lancet* **1995**, *338*, 959-964.
(27) Zober, A.; Messerer, P.; Huber, P. *Arch. Occup. Environ. Health* **1990**, *62*, 138-157.
(28) Saracci, R.; Kogevinas, M.; Bertazzi, P. A.; Bueno de Mesquita, B. H.; Coggon, D.; Green, L. M.; Kauppinen, T.; L'Abbe´, K. A.; Littorin, M.; Mathews, J. D.; Neuberger, M.; Osman, J.; Pearce, N.; Winkelman, R. *Lancet* **1991**, *338*, 1027-1032.
(29) Bertazzi, P. A. *Sci. Total Environ.* **1991**, *106*, 5-20.
(30) Bertazzi, P. A.; Pesatori, A. C.; Consonni, D.; Tironi, A.; Landi, M. T.; Zochetti, C. *Epidemiol*ogy **1993**, *4*, 398-406.
(31) Rose, J.Q.;Ramsey, J.C.;Wentzler,T.H.;Hummel,R.A.;Gehring, P. J. *Toxicol. Appl. Pharmacol.* **1976**, *36*, 209-226.
(32) Patterson, D. G.; Needham, L. L.; Pirkle, J. L.; Roberts, D. W.; Bagby, J.; Garrett, W. A.; Andrews, J. S.; Falk, H.; Bernert, J. T.; Sampson, E. J.; Houk, V.N. *Arch. Environ. Contam. Toxicol.* **1988**, *17*, 139-143.
(33) Michalek, J. E.; Pirkle, J. L.; Tripathi, R. C.; Patterson, D. G.; Needham, L. L. *J. Toxicol. Environ. Health* **1996**, *47*, 209-220.
(34) Flesch-Janys, D.; Gurn, P.; Jung, D.; Konietzko, J.; Manz, A.; Papke, O. *Organohalogen Compd.* **1994**, *21*, 93-99.

(35) Needham, L. L.; Gerthoux, P. M.; Patterson, D. G.; Brambilla, P.; Pirkle, J. L.; Tramacere, P. L.; Turner, W. E.; Beretta,C.; Sampson, E.; Mocarelli, P. *Organohalogen Compd.* **1994**, *21*, 81-85.
(36) Hertzberg, R. In *Statistics in Toxicology*; Krewski, D., Franklin, C., Eds.; Gordon and Breach Science: New York, 1991; pp 629-652.
(37) Miller, B. A.; Ries, L. A. G.; Hankey, B. F.; Kosary, C. L.; Harras, A.; Devesa S. S.; Edwards, B. K. (eds). *SEER Cancer Statistics Review: 1973*-1990. National Cancer Institute, 1991; NIH Pub. No. 93-2789.
(38) Pitot, H. C.; Goldsworthy, T. L.; Moran, S.; Kennan, W.; Glauert, H. P.; Maronpot, R. R.; Campbell, H. A. *Carcinogenesis* **1987**, 8, 1491-1499.
(39) Maronpot, R. R.; Foley, J. F.; Takahashi, K.; Goldsworthy, T.; Clark, G.; Tritscher, A.; Portier, C.; Lucier, G. *Environ. Health Perspect.* **1993**, 101, 634-642.
(40) Lucier, G. W.; Clark, G.; Tritscher, A.; Foley, J.; Maronpot, R. *Chemosphere* **1992**, *25*, 177-180.
(41) Finley, B.; Paustenbach, D. J. *Organohalogen Compd.* **1995**, *25*, 23-28.
(42) Collins, J. J.; Acquavella, J. F.; Friedlander, B. R. *Epidemiology***1992**, *3*, 65-69.
(43) Carlo, G. L.; Sund, K. G. *N. Engl. J. Med.* **1991**, *324*, 1809-1810.
(44) Morgan, R. W. *N. Engl. J. Med.* **1991**, *324*, 1810.
(45) Fingerhut, M. A.; Steenland, K.; Sweeney, M. H.; Halperin, W. E.; Picacitelli, L. A.; Marlow, D. A. *Epidemiology* **1992**, *3*, 69-72.
(46) Leung, H. W.; Paustenbach, D. J.; Murray, F. J.; Andersen, M. E.
Toxicol. Appl. Pharmacol. **1990**, *103*, 399-410.
(47) Leung, H. W.; Poland, A.; Paustenbach, D. J.; Murray, F. J.; Andersen, M. E. *Toxicol. Appl. Pharmacol.* **1990**, *103*, 411-419.
(48) Kissel, J. C.; Robarge, G. M. *Chemosphere* **1988**, *17*, 2017-2027.
(49) Carrier, G.; Brunet, R. C.; Brodeur, J. *Toxicol. Appl. Pharmacol.* **1995**, *131*, 253-266.
(50) Carrier, G.; Brunet, R. C.; Brodeur, J. *Toxicol. Appl. Pharmacol.* **1995**, *131*, 267-276.
(51) Andersen, M. E.; Mills, J. J.;Gargas, M. L.; Kedderis, L.; Birnbaum, L. S.; Neubert, D.; Greenlee, W. F. *Risk Anal.* **1993**, *13*, 25-36.
(52) Halperin, W.; Kalow, W.; Sweeney, M. H.; Tang, B. K.; Fingerhut, M.; Timpkins, B.; Wille, K. *Occup. Environ. Med.* **1995**, *52*, 86-91.
(53) Facchetti, S.; Fornari, A.; Montagna, M. *Forensic Environ. Appl.* **1981**, *1*, 1405-1414.
(54) Mocarelli, P.; Needham, L.; Marocchi, A.; Patterson, D. G., Jr.; Brambilla, P.; Gerthoux, P. M.; Meazza, L.; Carreri, V. *J. Toxicol. Environ. Health* **1991**, *32*, 357-366.
(55) Boroush, M.; Gough, M. *Reg. Toxicol. Pharmacol.* **1994**, *20*, 198-210.

Chapter 3: Issues in Risk Assessment for Developmental Effects of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin and Related Compounds

Aylward, L.L., J.C. Lamb, and S. Lewis. 2005. Toxicol. Sci. 87(1):3-10.

Abstract

Recent risk assessments for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds have focused on adverse effects observed in rodent offspring exposed while in utero during critical gestational periods as among the most sensitive adverse effects attributable to TCDD exposure. In addition, these risk assessments have converged on the use of body concentration (or "body burden") of TCDD as a dose metric superior to administered dose for cross-species comparisons and risk assessments due to the interspecies differences in elimination kinetics and substantial persistence of these compounds. The detailed, although incomplete, data that are available on maternal-fetal distribution of TCDD and related compounds illustrate differences in distribution among these compounds that impact assessments on a body burden basis. These data also demonstrate differences in distribution after subchronic or chronic administration compared to acute administration. Some data are now also available addressing inconsistencies that may arise from the use of TCDD toxic equivalency factors (TEFs), which were derived on an administered dose basis, in evaluating responses to mixtures of dioxins on a body burden basis in the context of chronic exposure situations. Finally, the use of body burden as a dose metric does not account for or eliminate the substantial differences in sensitivity to dioxin observed across species or between different strains of the same species, and thus does not eliminate the need to consider the relative sensitivity of humans compared to laboratory animal models in risk assessments. Additional research areas that may increase the foundation for interspecies extrapolations are discussed.

Introduction

Several US and international groups have conducted recent risk assessments for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. The World Health Organization/UN Food and Agriculture Agency Joint Expert Committee on Food Additives (JECFA 2001) and the European Commission Scientific Committee on Food (ECSCF 2001) published assessments of tolerable intake levels for dioxins within a few months of each other in 2001, while the USEPA published another draft of its dioxin risk assessment in 2003. All of these assessments focus on a body of data that indicates that the most sensitive adverse responses to TCDD in experimental animal studies appear to be effects on the development of offspring following in utero exposure to TCDD.

In these and other recent assessments of dioxin toxicity and risk, the body concentration (or "body burden") of dioxin has been identified and used widely as a dose metric for assessment of dioxin response, one that is deemed superior to administered dose for interspecies comparisons (Birnbaum and Tuomisto 2000; JECFA 2001; ECSCF 2001). The kinetics for elimination of

TCDD and other dioxin-like compounds vary widely among species (Van den Berg et al. 1994). For example, the apparent first-order half-life for elimination of TCDD in rats is on the order of 25 days, while in humans at background exposure levels, it is approximately 100 times as long. The slower elimination in humans results in a correspondingly greater accumulation of the compounds after chronic administration compared to the accumulation in rodents. Given the wide variation in elimination kinetics among species, the use of body burden as a dose metric for evaluation and comparison of responses to dioxin is attractive, because it may provide a more toxicologically relevant basis than administered dose for comparing responses among species, particularly following chronic exposure. However, the use of body burden as a dose metric is not a substitute for consideration of several key issues, including:

- Possible differences in response to acute, as opposed to chronic, administration, and differences between TCDD and other congeners in kinetics and intrinsic biological activity.
- Validity of the application of TEF/TEQ assessments to body burden dose metrics rather than administered dose.
- Intrinsic interspecies variations in sensitivity

This article provides a critical review of the factors impacting the interpretation and extrapolation to humans of the available toxicological data on low-dose adverse effects on development of offspring following *in utero* exposure to TCDD.

Maternal-Fetal Distribution Issues: Variations Due to Dosing Regimen and Among Congeners

In general, the most sensitive adverse effect endpoints identified and evaluated in recent dioxin risk assessments are alterations in male rat reproductive system parameters following acute TCDD administration to dams on gestation day 15 (ECSCF 2001; JECFA 2001; UKCOT 2001; USEPA 2003). However, the database on these and related endpoints in rats or mice is incomplete, because no studies of these effects resulting from chronic rather than acute administration of either TCDD or representative TEQ mixtures exist. Instead, the risk assessments based on these data rely on maternal body burden of TCDD as the appropriate measure of exposure combined with extrapolation of these results in two ways:

- From acute experimental exposure scenarios (gavage of rat dams on gestation day 15) to chronic human exposure scenarios (dietary exposure)

- From effects of pure TCDD to the potential effects of the mixture of dioxin, furan, and dioxin-like PCB compounds present in the general population.

Recent data on differential organ and tissue distribution among congeners after either acute or chronic administration have not been fully accounted for in these extrapolations in risk assessments conducted to date (Hurst et al. 2000a; Hurst et al. 200b). Recent studies in laboratory rats provide substantial data showing wide variations in maternal-fetal distribution patterns among TCDD and related compounds and following chronic vs. acute gestation day 15 administration (Chen et al. 2001). Some of these data are highlighted in the text and figures below.

1. Distribution of TCDD to the fetus on gestation day 16 after chronic maternal administration is much less efficient than after acute administration on gestation day 15 (Figure 1). Distribution of TCDD to the fetus as a fraction of maternal body burden after subchronic exposure is two- to three-fold lower than that observed after acute administration, and the difference in transfer rates is greatest at the lowest dose levels, which are most relevant to background exposure (Hurst et al. 2000a,b). These data were incorporated into the evaluations by JECFA (2001) and ECSCF (2001). In these evaluations, the NOAEL and LOAEL values from studies using acute exposure regimens were adjusted upwards to account for attenuation of fetal distribution likely to result from body burdens arising from chronic exposure to the dam.

2. Distribution of a mixture of nine TEQ contributors (including substantial proportions of TCDD and PCB 126) to the fetus on gestation day 16 is much less efficient than transfer of pure TCDD after acute administration on gestation day 15 or subchronic maternal TCDD administration (Figure 1). The ratio of fetal to maternal TEQ body burden is approximately four-fold lower than the corresponding ratio from the study of TCDD administration alone (Chen et al. 2001). The difference in fetal transfer is apparent when the distribution to the fetus is examined on a congener-specific basis (Figure 2). Every other tested congener is distributed to the fetus less efficiently than TCDD, and most are transferred at only a small fraction of the efficiency of TCDD. These data were not available at the time of the ECSCF (2001) and JECFA (2001) evaluations, and were not included in the USEPA (2003) evaluations.

3. The tested TEQ mixture in Chen et al. (2001) results in a different pattern of maternal body burden than is seen in typical U.S. background body burdens and probably over-predicts the transfer of a mix more typical of U.S. background (Figures 3 and 4). TCDD and PCB 126 account for nearly 80% of the maternal body burden at the lowest tested dose in Chen

et al. (2001), but account for less than 20% of typical U.S. body burdens. For the two congeners that together constitute nearly 40% of U.S. body burdens (PeCDD and 4 PeCDF), fetal transfer was approximately ten-fold less efficient than for TCDD and PCB 126 at the lowest (and most relevant) tested dose (Figure 4). The congener group that is most prominent in U.S. background body burdens, HxCDDs, was not included in the Chen et al. (2001) study.

4. The lower fetal transfer of non-TCDD congeners is probably due to differential maternal distribution (Figure 5). Nearly all of the other tested congeners in Chen et al. (2001) have much higher affinity for hepatic tissue, relative to adipose tissue, than TCDD. This pattern of differential hepatic sequestration among congeners in rodents has been understood for some time (reviewed in Van den Berg et al. 1994). This hepatic sequestration probably reduces the proportion of maternal body burden that is available for transfer to the fetus. In addition, maternal hepatic sequestration may be more pronounced after chronic administration, compared to acute administration, because chronic administration would allow for increased induction of the CYP1A2 binding protein in maternal liver. However, data do not exist to confirm this potential effect of chronic vs. acute administration on distribution to the fetus.

It is important to keep the actual magnitude of the "low" doses used in these studies in perspective: the lowest single maternal dose of TCDD used in these studies is approximately 100,000 times greater than the daily TEQ intake dose to which humans are exposed from the diet. The physiological and toxicological response to a bolus dose may well be substantially different from the response to a slowly accumulating, essentially steady state body burden, even at the same instantaneous body burden, for a variety of pharmacokinetic and pharmacodynamic reasons, particularly during a sensitive and dynamic physiological period such as gestation.

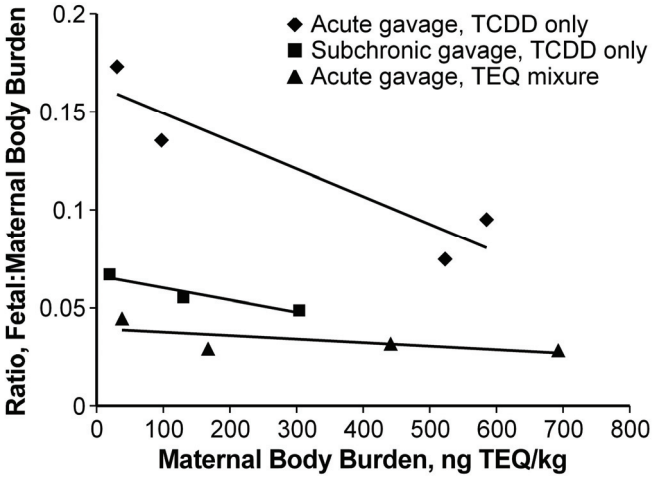


Figure 1. Ratio of fetal to maternal body burdens in Long-Evans rats on gestation day 16 across a range of doses. Data from Hurst et al. (2000a) study of acute administration of TCDD on gestation day 15; Hurst et al. (2000b) study of subchronic administration of TCDD; and Chen et al. (2001) study of acute administration of a mixture of TEQ contributors on gestation day 15. Maternal and fetal body burdens are represented either as reported by the authors (Hurst et al. 2000a, b) or were estimated using reported liver and adipose tissue burdens. Fetal burdens were reported by the authors. Transfer of TCDD to the fetus as a fraction of maternal body burden was much lower after subchronic administration than after acute administration. Transfer of a TEQ mixture to the fetus following acute maternal administration was less efficient still, with fetal transfer approximately 4-fold less efficient than after acute administration of TCDD. No data are available to document distribution of a TEQ mixture to the fetus following subchronic maternal exposure to a TEQ mixture.

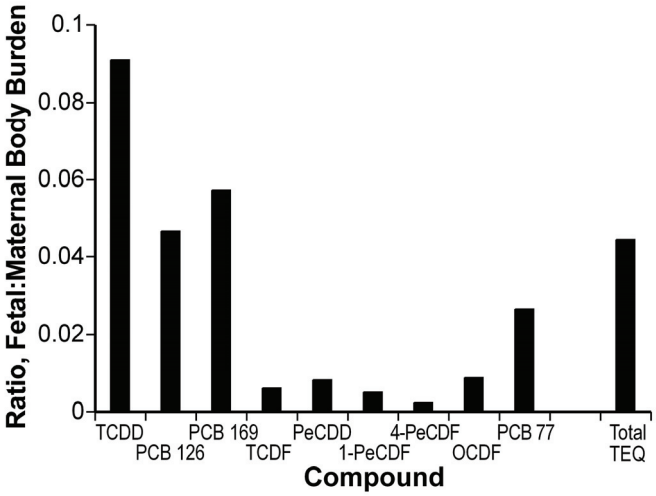


Figure 2. Congener-specific ratios of fetal to maternal body burdens in Long-Evans rats on gestation day 16 from Chen et al. (2001) for four dose groups (nominal TEQ dose levels) after acute administration of a mixture of TEQ contributors on gestation day 15. Fetal body burdens were reported by the authors; maternal body burdens were estimated as the sum of liver and adipose burdens. The overall TEQ transfer was heavily influenced by the transfer of TCDD and PCB 126, which together accounted for nearly 80% of the maternal body burden on gestation day 16 in this study. Fetal transfer of all other tested compounds was less efficient than the transfer of TCDD.

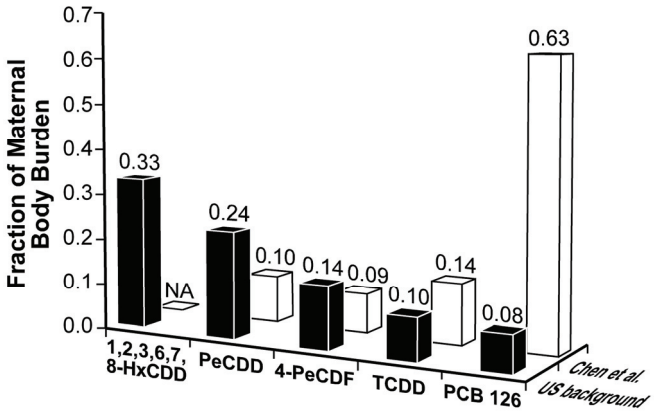


Figure 3. Comparison of maternal body burden TEQ contributors from Chen et al. (2001) (maternal body burdens in Long-Evans rats on gestation day 16 following acute administration on gestation day 15, lowest dose group) with pattern of congeners in typical U.S. background populations. Nearly 80% of the maternal TEQ body burdens in the Chen et al. (2001) study are due to TCDD and PCB 126, while these two compounds account for less than 20% of U.S. background body burdens. The U.S. background body burden profile was taken from a report of CDC data presented in USEPA (2003, Part I, Volume II, p. 4-96).

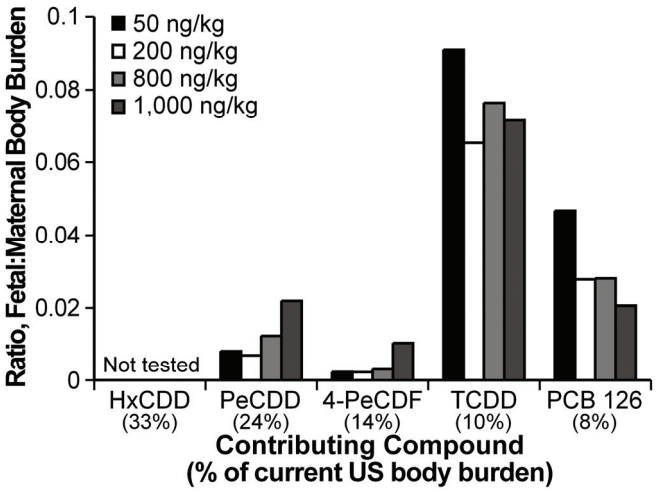


Figure 4. Ratio of fetal to maternal body burdens in Long-Evans rats on gestation day 16 from Chen et al. (2001) for the top five contributors to human background body burdens. Chen et al. (2001) did not include any HxCDD congeners, which are the largest contributors to U.S. background body burdens. The next two largest contributors to human body burdens, PeCDD and 4 PeCDF, showed very low transfer of compounds to the fetus in the Chen et al. (2001) study. The two congeners with the highest ratio of fetal to maternal body burdens in the Chen et al. (2001) study, TCDD and PCB 126, constitute less than 20% of U.S. background body burdens but constituted 80% of the maternal body burdens in the Chen et al. (2001) study.

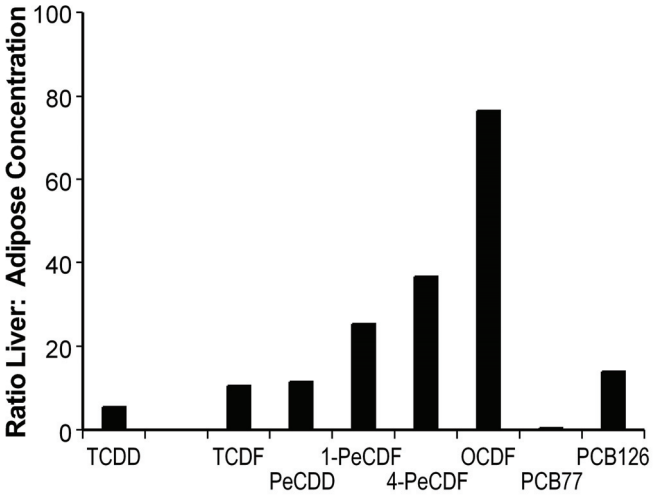


Figure 5. Ratio of maternal liver to adipose concentration in Long-Evans rats on gestation day 16 after acute administration of a mixture of TEQ contributors at the lowest tested dose, 50 ng TEQ/kg (Chen et al. 2001). All but one of the tested compounds had higher liver-to-adipose concentration ratios than TCDD, indicating increased hepatic sequestration of these compounds compared to TCDD, and the likelihood that a lower proportion of the maternal body burden is available for transfer to the fetus compared to TCDD.

Based on the available data, it is clear that there is a need for a comprehensive study of these endpoints and fetal distribution of these compounds employing chronic administration (preferably through diet, in order to mimic human exposure patterns) of a TEQ mixture that results in maternal body burdens with a congener pattern similar to that observed in the general population (that is, not dominated by TCDD). Based on the data to date, the fetal exposure after subchronic acute administration of TCDD is two- to three-fold lower than the fetal exposure resulting from acute maternal exposure to TCDD. In addition, distribution to the fetus following acute maternal exposure on gestation day 15 of a TEQ mixture is substantially lower than the fetal exposure following acute exposure to pure TCDD (at least four-fold lower). The combined impact of these two extrapolations suggests that the maternal body burdens in studies of acute TCDD administration on gestation day 15 may be four- to ten-fold lower than the chronic maternal body burdens of a representative TEQ mixture that would be required to result in equivalent distribution to the fetus in this animal model. Direct extrapolation of the results of the studies of acute administration of TCDD to the general

population exposure situation of chronic human exposure to a TEQ mixture consisting primarily of non-TCDD congeners on a maternal body burden basis may overstate substantially the risk of current maternal TEQ body burdens in the general population.

Application of TEF/TEQ Approach to Body Burden-Based Assessments

Recent risk assessments of dioxins have, by necessity, relied almost exclusively on studies of exposure to TCDD alone (rather than TEQ mixtures) in combination with a body-burden dose metric for hazard assessment. The results of the hazard assessments, whether for cancer or non-cancer endpoints, have been extended to predict risks to the human population due to a mixture of TCDD (accounting for 10% of TEQ) and other TEQ-contributing compounds, again on a body-burden basis. In some cases these body-burden based assessments have been re-converted to equivalent intake doses, but the extrapolations and applications of uncertainty factors have been conducted on a body burden basis. However, the TEF/TEQ scheme was *explicitly* developed based on assessment of the results of studies comparing potency between individual compounds and TCDD on an *administered dose* basis (Van den Berg et al. 1998). The pharmacokinetics of the various TEQ-contributing compounds vary widely, as do the distribution patterns of these compounds, and the relative kinetics among different congeners can be different in different species (Van den Berg et al. 1994). This was accounted for in the derivation of the TEF values on an administered dose basis.

However, a logical result of the differences among congeners in pharmacokinetics is that under conditions of chronic exposure, the relationship between body burden or tissue concentration and response may be quite different from the relationship between administered dose and response, and the potencies relative to TCDD may shift among the different compounds.

For example, DeVito et al. (1997), in a study in mice of disposition and enzyme induction potency for several dioxin and furan congeners, compared potency on the basis of both administered dose and tissue concentration and found significant differences in the two approaches. Based on their results, they concluded that:

> "These data suggest that two sets of TEF values may be useful in estimating risk of dioxinlike compounds. One set of values would be used for estimating intake equivalents and the other for estimating tissue equivalents."

The effect of this issue can be seen when examining the results of the recent two-year National Toxicology Program carcinogenicity bioassays of TCDD, 4-PeCDF, PCB-126, and a mixture of the three (NTP 2004a; NTP 2004b; NTP 2004c; NTP 2004d). Walker et al. (2005) have reported that for some of the tumor endpoints, the TEF/TEQ approach works reasonably well on an administered-dose basis. That is, the degree of tumor response is similar across the various compounds and the mixture for TEQ-equivalent administered doses (although 4-PeCDF showed little tumor response at any dose, and its potency was consistently over predicted by its TEF). However, the body burdens on a TEQ basis in these four bioassays vary dramatically. For TEQ-equivalent administered doses, the lifetime average TEQ body burdens in the 4-PeCDF, PCB 126, and TEQ mixture bioassays are approximately seven-fold, three-fold, and four-fold higher than in the TCDD bioassay, respectively. This indicates that the two tested compounds other than TCDD and the TEQ mixture, on a TEQ body burden basis, are at least several-fold less potent as carcinogens than TCDD. That is, on a body burden basis, the TEQ approach over-predicts the carcinogenicity of these compounds. This result is consistent with an earlier subchronic liver tumor promotion study which found that, on a tissue concentration basis, 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD) and 4-PeCDF had substantially lower potencies relative to TCDD than when assessed on an administered dose basis (Waern et al. 1991).

The practice of measuring the concentrations of PCDD/Fs in human serum lipid and converting those concentrations to TEQs has become routine, yet this conversion process explicitly applies relative toxicity estimates based on administered doses (the TEFs) to tissue concentrations to express serum lipid concentrations in TCDD "toxic equivalents." The recent cancer bioassay data from NTP highlight the pitfalls that can arise when the current TEFs, which were developed through comparisons of potency on an administered-dose basis, are applied to assessing and extrapolating risk on a body burden or tissue concentration basis.

Data available to date indicate that, for the risk endpoints of interest (developmental effects and, in the U.S., carcinogenic risk), the TEQ scheme consistently and substantially over predicts the activity of TEQ mixtures compared to TCDD alone on a body-burden or tissue-concentration basis. The International Programme on Chemical Safety, in coordination with the WHO, has recognized the emergence of this issue and the availability of new data and plans to explicitly address this topic at a meeting during the summer of 2005.

Intrinsic Interspecies Differences in Sensitivity

A hallmark of the toxicology of TCDD is substantial variability in sensitivity in response to TCDD among species and even among strains within a species. While differences in toxicokinetics (and thus, the resulting body burden from a given chronic intake dose) account for some portion of interspecies and strain differences in sensitivity, use of a body burden dose metric or adjustment for toxicokinetic differences does not eliminate or even meaningfully reduce these differences. Sensitivity to dioxin for specific endpoints can vary by orders of magnitude, even among different strains of mice and rats (Shen and Olson 1987; Tuomisto et al. 1999).

This can be illustrated by comparing benchmark dose modeling results on developmental endpoints for rats and mice presented by the USEPA (2003; Appendix III to Chapter 8 of Part II). Using the 34 data sets for which the endpoint of interest displayed a significant response at some tested dose and for which the EPA benchmark dose model gave an acceptable fit, the range of benchmark dose estimates for a 1% response level (ED_{01}) for two species can be compared (Figure 6). These data sets consist of 16 mouse and 18 rat developmental endpoints (with some overlap in endpoints across the two species) observed subsequent to single-dose administration of TCDD to dams at critical periods during gestation. Because these are single-dose studies, the maternal body burdens during the critical developmental periods are completely correlated with the administered doses and are not affected by the somewhat different kinetics between rats and mice. That is, a single dose of 100 ng/kg on the critical gestational day is presumed to result in a similar peak maternal body burden in both species, regardless of subsequent elimination rates. However, the responsiveness is substantially different between these two species.

The median modeled ED_{01} value for the two species differs by a factor of more than 300 (Figure 6). The modeled mouse ED_{01} values are all higher than the median rat ED_{01} value. These data sets illustrate that large interspecies differences in sensitivity to TCDD exist, even when body burden is used as the dose metric for comparison among species. Such interspecies variability still must be taken into account in extrapolations of risk among species, even when body burden is used as the dose metric.

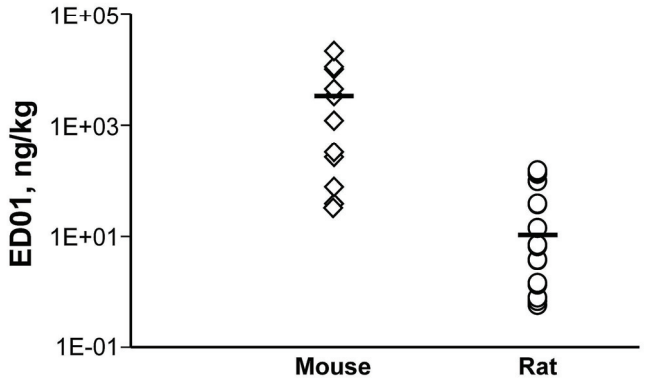


Figure 6. Plot of EPA-modeled ED_{01} values for mice and rats for developmental endpoints in mice (n=17) and rats (n=18) following administration of a single dose (ng/kg maternal bodyweight) of TCDD to dams during critical periods of gestation. Because these are single dose studies, the peak maternal body burden on the critical gestational day for each study is directly correlated with the single administered dose on a ng/kg bodyweight basis (i.e., peak body burden would differ from the administered dose only to the extent that the doses are not fully absorbed). Median ED_{01} values for the two species are indicated by the horizontal bars. The plotted ED_{01} values are based on EPA's benchmark dose modeling procedure, rather than risk-based benchmark doses. The median ED01 for these endpoints in mice, 3300 ng/kg, is more than 300 times greater than the corresponding median in rats, 10.5 ng/kg, and all benchmark doses for mice are above the median benchmark dose for rats, illustrating a fundamental difference in sensitivity to TCDD between mice and rats.

Recent risk assessments for dioxin have acknowledged the variability in species sensitivity and the likelihood that humans are in the mid-range of sensitivity (JECFA 2001; ECSCF 2001). For example, the USEPA (2003) stated that:

> "It is well known that individual species vary in their sensitivity to any particular dioxin effect… However, the evidence available to date indicates that humans most likely fall in the middle rather than at either extreme of the range of sensitivity for individual effects among animals. In other words, evaluation of the available data suggests that humans, in general, are neither extremely sensitive nor insensitive to the individual effects of dioxin-like compounds" (Part III, p. 6 2).

The basis for interspecies differences is almost certainly multi-factorial. One source of differences in sensitivity among strains and species has been identified. The DBA mouse is at least 10-fold less sensitive than the C57 mouse to the toxic effects of TCDD. The Ah receptor (AhR) of the DBA mouse carries two key mutations compared to that of the C57 mouse, one each in the binding domain and the transactivation domain. As a result, the AhR of the DBA mouse has a lower affinity for TCDD binding, and the bound receptor-ligand complex is less efficacious in producing toxicity (reviewed in Connor and Aylward, 2006). Humans share the same two key mutations in the AhR, and these mutations convey the same reduction in responsiveness to TCDD compared to C57 mice in a humanized chimeric mouse model in which a human AhR was inserted into a C57 AhR-knockout mouse (Moriguchi et al. 2003). The human AhR exhibits some polymorphisms, but the polymorphisms observed to date have not involved the key binding and transactivation domain mutations and have not been correlated with binding affinity (Harper et al. 2002; Nebert et al. 2004; Okey et al. 2005).

In addition, more recent research using gene-sequencing techniques has demonstrated interspecies differences in the numbers and identities of dioxin-responding genes. The human genome contains fewer than 40% of the active dioxin-responding genes found in mice and rats (Sun et al. 2004), raising the possibility that, in addition to quantitative reductions in sensitivity in humans due to the generally low-affinity AhR present in humans, there is a reduction in the number of responding genes in humans compared to the laboratory rodents. Depending on the specific differences in responding genes between rodents and humans, this reduction in responding genes could have a direct effect on toxic responses. These authors conclude that this research is likely to "fuel the debate regarding the suitability of rodent models to assess the potential human health risks associated with exposure to AhR ligands" (Sun et al. 2004, p. 4522).

These data suggest that direct extrapolation of the most sensitive adverse responses from sensitive rodent species to humans may be unnecessarily conservative. The ECSCF (2001) and JECFA (2001) risk assessments incorporated this understanding by applying minimal interspecies uncertainty factors to the most sensitive rodent responses evaluated on a body burden basis in their derivations of tolerable daily intakes. While the conventional approach to risk assessments may suggest a reliance on the most sensitive laboratory species in order to be protective of sensitive human subpopulation, the use of additional uncertainty factors in risk assessment for variations in human sensitivity may not be necessary if this approach is adopted for dioxin, because the most sensitive human may be less sensitive than the most sensitive rodents.

Conclusions and Research Needs

The focus of recent risk assessments for dioxin on potential adverse effects to offspring from *in utero* exposure to dioxins is appropriate. However, quantitative extrapolation of the animal data on these endpoints to potential human risks and in the context of identifying tolerable daily intakes should take into account the full range of available detailed data on kinetics and distribution, on interspecies sensitivity, and on the appropriateness of the TEF approach for body burden-based assessments. The rich database available on TCDD and related compounds, while still lacking key studies, allows a critical evaluation and possible modification of the conventional risk assessment assumptions for interspecies extrapolation. The available data on maternal to fetal transfer of non-TCDD congeners, the importance of dose regimen, limitations in the TEF approach on a body burden basis, and interspecies differences in sensitivity suggest that these conventional assumptions may result in overly conservative estimates of risk from general population exposures to dioxins.

Numerous areas of research could increase the reliability of and scientific foundation for risk extrapolations from animals to humans for dioxins.

- Chronic, low-dose administration studies of the developmental effects identified in studies employing acute gavage TCDD administration during gestation. These studies should include multiple low-dose groups employing administration of both TCDD-only and of a TEQ mixture that is representative of current human background exposures (i.e.,not dominated by TCDD). Ideally, this research should be carried out in more than one strain of rat, including Sprague-Dawley, and could include one or more mouse strain. Finally, these studies should include tissue analysis for the administered compounds during the critical gestation periods.
- Evaluation of human elimination kinetics for non-TCDD congeners. To date, only limited data are available for evaluation of human elimination kinetics of non-TCDD congeners. The relative pattern of elimination rates for different congeners across species is important in applying the results of subchronic studies across species using administered dose, and in understanding the relevance of tissue concentration-based evaluations for current human intake patterns. These studies may be very difficult or impossible to conduct in a consistent and structured way, but any opportunities should be seized on to develop further data on non-TCDD congeners, for example, in populations with previous known elevated exposures.
- Assessments of relative potency on a tissue concentration basis. A number of subchronic studies are now available that examine a

limited subset of responses using a range of congeners and that report tissue concentrations. For the major congeners of interest, these data sets could be used to develop a system of TEF values (even if incomplete) for use in assessing tissue concentrations. Studies of toxic responses *in vivo* should routinely include measurements of relevant tissue concentrations of the tested compounds. Efforts are underway by the IPCS and WHO to complete a reevaluation of the 1998 WHO TEF system during the summer of 2005. Explicitly under consideration during this meeting is an assessment of whether sufficient data exist to develop an alternative TEF scheme based on tissue concentrations. Even if sufficient data are not available to establish internal TEFs, this effort should provide further guidance on appropriate application of the current, intake-based TEFs.

- Proteomic and genomic evaluations. The expanding field of genomics and proteomics should continue to develop information on gene expression in response to administration of TCDD and related compounds between species (including humans) in order to begin to elucidate the basis for interspecies differences and to provide further basis for understanding and assessing relative human responsiveness. These efforts should include identification and characterization of responding genes and their functions.

Acknowledgements
Funding for this review was provided by the Chlorine Chemistry Council.

References

Birnbaum, L. S., and Tuomisto, J. (2000). Non-carcinogenic effects of TCDD in animals. *Food Addit. Contam*. **17(4)**, 275–288.

Chen, C. Y., Hamm, J. T., Hass, J. R., and Birnbaum, L. S. (2001). Disposition of polychlorinated dibenzo-p-dioxins, dibenzofurans, and non-ortho polychlorinated biphenyls in pregnant Long Evans rats and the transfer to offspring. *Toxicol. Appl. Pharmacol*. **173(2)**, 65–88.

Connor, K.T., and Aylward, L.L. (2006). Human response to dioxin: Aryl hydrocarbon receptor (AhR) molecular structure, function, and dose-response data for enzyme induction indicate an impaired human AhR. *J. Toxicol. Environ Health Crit. Rev. Part B,* 9:147-171.

DeVito, M. J., Diliberto, J. J., Ross, D. G., Menache, M.G., and Birnbaum, L. S. (1997). Dose-response relationships for polyhalogenated dioxins and dibenzofurans following subchronic treatment in mice. I. CYP1A1 and CYP1A2

enzyme activity in liver, lung, and skin. *Toxicol. Appl. Pharmacol*. **147(2)**, 267–280.

European Commission Scientific Committee on Foods (ECSCF) (2001). Opinion of the Scientific Committee on Food on the risk assessment of dioxins and dioxin-like PCBs in food. Update based on new scientific information available since the adoption of the SCF opinion of 22nd November 2000. Rep. CS/CNTM/DIOXIN/20 final. European Commission Scientific Committee on Foods, Brussels, Belgium. Avaliable at: http://europa.eu.int/comm/food/fs/sc/scf/out90_en.pdf.

Harper, P.A., Wong, J. Y., Lam, M. S., and Okey, A. B. (2002). Polymorphisms in the human AH receptor. *Chem Biol Interact.* **141(1-2)**, 161-187.Hurst, C. H., DeVito, M. J., and Birnbaum, L. S. (2000). Tissue disposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in maternal and developing Long-Evans rats following subchronic exposure. *Toxicol. Sci*. **57(2)**, 275–283.

Hurst, C.H., DeVito, M. J., Setzer, R. W., and Birnbaum, L. S. (2000). Acute administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in pregnant Long Evans rats: Association of measured tissue concentrations with developmental effects. *Toxicol. Sci*. **53(2)**, 411–420.

JECFA. 2001. Joint FAO/WHO Expert Committee on Food Additives, fifty-seventh meeting, Rome, 5–14 June. Summary and conclusions. Available at: http://www.who.int/ipcs/food/jecfa/summaries/en/summary_57.pdf.

Moriguchi, T., Motohashi, H., Hosoya, T., Nakajima, O., Takahashi, S., Ohsako, S., Aoki, Y., Nishimura, N., Tohyama, C, Fujii-Kuriyama, Y., and Yamamoto, M. (2003). Distinct response to dioxin in an arylhydrocarbon receptor (AHR)-humanized mouse. *Proc. Natl. Acad. Sci. USA* **100(10)**, 5652–5657.

National Toxicology Program (NTP). (2004a). *DRAFT NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4) in Female Harlan Sprague-Dawley Rats (Gavage Study) (NTP TR 525)*, National Toxicology Program.

National Toxicology Program (NTP). (2004b). *DRAFT NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (CAS No. 1746-01-6) in Female Harlan Sprague-Dawley Rats (Gavage Study) (NTP TR 521)*, National Toxicology Program.

National Toxicology Program (NTP). (2004c). *DRAFT NTP Technical Report on the Toxicology and Carcinogenesis Studies of 3,3'4,4',5-Pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in Female Harlan Sprague-Dawley Rats (Gavage Study) (NTP TR 520)*, National Toxicology Program.

National Toxicology Program (NTP). (2004d). *DRAFT NTP Technical Report on the Toxicology and Carcinogenesis Studies of a Mixture of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (CAS No. 1746.01-6), 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4), and 3,3'4,4',5-Pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in Female Harlan Sprague-Dawley Rats (Gavage Studies) (NTP TR 526)*, National Toxicology Program.

Nebert, D.W., Dalton, T. P., Okey, A. B., and Gonzalez, F. J. (2004). Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer*. J Biol Chem.* **279(23)**, 23847-23850.

Okey, A.B., Boutros, P. C., and Harper, P.A. (2005). Polymorphisms of human nuclear receptors that control expression of drug-metabolizing enzymes. *Pharmacogenet Genomics.* **15(6),** 371-379.

Shen, E. S., and Olson, J. R. (1987). Relationship between the murine Ah phenotype and the hepatic uptake and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Drug Metab. Dispos*. **15(5)**, 653–660.

Sun, Y. V., Boverhof, D. R., Burgoon, L. D., Fielden, M. R., and Zacharewski, T. R. (2004). Comparative analysis of dioxin response elements in human, mouse and rat genomic sequences. *Nucleic Acids Res*. **32(15)**, 4512–4523.

Theobald, H. M., and Peterson, R. E. (1997). In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-rho-dioxin: Effects on development of the male and female reproductive system of the mouse. *Toxicol. Appl. Pharmacol*. **145(1)**, 124–135.

Tuomisto, J. T., Viluksela, M., Pohjanvirta, R, and Tuomisto, J. (1999). The Ah receptor and a novel gene determine acute toxic responses to TCDD: Segregation of the resistant alleles to different rat lines. *Toxicol. Appl. Pharmacol*. **155(1)**, 71–81.

United Kingdom Committee on Toxicology (UKCOT) (2001). Statement on the tolerable daily intake for dioxins and dioxin-like polychlorinated biphenyls. http://www.food.gov.uk/science/ouradvisors/toxicity/statements/cotstatements2001/dioxinsstate.

United States Environmental Protection Agency (USEPA). (2003). Exposure and human health reassessment of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds. NAS Review Draft. U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC.

Van den Berg, M., De Jongh, J., Poiger, H., and Olson, J. R. (1994). The toxicokinetics and metabolism of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity. *Crit Rev Toxicol.* **24(1),** 1-74.

Van den Berg, M., Birnbaum, L., Bosveld, A. T., Brunstrom, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., van Leeuwen, F. X., Liem, A. K., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., and Zacharewski, T. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect*. **106(12)**, 775–792.

Waern, F., Flodstrom, S., Busk, L., Kronevi, T., Nordgren, I., and Ahlborg, U.G. (1991). Relative liver tumour promoting activity and toxicity of some polychlorinated dibenzo-p-dioxin- and dibenzofuran-congeners in female Sprague-Dawley rats. *Pharmacol Toxicol.* **69(6),** 450-458.

Walker, N. J., Crockett, P., Nyska, A., Brix, A., Jokinen, M. P., Sells, D. M., Hailey, J. R., Easterling, M., Haseman, J. K., Yin, M., Wyde, M. E., Bucher, J. R., and Portier, C. J.. (2005). Dose-additive carcinogenicity of a defined mixture of "dioxin-like compounds." *Environ. Health Perspect*. **113(1)**, 43-48.

Chapter 4: Relative cancer potencies of selected dioxin-like compounds on a body burden basis: Comparison to current Toxic Equivalency Factors (TEFs)

Gray, M.N., L.L. Aylward, and R.E. Keenan. 2006. J. Toxicol. Environ. Health. 69:907-917.

http://www.informaworld.com/smpp/content~db=all?content=10.1080/15287390500360042

Abstract

Recent National Toxicology Program (NTP) cancer bioassay data for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), 3,3',4,4',5-pentachlorobiphenyl (PCB 126), and a mixture of these three compounds offer opportunities to assess the accuracy of current World Health Organization (WHO) 1998 toxic equivalency factors (TEFs) for these compounds under a variety of assumptions. An evaluation of the current TEF values for these compounds using body burden in ng/kg as the dose metric is presented. Average lifetime body burdens were estimated for all compounds at all dose groups based on measured tissue concentrations at 4 time points during the two-year NTP studies. Poly-3 adjusted tumor incidences for hepatocellular adenomas, cholangiocarcinomas, and the two tumors combined were modeled using a quantal multistage model and the Hill model with lifetime average body burden as the dose metric. Benchmark doses for a 10% response (BMD_{10}) for each compound and the mixture were estimated. With TCDD as the reference standard, relative potency (REP) estimates were derived from ratios of the BMD_{10} estimates for PCB 126, 4-PeCDF, and for the TEQ mixture. On a body burden basis, PCB 126 and 4-PeCDF were 2-to-3 fold and 10-to-12 fold less potent than predicted based on the WHO TEFs, respectively, while the TEQ mixture was approximately 3-to-5 fold less potent than predicted by the TEFs. The current WHO TEF values, which were derived from data on non-cancer endpoints evaluated on an administered dose basis, over predict the carcinogenic potency of these compounds on a body burden basis compared to TCDD.

Introduction

The National Toxicology Program (NTP) recently published the results of detailed carcinogenicity bioassays for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), 3,3',4,4',5-pentachlorobiphenyl (PCB 126), and a mixture of these three compounds using female Sprague-Dawley rats. These bioassays offer the opportunity to evaluate the World Health Organization (WHO) scheme of toxic equivalency factors (TEFs) (Van den Berg et al., 1998), which present estimates of the relative potency of dioxin-like compounds compared to TCDD. Walker et al. (2005) published an analysis of the tumor responses in these bioassays on an administered dose basis and concluded that the data supported the assumption of additive response underlying the TEF scheme, although the potency of 4-PeCDF was lower than predicted by the TEFs.

Due to wide interspecies differences in the pharmacokinetics of TCDD, the current focus in human risk assessment for dioxin-like compounds is on assessing toxicity and exposure on a body burden basis. The current TEF/TEQ scheme, although developed on an administered dose basis, has been widely

applied in such body burden-based risk assessments (JECFA, 2001; ECSCF, 2001; EPA, 2003a). This paper reports an evaluation of the accuracy of the current TEFs at predicting the relative potency of 4-PeCDF, PCB 126, and the TEQ mixture tested in the NTP bioassays on a body burden basis.

Methods

Tumor incidence and tissue concentration data from NTP bioassays for TCDD, 4-PeCDF, PCB 126, and a mixture of the three compounds (NTP, 2004a; 2004b; 2004c; 2004d) were used to evaluate dose-response. Tissue concentration data for the components of the TEQ mixture bioassay were first converted to TEQs using the WHO TEFs (Van den Berg et al., 1998). For each bioassay, body concentrations, C_b, in ng/kg were estimated for each dose group at weeks 14, 31, 53, and 104 using reported liver, adipose, blood, and lung tissue concentrations of the tested compounds (C_h, C_a, C_{bl}, and C_l, respectively), as follows (results below limits of quantitation or for tissue/time points that were not analyzed were set to zero):

$$C_b = C_h w_h + C_a w_a + C_l w_l + C_{bl} w_{bl} \qquad (1)$$

Liver and lung weights as a fraction of bodyweight (w_h and w_l) were available from interim sacrifice data in the bioassay reports for weeks 14, 31, and 53; for week 104, the same tissue weight fractions reported from week 53 were used. Blood and adipose tissue weight fractions (w_{bl} and w_a) of 0.0176 and 0.07, respectively, were assumed to apply throughout the experiment, consistent with the assumptions made in the pharmacokinetic modeling reported in the NTP bioassays. The time-weighted average lifetime body burden ($C_{b,avg}$) was estimated for each dose group based on the estimated body concentrations at each of the four time points (t_1 to t_4 in weeks), as follows:

$$C_{b,avg} = \frac{\left[t_1 \frac{(C_{b,t_1})}{2} + (t_2 - t_1)\frac{(C_{b,t_1} + C_{b,t_2})}{2} + (t_3 - t_2)\frac{(C_{b,t_2} + C_{b,t_3})}{2} + (t_4 - t_3)\frac{(C_{b,t_3} + C_{b,t_4})}{2} \right]}{104} \qquad (2)$$

Body concentration at the beginning of the experiment was assumed to be zero.

This procedure is likely to underestimate actual body burdens for several reasons. First, rodent skin and muscle generally contain a few % of body burden (Hurst et al., 2000; Diliberto et al., 2000), but the NTP bioassays did not include data on the compound concentrations in these tissues. Second, the use of a constant estimate of adipose tissue weight fraction of 0.07 probably underestimates the true adipose bodyweight fraction at the end of

the experiment because of physiological changes due to aging, and thus would result in an underestimate of body concentrations. Finally, use of zero for results below the limit of quantitation and for samples that were not analyzed also would tend to result in an underestimate of true tissue and body concentrations.

Tumor incidences for hepatocellular adenomas, cholangiocarcinomas (the most sensitive tumor types observed in the bioassays) and the combination of these two hepatic tumors (with animals bearing either or both tumor(s) counted only once) were estimated incorporating the use of a poly-3 adjustment for survival (Bailar and Portier, 1988). Tumor incidences were modeled as a function of estimated body burden using the quantal multistage model (EPA benchmark dose modeling software version 1.3.2) and using a Hill model (fit using Microsoft Excel®) of the form:

$$P = b_0 + (1 - b_0)\frac{d^n}{b_1^n + d^n}, \qquad (3)$$

where P is the probability of tumor development, b_0 is the background tumor incidence, b_1 is the body burden for half-maximal response, d is the "dose" in units of body burden (ng/kg), and n is the Hill coefficient or shape parameter. The Hill model as formulated here is constrained to force a maximum tumor response of 100%.

Data used in the benchmark dose model included the same estimates of body burden, poly-3 adjusted number of animals, and tumor incidences as used in the Hill model. In the EPA benchmark dose software the parameter settings that were selected included a dichotomous multistage model, smoothing option set to *unique*, risk type set as *extra*, benchmark response set at 0.10, confidence level set at 0.95, and beta values restricted to values greater than or equal to zero.

Benchmark doses associated with an extra risk of 10% for tumors (BMD_{10}) were chosen for dose-response comparisons across compounds because this level was generally within the experimentally observed range of response for these tumors in these bioassays and thus did not require extrapolation either above or below the range of the observed responses (except for 4-PeCDF, which failed to produce a 10% response for cholangiocarcinomas at any tested dose). The relative potencies (REPs) of 4-PeCDF, PCB 126, and the TEQ mixture were estimated by comparing their respective BMD_{10} estimates to the BMD_{10} for TCDD. The REPs for 4-PeCDF and PCB 126 were compared to the WHO 1998 TEFs for these compounds (0.5 and 0.1, respectively). The REP for the mixture bioassay was compared directly to the TEF for TCDD, 1.0, as the body burden estimates for this bioassay were calculated and expressed in terms of TEQs for the combination of the three administered compounds.

Results

The measured tissue concentrations and tissue weight fractions for the bioassay of TCDD as reported by NTP (2004a) are detailed in Table 1. Table 1 also presents the calculated interim and lifetime average body burdens for all TCDD dose groups; similar calculations were performed for all dose groups in the other three bioassays (data not shown). Figure 1 illustrates the estimation of lifetime average body burden using equation 2 for one dose group from the TCDD bioassay. Interpolated average body concentrations between each of the measured time points were weighted by the number of weeks between each measured time point to derive the lifetime average concentration.

The lifetime average body concentrations and survival-adjusted tumor incidences for hepatocellular adenomas, cholangiocarcinomas, and the combination of the two tumors are presented in Table 2.

The fitted Hill model parameters for each tumor type and bioassay are presented in Table 3. The cholangiocarcinoma response in the 4-PeCDF bioassay did not exceed 6%. As a result, a best fit of the Hill model could not be determined for this tumor endpoint for that bioassay. A range of more linear (shape parameter $n<1.5$) and non-linear ($n>1.5$) responses were observed. For TCDD, the shape parameters for all three model fits for both tumor types and for the combined tumors were greater than 3, indicating a highly non-linear dose-response. The PCB 126 hepatocellular adenoma data suggested a more linear response, as did the data for the cholangiocarcinoma response to the TEQ mixture. For the combined tumor types the responses were generally non-linear in all four bioassays.

Table 1: Average tissue concentration and tissue weight fraction data from the NTP (2004a) bioassay of TCDD and calculated interim and lifetime average TCDD body concentrations.

Dose group, ng/kg-d[a]	Tissue concentrations, ng/kg				Tissue weight fraction relative to body weight			
	Week 14	Week 31	Week 53	Week 104	Week 14	Week 31	Week 53	Week 104[b]
Liver								
0	0	0	249	0	0.029	0.030	0.032	0.032
3	676.2	598.9	499.38	680.71	0.035	0.035	0.033	0.033
10	2,435	2,079	1,940	2,213	0.036	0.036	0.035	0.035
22	4,931	4,489	4,437.5	4,364	0.035	0.037	0.038	0.038
46	9,970	9,522	7,818.75	6,413	0.038	0.039	0.041	0.041
100	18,280	21,180	15,762.5	9,325	0.040	0.040	0.045	0.045
Adipose								
0	0	0	0	0				
3	295.33	378.1	304.29	505.38				
10	708.6	767.5	633.5	753	Not measured; assumed to be 0.07 for all dose groups and times[c]			
22	1,272.8	1,572	1,272.63	1,403.8				
46	2,620	3,089	2,502.5	1,996				
100	6,395	7,848	5,816.25	3,177				
Lung								
0	BLOQ	BLOQ	BLOQ	BLOQ	0.006	0.006	0.006	0.006
3	BLOQ	BLOQ	BLOQ	BLOQ	0.007	0.008	0.006	0.006
10	BLOQ	10500	BLOQ	BLOQ	0.007	0.008	0.006	0.006
22	BLOQ	BLOQ	BLOQ	BLOQ	0.006	0.007	0.006	0.006
46	BLOQ	BLOQ	963	BLOQ	0.007	0.008	0.007	0.007
100	BLOQ	BLOQ	BLOQ	BLOQ	0.007	0.008	0.009	0.009
Blood								
0	NA	NA	BLOQ	BLOQ				
3	NA	NA	NA	BLOQ				
10	NA	NA	NA	BLOQ	Not measured; assumed to be 0.018 for all dose groups and times[c]			
22	NA	NA	NA	NA				
46	NA	NA	NA	NA				
100	NA	NA	NA	NA				
Body concentration[d]					Time-weighted lifetime average body concentration[e]			
0	0	0	8	0	3			
3	44	48	38	58	43			
10	138	213	113	131	132			
22	262	275	258	265	246			
46	561	591	503	403	470			
100	1,184	1,398	1,114	640	987			

BLOQ: Below limit of quantitation
NA: Not analyzed.
[a] Corn oil gavage, 5 d/wk
[b] Tissue weights not reported for week 104; values from week 53 were used in these calculations.
[c] As assumed in NTP pharmacokinetic modeling (NTP 2004a).
[d] Calculated using equation 1.
[e] Calculated using equation 2.

Table 2: Estimated lifetime average body concentrations and survival-adjusted tumor incidences for hepatic adenomas, cholangiocarcinomas, and the two tumor types combined for bioassays of TCDD, 4-PeCDF, PCB 126, and the TEQ mixture.

			Tumor incidence[b]		
Compound	**Dose group ng/kg-d[a]**	**Lifetime average body conc. (ng/kg)**	**Hepato-cellular adenoma (HA)**	**Cholangio-carcinoma (CC)**	**Combined HA, CC**
TCDD	0	3	0.00	0.00	0.00
	3	43	0.00	0.00	0.00
	10	132	0.00	0.00	0.00
	22	246	0.00	0.03	0.03
	46	470	0.03	0.10	0.13
	100	987	0.28	0.54	0.62
4-PeCDF	0	13	0.02	0.00	0.02
	6	499	0.00	0.00	0.00
	12	1,734	0.03	0.00	0.03
	44	3,753	0.00	0.03	0.03
	92	7,920	0.06	0.03	0.08
	200	16,306	0.11	0.05	0.16
PCB-126	0	37	0.03	0.00	0.03
	30	1,316	0.05	0.00	0.05
	100	3,703	0.03	0.03	0.05
	175	6,101	0.00	0.00	0.03
	300	10,118	0.05	0.14	0.19
	550	17,306	0.09	0.14	0.26
	1000	32,813	0.19	0.59	0.67
TEQ mixture	0	2 [c]	0.00	0.00	0.00
	10	454 [c]	0.02	0.00	0.02
	22	948 [c]	0.02	0.05	0.07
	46	2,040 [c]	0.02	0.17	0.20
	100	4,393 [c]	0.28	0.23	0.49

[a] Corn oil gavage, 5 d/wk
[b] Poly-3 survival adjustment (Bailar and Portier 1988)
[c] Units are ng TEQ/kg

Table 3: Hill model fitted parameters

Tumor type	Parameter	Parameter values			
		TCDD	4-PeCDF	PCB 126	TEQ Mixture
Hepatocellular adenoma	b_0	0.000	0.012	0.020	0.016
	b_2	1,454	71,500	133,300	5,539
	n	3.56	1.53	1.16	4.69
	R^2	.99	.97	.94	.99
Cholangio-carcinoma	b_0	0.000	undefined	0.000	0.000
	b_2	1 062	undefined	26,160	13,355
	n	3.12	undefined	2.23	1.02
	R^2	.99	--	.99	.97
Combined HA, CC	b_0	0.002	0.012	0.040	0.005
	b_2	846	59,000	25,659	4,538
	n	3.27	1.33	2.53	1.75
	R^2	.99	.99	.97	.99

[a] The observed tumor incidences are too low to define a fit for the Hill model.

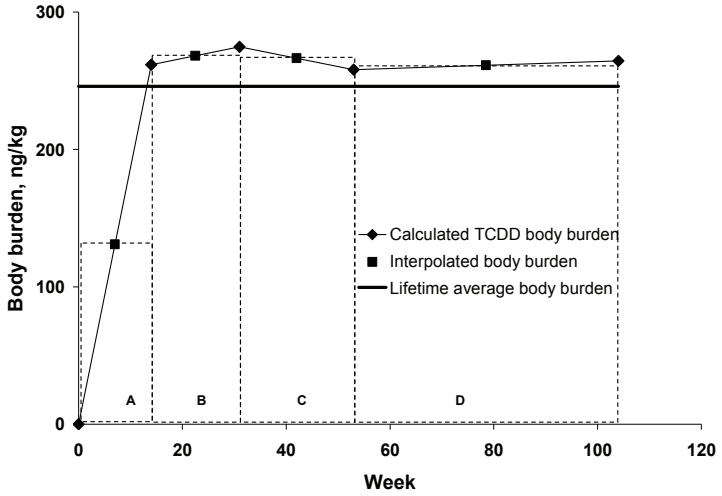


Figure 1: Illustration of trapezoidal interpolation to derive lifetime average body burden based on calculated body burdens at weeks 14, 31, 53, and 104 in animals in the TCDD 22 ng/kg-d dose group. Average concentrations between each of the measured time points are used to calculate the area of rectangles A, B, C, and D above in ng/kg*wks. The areas are summed and then divided by the total number of weeks in the experiment (104) to obtain the lifetime average body burden in ng/kg.

The BMD_{10} estimates from both models for both tumor types individually and combined and the respective REPs for 4-PeCDF, PCB 126, and the TEQ mixture are reported in Table 5. The REPs were all substantially lower than predicted based on the WHO TEFs. Similar results were obtained across the 4 bioassays for each of the individual hepatic tumor sites, using either the quantal multistage or Hill model. Relative potencies change somewhat if different response levels (e.g., 1% or 50% rather than 10%) are used for comparison, but the overall pattern of results is similar (results not shown). Using the 10% response benchmark, the common point of departure suggested for use in cross-chemical hazard ranking comparisons (EPA 2003b), the current WHO TEFs over estimate potency of 4-PeCDF and PCB 126 by factors of approximately 10 to 12 and 2 to 3, respectively, relative to the potency of TCDD expressed in the NTP bioassay (see Figure 2). The TEQ mixture, which was composed of equal amounts of the three compounds on a TEQ administered dose basis, displayed REPs ranging from 0.21 to 0.35 on a body burden basis depending on tumor type. The REP of 0.35 for the combined tumors of the TEQ mixture study was mathematically similar to the mean of the body burden based REPs for the three individual compounds.

Table 4: Benchmark dose estimates (ng/kg lifetime average body burden) and estimates of relative potency for 4-PeCDF, PCB 126, and the TEQ mixture bioassay compared to TCDD.

		Hepatocellular adenoma				Cholangiocarcinoma				Combined			
		Hill model		LMS		Hill model		LMS		Hill model		LMS	
Compound	**TEF[a]**	**BMD_{10}**	**REP**	**BMD_{10}**	**REP**	**BMD_{10}**	**REP**	**BMD_{10}**	**REP**	**BMD_{10}**	**REP**	**BMD_{10}**	**REP**
TCDD	1	785	1	727	1	525	1	469	1	430	1	439	1
4-PeCDF	0.5	15,700	0.05	16,100	0.04	[c]	[c]	28,800	0.02	10,300	0.04	11,200	0.04
PCB 126	0.1	16,400	0.05	24,400	0.03	9770	0.05	11,600	0.02	8,780	0.05	10,300	0.04
TEQ Mixture	1	3,340[d]	0.23	3,500[d]	0.21	1560[d]	0.34	1,660[d]	0.28	1,260[d]	0.34	1,260[d]	0.35

[a] WHO 1998 TEF values. [b] Relative potency = $BMD_{TCDD}/BMD_{Compound}$. [c] Model fit undefined. [d] Units are ng TEQ/kg.

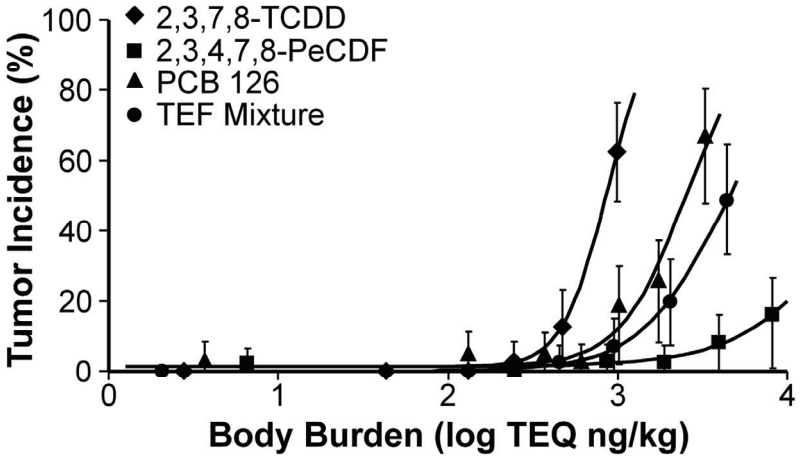


Figure 2: Poly-3 adjusted tumor incidence (combined hepatocellular adenomas and cholangiocarcinomas) v. log (TEQ body burden) for all 4 bioassays with Hill model-fit curves. Body burdens are plotted on a TEQ basis using the WHO 1998 TEF values. The Hill model fits are generally non-linear, with Hill coefficients of 3.4, 1.3, 2.2, and 1.7 for TCDD, 4-PeCDF, PCB 126, and the TEF mixture, respectively. If the TEF values adequately accounted for differences in relative potency on a body burden basis, the curves should be coincident. Instead, the curves are displaced to the right compared to the TCDD curve, indicating lower potency on a body burden basis for both individual compounds and for the TEQ mixture than predicted by the WHO 1998 TEF values.

Discussion

Walker et al. (2005) evaluated the 2004 NTP bioassay data and found that the potencies of PCB 126 and the TEQ mixture were similar to TCDD when applying the WHO 1998 TEF values to administered dose, although 4-PeCDF was less potent than predicted even on an administered dose basis. In contrast, in this analysis based on body burden, the relative cancer potencies of PCB 126, 4-PeCDF, and the TEQ mixture were lower than predicted using the current WHO TEF scheme.

The relationship between administered dose and body burden following subchronic or chronic exposure to a compound depends upon the pharmacokinetic properties of that compound. The pharmacokinetic and distribution patterns for other TEQ-contributing compounds are substantially different from those of TCDD (DeVito et al., 1998), and these differences would be expected to have their greatest impact under chronic exposure

conditions and for endpoints such as carcinogenesis that require sustained exposure.

The current TEF values were developed primarily on the basis of relative potencies estimated by *administered dose* or based on *in vitro* studies of less complex responses such as enzyme induction (Van den Berg et al., 1998). The database used in the development of the 1998 WHO TEF values has never been published by the WHO. However, selected studies are cited in the publication presenting these values (Van den Berg et al., 1998). Subchronic studies were available for both 4-PeCDF and PCB 126, and some of these studies assess relative potency on a tissue concentration basis as well as on an administered dose basis. Waern et al. (1991) reported that in a 20 week study of liver tumor promotion, the REP for 4-PeCDF was approximately 0.007 (compared to the WHO TEF based on administered dose of 0.5). In a study of hepatic porphyrin accumulation and enzyme induction, relative potencies of 4-PeCDF and PCB 126 were generally lower on a tissue concentration basis than on an administered dose basis (van Birgelen et al., 1996). The role of hepatic sequestration observed in these and other studies due to binding of TCDD and other dioxin-like compounds to the CYP1A2 protein (Diliberto et al., 1999) in the observed tumor responses is unknown. If irreversible, binding to the CYP1A2 protein might serve as a protective mechanism for hepatic responses. However, if the binding occurs in a reversible equilibrium with "free" unassociated compound, the increased hepatic concentrations could result in increased responses compared to what would occur without the presence of the binding protein (Liang et al., 1997). Experimental studies in CYP1A2 knockout mice have not demonstrated increased hepatic responses or toxicity compared to parental mice, but the endpoints assessed have been limited (Slezak et al., 1999; Liang et al., 1997).

These findings of decreased relative carcinogenic potencies for two key TEQ contributors have significant implications for risk assessment approaches being used in the US and Europe. These approaches have generally relied upon toxicity assessments from studies of TCDD alone, and use of a body burden dose metric to quantify LOAEL/NOAELs (JECFA, 2001; ECSCF, 2001;) or cancer potencies (EPA, 2003a). Human biomonitoring through sampling of human serum for dioxin-like compounds has become more prevalent and is being used for population exposure monitoring. These biomonitoring results are routinely expressed in terms of TEQs, and formal and informal assessments of population risks have been made on this basis (EPA, 2003a).

However, human TEQ body burdens in the general population are typically composed of less than 10% TCDD (EPA, 2003a). Several other PCDD/Fs and PCB compounds, including 4-PeCDF, PCB 126, 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD), and a mixture of 2,3,7,8-substituted hexachlorodibenzo-p-dioxin (HxCDD) congeners, contribute to background human TEQ body

burdens at levels as great as or greater than TCDD when estimated using current WHO TEF values. Thus, these NTP bioassay data indicate that, when assessed on a TEQ body burden basis using the current WHO TEFs, at least two of these key compounds are substantially less potent than predicted by the TEFs. Comparable data on body burden and response are not available for PeCDD or HxCDD compounds.

This analysis suggests that for carcinogenesis, the current WHO TEF values substantially overpredict the cancer potency of 4-PeCDF and PCB 126 on a body burden basis. Thus, comparisons of measured human body burdens expressed in terms of TEQ to body burdens in animal studies using TCDD alone may provide an overestimate of carcinogenic risk. The TEF approach is routinely presented as an "order of magnitude" estimate of relative potencies. Because of the importance of cancer risk estimates in public health evaluations and decisions regarding dioxins in the U.S., the high quality data available from the NTP bioassays should be used to reexamine the current intake-based TEF values for any assessments of cancer risk of these compounds on a body burden basis.

Acknowledgements
This analysis was partially funded by the General Electric Company.

References

Bailer, A. J. and Portier, C. J. 1988. Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* 44:417-431.

DeVito, M.J., Ross, D.G., Dupuy, A.E., Ferrario, J., McDaniel, D., Birnbaum, L. S.1998. Dose-response relationships for disposition and hepatic sequestration of polyhalogenated dibenzo-p-dioxins, dibenzofurans, and biphenyls following subchronic treatment in mice. *Toxicol. Sci.* 46:223-234.

Diliberto, J. J., Burgin, D. E., Birnbaum, L. S. 1999. Effects of CYP1A2 on disposition of 2,3,7, 8-tetrachlorodibenzo-p-dioxin, 2,3,4,7,8-pentachlorodibenzofuran, and 2,2',4,4',5,5'-hexachlorobiphenyl in CYP1A2 knockout and parental (C57BL/6N and 129/Sv) strains of mice. *Toxicol Appl Pharmacol* 159:52-64.

Diliberto, J. J., DeVito, M. J., Ross, D. G., Birnbaum, L. S. 2001. Subchronic exposure of [3H]- 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in female B6C3F1 mice: relationship of steady-state levels to disposition and metabolism. *Toxicol. Sci.* 61:241-55.

European Commission Scientific Committee on Foods (ECSCF). 2001. *Opinion of the Scientific Committee on Food on the risk assessment of dioxins and dioxin-like PCBs in food. Update based on new scientific information available since the adoption of the SCF opinion of 22nd November 2000. Rep. CS/CNTM/DIOXIN/20 final.* European Commisssion, Brussels, Belgium

Hurst, C. H., DeVito, M. J., Birnbaum, L. S. 2000. Tissue disposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in maternal and developing Long-Evans rats following subchronic exposure. *Toxicol. Sci.* 57:275-283.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2001. *Fifty-seventh meeting Rome, 5-14 June 2001. Summary and conclusions. Rep. http://www.who.int/ipcs/food/jecfa/summaries/en/summary_57.pdf*

Liang, H. C., McKinnon, R. A., Nebert, D. W. 1997. Sensitivity of CYP1A1 mRNA inducibility by dioxin is the same in Cyp1a2(+/+) wild-type and Cyp1a2(-/-) null mutant mice. *Biochem Pharmacol* 54:1127-31.

National Toxicology Program (NTP). 2004a. *DRAFT NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4) in Female Harlan Sprague-Dawley Rats (Gavage Study) (NTP TR 525)*, National Toxicology Program.

National Toxicology Program (NTP). 2004b. *DRAFT NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (CAS No. 1746-01-6) in Female Harlan Sprague-Dawley Rats (Gavage Study) (NTP TR 521)*, National Toxicology Program.

National Toxicology Program (NTP). 2004c. *DRAFT NTP Technical Report on the Toxicology and Carcinogenesis Studies of 3,3'4,4',5-Pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in Female Harlan Sprague-Dawley Rats (Gavage Study) (NTP TR 520)*, National Toxicology Program.

National Toxicology Program (NTP). 2004d. *DRAFT NTP Technical Report on the Toxicology and Carcinogenesis Studies of a Mixture of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (CAS No. 1746.01-6), 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4), and 3,3'4,4',5-Pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in Female Harlan Sprague-Dawley Rats (Gavage Studies) (NTP TR 526)*, National Toxicology Program.

Slezak, B. P., Diliberto, J. J., Birnbaum, L. S. 1999. 2,3,7,8-Tetrachlorodibenzo-p-dioxin-mediated oxidative stress in CYP1A2 knockout (CYP1A2-/-) mice. *Biochem Biophys Res Commun* 264:376-9.

United States Environmental Protection Agency (USEPA). 2003a. *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin and Related Compounds. NAS Review Draft.*, National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, D.C.

United States Environmental Protection Agency (USEPA). 2003b. *Draft Final Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001A. www.epa.gov/ncea/raf/cancer2003.htm*, National Center for Environmental Assessment, U.S. Environmental Protection Agency, Washington, D.C.

van Birgelen, A. P., DeVito, M. J., Akins, J. M., Ross, D. G., Diliberto, J. J., Birnbaum, L. S. 1996. Relative potencies of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls derived from hepatic porphyrin accumulation in mice. *Toxicol Appl Pharmacol* 138:98-109.

Van den Berg, M., Birnbaum, L., Bosveld, A. T., Brunstrom, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., van Leeuwen, F. X., Liem, A. K., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* 106:775-792.

Waern, F., Flodstrom, S., Busk, L., Kronevi, T., Nordgren, I., Ahlborg, U. G. 1991. Relative liver tumour promoting activity and toxicity of some polychlorinated dibenzo-p-dioxin- and dibenzofuran-congeners in female Sprague-Dawley rats. *Pharmacol Toxicol* 69:450-8.

Walker, N. J., Crockett, P., Nyska, A., Brix, A., Jokinen, M. P., Sells, D. M., Hailey, J. R., Easterling, M., Haseman, J. K., Yin, M., Wyde, M. E., Bucher, J. R., Portier, C. J. 2005. Dose-additive carcinogenicity of a defined mixture of "dioxin-like compounds". *Environ. Health Perspect.* 113:43-48.

Chapter 5: Toxicokinetic Modeling for the NTP Bioassays of TCDD, 4-PeCDF, PCB-126, and a TEQ Mixture: Application of the Carrier et al. (1995) Model to Rat Distribution Data

L. L. Aylward; C. A. Cushing; R. Brunet

Sponsored by, prepared for, and included by permission of the Chlorine Chemistry Division of the American Chemistry Council, 1300 Wilson Boulevard, Arlington, VA 22209

Introduction

Results from chronic carcinogenesis bioassays carried out on female Sprague-Dawley rats for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), 3,3',4,4',5-pentachlorobiphenyl (PCB-126), and a mixture of these three compounds were recently published by the National Toxicology Program (NTP 2004a–d). One of the major goals of these studies was to provide a basis for assessing the dose-response for carcinogenesis of these compounds and for assessing the applicability of the toxic equivalency (TEQ) approach to risk assessment for TCDD and mixtures of TCDD and other compounds that are considered to have similar activity. The TEQ approach relies on the premise that each of the related dioxin, furan, and PCB compounds will behave identically to TCDD with only the relative potency differing among compounds. Data from the bioassays also allows assessment of the distribution of these compounds in the body, and provides for dose-response assessments based on tissue concentrations. This project was designed to apply a simple toxicokinetic model to the NTP bioassay data to facilitate tissue-based dose metric estimation for the dose groups and tumor response data sets from these four bioassays.

The toxicokinetic model used in this work is the model developed by Carrier et al. (1995a, b). The model is based on the assumption that distribution to the liver is concentration-dependent, with an increasing fraction of the body burden of TCDD or related compounds distributed to the liver as binding proteins (presumably CYP1A2) are induced. The balance of the body burden is assumed to distribute to adipose and lipid tissue throughout the body, resulting in a two-compartment model. Elimination of the compounds is presumed to occur in the liver through a first-order process. The full mathematical structure of the model is presented in Carrier et al. (1995a,b). The concentration dependence of distribution to the liver is represented in the model by the following relationship (variables as defined in Table 1):

$$f_h\left(C_b(t)\right) = f_{h\min} + \frac{(f_{h\max} - f_{h\min}) * C_b(t)}{K + C_b(t)} \qquad (1)$$

The function f_h is the fraction of the body burden that is distributed to hepatic tissue for a given body concentration $C_b(t)$. The function results in an increasing, saturating curve beginning with a minimal fraction of body burden distributed to hepatic tissue (f_{hmin}) at the lowest body concentrations, rising to a maximal fraction of body burden (f_{hmax}) at higher body burdens. The parameter K represents the body concentration at which the fraction of body burden distributed to hepatic tissue is half of the maximum fraction. This formulation of the distribution function follows the general form of a Michaelis-Menten relationship, which itself is a special case of the Hill function with the

shape parameter set to one. The Hill function is widely used in modeling receptor-mediated responses and has been extensively used in modeling dose-response for TCDD and related compounds (USEPA 2000; Walker et al. 1999; Toyoshiba et al. 2004).

This report describes the parameterization of the model for the individual congener bioassays and an attempt to extend the model to the mixture bioassay based on the TEQ approach and an assumed additivity for the compounds in the mixture. The results of the model include concentration vs. time profiles for liver, adipose, and whole-body for each single-congener bioassay and dose group. The results also include an assessment of the applicability of the TEQ approach on a tissue concentration basis for the mixture bioassay. These profiles are intended for use in subsequent evaluations of dose metrics and dose-response for the tumors observed in the NTP studies by Dr. Russell Keenan and his colleagues at AMEC, Inc.

Methods

The model was implemented in a Microsoft Excel® spreadsheet using numerical methods. Body concentration as a function of time was derived based on the following general function (variables defined in Table 1):

$$C_b(t_{i+1}) = C_b(t_i) + g(t_i) - [k_e * f_h(C_b(t_i)) * C_b(t_i)] - [C_b(t_i) * \frac{(BW(t_{i+1}) - BW(t_i)}{BW(t_i)}] \qquad (2)$$

The time step used in the modeling was one week.

Physiological Parameters

The physiological inputs for the model (bodyweight, liver weight, and adipose/lipid tissue weight) were derived from congener- and dose group-specific data in the bioassay reports. Reported bodyweights over time during the bioassays for each dose group were fit with a function of the form:

$$BW(t) = BW_0 * t^x \qquad (3)$$

The resulting functions were used in the modeling to provide a week-by-week bodyweight profile throughout the two-year bioassays for each dose group.

The relative liver weights w_h were measured in the bioassays at weeks 14, 31, and 53 for each dose group. However, liver weights at terminal sacrifice were not measured (Walker 2004, pers. comm.). The relative liver weights at the interim sacrifices for each dose group were fit with linear functions with time, and these functions were used to estimate relative liver weights over the entire course of the studies.

Finally, the fraction of body adipose or lipid tissue as a function of bodyweight w_a was estimated using a relationship from Brown et al. (1997) based on data for male Sprague-Dawley rats developed by Bailey et al. (1980; as cited by Brown et al. 1997). Although the animals used in the NTP bioassays were female, no female-specific data or relationships for percent adipose tissue were identified, so the following relationship, developed based on data from male rats, was used:

$$w_a = (0.0199 * BW + 1.664)/100 \qquad (4)$$

Model Parameters

Key model parameters for the modeling include f_{hmax}, K, and k_e. The values for f_{hmax} and K for each individual compound were derived based on the estimated liver weights and the measured congener concentrations at the three interim sacrifice periods (14, 31, and 53 weeks) using equation (1). The body burden of the compound at each time point for a given dose group was calculated by assuming that all compound resides in either hepatic or adipose tissue:

$$Q_b = C_h * w_h * BW + C_a * w_a * BW \qquad (5)$$

and the body concentration was then calculated as:

$$C_b = \frac{Q_b}{BW} \qquad (6)$$

The fraction of the body burden measured in liver was then plotted at each time point and for each dose group and plotted vs. C_b. These plots were fitted on a least square deviations basis with a function as in equation 1 to estimate f_{hmax} and K. Using these parameters, and equation 1 for distribution, the hepatic elimination rate, k_e, was estimated from fits for each compound across all dose groups to provide the best fit to the interim and terminal measured hepatic tissue concentrations of the bioassay compounds by minimizing the following function:

$$L(k_e) = \sum_{i=1}^{N} \sum_{j=1}^{4} (\ln(C^{ij}{}_{h\_measured}) - \ln(C^{ij}{}_{h\_predicted}))^2 \qquad (7)$$

where *i* identifies the dose groups (1 to N) and *j* identifies the interim and terminal measurements of hepatic tissue concentration.

Concentration vs. time profiles for whole body, hepatic, and adipose concentrations were derived using the model and the above fitted parameters. The model postulates that all compound resides in hepatic or adipose/lipid tissue. However, because many organs contain measurable lipid tissue, this does not preclude distribution to organs on the basis of their lipid content. The hypothesis of the model would suggest that such organ concentrations can be represented as a constant fraction of the adipose tissue concentration. We evaluated this hypothesis by comparing the lung and adipose tissue concentrations for those time points and dose levels for which both concentrations were above the limit of quantitation (LOQ).

For the mixture bioassay, several methods were tried to apply the TEQ approach to model the measured tissue concentrations assuming strict additivity and identical compound behavior compared to TCDD when adjusted for relative potency. The most successful of these approaches was to interpret tissue concentrations on a TEQ basis, assess the $f_h(C_b TEQ)$ curve using the measured tissue TEQ concentrations, and model intake and elimination on a TEQ basis; results from this approach are presented and discussed briefly.

Results and Discussion

The bioassay data on bodyweights and relative liver weights and the fitted functions used to represent those parameters are presented in Figures A-1 through A-8 in Appendix A. Data from the 100 ng TEQ/kg/day dose group of the mixture bioassay indicated a significant drop in bodyweight during the last 25 weeks of the study, so an additional piece-wise linear fit to the bodyweight data was used for weeks 74 forward in this dose group (Figure A-7). Relative liver weights for some dose groups demonstrated a strong increasing trend during the interim sacrifices. This behavior was extrapolated to terminal sacrifice, but may not accurately represent actual relative liver weights one year after the last relative liver weight determination was made.

Figure 1 illustrates the calculated fraction of body burden in liver as a function of body concentration for each of the individual compound bioassays and the mixture bioassay on the basis of TEQ tissue and body concentration and presents the fitted $f_h(C_b)$ function (see equation 1) for each compound and the TEQ mixture. If strict TEQ behavior were being exhibited, the curves would

have identical f_{hmax} values, and the half-maximum body concentration for each compound, $K_{compound}$, would be related to K_{TCDD} by the following relationship:

$$K_{compound} = \frac{K_{TCDD}}{TEF_{compound}} \qquad (8)$$

However, the f_{hmax} and K values for the individual congener bioassays and the mixture bioassay deviate substantially from this predicted behavior. Table 2 presents the fitted f_{hmax}, K, and k_e values for each bioassay in comparison with the predicted values that would result from a strict TEQ hypothesis based on the results of the TCDD bioassay.

Figures 2 and 3 illustrate the measured (± 1 S.D.) and modeled liver and adipose concentrations (respectively) vs. time for each dose group in the TCDD bioassay. Figures 4/5, 6/7, and 8/9 present the same information for the 4-PeCDF, PCB-126, and the TEQ mixture bioassays, respectively.
The models generally were able to match the measured tissue concentration data within 1 standard deviation, with a few exceptions. The measured hepatic tissue concentrations in the 4-PeCDF bioassay at 31 weeks were consistently above the modeled values. Terminal adipose tissue concentrations in the PCB-126 bioassays were much higher than modeled values for several dose groups, and a similar pattern of underestimated terminal adipose concentration is seen in the TEQ mixture bioassay data, due largely to higher-than-predicted PCB-126 concentrations. Obtaining an accurate match to the terminal time point concentration data may have been hampered by the lack of measured organ weight data at this time point. Because the concentration of the test compounds in liver and adipose is calculated by dividing the distributed quantity by the organ mass, small differences in the actual organ weights compared to the modeled weights at the terminal time point can have a substantial impact on the agreement between modeled and measured tissue concentrations. A similar issue may occur in the estimation of adipose tissue weight fractions as a function of body weight, and therefore in the estimation of adipose tissue concentrations. No specific data were available for this parameter from the NTP bioassays, and the only relationship identified in the literature was based on male rather than female rats.

An evaluation of lung versus adipose tissue concentrations from the bioassays for the individual compounds was made to determine whether the lung tissue concentrations can be represented as a constant fraction of adipose tissue levels. Table 3 presents the ratio between lung and adipose tissue concentrations among all of the bioassays for all time points and dose levels for which both concentrations were above the LOQ. For TCDD, lung tissue concentrations were generally not detectable. Lung tissue concentrations of 4-

PeCDF were generally in the range of 3 to 8 percent of those in adipose, while lung tissue concentrations of PCB-126 were generally 1 to 7 percent of corresponding adipose concentrations. No clear patterns with time or dose were apparent.

Use of a fitted $f_h(C_b$ TEQ) function for the mixture bioassay allowed modeling of the mixture bioassay data with acceptable but not perfect results. However, the parameters of the $f_h(C_b$ TEQ) function were not what would be expected based on the TEQ concept, and were clearly influenced by the high f_{hmax} exhibited by 4-PeCDF and the much higher than predicted half-maximum value, *K*, for PCB-126.

Conclusions

The modeling presented here and in the accompanying spreadsheets will make possible a variety of tissue-based dose estimates for the recently completed NTP bioassay data for TCDD, 4-PeCDF, PCB-126, and TEQ mixtures. A major focus of these bioassays was to evaluate the TEQ hypothesis. Simply stated, the TEQ hypothesis is that each of the related dioxin, furan, and PCB compounds will behave identically to TCDD with only the relative potency differing among compounds. However, this analysis confirms that each of the individual compounds display distribution and elimination behavior that is distinct from that predicted by the strict application of the TEQ hypothesis.

4-PeCDF displays a much higher affinity for liver tissue and much slower elimination than would be expected from the TEQ hypothesis, while PCB-126 requires much higher body concentrations to reach half-maximal liver concentration than predicted by the TEQ approach. The TEQ mixture requires higher body concentrations to reach half-maximal distribution to liver compared to TCDD, but then accumulates to a greater degree in liver than TCDD. The elimination rate as a fraction of hepatic quantity was much slower for all of the tested compounds and the TEQ mixture compared to TCDD, with 4-PeCDF demonstrating the slowest elimination rate of all of the tested compounds and the mixture.

These results are consistent with those presented by Toyoshiba et al. (2004) based on enzyme induction data from these NTP bioassays. These authors also found significantly higher induction due to 4-PeCDF and a less potent response of the TEQ mixture compared to TCDD. These differences in kinetic and distribution behavior have important implications for dose-response assessment under conditions of chronic intake. They suggest that the TEQ approach can lead to important mis-estimation of body burden, distribution, and elimination behavior under chronic administration conditions.

References

Bailey, J.W., D.B. Andersen, M.W.A. Verstegen, and S.E. Curtis. 1980. Relative growth rates of various fat depots in Sprague Dawley rats. Growth 44:220–229 (not seen, as cited in Brown et al. 1997).

Brown, R.P., M.D. Delp, S.L. Lindstedt, L.R. Rhomberg, and R.P. Beliles. 1997. Physiological parameter values for PBPK models. Toxicol Ind. Health 13(4):407–484.

Carrier, G., R.C. Brunet, and J. Brodeur. 1995. Modeling of the toxicokinetics of polychlorinated dibenzo-p-dioxins and dibenzofurans in mammalians, including humans. I. Nonlinear distribution of PCDD/PCDF body burden between liver and adipose tissues. Toxicol Appl Pharmacol. 131(2):253–266.

Carrier, G., R.C. Brunet, and J. Brodeur. 1995. Modeling of the toxicokinetics of polychlorinated dibenzo-p-dioxins and dibenzofurans in mammalians, including humans. II. Kinetics of absorption and disposition of PCDDs/PCDFs. Toxicol Appl Pharmacol. 131(2):267–276.

NTP. 2004a. DRAFT NTP technical report on the toxicology and carcinogenesis studies of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (CAS No. 1746-01-6) in female Harlan Sprague-Dawley rats (gavage study) (NTP TR 521). National Toxicology Program.

NTP. 2004b. DRAFT NTP technical report on the toxicology and carcinogenesis studies of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4) in female Harlan Sprague-Dawley rats (gavage study) (NTP TR 525). National Toxicology Program.

NTP. 2004c. DRAFT NTP technical report on the toxicology and carcinogenesis studies of 3,3'4,4',5-pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in female Harlan Sprague-Dawley rats (gavage study) (NTP TR 520). National Toxicology Program.

NTP. 2004d. DRAFT NTP technical report on the toxicology and carcinogenesis studies of a mixture of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (CAS No. 1746.01-6), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4), and 3,3'4,4',5-pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in female Harlan Sprague-Dawley rats (gavage studies) (NTP TR 526). National Toxicology Program.

Toyoshiba, H., N.J. Walker, A.J. Bailer, and C.J. Portier. 2004. Evaluation of toxic equivalency factors for induction of cytochromes P450 CYP1A1 and

CYP1A2 enzyme activity by dioxin-like compounds. Toxicol Appl Pharmacol. 194(2):156–168.

USEPA 2000. Exposure and human health reassessment of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds. Draft Final. Washington, D.C.: National Center for Environmental Assessment, U.S. Environmental Protection Agency. EPA/600/P-00/001Be.

Walker, N.J., C.J. Portier, S.F. Lax, F.G. Crofts, Y. Li, G.W. Lucier, and T.R. Sutter. 1999. Characterization of the dose-response of CYP1B1, CYP1A1, and CYP1A2 in the liver of female Sprague-Dawley rats following chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol. 154(3):279–286.

Walker, N.J. Personal communication (email to L. Aylward, Exponent, Alexandria, VA, on March 29, 2004). National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Table 1. Model parameters, definitions, and values

Model Parameter	Description, Units	Value
f_{hmin}	Minimum proportion of body burden distributed to liver, unitless	0.01
f_{hmax}	Maximum proportion of body burden distributed to liver, unitless	Fit to distribution data
K	Body concentration for half-maximum increase in liver distribution proportion, ng/kg	Fit to distribution data
k_e	Rate constant for hepatic elimination, yr^{-1}	Fit to measured concentrations
BW	Body weight, kg	Time-dependent function derived from measured values
w_a	Fraction body weight: adipose/lipid tissue, unitless	Equation from Brown et al. (1997)
w_h	Fraction body weight: liver, unitless	Time-dependent function derived from measured values
f_h	Fraction of body burden in liver, unitless	See Eq. 1
f_a	Fraction of body burden in adipose/lipid tissue, unitless	$1-f_h$
Q_a	Quantity of TCDD in adipose/lipid tissue, ng	calculated
Q_h	Quantity of TCDD in hepatic tissue, ng	calculated
Q_b	Quantity of TCDD in body tissue, ng	calculated
C_a	Concentration of TCDD in adipose/lipid tissue, ng/kg	Measured & simulated
C_h	Concentration of TCDD in hepatic tissue, ng/kg	Measured & simulated
C_b	Concentration of TCDD in body tissue, ng/kg	Measured & simulated
g	Absorbed weekly intake from dosing (assumed 90% absorption from gavage), ng/kg-bw	Dosing regimen

Table 2. TEQ predicted and fitted f_{hmax}, K, and fitted k_e parameters for each individual compound bioassay and for the TEQ mixture bioassay

		f_{hmax}, unitless		K, ng/kg		k_e, wk^{-1}
Compound	**TEF**	**TEQ Prediction**	**Fitted**	**TEQ Prediction**	**Fitted**	**Fitted**
TCDD	1	--	0.65	--	19.8	0.61
4-PeCDF	0.5	0.65	0.96	39.6	28.7	0.04
PCB-126	0.1	0.65	0.76	198	1,137	0.18
TEQ Mixture	--	0.65	0.88	19.8	44.8	0.10

Table 3. Ratios of lung to adipose tissue concentrations

Week	Dose Group 1	Dose Group 2	Dose Group 3	Dose Group 4	Dose Group 5	Dose Group 6	Dose Group 7	Dose Group 8
TCDD	3 ng/kg	10 ng/kg	22 ng/kg	46 ng/kg	100 ng/kg	100 ng/kg (Stop)		
14	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	--	--	--
31	BLOQ	13.68078	BLOQ	0.311751	BLOQ	--	--	--
53	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	--	--	--
104	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	--	--
PeCDF	6 ng/kg	20 ng/kg	44 ng/kg	92 ng/kg	200 ng/kg	200 ng/kg (Stop)		
14	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	--	--	--
31	BLOQ	0.062768	0.060651	0.058078	0.060565	--	--	--
53	BLOQ	0.055478	0.057122	0.048866	0.043085	--	--	--
104	0.136915	0.085075	0.084498	0.051894	0.036334	0.060648	--	--
PCB 126	10 ng/kg	30 ng/kg	100 ng/kg	175 ng/kg	300 ng/kg	550 ng/kg	1,000 ng/kg	1,000 ng/kg (Stop)
14	BLOQ	0.144710	0.027017	0.034595	0.025859	0.026771	0.014963	--
31	BLOQ	0.018521	0.016500	0.015418	0.015695	0.021515	0.018364	--
53	0.144013	0.035167	0.031364	0.108223	0.112588	0.030892	0.070128	--
104	--	0.016745	0.013615	0.016972	0.007394	0.012009	0.014114	0.029181

Notes:

Values represent the lung tissue concentration divided by the adipose tissue concentration

BLOQ = Either the lung or adipose concentration (or both) were below the limit of quantitation

-- = no data available

Stop = stop-exposure dose group

Figures

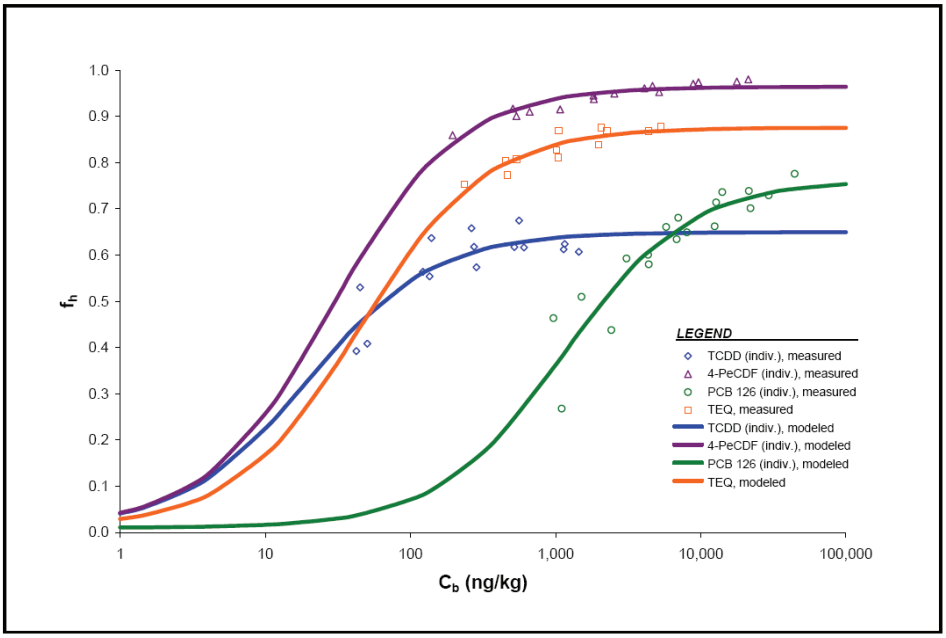
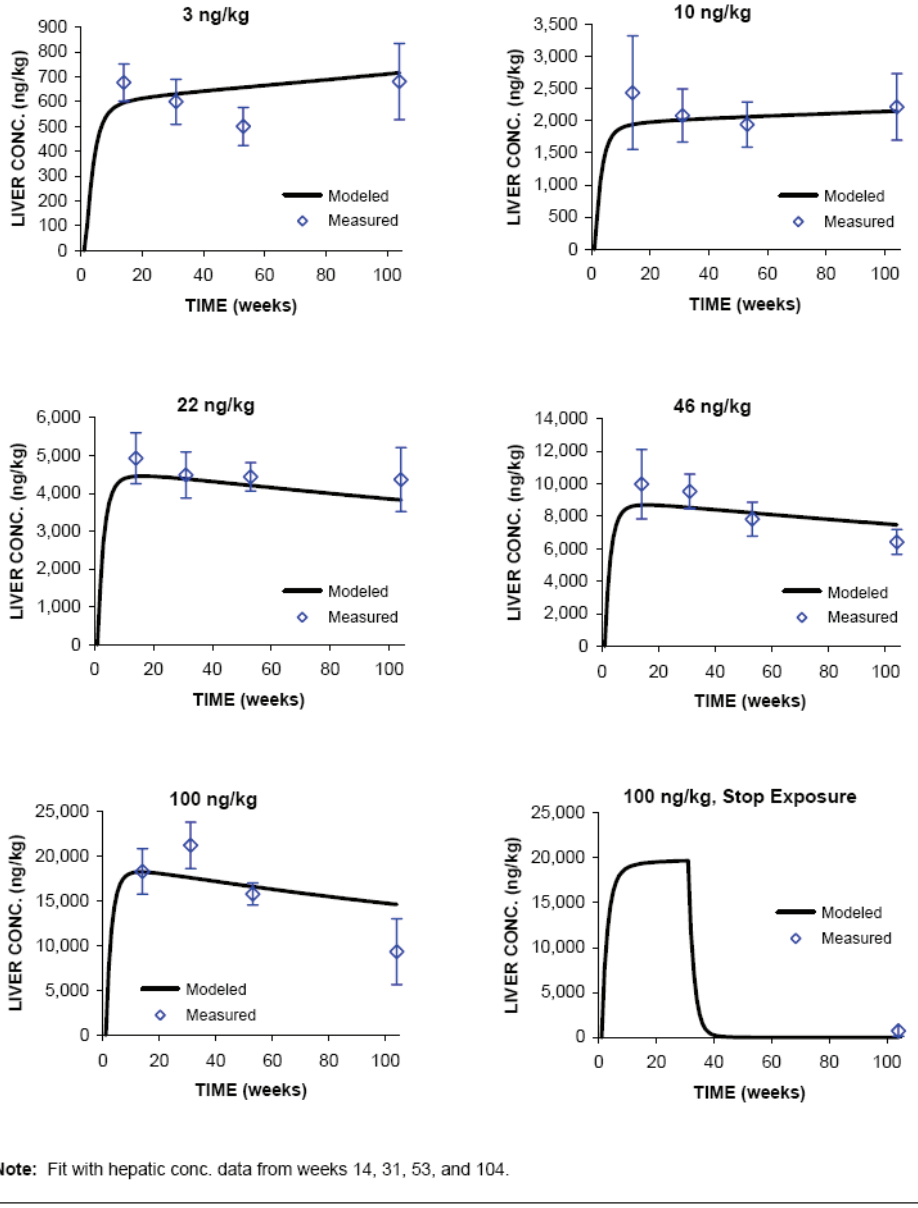
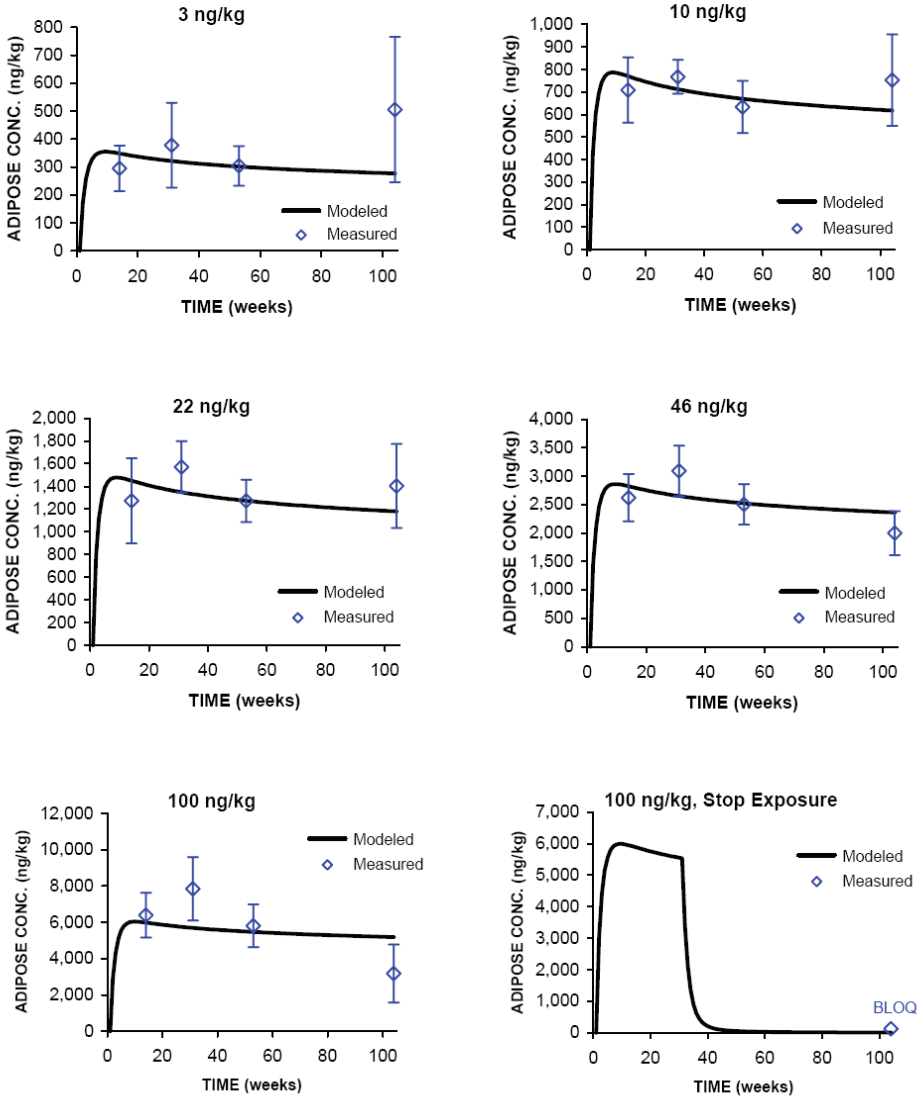


Figure 1. Fraction of compound in liver vs. body concentration: TCDD, 4-PeCDF, PCB 126 (individual bioassays), and TEQ mixture.



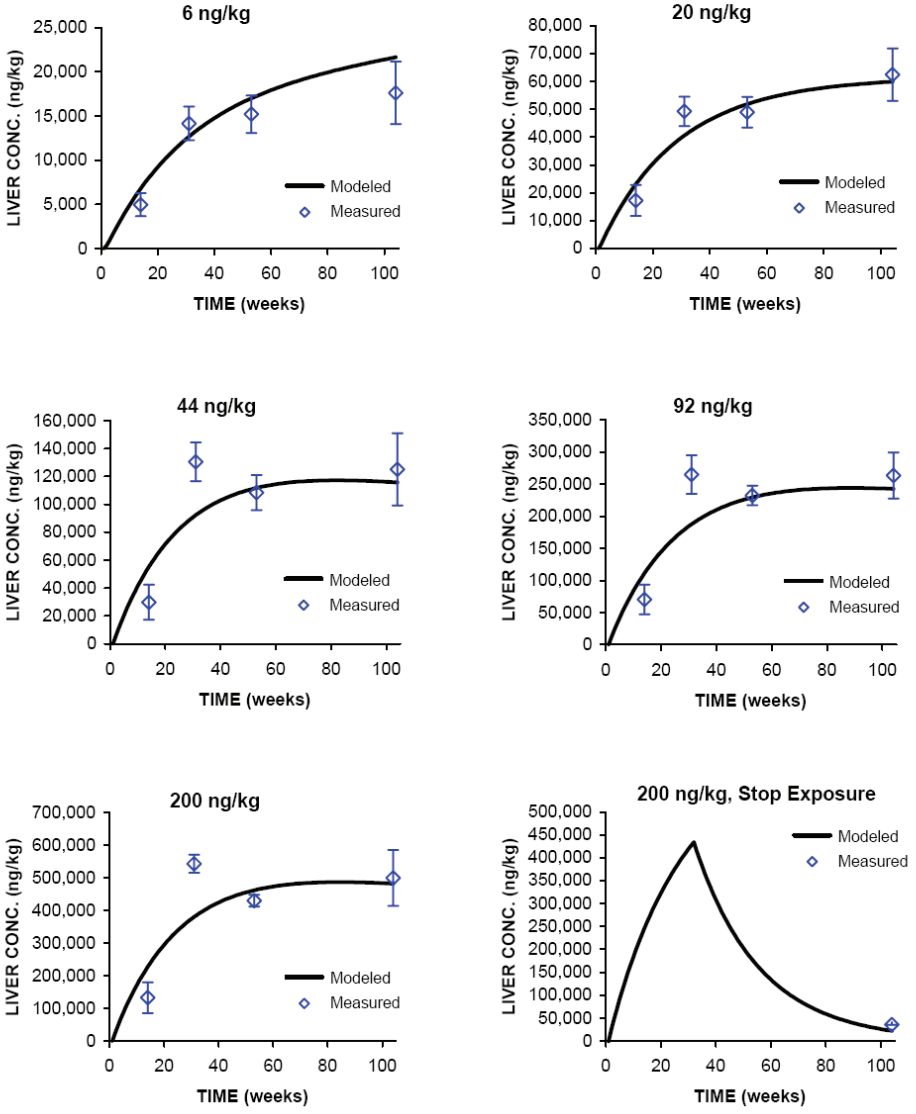
Note: Fit with hepatic conc. data from weeks 14, 31, 53, and 104.

Figure 2. Measured and modeled TCDD liver concentrations (individual bioassay).



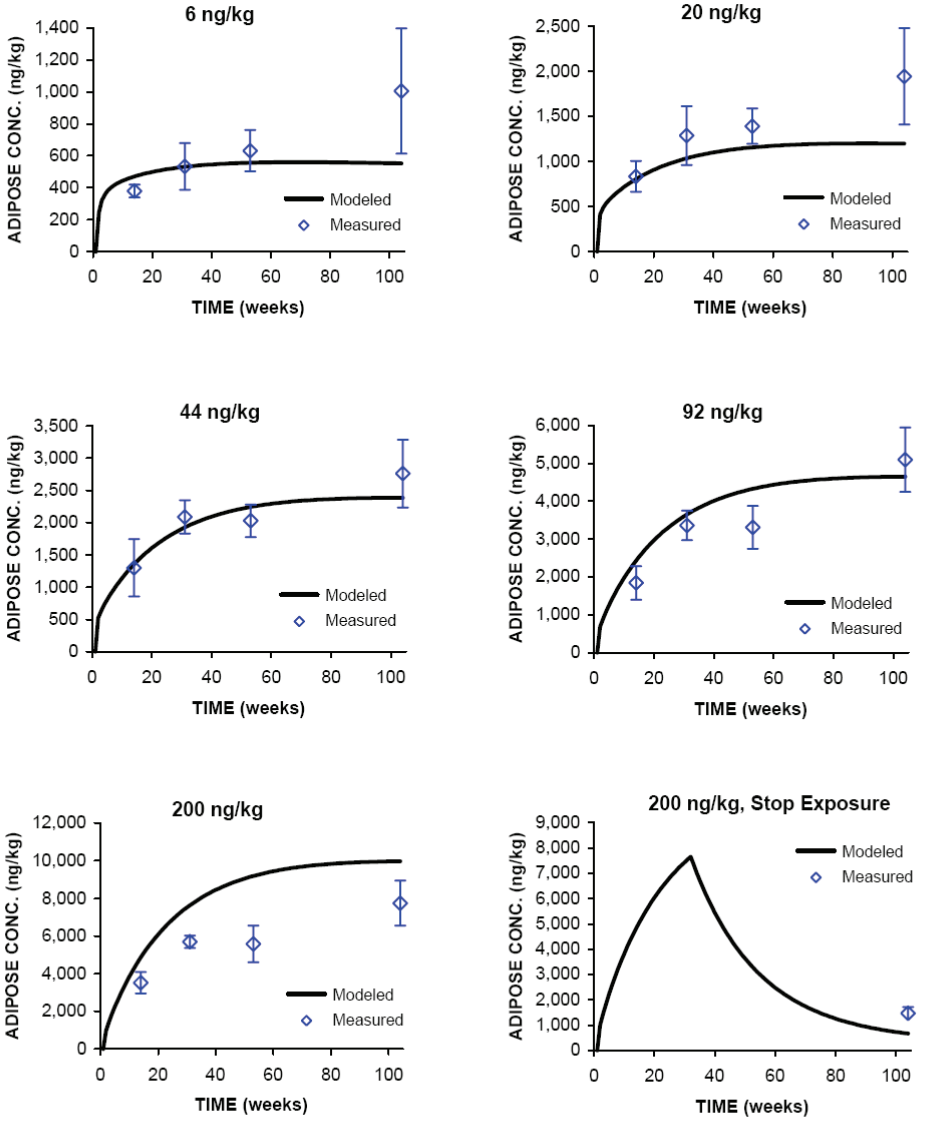
Note: Fit with hepatic conc. data from weeks 14, 31, 53, and 104.

Figure 3. Measured and modeled TCDD adipose/lipid concentrations (individual bioassay).



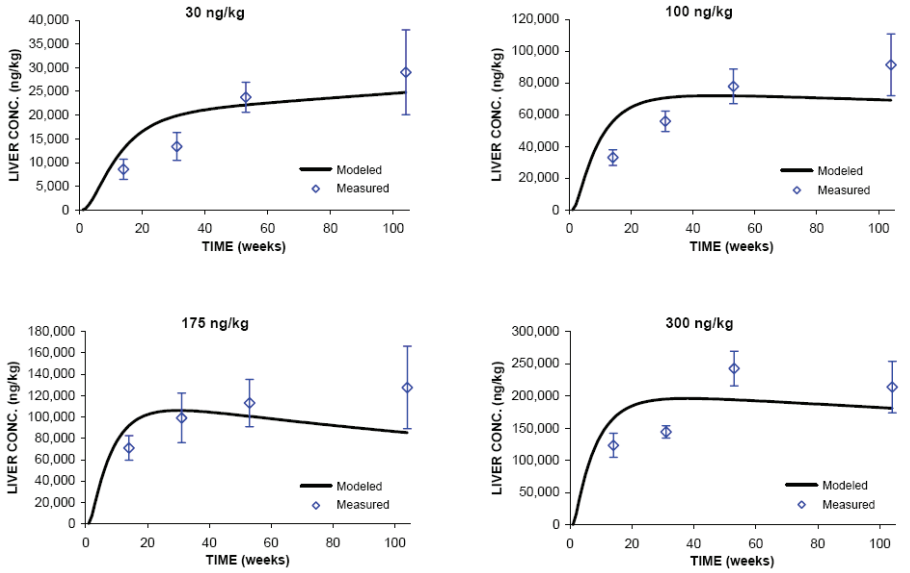
Note: Fit with hepatic conc. data from weeks 14, 31, 53, and 104.

Figure 4. Measured and modeled 4-PeCDF liver concentrations (individual bioassay).



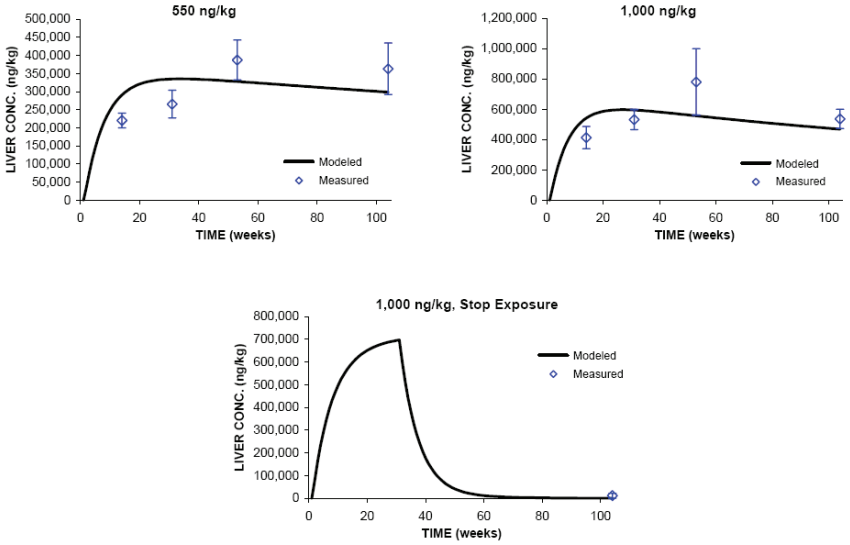
Note: Fit with hepatic conc. data from weeks 14, 31, 53, and 104.

Figure 5. Measured and modeled 4-PeCDF adipose/lipid concentrations (individual bioassay).



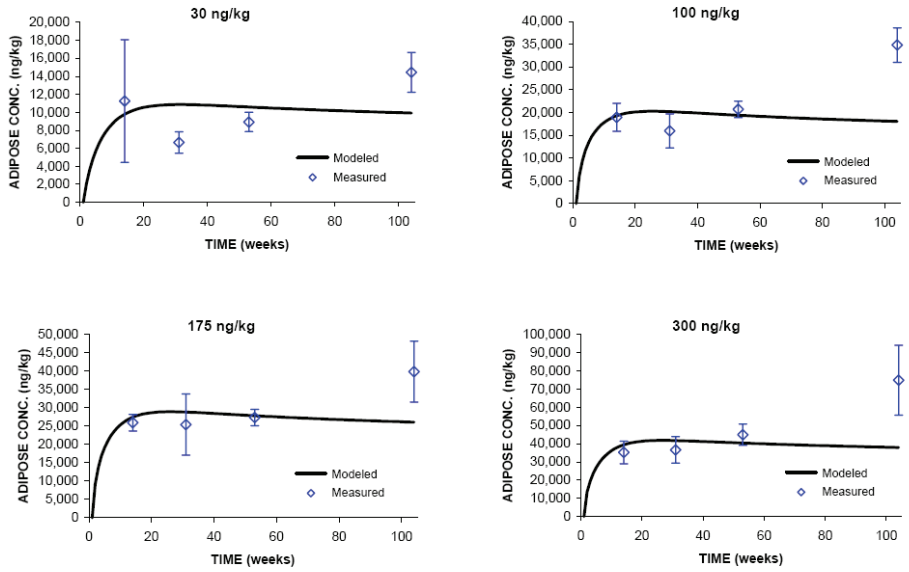
Note: No body weight data were provided for Dose Group 1 (10 ng/kg), and therefore, no modeling was done. Fit with hepatic conc. data from weeks 14, 31, 53, and 104.

Figure 6a. Measured and modeled liver concentrations of PCB 126 (individual bioassay).



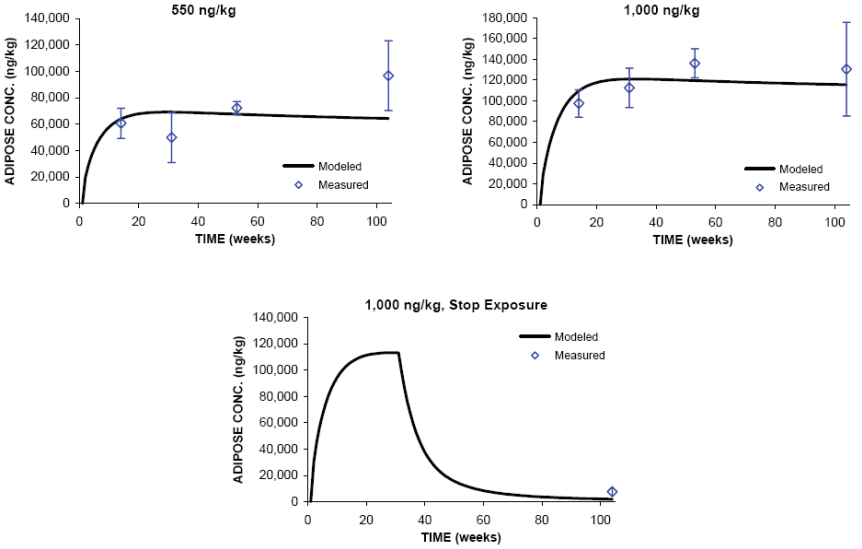
Note: No body weight data were provided for Dose Group 1 (10 ng/kg), and therefore, no modeling was done. Fit with hepatic conc. data from weeks 14, 31, 53, and 104.

Figure 6b. Measured and modeled liver concentrations of PCB 126 (individual bioassay).



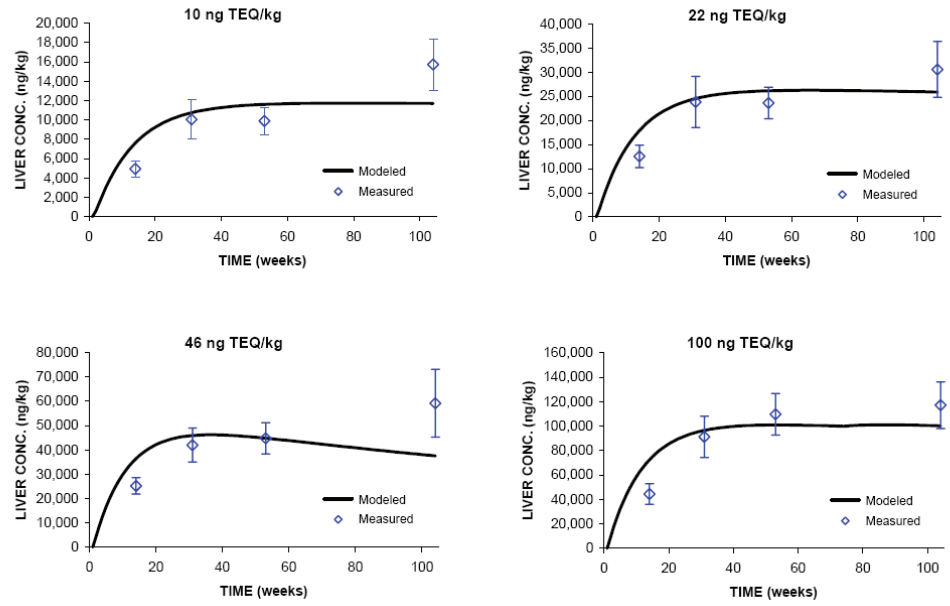
Note: No body weight data were provided for Dose Group 1 (10 ng/kg), and therefore, no modeling was done. Fit with hepatic conc. data from weeks 14, 31, 53, and 104.

Figure 7a. Measured and modeled adipose/lipid concentrations of PCB 126 (individual bioassay).



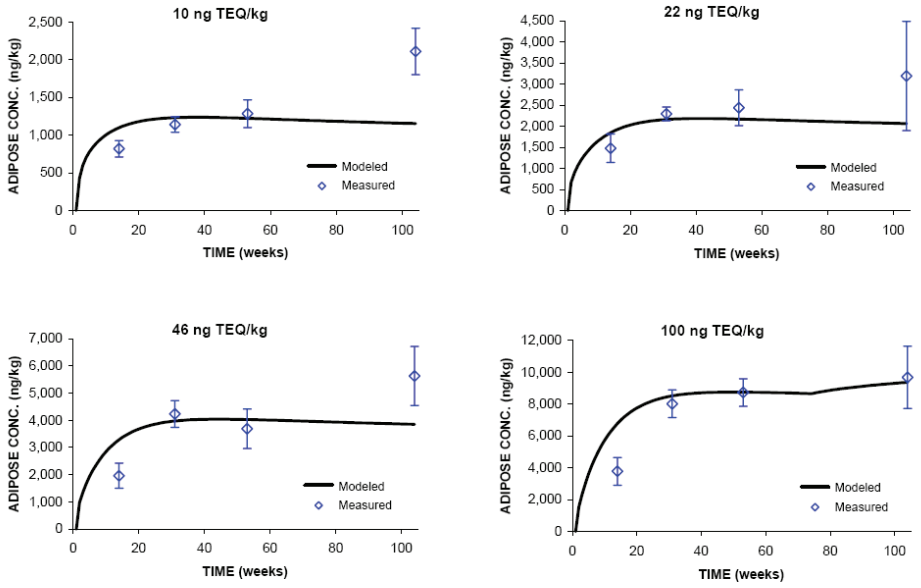
Note: No body weight data were provided for Dose Group 1 (10 ng/kg), and therefore, no modeling was done. Fit with hepatic conc. data from weeks 14, 31, 53, and 104.

Figure 7b. Measured and modeled adipose/lipid concentrations of PCB 126 (individual bioassay).



Note: Model fit with hepatic data from weeks 14, 31, 53, and 104
BW function for 100 ng/kg group adjusted at wk 74 for reduced BW

Figure 8. Measured and modeled TEQ concentrations in the liver.



Note: Model fit with hepatic data from weeks 14, 31, 53, and 104
BW function for 100 ng/kg group adjusted at wk 74 for reduced BW

Figure 9. Measured and modeled TEQ concentrations in the adipose/lipid.

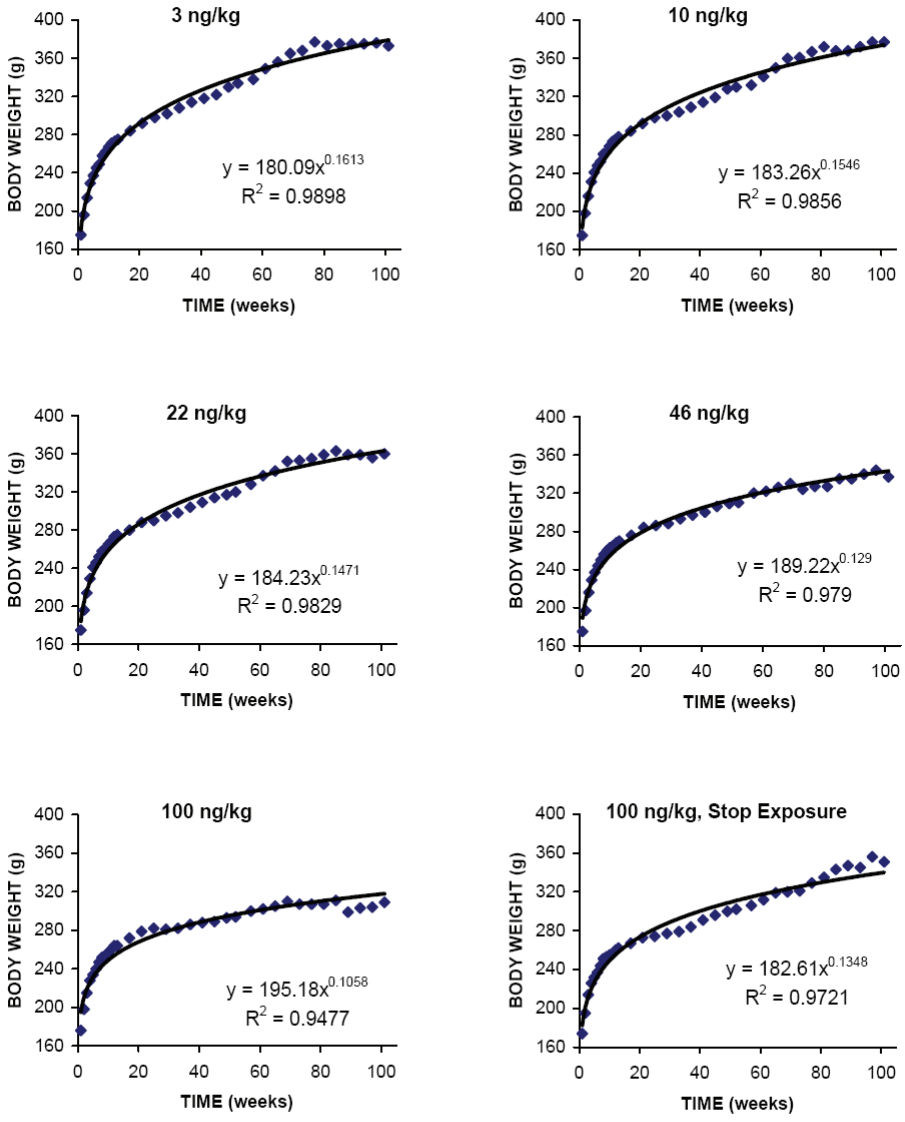
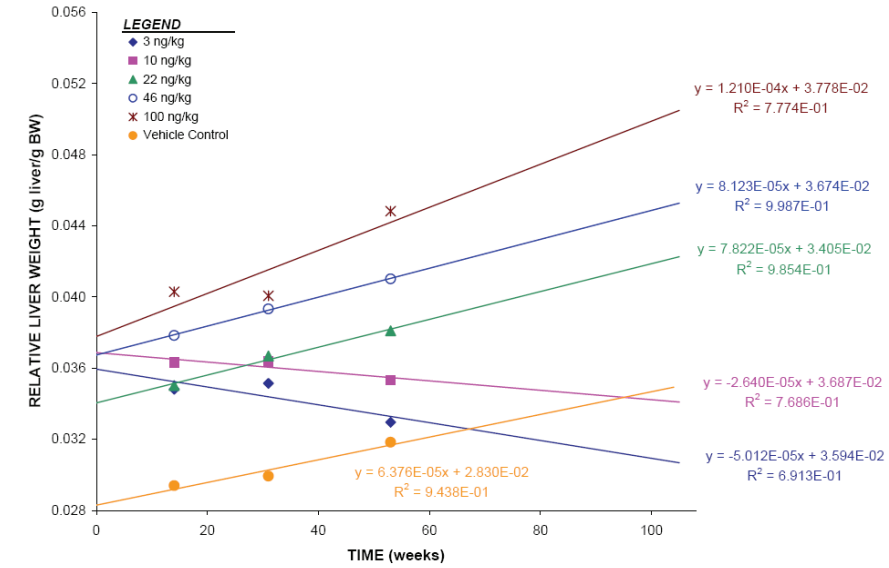


Figure A-1. Measured and best-fit function for body weights from the TCDD bioassay.



Note: Measured values from weeks 14, 31, and 53

Figure A-2. Measured and best-fit function for liver weights from the TCDD bioassay.

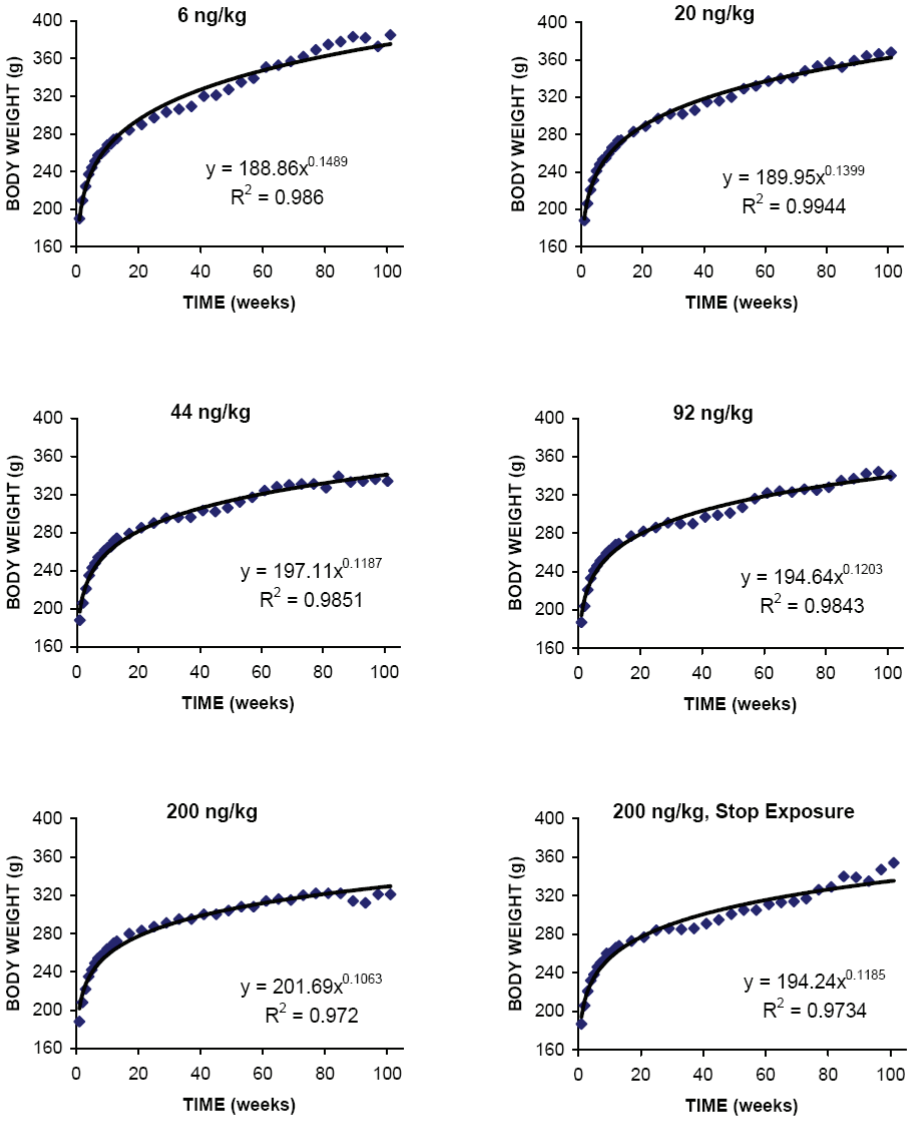
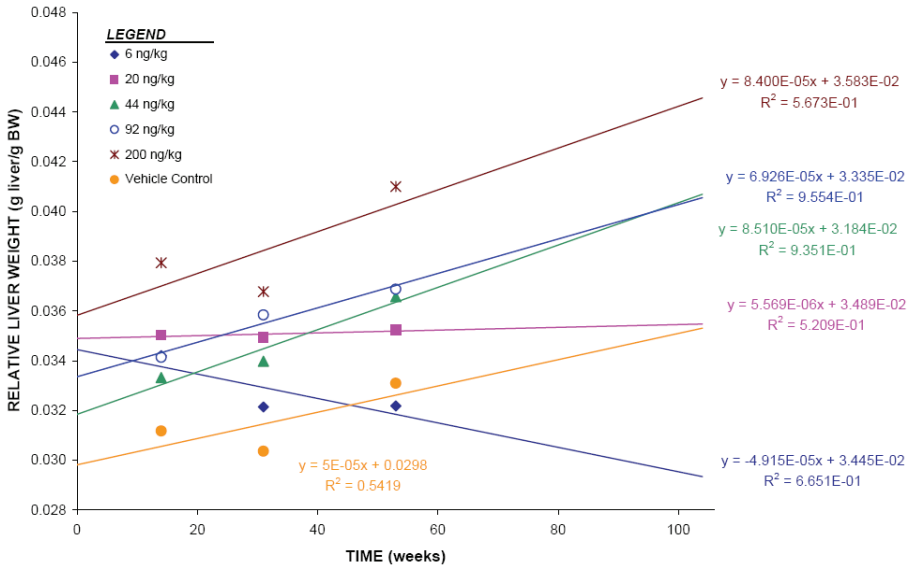


Figure A-3. Measured and best-fit function for body weights from the 4-PeCDF bioassay.



Note: Measured values on weeks 14, 31, and 53

Figure A-4. Measured and best-fit function for liver weights from the 4-PeCDF bioassay.

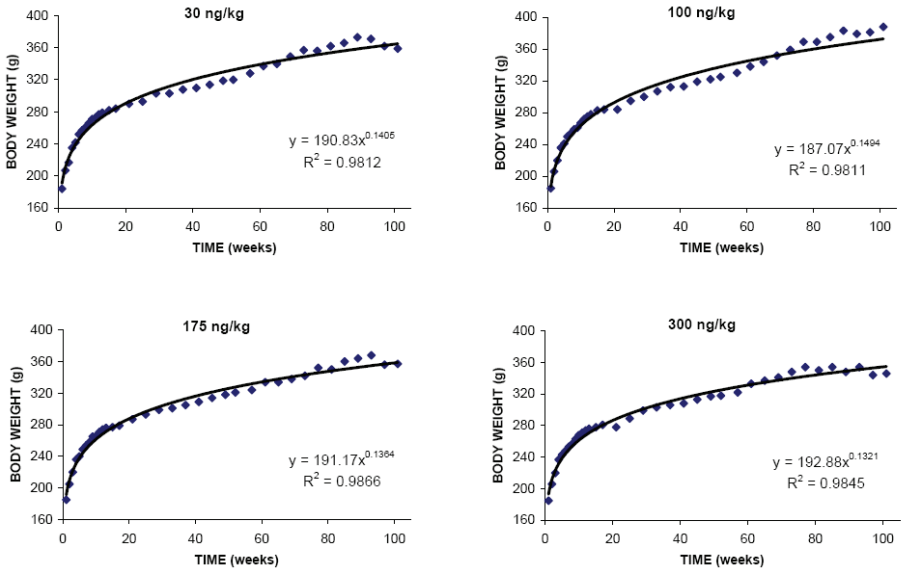


Figure A-5a. Measured and best-fit function for body weights from the PCB 126 bioassay.

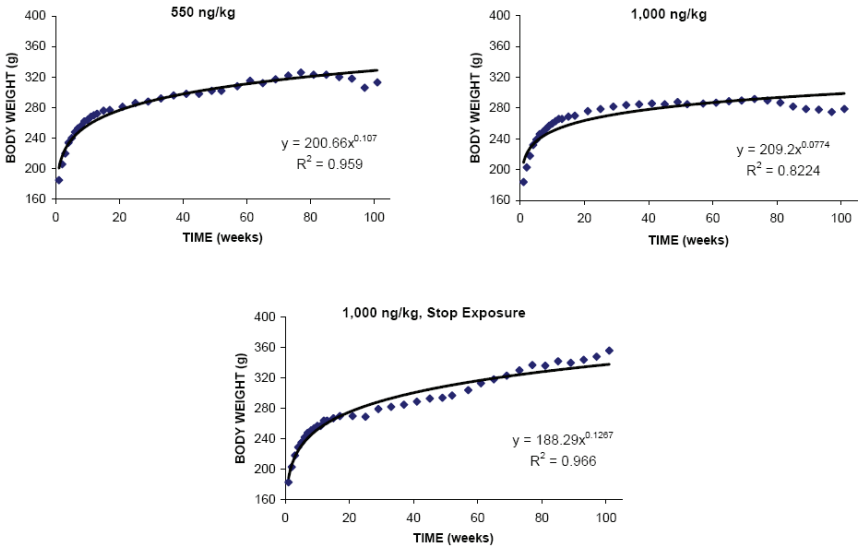
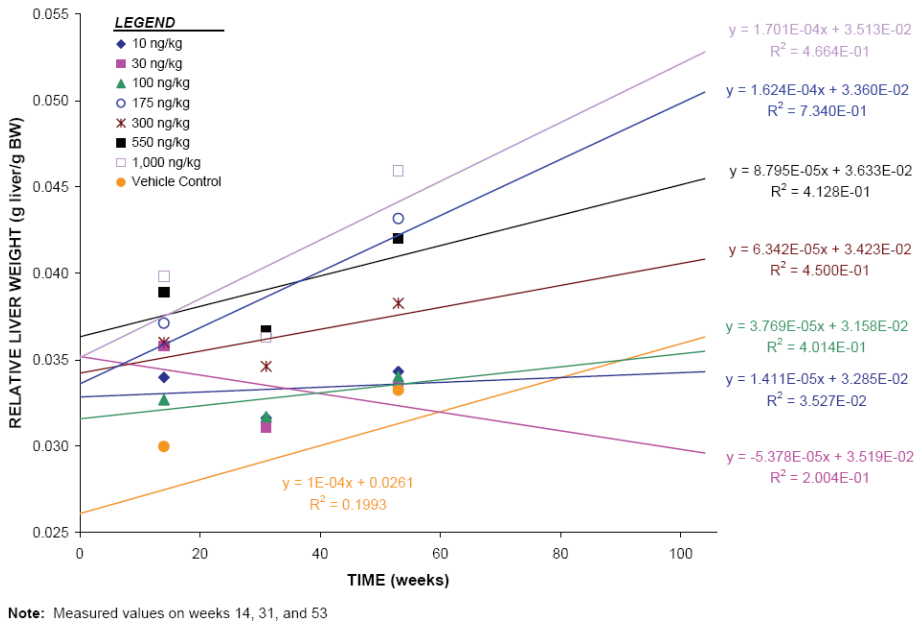


Figure A-5b. Measured and best-fit function for body weights from the PCB 126 bioassay.



Note: Measured values on weeks 14, 31, and 53

Figure A-6. Measured and best-fit function for liver weights from the PCB 126 bioassay.

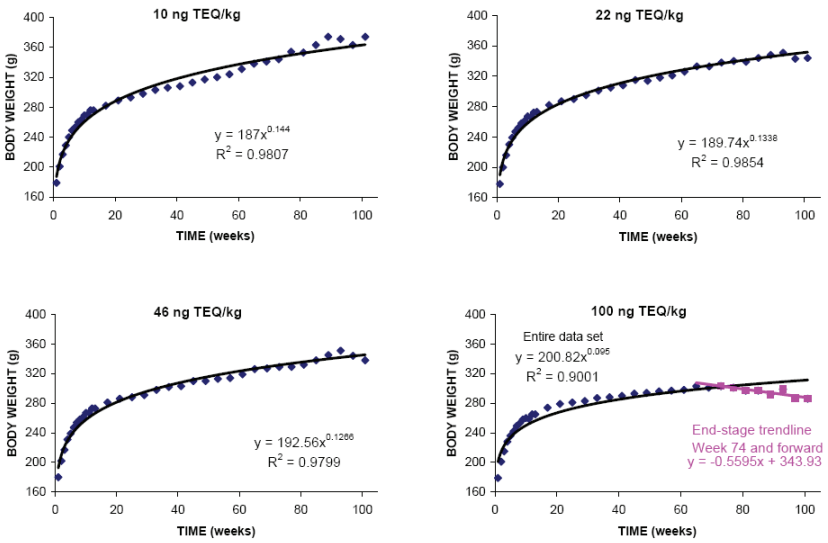
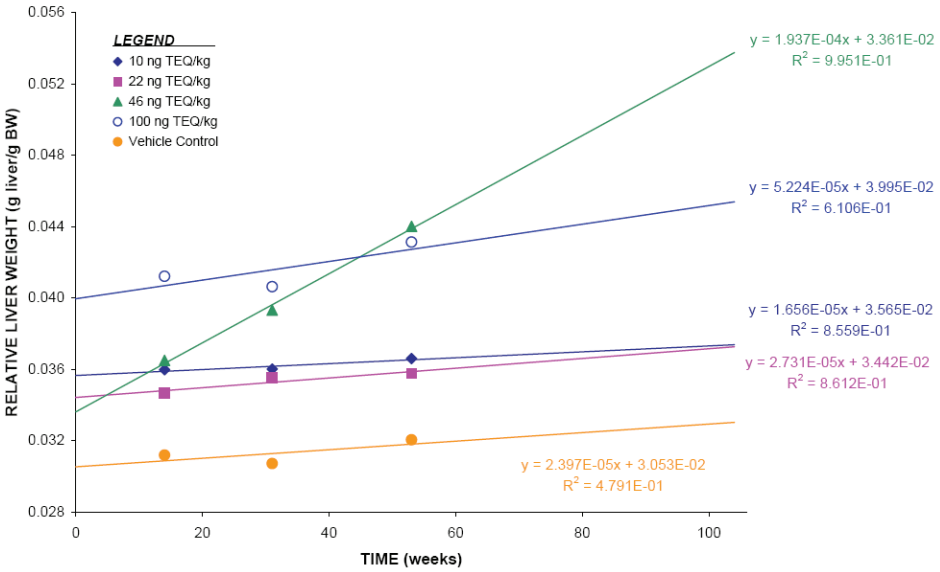


Figure A-7. Measured and best-fit function for body weights from the TEQ mixture bioassay.



Note: Measured values on Weeks 14, 31, and 53

Figure A-8. Measured and best-fit function for liver weights from the TEQ mixture

Chapter 6: Concentration-Dependent TCDD Elimination Kinetics in Humans: Toxicokinetic Modeling for Moderately to Highly Exposed Adults from Seveso, Italy, and Vienna, Austria, and Impact on Dose Estimates for the NIOSH Cohort

Lesa L. Aylward, Robert C. Brunet, Gaétan Carrier, Sean M. Hays, Colleen A. Cushing, Larry L. Needham, Donald G. Patterson, Jr., Pier Mario Gerthoux, Paolo Brambilla, Paolo Mocarelli. 2005. J. Exposure Analysis and Environmental Epidemiology 15:51-65 doi:10.1038/sj.jea.7500370.

http://www.nature.com/jes/journal/v15/n1/abs/7500370a.html

Abstract

Serial measurements of serum lipid 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) concentrations in 36 adults from Seveso, Italy, and three patients from Vienna, Austria, with initial serum lipid TCDD concentrations ranging from 130 ppt to 144,000 ppt, were modeled using a modified version of a previously published toxicokinetic model for the distribution and elimination of dioxins (Carrier et al. 1995a, b). The original model structure accounted for a concentration-dependent increase in overall elimination rate for TCDD due to non-linear distribution of TCDD to the liver (secondary to induction of the binding protein CYP1A2), from which elimination takes place via a first-order process. The original model structure was modified to include elimination due to lipid partitioning of TCDD from circulation into the large intestine, based on published human data. We optimized the fit of the modified model to the data by varying the hepatic elimination rate parameter for each of the 39 persons. The model fits indicate that there is significant inter-individual variability of TCCD elimination efficiency in humans and also demonstrate faster elimination in males compared to females, and in younger vs. older persons. The data and model results indicate that, for males, the mean apparent half-life for TCDD (as reflected in changes in predicted serum lipid TCDD level) ranges from less than 3 years at serum lipid levels above 10,000 ppt to over 10 years at serum lipid levels below 50 ppt. Application of the model to serum sampling data from the cohort of U.S. herbicide manufacturing workers assembled by the National Institute of Occupational Safety and Health (NIOSH) indicates that previous estimates of peak serum lipid TCDD concentrations in dioxin-exposed manufacturing workers, based on first-order back-extrapolations with half-lives of 7 to 9 years, may have underestimated the maximum concentrations in these workers and other occupational cohorts by several-fold to an order of magnitude or more. Such dose estimates, based on a single sampling point decades after last exposure, are highly variable and dependent on a variety of assumptions and factors that cannot be fully determined, including inter-individual variations in elimination efficiency. Dose estimates for these cohorts should be re-evaluated in light of the demonstration of concentration-dependent elimination kinetics for TCDD, and the large degree of uncertainty in back-calculated dose estimates should be explicitly incorporated in quantitative estimates of TCDD's carcinogenic potency based on such data.

Introduction

Recent reports providing data on elimination rates for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in persons with moderate to very high exposures suggest that at substantially elevated body burdens, elimination rates are much higher than previously estimated. Elimination half-lives of less

than 1 year to 3.6 years were estimated in two women and one man who were exposed to very high levels of TCDD in Vienna, Austria, in 1997 (peak measured serum lipid levels of 144,000, 26,000, and 856 ppt) (Abraham et al., 2002; Geusau et al., 2002) and in adults during the first 3 years after the Seveso, Italy, accident (initial levels typically over 2,000 ppt) (Michalek et al., 2002). Previous estimates of the half-life of elimination for TCDD have ranged from about 7 to 9 years based on serial measurements in the Ranch Hand population, or occupationally exposed workers (Flesch-Janys et al., 1996; Michalek and Tripathi 1999; Rohde et al., 1999). These studies have typically involved 2 to 4 serial samples taken over several years from persons with serum lipid TCDD levels of less than about 500 ppt and have relied on an explicit assumption of first-order elimination behavior. Earlier estimates based on a subset of the Seveso data sets analyzed here also found average half-lives in the range of 7 to 9 years based on an assumption of first-order elimination behavior (Needham et al., 1994; Needham et al., 1997/98).

A dependence of TCDD elimination rate on body burden has been observed in rodents (reviewed by Carrier et al., 1995a, b), and a similar increased elimination rate at high concentrations was reported for polychlorinated dibenzofurans in humans (Ryan et al., 1993). The dose-dependence of elimination rate in rodents has been hypothesized to occur secondary to induction of CYP1A2 in the liver, and data demonstrating CYP1A2 induction support this possibility in the Austrian patients (Abraham et al., 2002).

Carrier et al. (1995a, b) constructed a toxicokinetic model to address these dose-dependent mechanisms of distribution and elimination, and implemented the model for several sets of animal data and on serial sample data for 2,3,4,7,8-pentachlorodibenzofuran from one Yu-cheng patient. The model predicted a strong dose dependence of apparent elimination half-life. The model is based on the assumption that elimination of TCDD is directly proportional to the current concentration in the liver, but that the concentration of TCDD in the liver increases with increasing body burden in a non-linear, saturable fashion as a consequence of induction of the binding protein CYP1A2. For the first time in humans, significant induction of CYP1A2 activity has been clearly observed in the Austrian patients with high TCDD exposure (Abraham et al., 2002), so the model's reliance on the induction of this protein as a basis for non-linear distribution of TCDD in humans is supported.

The original model aptly describes high and moderate dose distribution and elimination of TCDD in both rodents and humans (Carrier et al., 1995a; Carrier et al., 1995b; Carrier et al., 1999). However, the original model structure predicts increasingly longer half-lives for elimination of TCDD as body and lipid concentrations approach background. The predicted half-lives (more than 20 years) exceed substantially the observed half-lives (range of about 6 to 9

years) (Michalek and Tripathi 1999; Schlatter 1991) in humans at lipid concentrations below about 100 ppt (depending on model parameters). This divergence of the model from observed elimination behavior in humans may be largely remedied by consideration of an additional mechanism for elimination of TCDD, a mechanism that is relatively unimportant at elevated body burdens but becomes relatively important at low body burdens. Recent experimental assessments of the fecal elimination of highly lipid soluble persistent organochlorines, including TCDD, have observed elimination due to simple lipid partitioning from the circulation across the intestinal lumen into fecal contents (reviewed by Moser and McLachlan, 2002).

More recently, this lipid-based elimination mechanism has been demonstrated through experiments to enhance elimination of TCDD by administration of Olestra, a non-absorbed dietary fat substitute (Moser and McLachlan 1999; Geusau et al., 1999; 2002). Because Olestra is not absorbed, there is no plausible mechanism by which an increase in hepatic elimination could be the mechanism for the increased TCDD excretion, while simple lipid partitioning into the "clean" fat in the intestine is plausible and would be expected. In addition, there is evidence that unchanged TCDD partitions on a lipid basis into bile and thus is excreted into feces as well (Kitamura et al., 2001b). Five recent studies provide data that allow assessment of the rate of partitioning of TCDD from circulating lipids into fecal contents (Geusau et al., 2002; Kitamura et al., 2001a; Moser and McLachlan 2001; Rohde et al., 1999; Schlummer et al., 1998).

The original formulation of the model did not specify whether the elimination mediated in the liver occurred as a result of elimination of unchanged compound or through metabolism. However, recent data from human populations (Rohde et al. 1999; Geusau et al. 2002; Moser and McLachlan 2002) suggest that the amount of unchanged TCDD eliminated cannot account for the rate of disappearance of TCDD in moderately to highly exposed persons. In combination with data that show inducible hepatic metabolism of TCDD in animals (Hu and Bunce 1999; Olson et al., 1994), these data suggest that the hepatic elimination of TCDD modeled in the Carrier et al. (1995a, b) model is through hepatic metabolism and not elimination of the unchanged parent compound. This suggests that addition of a model component to account for elimination of unchanged compound through lipid partitioning into the contents of the large intestine would improve the performance of the model at lower body concentrations.

This paper describes a modification to the original Carrier et al. (1995a, b) toxicokinetic model structure to account for lipid-based elimination. The modified model was fit to serial serum lipid TCDD sampling data for three persons from Vienna, Austria, whose TCDD exposure has been described in the literature (Abraham et al., 2002; Geusau et al., 2002) and to 36 adults exposed during the Seveso accident. The resulting range of fitted parameters

for all 39 adults provides an indication of the variability in elimination behavior of TCDD in the adult population. The modeling presented in this paper addresses the kinetic behavior of TCDD only. Other compounds that contribute to dioxin toxic equivalency (TEQ) exposure could conceivably be addressed through the same model structure, but such an effort is outside the scope of this paper.

An increased rate of TCDD elimination in persons with elevated body burdens could have a significant impact on the estimates of exposure for the cohorts of industrial workers who were exposed to TCDD during the manufacture of herbicides. Estimates of exposure for these workers have generally been based on serum lipid levels of TCDD measured a decade or more after their last industrial exposure. These measured levels were used by several research groups (Aylward et al., 1996; Flesch-Janys et al., 1996; Steenland et al., 2001) to estimate, through back-calculations, peak levels at time of last exposure, assuming a simple first-order elimination rate with a fixed half-life ranging from 7.1 to 8.7 years. In this paper, we apply the modified toxicokinetic model (with parameters based on fits to the elimination data from the male Seveso patients) to measured serum lipid TCDD concentration and data on dates of employment for the 250 NIOSH workers for whom such data are available. We compared the dose estimates using the concentration-dependent elimination rate model to those obtained using the assumption of constant first-order elimination to assess the potential impact of these different approaches on estimates of exposure levels for these workers. Recent efforts by the U.S. Environmental Protection Agency (USEPA) and others to assess the cancer potency of TCDD rely on estimated exposure levels for the occupational cohorts (Becher et al., 1998; Crump et al., 2003; Steenland et al., 2001; United States Environmental Protection Agency (USEPA) 2000). Changes in exposure estimates for these cohorts would directly influence the cancer dose-response assessments for TCDD.

Methods

Original model structure. In the original Carrier et al. toxicokinetic model, the elimination of TCDD is modeled as a simple first-order elimination process that is a function of the current amount of TCDD in the liver, $Q_h(t)$:

$$\frac{dQ_b(t)}{dt} = -k_e * Q_h(t) \qquad (1)$$

where the amount in liver is a fraction f_h of the total body burden Q_b:

$$Q_h = f_h Q_b \qquad (2)$$

(model parameter nomenclature and definitions are presented in Table 1). However, this fraction of the body burden in liver, f_h, increases in a non-linear, saturable manner as body concentration increases (following a Michaelis-Menten relationship), theoretically as a result of the induction of the binding protein CYP1A2 in the liver:

$$f_h\left(C_b(t)\right) = f_{h\min} + \frac{(f_{h\max} - f_{h\min}) * C_b(t)}{K + C_b(t)} \qquad (3)$$

As implemented, the model assumes that the remaining fraction of body burden is distributed in lipid (designated as "adipose" in the nomenclature used here) tissue throughout the body. The key parameters for the model are k_e, the hepatic elimination rate; f_{hmin} and f_{hmax}, the minimum and maximum fractions of body burden that distribute to the liver; and K, the concentration at which the proportion distributing to liver reaches half-maximum. The model predicts the time-dependent TCDD concentrations in the body and in liver and adipose tissue. The model can incorporate changes in body weight and body composition (percent adipose tissue), which can have significant effects on tissue concentrations.

Model structure modification. The structure of the Carrier et al. model was altered by adding a term to account for the amount of TCDD eliminated through partitioning from circulating lipids across the lumen of the large intestine into the fecal content. The change in body quantity of TCDD as a function of time in the modified model is of the general form:

$$\frac{dQ_b(t)}{dt} = \frac{dQ_a(t)}{dt} + \frac{dQ_h(t)}{dt} + g(t) \qquad (4)$$

where $g(t)$ is the rate of absorbed intake, $dQ_h(t)/dt$ is the hepatic elimination as modeled in the original construction of the Carrier et al. (1995a, b) model (see equations 1–3 above), and the rate of elimination through lipid partitioning in the gut is represented by a first-order elimination function:

$$\frac{dQ_a(t)}{dt} = -k_a * Q_a(t) \qquad (5)$$

where $Q_a(t)$ is the TCDD burden in adipose tissue and is given by:

$$Q_a(t) = BW(t) * w_a(t) * C_a(t) \qquad (6)$$

The original structure of the model predicting distribution between adipose (or lipid) tissue and hepatic tissue as a function of body concentration remains unchanged, as does the structure of the model representation of hepatic elimination rate. Figure 1 illustrates the modified model structure.

Derivation of lipid-partitioning elimination rate constant k_a. To derive from experiments the rate constant k_a (units of yr^{-1}), we propose a simple dynamic model to account for the relationship among dietary intake of TCDD, existing serum lipid levels of TCDD, and measured fecal excretion of unchanged TCDD observed in individuals from several studies (Geusau et al., 2002; Kitamura et al., 2001a; Moser and McLachlan 2001; Rohde et al., 1999; Schlummer et al., 1998). This model assumes that unchanged fecal TCDD stems from two sources: 1) elimination from circulating blood lipid through partitioning in the larger intestine (proportional to the adipose-lipid TCDD burden Q_a in ng), and 2) pass-through elimination of unabsorbed TCDD from dietary intake (Figure 2). Thus:

$$F = \frac{k_a}{365} * Q_a * 1000 + I * (1 - f_{abs}) \qquad (7)$$

where F is the amount of unchanged TCDD eliminated in feces in pg/day, I is the amount of TCDD intake in diet in pg/day, and f_{abs} is the fraction of TCDD in food that reaches the systemic circulation across the membrane of the small intestine. Experiments comparing fecal elimination of unchanged TCDD after high daily intakes to excretion after low daily intakes (four individuals) provided data to estimate this absorption efficiency (Moser and McLachlan 2001). Comparing the results F_{hi} and F_{lo} of equation (7) for high and low intakes I_{hi} and I_{lo} on a given individual within a short time span, one can assume that the slowly evolving Q_a is nearly constant over that time span. By subtraction we have:

$$(1 - f_{abs}) = \frac{F_{hi} - F_{lo}}{I_{hi} - I_{lo}} \qquad (8)$$

Table 1: Model parameters, definitions, and values

Model Parameter	Description, units	Value
f_{hmin}	Minimum proportion of body burden distributed to liver, unitless	0.01[a]
f_{hmax}	Maximum proportion of body burden distributed to liver, unitless	0.7 [a]
K	Body concentration for half-maximum increase in liver distribution proportion, ng/kg	100 [a]
k_a	Rate constant for elimination based on partitioning from circulating lipids into large intestine, yr^{-1}	0.03 [b]
k_e	Rate constant for hepatic elimination, yr^{-1}	fitted
BW	Body weight, kg	patient data
w_a	Fraction body weight adipose/lipid tissue	patient data
w_h	Fraction body weight liver	0.03
f_h	Fraction of body burden in liver, unitless	Eq. 3
f_a	Fraction of body burden in adipose/lipid tissue, unitless	$1-f_h$
Q_a	Quantity of TCDD in adipose/lipid tissue, ng	calculated
Q_h	Quantity of TCDD in hepatic tissue, ng	calculated
Q_b	Quantity of TCDD in body tissue, ng	calculated
C_a	Concentration of TCDD in adipose/lipid tissue, ng/kg	calculated
C_h	Concentration of TCDD in hepatic tissue, ng/kg	calculated
C_b	Concentration of TCDD in body tissue, ng/kg	calculated

[a] Derived from earlier modeling of animal and human data, Carrier et al. (1995, 1999)
[b] Calculated from data, this work (see results section and Table 3)

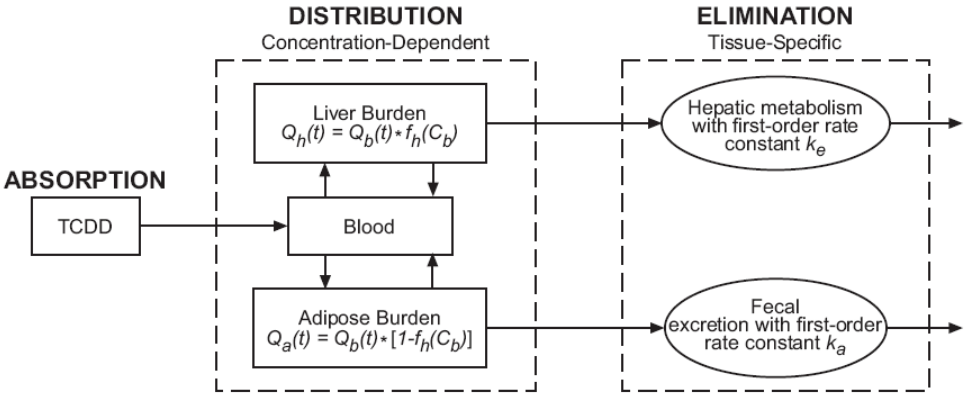
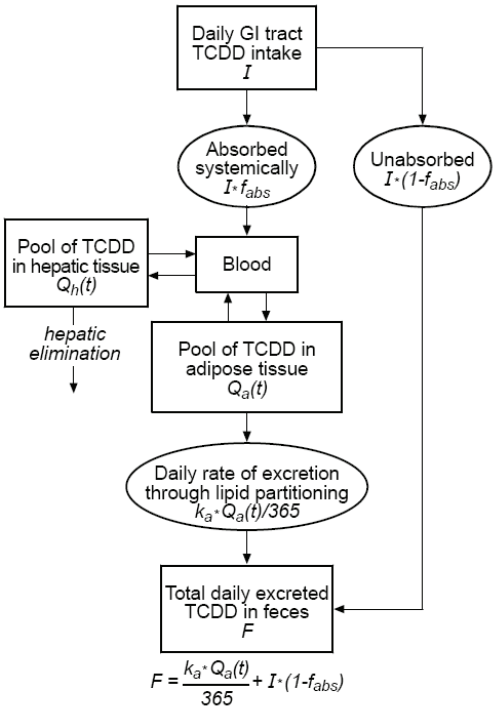


Figure 1: Schematic of the model structure. The model postulates that absorbed TCDD is rapidly distributed to hepatic and adipose tissue. The hepatic fraction, $f_h(C_b)$, of the total body burden, $Q_b(t)$, is continually adjusted through blood exchanges according to body concentration $C_b(t)$ (see equation 3 for details). Metabolism and elimination occur from the hepatic and adipose tissues, respectively, according to equations 1, 4, and 5.

Figure 2: Schematic model of gastrointestinal tract uptake and excretion of unchanged TCDD. Rate constant for elimination of unchanged TCDD from circulating lipids estimated according to this model from data in Table 3.



Using equation (8), the average absorption fraction from these four individuals was found to vary between 95 and 99 percent, with an average of 97 percent. This average value was applied to the intake and excretion data on the other individuals considered for the determination of k_a. Data on intake and excretion of unchanged TCDD for 18 individuals (serum lipid TCDD concentrations ranging from 2.8 to 80,900 ppt) from five studies (Geusau et al., 2002; Kitamura et al., 2001a; Moser and McLachlan 2001; Rohde et al., 1999; Schlummer et al., 1998) were used to evaluate k_a from equation (7) using the f_{abs} estimated above. The values of k_a estimated from these individual data ranged from 0.013 yr^{-1} to 0.06 yr^{-1}, with a mean of 0.03 yr^{-1} (S.D. 0.014). The mean value from these individuals was set as a constant for modeling elimination from exposed individuals.

Elimination rate modeling. Serial measurements of serum lipid TCDD data for 54 adults (29 men and 25 women) from Seveso and the three Austrian patients were fitted using the modified Carrier et al. model. The Seveso data sets consisted of multiple measurements of serum lipid TCDD level (2 to 10 measurements), with the first serum samples taken for most patients within two weeks of the accident. The data set included body weight and height data (required in the modeling to estimate the adipose tissue fraction of body weight) for 43 of the 54 patients. Examination of the data sets revealed that for 7 of these 43 patients, the serial measurements were highly variable and/or inconsistent with elimination (in some cases with peak levels observed years after the accident). The variability may have been the consequence of small sample sizes, long storage time for samples, analytical variability, large unmeasured changes in body weight or fat levels, or other factors. Data for these 7 persons and the 11 persons with no body weight or height data were excluded from the model fitting and analysis, leaving data sets for 36 persons (17 women and 19 men) for analysis.

The available published serial serum lipid level measurements of TCDD for the three Austrian patients included 25 measurements for Patient 1 (initial TCDD level of 144,000 ppt), 19 measurements for Patient 2 (initial TCDD level of 26,000 ppt), and 3 measurements for Patient 3, all over a period of just under three years. Accompanying body-weight data at all time points were available for Patients 1 and 2 (Geusau et al. 2002; Abraham et al. 2002).

Table 2 summarizes the Seveso and Austrian data used in fitting the model. Percent body fat was estimated from body mass index values using an age- and sex- specific formula derived by Deurenberg and coworkers. The formula was recently validated in a multi-center European study of more than 400 individuals (Deurenberg et al., 2001; Deurenberg et al., 1991) and was thus considered to be appropriate for use in analyzing data from the Seveso, Italy, patients.

The model was implemented using Microsoft Excel® spreadsheets and numerical simulation of the differential equations that describe the time dependence of body concentration and associated adipose and hepatic concentrations. Specifically, the incremental changes in body concentration, C_b, were calculated as follows:

$$C_b(t_{i+1}) = C_b(t_i) - [k_e * f_h(C_b(t_i)) * C_b(t_i)] - [k_a * C_a(t_i) * w_a(t_i)] - [C_b(t_i) * \frac{(BW(t_{i+1}) - BW(t_i)}{BW(t_i)}] \quad (9)$$

Incremental absorbed doses could also be included but were not incorporated in the modeling for Seveso and Austrian patients, because background intake levels were expected to be insignificant compared to the elevated body burdens. Functions for changes in body weight and percent body fat over time were included in the simulations to correspond with the patient data.

The modified toxicokinetic model was fit to the time series measurements of serum lipid TCDD levels for each individual in the Seveso data set and three Austrian patients, starting from the initial measured serum lipid TCDD level, by minimizing the sum of squares of the differences between the natural logarithm of the measured and predicted values at time points where measurements exist. The model was fit by varying only k_e, the hepatic elimination rate constant, while other model parameters (f_{hmin}, f_{hmax}, and K) were held constant at values derived from animal data sets and previous human modeling (Carrier et al. 1995a, b; Carrier et al. 1999), and for k_a, to the value derived from the experimental data on fecal elimination of unchanged TCDD (see above; see Table 1 for details on model parameters and values). In addition, a simple first-order elimination model was applied to the serial serum lipid TCDD sampling data for each individual, for comparison with the concentration-dependent model. The same fitting procedure was used, again beginning with the initial measured serum lipid TCDD level, and results were compared to the results of the modified concentration-dependent elimination model.

Application of the model to back-extrapolated exposure levels for the NIOSH cohort. A database, including information on year of birth, dates of first and last employment, date of sampling, and measured serum lipid TCDD level for 253 workers, was obtained from NIOSH. Of the 253 records, three were missing one or more dates, so complete data were available for 250 workers. Table 3 summarizes the sampling database overall, and by exposure duration categories corresponding to those used in an early mortality study on the NIOSH cohort and a previous assessment of exposure levels (Aylward et al., 1996, respectively; Fingerhut et al., 1991). No person-specific data were available regarding body weight, body-fat level, changes in these parameters over time, or any other physiological parameters, so modeling was conducted

by assuming a constant body weight of 70 kg and either a) constant 20% body-fat level, or b) assuming that percent body fat increased with age, according to Deurenberg et al. (1991).

Using the toxicokinetic model, a concentration-vs.-time profile was estimated for each of the 250 workers using a three-step process illustrated in Figure 3. First, measured serum lipid TCDD levels were back-calculated from date of sampling to a peak exposure on the date of last employment (a minimum of 15 years). Because of the lack of data on body weight and changes in body weight, we assumed that body weight was constant over the entire time period for each person. Adipose (or lipid) and hepatic concentrations were calculated from the body concentrations at each time step using the following equations:

$$C_a(t_i) = \frac{C_b(t_i)}{w_a(t_i)} * \left[1 - f_h\left(C_b(t_i)\right)\right] \qquad (10)$$

$$C_h(t_i) = \frac{C_b(t_i)}{w_h(t_i)} * f_h\left(C_b(t_i)\right) \qquad (11)$$

Second, a concentration-vs.-time profile during the period of employment was estimated using a forward calculation based on two assumptions: 1) that the estimated peak at date of last employment, based on the back-calculation, was the highest body burden experienced by the person, and 2) that exposure during employment occurred at a constant dosing rate throughout the period of employment (assumptions incorporated in previous dose estimates for this cohort; Aylward et al., 1996). The dose rate during employment was estimated (taking into account ongoing elimination) through an iterative process in order to match the estimated peak concentration at date of last employment. Finally, a constant serum lipid TCDD level of 5 ppt was assumed for the period of time from birth to first date of employment, consistent with previous dose estimates for this cohort (Aylward et al., 1996). Values for the model parameters used in this modeling are presented in Table 1, except that values for the hepatic elimination rate constant, k_e, derived from fitting the data from male Seveso patients were used.

Table 2: Demographic information on Seveso and Austrian patients

Population	n	Age in 1976, yrs Mean (range)	Initial TCDD ppt lipid basis Mean (range)	Number of serial samples Mean (range)	Follow-up period, yrs Mean	Initial %body fat Mean (range)
Seveso male	19	33 (18, 45)	3056 (130, 9140)	4.2 (2,10)	16[a]	20 (15,29)[c]
Seveso female	17	33 (16, 47)	2544 (145, 6320)	5.2 (2,10)	16[b]	30 (24, 42)[c]
Austrian Patients						
Patient 1 (female)	1	30	144,000	25	3	15[d]
Patient 2 (female)	1	27	26,000	19	2.7	19[d]
Patient 3 (male)	1	26	856	3	2.7	NM

[a] All but five patients had a 16-year follow-up. The remaining five had a 17-year follow-up period.
[b] All but six patients had a 16-year follow-up. The remaining six had follow-up periods of 6, 3, 17, 3, 5, and 3 years.
[c] Percent body fat estimated from formula by Deurenberg et al. (1991)
[d] Percent body fat measured
NM – Not measured

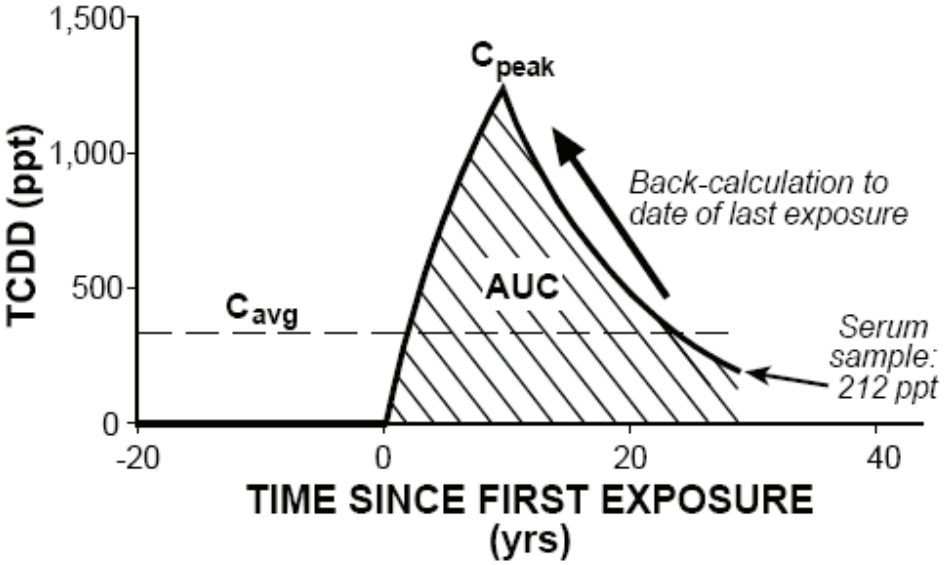


Figure 3: Illustration of a concentration-vs.-time curve for one worker derived from a single serum lipid TCDD measurement taken decades after last exposure. The dose metrics estimated for the NIOSH cohort members are illustrated: peak concentration (C_{peak}), AUC, and average concentration (C_{avg}). This figure illustrates the basic approach taken in quantifying historical exposures of the occupationally exposed cohorts from the U.S. and Germany based on serum samples taken years after last exposure.

Three estimated dose metrics were calculated for each of the 250 individuals based on the reconstructed serum lipid TCDD concentration-vs.-time curves, corresponding to dose metrics previously estimated for this cohort (Aylward et al., 1996; Steenland et al., 2001): peak concentration (C_{peak}), area under the curve (or *AUC*; also called the cumulative serum lipid concentration), and the average concentration (C_{avg}) over the lifetime through the time of sampling. Figure 3 illustrates these metrics on a theoretical concentration-vs.-time curve. We performed similar calculations using constant first-order elimination half-lives of 7.5 or 8.7 years, as used in previous dose estimates for this cohort (Aylward et al., 1996 and Steenland et al., 2001, respectively). Dose metric estimates for the 250 workers were summarized by exposure duration subcohort, as previously defined by Fingerhut et al. (1991) and as used by Aylward et al. (1996).

Results

Model fit results. Table 4 summarizes the results for the values of k_e obtained by fitting the modified model to the 36 Seveso and 3 Austrian data sets of serial measurements of serum lipid TCDD concentrations. The fitted values for k_e ranged from 0.04 to 1.00 yr^{-1}, with values generally higher in males than in females.

Table 5 presents the results of a multivariate linear regression model used to examine the influence of age (in 1976), initial serum lipid TCDD level, estimated percent body fat, and sex in the Seveso patients on fitted k_e values. The goodness of fit of the multivariate regression was confirmed via model diagnostics and an overall F-statistic ($p=0.0016$). For the parameter k_e, the regression indicated a significant negative relationship with age ($p=0.005$), indicating a likely decrease in hepatic elimination capacity with age, and a significant effect of sex, with males having higher elimination rates on average than females ($p<0.05$), while initial serum lipid TCDD and percent body fat were not statistically significant contributors to the variability in k_e. Together, the factors included in the regression accounted for a substantial proportion of the variability in k_e, but a significant amount of unexplained variability remained ($r^2 = 0.42$). The effect of sex was substantial, with an increase in k_e of about 0.2 yr^{-1} associated with being male vs. female. The effect of age was also substantial, with a decrease in k_e of approximately 0.1 yr^{-1} per 10-year increase in age.

The elimination rate constants obtained by fitting each patient data set to a simple first-order elimination function were also evaluated using the multivariate regression. The fitted first-order elimination rate constants showed a strong, statistically significant relationship with initial serum lipid TCDD level (Table 5, Figure 4). If the elimination of TCDD were actually occurring via such a first-order process, the rate of elimination should be independent of TCDD level. The strong relationship is direct evidence that the elimination of TCDD in this population violates the assumption of first-order behavior, and supports the use of a concentration-dependent model. The sum of squares fitting assessment showed a small but consistent improvement in fit for nearly all of the data sets with more than two data points for the modified concentration-dependent model compared to the first-order elimination model. Data sets with only two data points were fit equally well by either model, as would be expected.

Table 4: Results of model fitting to serial serum lipid TCDD sampling data for Seveso and Austrian patients

		Fitted value of k_e, yr^{-1}		
Population	**n**	**Mean (mean +/- 2 S.E.)**	**Median**	**Range**
Seveso male	19	0.42 (0.31-0.53)	0.42	0.12-1.00
Seveso female	17	0.25[a] (0.17-0.34)	0.18	0.04-0.63
Austrian Patients				
Patient 1 (female)[b]		0.94		
Patient 2 (female)[b]		0.63		
Patient 3 (male)		0.77		

[a] Significantly different from mean value for males, $p<0.05$

[b] Data fit without taking into account the effect of Olestra administration on elimination rate.

Table 5: Results of multivariate linear regression on best-fit first-order elimination-rate constant and hepatic elimination-rate constant (k_e) values for 36 Seveso patients

	Best-fit first-order elimination rate constant		**Best-fit k_e hepatic elimination rate constant**	
Variable	**Regression coefficient**	**p-value**	**Regression coefficient**	**p-value**
Age in 1976	-0.001	0.306	-0.012	0.005
Initial Serum lipid TCDD (ppt)	0.000018	<0.0001	0.000003	0.058
Estimated %BF	-0.001	0.477	0.004	0.542
Sex	0.018	0.375	0.199	0.033

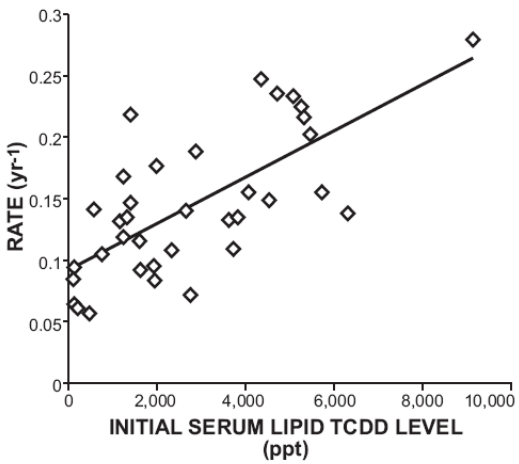


Figure 4: First-order elimination rate fits to 36 sets of serial TCDD sampling data from Seveso patients as a function of initial serum lipid TCDD. The best-fit first-order elimination rates show a clear dependence on initial serum lipid TCDD level (linear regression $R^2=0.49$, $p<0.0001$), with more rapid elimination associated with increasing TCDD level. This result indicates that the TCDD elimination behavior violates the first-order assumption, and demonstrates clearly a concentration- dependent elimination rate for TCDD.

The lack of a significant relationship between the concentration-

dependent model's fitted parameter k_e and initial TCDD level is what would be expected if the model formulation represents accurately the biological processes governing the disposition and elimination of TCDD. The finding of a very small but borderline statistically significant relationship between fitted k_e and initial TCDD levels suggests that the current model structure is substantially capturing the observed elimination behavior, but may still be slightly underestimating the concentration-dependence of elimination rate.
Figures 5 and 6 present illustrations of the measured data and model predictions for Austrian patients 1 and 2 and for four representative Seveso patients. For all of these sets of data, there was considerable fluctuation in reported values from one measurement to the next. In the case of the Austrian patients, the serum samples were quite small, which may have contributed to analytical variability. As discussed above for the Seveso data, there are a number of factors that could contribute to the variability in these data as well.

The model predicts that, as TCDD is eliminated from the body, the net rate of elimination varies continuously with time as a function of the serum lipid TCDD level. Figure 7 illustrates this using an "apparent" half-life calculated from the instantaneous rate of change in predicted serum lipid TCDD levels. Figure 7a compares the mean elimination behavior for males and females, and Figure 7b illustrates the range of elimination behavior associated with the upper and lower 95^{th} percent confidence intervals on the mean k_e for the males from Seveso.

In establishing a mean parameter set for use in modeling the back-extrapolated exposure levels in the NIOSH cohort members, we did not include the values derived from the modeling of the Austrian patients. The medical and research team studying Austrian Patients 1 and 2 administered Olestra in varying amounts over time to these two patients to attempt to increase the clearance rate of TCDD (Geusau et al., 2002; Geusau et al., 1999). This treatment was successful in accelerating the overall elimination of TCDD. The researchers estimated that over the 3 years of follow-up to date, Olestra-related clearance of TCDD accounted for about 10 percent of total clearance in Patient 1, and about 15 percent of total clearance in Patient 2. In fitting the serial serum lipid TCDD measurement data for these patients, we did not attempt to adjust for the enhanced elimination due to Olestra administration, so the fitted elimination rate includes this artificial acceleration of elimination, and the fitted rate constants are not directly comparable to the Seveso data. However, as presented in Table 3, the results of the fitting for the Austrian patients are not inconsistent with the results from the Seveso population. Although the hepatic elimination rate parameter estimates for the Austrian women were above (Patient 1) or at the upper end (Patient 2) of the range of values found for women from Seveso, this is not surprising in light of the

documented effect of Olestra administration in accelerating TCDD clearance in these patients.

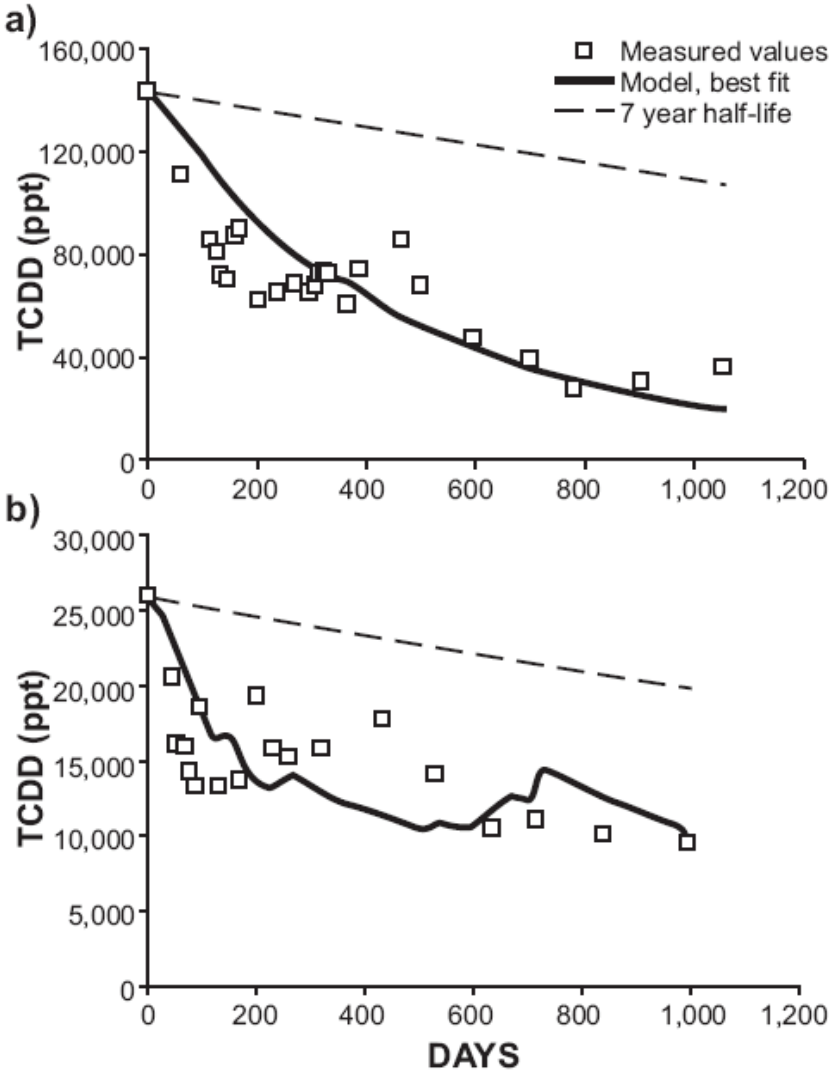


Figure 5: Measured serum lipid TCDD levels and best-fit model results for the two female Austrian patients. Body-weight changes were incorporated in the model simulation. Elimination predicted using a 7-year constant half-life illustrated for comparison purposes. a) Patient 1, best-fit k_e =0.94 yr^{-1}. b) Patient 2, best-fit k_e =0.63 yr^{-1}. Other model parameter values as listed in Table 1.

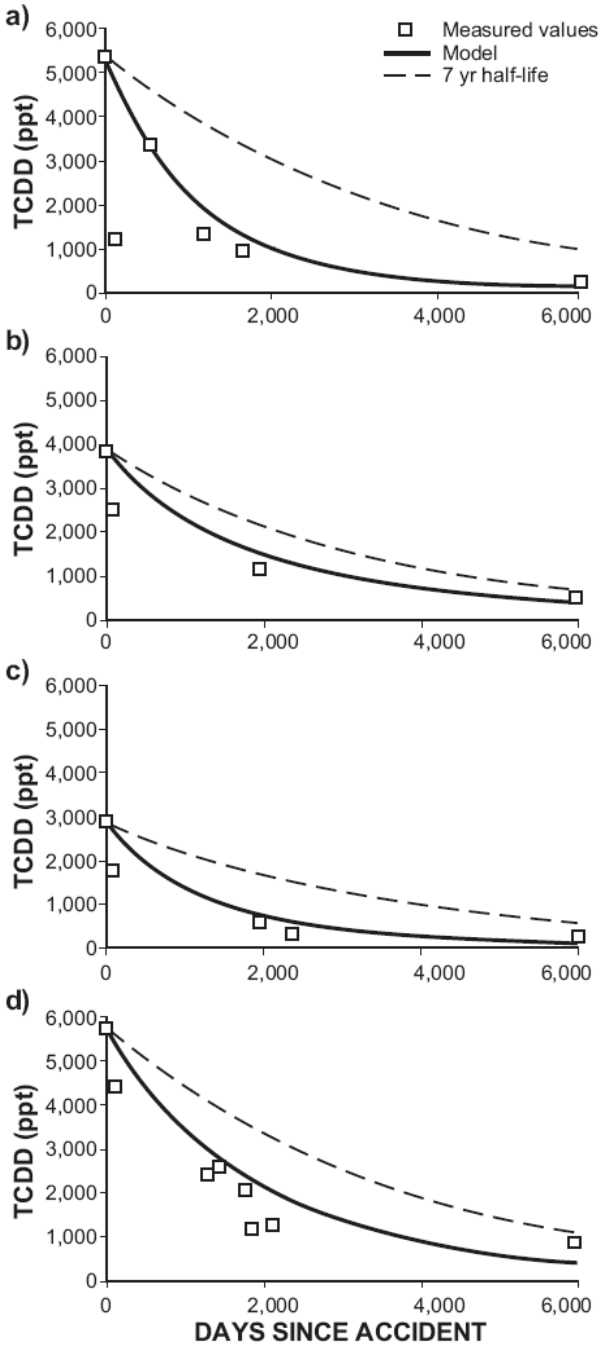


Figure 6: Illustration of model fits compared to elimination predicted by a 7-year first-order half-life for four Seveso patients. Model parameter values as in Table 1 with best-fit k_e for each patient and patient-specific data for body weight. Note that, at the highest concentrations, the model still somewhat underpredicts the apparent elimination rate, but matches the elimination behavior more closely than the constant first-order elimination rate. At lower concentrations, the model predicts elimination rates close to or even slower than the rate resulting from a first-order elimination process with 7-year half-life. a) Male, age in 1976, 45 years. Best-fit k_e = 0.47 yr^{-1}. b) Male, age in 1976, 41 years. Best-fit k_e = 0.23 yr^{-1}. c) Female, age in 1976, 16 years. Best-fit k_e = 0.44 yr^{-1}. d) Female, age in 1976, 40 years. Best-fit k_e = 0.23 yr^{-1}.

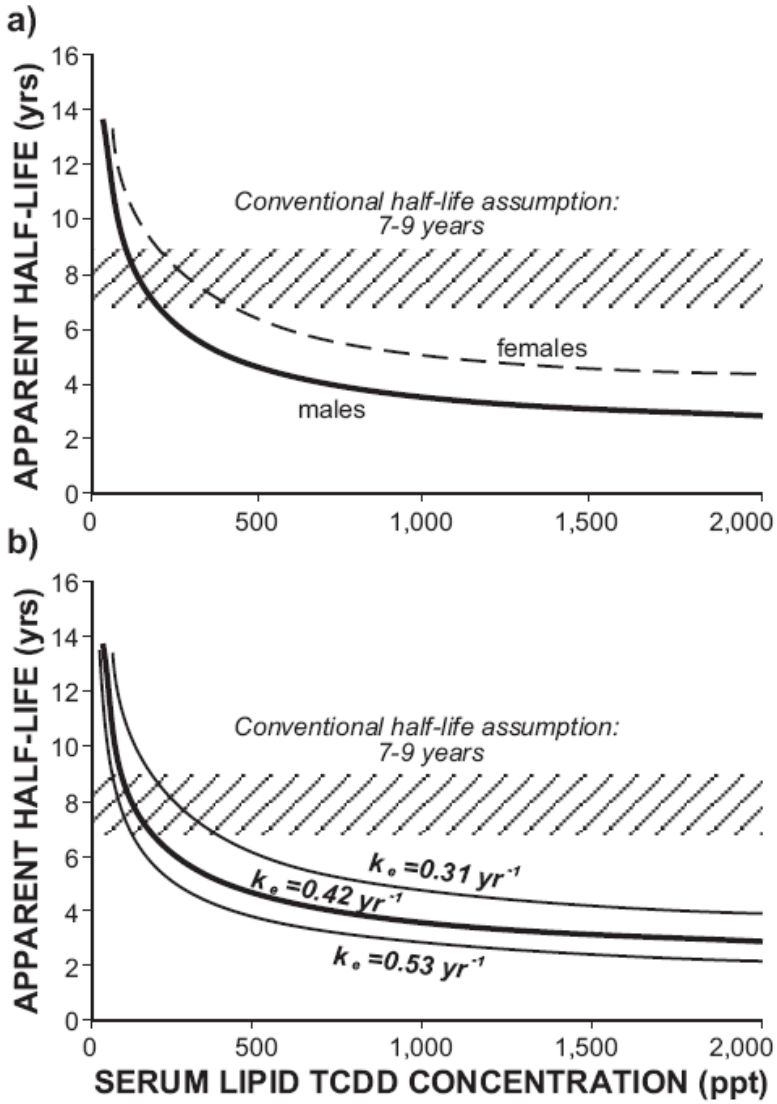


Figure 7: Apparent half-life for elimination of TCDD based on instantaneous changes in serum lipid TCDD concentration as a function of serum lipid TCDD concentration based on the model fits (parameter values as in Table 1). a) Concentration-dependence predicted by the model for the mean of the best-fit k_e values for males versus females from Seveso. b) Range of apparent half-life behavior predicted for males for the mean k_e and 95 percent confidence interval on the mean for males from Seveso.

Impact of concentration-dependent model on back-extrapolated estimates of NIOSH cohort member exposures. Figure 8 presents the estimated dose metrics for the NIOSH exposure duration subcohorts derived using the concentration-dependent model compared to the estimated doses derived from conventional first-order elimination assumptions. The concentration-dependent model was implemented for the NIOSH cohort using two sets of model parameters (one corresponding to the mean hepatic elimination rate from Seveso males, the other to the 95 percent confidence interval [LCI] of the mean). The first-order elimination estimates were derived using either a 7.5- or an 8.7-year half-life, corresponding to values used in previous estimates for the cohort (Aylward et al., 1996; Steenland et al., 2001).

The estimated doses vary over orders of magnitude for each of the methods used, and the estimated arithmetic means are greatly influenced by the upper range of each set of estimates. Estimates of dose metrics for the shortest duration exposure group using the concentration-dependent model were similar to or somewhat lower than those derived using the constant half-life assumption, depending on the summary measure used. This is because the concentration-dependent model actually predicts elimination rates that are lower than the conventional first-order estimates at serum lipid TCDD levels below about 50 ppt (see Figure 7). However, for individuals with higher measured serum lipid TCDD levels, the concentration-dependent model predicts much higher peak and total exposures than are predicted using the first-order models. For the subcohorts with longer exposure durations, the exposure estimates are greatly increased (several-fold to more than 25-fold) using the concentration-dependent model, compared to the assumption of first-order elimination with the conventional half-life parameters.

Figure 9 shows the impact of the different methods of reconstruction on the concentration-vs.-time curve for one individual from the NIOSH cohort, including an assessment of the impact of assuming age-dependence for the value of k_e. For persons with higher measured serum lipid TCDD levels in 1987–1988, and as the time of back-calculation increases, the estimates derived from the various methods diverge more widely. Some individuals in the cohort ceased employment more than 35 years before their serum samples were taken and measured for TCDD; for such individuals, even small differences in assumptions regarding elimination-rate behavior have large impacts on dose estimates.

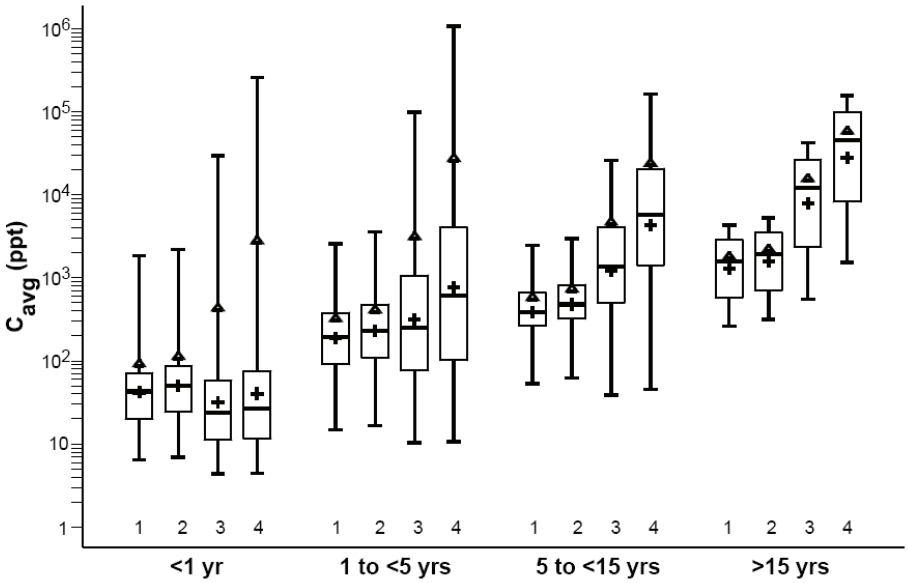


Figure 8: Box plots of estimated average serum lipid concentrations for the NIOSH cohort using the first-order elimination model with half-lives of 8.7 or 7.5 years (labeled 1 and 2, respectively) or the concentration-dependent toxicokinetic model with hepatic elimination rates equal to the LCI on the mean or the mean from Seveso males (labeled 3 and 4, respectively) for each of the NIOSH exposure duration subcohorts (exposed <1 year, 1 to <5 years, 5 to <15 years, and 15+ years). For each box plot, the interquartile range is included in the box, with the median indicated by the horizontal line. Geometric mean is indicated by the + symbol, and the arithmetic mean by the ▲. Whiskers encompass the entire range of the estimated levels.

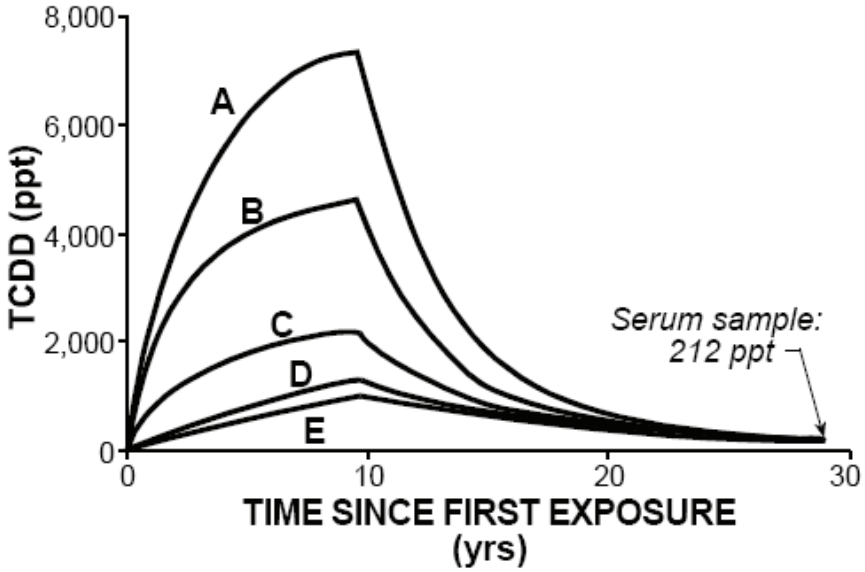


Figure 9: Illustration of the reconstructed TCDD serum lipid concentration-vs.-time curve for one individual resulting from five approaches. Lines illustrate reconstructed curves under the following approaches and assumptions: A: concentration-dependent toxicokinetic model, k_e = 0.42 yr^{-1} (mean of Seveso males); B: concentration-dependent model with age-dependent function for k_e (k_e=*0.85-0.01*age)* as found in Seveso males; C: concentration-dependent model with k_e=0.31 yr^{-1} (the 95 percent LCI on the mean for Seveso males). Lines D and E illustrate the results from assuming first-order elimination with 7.5- and 8.7-year half-lives, respectively. All modeling assumes constant body weight and body-fat level except B, which incorporates an age-dependent increase in percent body fat per Deurenberg et al. (1991). The estimated peak concentration varies from less than 1,000 ppt to over 7,000 ppt, depending on the back-calculation procedure used.

Table 6 presents the dose estimates used by the USEPA (2000) in the cancer dose-response assessment for the NIOSH cohort and the corresponding dose estimates derived using the concentration-dependent elimination model. Even the use of the LCI on the mean k_e parameter, based on fits from the Seveso males, results in about four- to seven-fold increases over the previously estimated dose levels; use of the mean k_e parameter results in increases of 25-fold or more in the dose estimates. Only with the use of the lowest hepatic elimination rate parameter k_e, from the Seveso male with the ***slowest*** elimination behavior, do the results in summary dose estimates for the highest exposed groups become similar to those resulting from the first-order model with a half-life of 8.7 years used by Steenland et al. (2001) (data not shown). As noted above, the large degree of variability in dose estimates within each exposure duration subcohort makes selection of a representative summary measure (mean, median, etc.) for these subcohorts problematic. Dose-response assessments based on such back-calculated exposure estimates need to acknowledge and account for this variability within subcohorts.

Discussion

These data sets of serial measurements of serum lipid TCDD concentration in persons with elevated exposures to TCDD confirm that in humans, as in experimental animals, the elimination rate for TCDD varies with body concentration, with substantially faster elimination at elevated body concentrations compared to lower body concentrations. The modified Carrier et al. toxicokinetic model provides a conceptual, quantitative framework for modeling this elimination based on a mechanistic understanding of the distribution and elimination of TCDD in experimental animals and measurements of elimination of unchanged TCDD in human subjects with a wide range of body burdens. The model now accounts for hepatic-mediated elimination (probably metabolism) and for elimination via partitioning based on lipophilicity from the circulation, across the intestinal lumen, and into the contents of the large intestine (Moser and McLachlan 2002). The results of the modeling of the Seveso serial serum lipid TCDD concentration data with a concentration-dependent model are consistent with the results reported by Michalek et al. (2002) for a subset of these data. They found much faster elimination in the first 3 months and 3 years after the accident than in the period from 3 to 16 years after the accident. The proposed concentration-dependent model places these findings into the context of a physiological explanation for the varying elimination rates and provides a framework for assessing concentration-vs.-time profiles for other populations.

Table 6: Comparison of dose estimates used by USEPA (2000) for NIOSH subcohorts (group mean C_{avg}) to dose estimates resulting from use of the concentration-dependent model with hepatic elimination-rate parameter k_e equal to the mean or lower 95th confidence interval on the mean based on measured and modeled elimination in Seveso males

Exposure duration, years	First-order estimate, ppt USEPA (2000)[a]	Concentration-dependent model estimates,[b] ppt k_e=0.31 yr^{-1}	k_e=0.42 yr^{-1}	Fold increase
<1	111	437	2 830	4–25
1–<5	413	3 170	27 700	8–67
5–<15	738	4 490	24 000	6–32
15+	2 220	15 800	58 800	7–26

[a] Dose estimates used in USEPA (2000) were based on NIOSH subcohort mean C_{avg} values as estimated by Aylward et al. (1996) using a constant first-order half-life of 7.5 years. The lipid-based concentrations were converted to body burdens by dividing by 4, based on the assumption that a) all TCDD was distributed to lipid tissue in the body, and b) average body-fat level was 25%.
[b] Model parameters as described in Table 1.

Despite the fact that there is a physical interpretation associated with the various parameters in the model, specific values of the individual parameters used in this modeling should be interpreted with caution. Human data are insufficient at present to determine the exact shape and parameters of the dose-response curve for the liver fraction due to induction of CYP1A2 in the liver. The values for f_{hmin}, f_{hmax}, and K used in this modeling were those based on fits to animal data and previous modeling of human data (Carrier et al., 1995b; Carrier et al., 1999); the fitting procedure in this paper varied only the basic hepatic elimination rate k_e. However, due to the structure of the model, similar results (in terms of elimination behavior and fits to the serial sampling data) could be obtained by co-varying k_e and K. Similarly, f_{hmax} and k_e could be adjusted in opposite directions to result in similar elimination behavior. A unique estimation of the value for any specific model parameter relating to hepatic elimination is thus not actually possible; the values proposed for the hepatic parameters should be regarded as a package.

For example, although a value of 100 ng/kg was used as the value of K (the whole-body concentration at which the body burden fraction in the liver is half-maximum), based on fits to animal data sets and one earlier human data set (Carrier et al., 1995a; Carrier et al., 1995b; Carrier et al., 1999), these data are not sufficient to conclude that a value of 100 ng/kg for K is the actual body burden at which humans generally exhibit half-maximal liver fraction of body burden. Nonetheless, induction data for the Austrian patients tend to support this general range of body burden for this parameter (Abraham et al., 2002). We varied the values for K from 50 to 1000 ng/kg and refit the Seveso data sets (results not shown). Although the fitted values of k_e were shifted, they displayed similar age and sex dependence, and the shape of the elimination curves was indistinguishable from the original fits (results not shown). Thus, although the absolute value of k_e is sensitive to the value chosen for K, taken as a whole, the given set parameter values and fitted k_e values accurately reflect the concentration dependence of the elimination behavior for TCDD in these data sets, and the overall elimination behavior predicted by parameters sets keyed to different values of K are indistinguishable. In contrast, the value for k_a, the lipid-based elimination-rate constant parameter, was determined from data on 18 individuals with a wide range of body burdens and other characteristics. The estimate of this parameter value certainly could be improved with additional data, but the value is based directly on observations of intake, excretion, and measured serum lipid TCDD levels in humans.

The model fits to the elimination data for the 36 Seveso patients confirm earlier observations that elimination is slower in females than in males, on average (Landi et al., 1998; Needham et al., 1994; Needham et al., 1997/98).

This is also consistent with animal data indicating that adult female rats eliminate TCDD more slowly than adult males (Jackson et al., 1998). In addition, the fitted values of k_e also demonstrate a statistically significant inverse correlation with age. That is, younger persons appear to metabolize TCDD more rapidly than older persons, on average. This relationship held true for both males and females and was also similar to previous reports for humans (Flesch-Janys et al., 1996) and to the elimination behavior observed in rats (Jackson et al., 1998).

The fitted values of k_e varied over a substantial range for both males and females in the Seveso population, even after controlling for the associations with age and sex. These variations may provide an indication of the intrinsic range of variability in elimination efficiency in humans. There is some indication that elimination due to hepatic metabolism at background body burdens of TCDD may be underestimated by the model. Evaluations of population trends in TCDD levels are consistent with elimination half-lives of under 10 years at background exposure levels, although such studies cannot address individual elimination rates (Aylward and Hays 2002; Wittsiepe et al., 2000). By relying solely on the level of one congener (TCDD) to determine the liver fraction of burden, $f_h(C_b)$, the model may underestimate *de facto* elimination rates at background body burdens due to failure to account for possible hepatic CYP1A2 induction by other TEQ contributors (Kitamura et al., 2001b) or non-dioxin exposures (e.g., tobacco smoking or alcohol consumption).

The results from this modeling are not inconsistent with previous estimates based on simple first-order elimination for persons with moderately elevated body burdens. This is a result of limited numbers of serial sampling data points over a relatively narrow range of serum lipid TCDD concentrations available in these earlier studies. For example, Rohde et al. (1999) reported an average elimination half-life for six occupationally exposed workers of 7.9 years, based on two serum samples for each person taken 4 to 6 years apart. The final serum lipid TCDD concentrations in these workers ranged from 85 to 505 ppt. The model presented here (using the mean k_e value derived from the fits to male Seveso patients) would predict apparent elimination half-lives ranging from 10 to about 4.6 years over the same concentration range. This range is entirely consistent with the estimate of average elimination half-life of 7.9 years derived by Rohde et al. (1999). Without serial serum lipid TCDD measurements taken over a relatively wide range of concentrations and long time period for a single individual (as provided by the Seveso data), the departure from first-order elimination behavior might not be apparent.

Previous analyses of a subset of the Seveso data analyzed here relied on an assumption of first-order elimination to fit the data sets (Needham et al., 1994; Needham et al., 1997/98), but as discussed above, the best-fit first-

order elimination rates for the data set are strongly concentration-dependent, as predicted by the model.

NIOSH cohort dose estimates. Application of the model with parameters derived from the Seveso male population to the back-calculation of exposures for a subset of the NIOSH cohort of herbicide manufacturing workers indicates that previous dose estimates for this cohort greatly underestimated actual exposure levels for these workers. Depending on the dose metric and summary measure selected, dose estimates obtained for the more highly exposed subcohorts using the concentration-dependent model with the mean parameter set based on elimination behavior in males from Seveso are 25-fold or more higher than those obtained using a first-order model with an elimination half-life of 7.5 years.

It is important to consider the inter-individual variability in elimination behavior observed in the analysis of the Seveso data. For an individual in the NIOSH cohort with a serum lipid TCDD level of several hundred ppt in a sample taken decades after last exposure, we can conclude that two factors are likely to be operating: a) the individual was highly exposed, and/or b) the individual may have a lower hepatic elimination rate. Conversely, some individuals with lower measured levels may have had high exposures and intrinsically rapid clearance, or lower exposure and more moderate clearance. However, based on a single sampling point for these individuals, we cannot determine the relative contribution of each factor (exposure magnitude vs. individual clearance rate). Thus, use of the mean elimination rate parameters based on the fits to data from male Seveso patients in the concentration-dependent toxicokinetic model will result in overestimates of exposure for some persons and underestimates for others. The degree of over or underestimate for each individual may be substantial and is essentially unknowable with the limited data available on this population.

The overall uncertainty introduced into the dose estimates due to the inter-individual variability in elimination behavior is exacerbated by the length of time of back-calculation, which is critical due to the non-linear nature of the process. As the estimated body level, and therefore concentration-dependent elimination rate, increases, the "doubling time" for the back-calculated levels decreases. There are many other sources of uncertainty in the dose estimates as well. The concentration-dependent toxicokinetic model results can be affected significantly by changes in body weight and body-fat levels, regarding which we have no data for this population. For example, a doubling of the volume of adipose tissue in an individual would result in a dilution by half of lipid TCDD concentrations, thus leading to an important underestimation of the original exposure dose. In addition, the results from modeling the Seveso serial TCDD measurement data indicate that the elimination rate generally slows with increasing age; this effect was not incorporated in the modeling

presented in Figure 6, although Figure 9 illustrates the effect of this age-dependence on the concentration-vs- time curve for one individual. Another factor affecting the dose estimates is the precision of the measured TCDD levels from the serum samples taken in 1987–1988; the measured values may have been subject to some analytical variability. Figure 10 illustrates the effect of a 20% error in the measured TCDD level in either direction on the estimated peak concentration for one individual. Again, the magnitude of uncertainty increases with increasing back-calculation time, and variations of several-fold can occur.

The dose estimates for the NIOSH cohort that are derived here are not directly comparable to the dose estimates by Steenland et al. (2001) for the NIOSH cohort. That analysis relied on a detailed exposure index assessment for each worker in the NIOSH cohort, based on work history and industrial hygiene data (Piacitelli et al., 2000), to customize the intake portion of the concentration-vs.-time curve. However, the exposure index process does not reduce the uncertainty associated with the back-calculation part of the estimation or with the kinetic treatment of doses estimated to have been received by each individual during their employment.

Other estimates of exposures and assessments of cancer dose-response for the occupational cohorts (Crump et al., 2003; Flesch-Janys et al., 1998; Ott and Zober 1996; Starr 2001) all rely on cumulative exposure estimates that are fundamentally based on back-calculations of measured levels over decades, assuming constant first-order elimination rates ranging from 7 to 9 years—the best available estimates at the time the studies were conducted. The conventional back-calculated exposures for these cohorts have probably been underestimated for the most highly exposed subcohorts by several-fold, and perhaps by more than an order of magnitude (Table 6). Estimates of exposure obtained through long-term back-calculation for any individual are highly uncertain, whether using a concentration-dependent model or assuming constant-half-life first-order elimination, and the degree of uncertainty increases with increasing duration of back-calculation. The large uncertainty inherent in exposure estimates based on long-term back-calculations has never been explicitly accounted for in such cancer dose-response assessments.

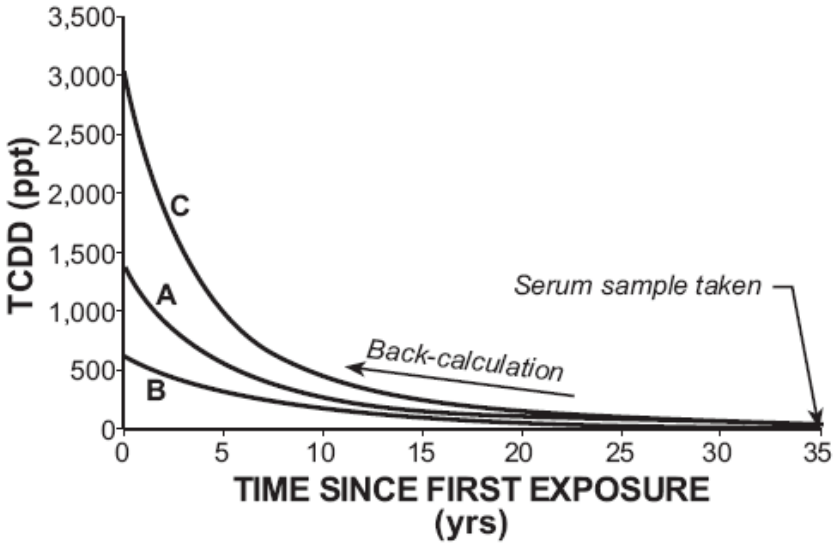


Figure 10: Effect of possible analytical variability in measured serum lipid TCDD levels on resulting back-calculations. Lines represent back-calculated serum lipid TCDD profiles for one NIOSH worker using mean parameter set from the Seveso males. Line A: Back-calculation begins from measured level of 42.9 ppt in October 1987 back to date of last exposure in 1952. Lines B and C illustrate the effect of a 20% error in measured serum lipid TCDD level (34.3 and 51.5 ppt, respectively) on back-calculated peak level. Estimated peak levels are affected by more than two-fold in either direction based on the hypothesized 20% variation in measured level in 1987. The degree of divergence increases as the back-calculation time increases.

The modeling conducted here addresses the pharmacokinetic behavior only of TCDD. However, as originally envisioned by Carrier et al. (1995a, b), the model could be adapted to address other dioxin or furan contributors to dioxin toxic equivalency (TEQ) exposures. Application of the model to other compounds would require assuming or demonstrating for each compound that a) the compound induces CYP1A2 in the liver, and b) the compound is bound by CYP1A2 in the liver. Parameter values for both the hepatic and the lipid elimination functions would likely be compound specific. Such parameters could be estimated from animal experiments, but from a practical standpoint, serial elimination data from high exposures (similar to that modeled here from the Seveso accident, or from the limited data available for selected chlorinated furan compounds from the Yusho and Yu-cheng incidents) would be required to confirm the overall model performance in humans. Such data may never be available for most other TEQ contributors. Because most TEQ exposures of interest occur at or near background exposure levels, the change in elimination behavior at higher exposures may be of limited practical interest.

The data and analysis presented here clearly indicate that, for human dose estimations back-extrapolated over long times and to elevated body burdens, the assumption of simple first-order elimination kinetics is not valid. This toxicokinetic model and parameter set based on serial serum lipid TCDD measurements from 36 adults from Seveso provides a critical tool for evaluating historical dioxin exposures, predicting future elimination behavior, and assessing the human health risks from a variety of exposure profiles. The patterns in elimination behavior observed with age and sex will also be important in future assessments of human exposures to and risks from dioxins.

The exposure assessment for the NIOSH cohort presented in this analysis indicates that the difference in TCDD exposure levels experienced by the occupational cohorts compared to the general population is likely substantially greater than previously thought. The uncertainty in dose estimates, combined with the other uncertainties inherent in estimates of the carcinogenic potency of TCDD, based on the mortality data from the occupational cohorts, suggests that quantitative estimates of cancer risk at general-population exposure levels derived from these data are highly uncertain. The concentration-dependent elimination model presented here could be used to reassess exposure estimates for the entire NIOSH cohort over a range of possible elimination behavior, providing a tool for characterizing the uncertainty in the dose estimates associated with observed mortality rates in this population. Cancer dose-response assessments based on the revised exposure estimates could incorporate a probabilistic approach to the exposure estimates, allowing for a more explicit characterization of this source of uncertainty in the cancer dose-response assessment.

Acknowledgements
We thank Alex Exuzides for assistance with statistical analyses, and Joel Michalek for assistance with data. This research was supported in part by grant 2896 from Regione Lombardia, Milano, Italy, and by funding from the Chlorine Chemistry Council and Tierra Solutions, Inc. Most serum TCDD analyses for female Seveso residents were funded by grants (P.I. B. Eskenazi) from the National Institutes of Health (R01 ES07171) and the U.S. Environmental Protection Agency (R82471).

References

Abraham K., Geusau A., Tosun Y., Helge H., Bauer S., Brockmoller J. Severe 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) intoxication: insights into the measurement of hepatic cytochrome P450 1A2 induction. *Clin Pharmacol Ther.* 2002; 72(2):163-174.

Aylward L. L., Hays S. M. Temporal trends in human TCDD body burden: Decreases over three decades and implications for exposure levels. *J Expo Anal Environ Epidemiol.* 2002; 12:319-328.

Aylward L. L., Hays S. M., Karch N. J., Paustenbach D. J. Relative susceptibility of animals and humans to the cancer hazard posed by 2,3,7,8-tetrachlorodibenzo-p-dioxin using internal measures of dose. *Environmental Science & Technology.* 1996; 30(12):3534-3543.

Becher H., Steindorf K., Flesch-Janys D. Quantitative cancer risk assessment for dioxins using an occupational cohort. *Environ Health Perspect.* 1998; 106(2):663-670.

Carrier G., Brunet R. C., Brodeur J. Modeling of the toxicokinetics of polychlorinated dibenzo-p-dioxins and dibenzofurans in mammalians, including humans. I. Nonlinear distribution of PCDD/PCDF body burden between liver and adipose tissues. *Toxicol Appl Pharmacol.* 1995a; 131(2):253-266.

Carrier G., Brunet R. C., Brodeur J. Modeling of the toxicokinetics of polychlorinated dibenzo-p-dioxins and dibenzofurans in mammalians, including humans. II. Kinetics of absorption and disposition of PCDDs/PCDFs. *Toxicol Appl Pharmacol.* 1995b; 131(2):267-276.

Carrier G., Brunet R. C., Mocarelli P., Brambilla G., Gerthoux P. M., Needham L. L., Patterson D. G. TCDD kinetics in Seveso patients. *Organohalogen Compounds.* 1999; 42:159-164.

Crump K. S., Canady R., Kogevinas M. Meta-analysis of dioxin cancer dose response for three occupational cohorts. *Environ Health Perspect.* 2003; 111(5):681-687.

Deurenberg P., Andreoli A., Borg P., Kukkonen-Harjula K., de Lorenzo A., van Marken Lichtenbelt W. D., Testolin G., Vigano R., Vollaard N. The validity of predicted body fat percentage from body mass index and from impedance in samples of five European populations. *Eur J Clin Nutr.* 2001; 55(11):973-979.

Deurenberg P., Weststrate J. A., Seidell J. C. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. *Br J Nutr.* 1991; 65(2):105-114.

Fingerhut M. A., Halperin W. E., Marlow D. A., Piacitelli L. A., Honchar P. A., Sweeney M. H., Greife A. L., Dill P. A., Steenland K., Suruda A. J. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p- dioxin [see comments]. *N Engl J Med.* 1991; 324(4):212-218.

Flesch-Janys D., Becher H., Gurn P., Jung D., Konietzko J., Manz A., Papke O. Elimination of polychlorinated dibenzo-p-dioxins and dibenzofurans in occupationally exposed persons. *J Toxicol Environ Health.* 1996; 47(4):363-378.

Flesch-Janys D., Steindorf K., Gurn P., Becher H. Estimation of the cumulated exposure to polychlorinated dibenzo-p-dioxins/furans and standardized mortality ratio analysis of cancer mortality by dose in an occupationally exposed cohort. *Environ Health Perspect.* 1998; 106 Suppl 2:655-662.

Geusau A., Schmaldienst S., Derfler K., Papke O., Abraham K. Severe 2,3,7,8-tetrachlorodibenzo- p-dioxin (TCDD) intoxication: kinetics and trials to enhance elimination in two patients. *Arch Toxicol.* 2002; 76(5-6):316-325.

Geusau A., Tschachler E., Meixner M., Sandermann S., Papke O., Wolf C., Valic E., Stingl G., McLachlan M. Olestra increases faecal excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Lancet.* 1999; 354(9186):1266-1267.

Hu K., Bunce N. J. Metabolism of polychlorinated dibenzo-p-dioxins and related dioxin-like compounds. *J Toxicol Environ Health, Part B.* 1999; 2:183-210.

Jackson J. A., Birnbaum L. S., Diliberto J. J. Effects of age, sex, and pharmacologic agents on the biliary elimination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in F344 rats. *Drug Metab Dispos.* 1998; 26(7):714-719.

Kitamura K., Nagahashi M., Sunaga M., Watanabe S., Nagao M. Balance of intake and excretion of 20 congeners of polychlorinated dibenzo-p-dioxin, polychlorinated dibenzofuran and coplanar polychlorinated biphenyl in healthy Japanese men. *J. Health Sci.* 2001a; 47(2):145-154.

Kitamura K., Nagao M., Yamada T., Sunaga M., Hata J., Watanabe S. Dioxins in bile in relation to those in the human liver and blood. *J Toxicol Sci.* 2001b; 26(5):327-336.

Landi M. T., Consonni D., Patterson D. G., Jr., Needham L. L., Lucier G., Brambilla P., Cazzaniga M. A., Mocarelli P., Pesatori A. C., Bertazzi P. A., Caporaso N. E. 2,3,7,8-Tetrachlorodibenzo-p-dioxin plasma levels in Seveso 20 years after the accident. *Environ Health Perspect.* 1998; 106(5):273-277.

Michalek J. E., Pirkle J. L., Needham L. L., Patterson D. G., Caudill S. P., Tripathi R. C., Mocarelli P. Pharmacokinetics of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Seveso adults and veterans of operation Ranch Hand. *J Expo Anal Environ Epidemiol.* 2002; 12(1):44-53.

Michalek J. E., Tripathi R. C. Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 15-year follow-up. *J Toxicol Environ Health A.* 1999; 57(6):369-378.

Moser G. A., McLachlan M. S. A non-absorbable dietary fat substitute enhances elimination of persistent lipophilic contaminants in humans. *Chemosphere.* 1999; 39(9):1513-1521.

Moser G. A., McLachlan M. S. The influence of dietary concentration on the absorption and excretion of persistent lipophilic organic pollutants in the human intestinal tract. *Chemosphere.* 2001; 45:201-211.

Moser G. A., McLachlan M. S. Modeling digestive tract absorption and desorption of lipophilic organic contaminants in humans. *Environ Sci Technol.* 2002; 36(15):3318-3325.

Needham L. L., Gerthoux P. M., Patterson D. G., Brambilla G., Pirkle J. L., Tramacere P. L., Turner W. E., Beretta C., Sampson E. J., Mocarelli P. Half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin in serum of Seveso adults: Interim report. *Organohalogen Compounds.* 1994; 21:81-85.

Needham L. L., Gerthoux P. M., Patterson D. G., Brambilla G., Turner W. E., Beretta C., Pirkle J. L., Colombo L., Sampson E. J., Tramacere P. L., Signorini S., Meazza L., Carreri V., Jackson R. J., Mocarelli P. Serum dioxin levels in Seveso Italy, population in 1976. *Teratog Carcinog Mutagen.* 1997/98; 17:225-240.

Olson J. R., McGarrigle B. P., Gigliotti P. J., Kumar S., McReynolds J. H. Hepatic uptake and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran. *Fundam Appl Toxicol.* 1994; 22(4):631-640.

Ott M. G., Zober A. Cause specific mortality and cancer incidence among employees exposed to 2,3,7,8-TCDD after a 1953 reactor accident. *Occup Environ Med.* 1996; 53(9):606-612.

Piacitelli L., Marlow D., Fingerhut M., Steenland K., Sweeney M. H. A retrospective job exposure matrix for estimating exposure to 2,3,7, 8-tetrachlorodibenzo-p-dioxin. *Am J Ind Med.* 2000; 38(1):28-39.

Rohde S., Moser G. A., Papke O., McLachlan M. S. Clearance of PCDD/Fs via the gastrointestinal tract in occupationally exposed persons. *Chemosphere.* 1999; 38(14):3397-3410.

Ryan J. J., Levesque D., Panopio L. G., Sun W. F., Masuda Y., Kuroki H. Elimination of polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) from human blood in the Yusho and Yu-Cheng rice oil poisonings. *Arch Environ Contam Toxicol.* 1993; 24(4):504-512.

Schlatter C. Data on kinetics of PCDDs and PCDFs as a prerequisite for human risk assessment. In: Gallo MA, Scheuplein RJ, Van der Heijden KA, eds. *Biological Basis for Risk Assessment of Dioxins and Related Compounds*. Plainview, NY: Cold Spring Harbor Laboratory Press; 1991:215-228.

Schlummer M., Moser G. A., McLachlan M. S. Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans: mass balances and mechanistic considerations. *Toxicol Appl Pharmacol.* 1998; 152(1):128-137.

Starr T. B. Significant shortcomings of the U.S. Environmental Protection Agency's latest draft risk characterization for dioxin-like compounds. *Toxicol Sci.* 2001; 64(1):7-13.

Steenland K., Deddens J., Piacitelli L. Risk assessment for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) based on an epidemiologic study. *Am J Epidemiol.* 2001; 154(5):451-458.

United States Environmental Protection Agency (USEPA). *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin and Related Compounds. Draft Final.* Washington, D.C.: National Center for Environmental Assessment, U.S. Environmental Protection Agency; 2000. EPA/600/P-00/001Be.

Wittsiepe J., Schrey P., Ewers U., Wilhelm M., Selenka F. Decrease of PCDD/F levels in human blood--trend analysis for the German population, 1991-1996. *Environ Res.* 2000; 83(1):46-53.

Chapter 7: Exposure Reconstruction for the TCDD-Exposed NIOSH Cohort Using a Concentration- and Age-Dependent Model of Elimination

Aylward, L.L., R. Brunet, T. Starr, G. Carrier, E. Delzell, H. Cheng, and C. Beall. 2005. Risk Anal. 25(4):945-956.

The definitive version is available at www.blackwell-synergy.com.

Abstract

Recent studies demonstrating a concentration dependence of elimination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) suggest that previous estimates of exposure for occupationally exposed cohorts may have underestimated actual exposure, resulting in a potential overestimate of the carcinogenic potency of TCDD in humans based on the mortality data for these cohorts. Using a database on U.S. chemical manufacturing workers potentially exposed to TCDD compiled by the National Institute for Occupational Safety and Health (NIOSH), we evaluated the impact of using a concentration- and age-dependent elimination model (CADM) (Aylward *et al.*, 2005) on estimates of serum lipid area under the curve (AUC) for the NIOSH cohort. These data were used previously by Steenland *et al.* (2001) in combination with a first-order elimination model with an 8.7-year half-life to estimate cumulative serum lipid concentration (equivalent to AUC) for these workers for use in cancer dose-response assessment. Serum lipid TCDD measurements taken in 1988 for a subset of the cohort were combined with the NIOSH job exposure matrix and work histories to estimate dose rates per unit of exposure score. We evaluated the effect of choices in regression model (regression on untransformed vs. ln-transformed data and inclusion of a non-zero regression intercept) as well as the impact of choices of elimination models and parameters on estimated AUCs for the cohort. Central estimates for dose rate parameters derived from the serum-sampled subcohort were applied with the elimination models to time-specific exposure scores for the entire cohort to generate AUC estimates for all cohort members. Use of the CADM resulted in improved model fits to the serum sampling data compared to the first-order models. Dose rates varied by a factor of 50 among different combinations of elimination model, parameter sets, and regression models. Use of a CADM results in increases of up to 5-fold in AUC estimates for the more highly exposed members of the cohort compared to estimates obtained using the first-order model with 8.7-year half-life. This degree of variation in the AUC estimates for this cohort would affect substantially the cancer potency estimates derived from the mortality data from this cohort. Such variability and uncertainty in the reconstructed serum lipid AUC estimates for this cohort, depending on elimination model, parameter set, and regression model, have not been described previously and are a critical component in evaluating the dose-response data from the occupationally exposed populations.

Keywords: Dioxin, occupational exposure reconstruction, toxicokinetic models

Introduction

Estimates of cumulative exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have been developed for several occupationally exposed cohorts by

integrating job-specific indices of exposure intensity based on work histories with measured serum lipid TCDD levels (Flesch-Janys *et al.*, 1998; Ott & Zober, 1996; Steenland *et al.*, 2001). These efforts have relied on the assumption that TCDD elimination occurred via a first-order process with a half-life of 7.1 to 8.7 years. Serum lipid levels measured years after last exposure for a subset of each cohort were combined with estimates of exposure intensity over time during employment to derive central estimates of the absorbed dose associated with one unit of the exposure score. These dose rates were then applied to the job exposure histories of each member of the full cohort to estimate cumulative exposures (termed either area under the curve [AUC] or cumulative serum lipid concentration, depending on researcher). A meta-analysis of three occupational cohorts employed such dose estimates and standardized mortality ratios (SMRs) to estimate the carcinogenic potency of TCDD in these populations (Crump *et al.*, 2003).

Recent studies have demonstrated that the elimination of TCDD in humans occurs via a concentration-dependent process: elimination occurs at a faster rate when body concentrations are relatively high, with effective elimination half-lives of less than 3 years at serum lipid levels above 1,000 ppt (Aylward *et al.*, 2005; Geusau *et al.*, 2002; Michalek *et al.*, 2002). In addition, analysis of serial sampling data from subjects exposed to TCDD during the 1976 chemical reactor accident in Seveso, Italy, has demonstrated an age-dependent slowing of elimination (Aylward *et al.*, 2005). These results are consistent with similar observations in laboratory rodents (Abraham *et al.*, 1988; Diliberto *et al.*, 2001) and suggest that back-calculations of serum lipid TCDD concentrations based on the assumption of a first-order elimination process with a constant half-life of 7 to 9 years may underestimate actual serum lipid concentrations and exposure levels in the occupationally exposed populations, depending on the level and and timing of exposure.

Here we present results from application of a concentration- and age-dependent model of elimination (CADM) (originally developed by Carrier *et al.* (Carrier *et al.*, 1995a, b), modified and parameterized by Aylward et al. (Aylward *et al*., 2005) to the data for the cohort of TCDD-exposed chemical manufacturing workers studied by the United States (U.S.) National Institute for Occupational Safety and Health (the "NIOSH cohort"; Fingerhut *et al.*, 1991; Steenland *et al.*, 2001; Steenland *et al.*, 1999). We describe the process of dose reconstruction, the impact of the CADM on dose estimates for the cohort, and the variability in the dose estimates obtained depending on the model and regression procedure used, the model parameters chosen, and the use of an exposure lag period.

Methods

Cohort data

A job exposure matrix (JEM) was developed by NIOSH for a subset (n=3,538) of the original cohort of 5,132 U.S. chemical plant workers with potential exposure to TCDD. The subset came from eight of the 12 plants originally included in the cohort (Piacitelli *et al.*, 2000). The JEM was used in the 1999 update of the mortality experience of this subset of the NIOSH cohort (Steenland *et al.*, 1999). We obtained from NIOSH a database that included information on subjects' vital status at end of follow-up, work histories, the JEM, birth dates, chloracne status, and, for deceased workers, cause and date of death. The JEM provided TCDD exposure scores that were specific to each plant, department, job, and time period. Linkage of the work history of each subject with the JEM allowed us to compute a set of time-specific exposure scores for each worker, designated E(t). The exposure score for a particular job held by a subject was the product of the estimated TCDD concentration (μg/g) in the process materials used in the job, a qualitative contact factor that scores the extent of dermal contact, exposure to airborne particulates in the job, and the estimated time spent actively engaged in the job during the course of a day. A subset of the workers (n=193), all from a single plant, underwent physical examination in 1987/88, and for these workers, the database also contained information on smoking status, height, weight, and measured serum lipid TCDD concentrations.

Elimination modeling

Two models of elimination were employed in this analysis—the conventional first-order model and the CADM, each with two parameter sets representing a range of observed elimination behaviors. Definitions of modeling terms and parameters for both models are presented in Table 1, and a schematic of the CADM is presented in Figure 1.

Table 1: Model parameters, definitions, and values

Model Parameter	Description, units	Value
First-order model		
k	Elimination rate constant, yr^{-1}	8.7-yr HL: 0.0796 7.1-yr HL: 0.0976
BW	Body weight	70
w_a	Fraction body weight adipose/lipid tissue	0.25
Concentration- and age-dependent model		
f_{hmin}	Minimum proportion of body burden distributed to liver, unitless	0.01[a]
f_{hmax}	Maximum proportion of body burden distributed to liver, unitless	0.7 [a]
K	Body concentration for half-maximum increase in liver distribution proportion, ng/kg	100 [a]
k_a	Rate constant for elimination based on partitioning from circulating lipids into intestine, yr^{-1}	0.03 [a]
k_e	Rate constant for hepatic elimination, yr^{-1}	Eq. 7
k_{e0}	Base hepatic elimination rate at age zero, yr^{-1}	CADM-Mean: 0.85 CADM-LCI: 0.76
$k_{e\ min}$	Minimum hepatic elimination rate, yr^{-1}	CADM-Mean: 0.2 CADM-LCI: 0.11
BW	Body weight, kg	70
w_a	Fraction body weight adipose/lipid tissue	Age-dependent[b]
w_h	Fraction body weight liver	0.03
f_h	Fraction of body burden in liver, unitless	Eq. 3
f_a	Fraction of body burden in adipose/lipid tissue, unitless	$1-f_h$
Q_a	Quantity of TCDD in adipose/lipid tissue, ng	calculated
Q_h	Quantity of TCDD in hepatic tissue, ng	calculated
Q_b	Quantity of TCDD in body tissue, ng	calculated
C_a	Concentration of TCDD in adipose/lipid tissue, ng/kg	calculated
C_h	Concentration of TCDD in hepatic tissue, ng/kg	calculated
C_b	Concentration of TCDD in body tissue, ng/kg	calculated
Estimated parameters		
β_0	Regression intercept, ppt	Maximum likelihood estimate
β_1	Dose rate, ng per unit of exposure score	Maximum likelihood estimate
β_2	Background dose rate unrelated to occupational exposure, ng/kg/month	Maximum likelihood estimate

[a] See Aylward *et al.* (2005) for full description of these parameters.

[b] Estimated from formula from Deurenberg *et al.* (1991) and an assumption of constant body mass index (BMI) of 24: w_a = [(1.2*BMI) + (0.23*Age) – (10.8*sex) – 5.4]/100 where sex=1 for males, 0 for females.

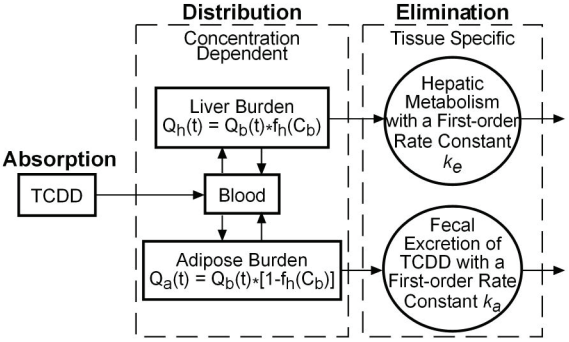


Figure 1: Schematic of the CADM structure (Aylward *et al.*, 2005). Distribution in the body is modeled to occur between hepatic and adipose/lipid tissues, with the fraction of body burden in liver increasing according to a function that parallels the induction of the binding protein CYP1A2 (Eq. 3). Elimination is modeled to occur through hepatic metabolism (modeled as a first-order process with rate constant k_e, which is modeled to decrease with age; see Eq. 7) and through lipid-based partitioning of unmetabolized TCDD across the intestinal lumen into the gut, also modeled as a first-order process. As the body burden increases, the amount of compound in the liver increases non-linearly, resulting in an increased overall elimination rate.

For the first-order model, the serum lipid concentrations (C_a) were modeled numerically in Microsoft Excel® using the following equation and one-month time increments:

$$C_a(t_{i+1}) = C_a(t_i) + \frac{\beta_1 * E(t_{i+1})}{BW} - k * C_a(t_i) \qquad (1)$$

(see Table 1 for definitions of terms). The first-order model was implemented with two values for the elimination rate, k, corresponding to half-lives of 8.7 or 7.1 years. These values span the range of values used previously in dose reconstruction for occupationally exposed cohorts (Flesch-Janys *et al.*, 1998; Ott & Zober, 1996; Steenland *et al.*, 2001) and are consistent with other recent estimates (Michalek *et al.* 2002).

The mathematical structure and biological basis of the CADM is presented in detail elsewhere (Aylward *et al.*, 2005). Briefly, the model is predicated on the assumption that all dioxin in the body (Q_b) is distributed between hepatic (Q_h) and adipose/lipid (Q_a) tissue:

$$Q_b = Q_h + Q_a \qquad (2)$$

Distribution to the liver is postulated to occur due to induction of hepatic binding proteins (CYP1A2), which is dependent on body concentration. The fraction of body burden in the liver (f_h) as a function of body concentration (C_b) parallels the induction curve for CYP1A2 and is given by:

$$f_h\left(C_b(t)\right) = f_{h\min} + \frac{(f_{h\max} - f_{h\min}) * C_b(t)}{K + C_b(t)} \quad (3)$$

so that

$$Q_h = f_h Q_b \quad (4)$$

And

$$Q_a = (1 - f_h) Q_b \quad (5)$$

Elimination occurs through two first-order processes that operate independently on TCDD in hepatic and adipose tissue (as illustrated in Figure 1):

$$\frac{dQ_b(t)}{dt} = -k_e Q_h(t) - k_a Q_a(t) \quad (6)$$

Finally, the hepatic elimination rate, k_e, was observed to have a strong negative relationship with age in modeling of serial serum lipid TCDD measurements taken from subjects exposed to TCDD in Seveso, Italy (Aylward *et al*., 2005) (see Figure 2). Based on the data from male subjects from Seveso, k_e was modeled as follows:

$$k_e = k_{e0} - 0.011 * age \quad (7)$$

with an imposed minimum hepatic elimination rate ($k_{e\ min}$; see Table 1) to avoid negative or zero elimination rates at older ages. This age-dependence is consistent with available data on CYP1A2 activity in humans, with children exhibiting caffeine metabolism rates approximately twice those of healthy adults, which in turn are approximately twice as rapid as those exhibited by elderly adults (Dorne *et al.*, 2001). Two parameter sets for the hepatic elimination rate function, representing the mean and the lower 95th confidence bound on the mean for males from the Seveso data set, were used in the dose reconstruction effort for the NIOSH cohort (see Figure 2 and Table 1 for details).

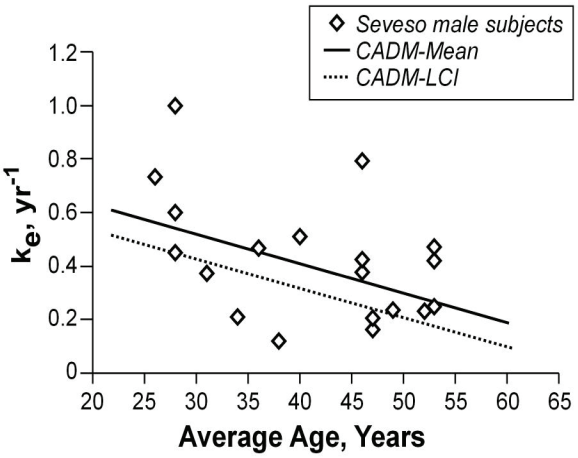


Figure 2: Age dependence of hepatic elimination rate observed in Seveso males, and age-dependent hepatic elimination rate functions used in the modeling in this analysis. A strong negative relationship between the fitted hepatic elimination rate, k_e, and average age during follow-up period was observed in Seveso subjects; slope = –0.011 per year, p <0.005 (Aylward *et al.* 2005). Elimination data sets from the male patients were refit with the hepatic elimination rate as a function of age (see Eq. 7). The resulting mean and lower 95% confidence interval (LCI) on the mean for k_{e0} (0.85 and 0.76 yr^{-1}, respectively) were used to derive the age-dependent hepatic elimination rate parameters for the CADM-Mean and CADM-LCI, respectively.

Numerically, the body concentration as a function of time for the CADM was estimated using the following relationship and one-month time increments:

$$C_b(t_{i+1}) = C_b(t_i) + \frac{\beta_1 * E(t_{i+1})}{BW} - [k_e * f_h(C_b(t_i)) * C_b(t_i)] - [k_a * C_a(t_i) * w_a(t_i)] \qquad (8)$$

Adipose/lipid (C_a) or hepatic (C_h) TCDD concentrations for an individual at a given time can be calculated from the corresponding modeled body concentration, C_b:

$$C_a(t_i) = \frac{C_b(t_i)}{w_a(t_i)} * \left[1 - f_h\left(C_b(t_i)\right)\right] \qquad (9)$$

$$C_h(t_i) = \frac{C_b(t_i)}{w_h(t_i)} * f_h\left(C_b(t_i)\right) \qquad (10)$$

Dose-rate regression

For individuals with measured serum lipid TCDD concentrations above 10 ppt in 1987/1988 (n = 172; mean TCDD = 296.1 ppt; median = 100.8 ppt; range: 10.1 to 3,388.5 ppt), two possible relationships were investigated with

non-linear regression methods using either the untransformed or ln-transformed measured serum lipid values. The regressions took the form of:

$$X_i = C_{ai}(E_i, \beta_1) + \beta_0 + \varepsilon_i, \quad (11a)$$

or

$$ln(X_i) = ln(C_{ai}(E_i, \beta_1) + \beta_0) + \varepsilon_i, \quad (11b)$$

where X_i is the measured serum lipid concentration in 1987 or 1988 for the i^{th} subject, and C_{ai} is the corresponding modeled serum lipid TCDD concentration at that time as modeled either using the first-order elimination model (with a half-life of either 7.1 or 8.7 years) or the CADM (with either the mean or lower-bound hepatic elimination rate parameter set) as described above. β_0 is an intercept parameter, expressed in parts per trillion (ppt), which can be interpreted as arising from exposure sources not accounted for by the JEM-derived exposure scores, either from background sources or from occupational sources not fully captured in the JEM. The residual errors ε_i were assumed to be independently, identically, and normally distributed random variables with zero mean and constant variance σ^2 for either the untransformed or ln-transformed data. Consistent with procedures used by Steenland *et al.* (2001), a "background" concentration of 6.1 ppt was subtracted from the measured values in 1987/88 before performing the regression, and then added back into the measured and modeled values after the occupational exposures were modeled. Central estimates of β_0 and β_1 were identified for each model case by minimizing the sum of the squared residuals between measured and modeled concentrations in either untransformed or ln-transformed space, as described above. In addition, a zero-intercept regression was also implemented, as used by Steenland *et al.* (2001), resulting in estimates for β_1 with β_0 fixed at zero.

Based on the results of the regression with intercept described above, the CADM was reimplemented with an additional term to represent exposures not accounted for by the JEM–derived exposure score:

$$C_b(t_{i+1}) = C_b(t_i) + \frac{\beta_1 * E(t_{i+1})}{BW} + \beta_2 - [k_e * f_h(C_b(t_i)) * C_b(t_i)] - [k_a * C_a(t_i) * w_a(t_i)] \quad (12)$$

(see Table 1 for parameter definitions). The regressions then took the form of:

$$X_i = C_a(E_i, \beta_1, \beta_2) + \varepsilon_i, \quad (13a)$$

or

$$ln(X_i) = ln(C_a(E_i, \beta_1, \beta_2)) + \varepsilon_i, \quad (13b)$$

Results for each model were assessed for goodness-of-fit using F-ratio tests for equality of the original and residual variances. Results from the 7.1-yr half-life and CADM models were compared to those from the 8.7-yr half-life model with F-ratio tests for equality of the residual variances. Confidence intervals for model parameters were determined with asymptotic likelihood ratio tests. Heteroskedasticity was assessed by simple linear regression of the squared residuals against the modeled values.

Dose estimation for full cohort

The estimated dose rates and intercepts associated with each model, parameter set, and regression procedure were combined with the individual time-specific exposure scores to construct TCDD body, adipose/lipid, and hepatic concentration estimates as a function of time and corresponding AUC estimates for each individual in the full cohort (hepatic concentration estimates were available only from the CADM). Cohort members were stratified into exposure septiles using the procedures outlined by Steenland *et al.* (1999, 2001). Briefly, for each model and parameter set, the cohort was sorted in order of increasing serum lipid AUC at death or end of follow-up, then divided into septiles based on approximately equal numbers of total deaths in each septile. Median AUC estimates for each septile were then calculated.

Results

We confirmed our implementation and interpretation of the JEM and work history database information by calculating cumulative exposure scores for each individual in the cohort and comparing the median values by plant and chloracne status for the cohort. Values we obtained agreed with those reported by Steenland *et al.* (1999) (results not shown).

Regression results

The regression model chosen (regression on untransformed or ln-transformed data) has a substantial impact on the estimates of β_0 and β_1. Figures 3a and 3b illustrate the sum of squared residuals as a function of β_1 for the first-order model (8.7-year half-life) for the untransformed and ln-transformed regression models (β_0 fixed at zero for this example). For the regression on untransformed data, β_1 is determined almost entirely by the persons with measured TCDD levels >100 ppt in 1987/88. In contrast, for the ln-transformed regression, β_1 depends on the whole range of measured TCDD values. Regression based on ln-transformed values thus allows for a more balanced weighting of all the serum data, which range over nearly three orders of magnitude. The heteroskedasticity of the residuals is also markedly

reduced or eliminated for regression on the ln-transformed data compared to that remaining after regression on untransformed data.
The central estimates and 90% confidence intervals for β_0 (ppt) and β_1 (ng TCDD/unit of the exposure index) for the different models are summarized in Table 2. The estimates of β_0 and β_1 vary widely from one model and parameter set to another, reflecting the impact on their estimated values of different assumptions regarding elimination kinetics and regression model assumptions. The CADM-Mean and CADM-LCI provide improved model performance over the first-order fixed-half-life models, as demonstrated by increased R^2 values compared to first-order models. The fit of the first order models is identical regardless of the half-life assumed, because the dose rate β_1 and the elimination rate vary inversely and the dose rate is determined by the choice of elimination rate or vice versa.

The regression intercept, β_0 (in ppt), may be interpreted as a contribution to body burden that arises from sources other than those accounted for in the occupational exposure scores. For the first-order models, a background term of 6.1 ppt (based on the measured TCDD concentrations in a control population, as reported by Steenland *et al.* 2001) was already subtracted from the data prior to regression and added back in after modeling (as in Steenland *et al.*, 2001). Thus, β_0 in the first-order model represents an additional contribution to measured values that is not accounted for by either the 6.1-ppt background body burden or the occupational exposure scores based on work history and the job exposure matrix.

Table 2: Central estimates and 90% confidence intervals for the intercept, β_0 (ppt in lipid); dose rate, β_1 (ng TCDD/unit of exposure index); and average background TCDD dose rate, β_2 (ng/kg/month) for evaluated models, parameter sets, and regression assumptions based on regression on data from the subcohort of NIOSH workers with measured serum lipid TCDD concentrations in 1987/88 >10 ppt (n=172)

	Regression on untransformed data with β0 =zeroa		Regression on ln-transformed data with β0 =zero		Regression on ln-transformed data with non-zero β0			Regression on ln-transformed data with background dose β2		
Model, Parameter Set	β1 (90% CI[b])	R^2 [c,d]	β1 (90% CI)	R^2	β0 (90% CI)	β1 (90% CI)	R^2	β1 (90% CI)	β2 (90% CI)	R^2
First-order, 8.7-yr HL	3.4 (2.9, 4.0)	0.19	7.3 (6.1, 8.7)	0.27 *	21 (13, 30)	3.8 (2.9, 4.8)	0.27 *	ND	ND	ND
First-order, 7.1-yr HL	5.3 (4.5, 6.1)	0.19	11.7 (9.8, 14.0)	0.27 *	21 (13, 31)	6.0 (4.6, 7.8)	0.27 *	ND	ND	ND
CADM, LCI	35 (25, 42)	0.24 *	48 (36, 65)	0.40 **†	12 (5, 22)	31 (20, 47)	0.44 **††	38 (27,53)	0.012 (0.004, 0.03)	0.43 **
CADM, Mean	140 (112, 170)	0.23 *	180 (130, 250)	0.41 **†	8.3 (1, 19)	134 (80, 210)	0.41 **†	156 (110, 220)	0.01 (0, 0.033)	0.41 **

[a] As used by Steenland *et al.* (2001).
[b] Asymptotic likelihood ratio test-based confidence intervals.
[c] Goodness of model fit: F-ratio test of equality of original and residual variance estimates: *p<0.05; **p<0.001.
[d] Performance compared to 8.7-yr first-order model: F-ratio test of equality of residual variance estimates †p<0.1; ††p<0.05
ND = not done.

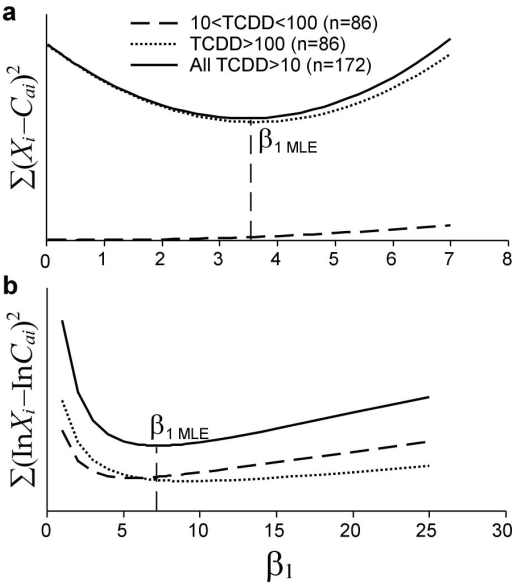


Figure 3: Sum of squared residuals (measured – modeled), for the 8.7-yr half-life first-order model comparing regression on a) untransformed values or b) ln-transformed values. In 3a, data from persons with measured TCDD concentrations below 100 ppt in 1987/88 contribute little to the determination of β_1, while those in the upper half of measured TCDD concentrations dominate. In 3b, data throughout the range of measured values contribute about equally. Modeling by Steenland *et al.* (2001) utilized regression on the untransformed data.

For the CADM models, the assumed background serum lipid level could not be subtracted and re-added due to the non-linearity of the model, so no initial term for background exposure was incorporated. For the CADM models, β_0 could reasonably represent background exposure as well as occupational exposures not captured in the occupational exposure scores, and the magnitude of the central estimates for β_0 (8.3 and 12 ppt for the CADM-Mean and CADM-LCI) are consistent with this interpretation. To explicitly include background doses (due to dioxin in food and the environment), the intercept term was omitted, and an independent constant dose term, β_2 (representing the background dose independent of occupational exposure; see equation 12 above) was added. The regression was repeated to derive central estimates of β_1 (in ng TCDD/unit of exposure index) and β_2 (units of ng/kg/month; see Table 2).

The central estimates for β_1, the dose associated with a unit of exposure index, varied by a factor of about 50 among the different model implementations, reflecting the impact of different assumptions regarding elimination behavior and different assumptions in the regression procedure. By extension, this reflects up to a 50-fold variation in the estimates of cumulative occupational dose for the members of this cohort. The central estimate for β_1 for the first-order model assuming an 8.7-yr half-life derived using regression on untransformed values with no intercept (method used in Steenland *et al*., 2001) is 3.44 ng/unit exposure score. For a 70-kg individual with 25% body fat (the values used in the first-order modeling), this would

correspond to an increment in serum lipid levels of approximately 0.197 ppt per unit of exposure score.

The central estimates for β_2, the independent monthly background dose, were 0.010 and 0.012 ng/kg/month for the CADM-Mean and CADM-LCI parameter sets, respectively. These values correspond to average daily intakes of 0.33 and 0.40 pg TCDD/kg/day. These values are consistent with estimates of average background TCDD intake rates due to its presence in the food chain during the middle decades of the 20th century (Aylward & Hays, 2002; Lorber, 2002; Pinsky & Lorber, 1998).

Figure 4 presents the ratio of measured to modeled values as a function of the modeled values for the serum-sampled subcohort. The results from the original 8.7-yr half-life model (central estimate for β_1 derived from regression on untransformed values with a zero regression intercept β_0, as used in Steenland *et al*., 2001) are compared to those of the CADM-Mean model (estimates for β_1 and β_2 based on regression on ln-transformed values). Use of the CADM reduces the systematic bias and variance of the residuals, but as discussed above, substantial discrepancies between measured and modeled values remain. The models resulted in similar Spearman rank correlation coefficients between measured serum lipid concentrations and modeled values (0.67 and 0.64 for the 8.7 yr first-order half-life model and the CADM-Mean model, respectively).

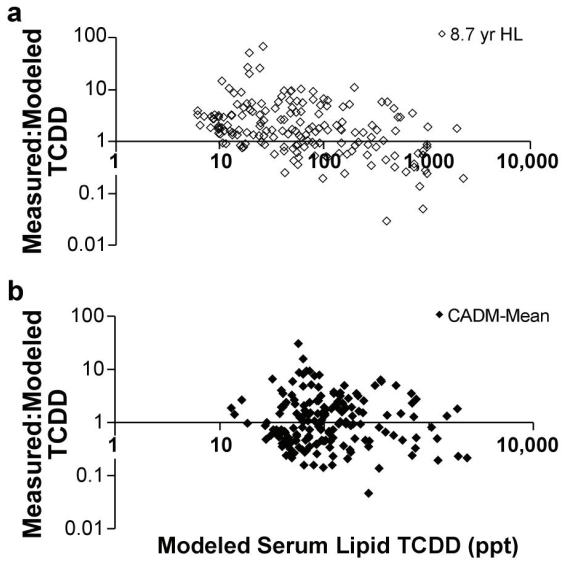


Figure 4: Ratio of measured to modeled serum lipid TCDD concentrations vs. modeled concentrations in the serum sampling subcohort (n=172) derived based on either a) the first-order model with 8.7-year half-life and an assumption of constant 6.1-ppt background, or b) based on the CADM-Mean model with background TCDD dose rate of 0.33 pg/kg/day. Use of the CADM reduces the bias in the model results and improves the model fit to the serum sampling data over that from the use of the first-order model (see Table 2).

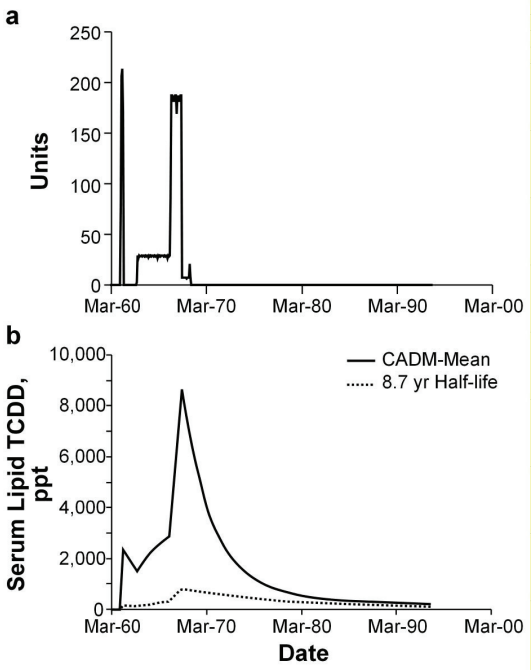


Figure 5: Illustrations of the estimated exposure over time for one individual in the NIOSH cohort. Figure 5a) Monthly cumulative exposure score; 5b) modeled serum lipid TCDD concentrations from the first-order model (8.7-yr half-life, β_1 from Table 2 based on regression on untransformed data with zero intercept, as used by Steenland *et al.* 2001) and from the CADM (mean parameter set, parameter set as reported in Table 2 with regression on ln-transformed data and background dose rate) on a month-by-month basis.

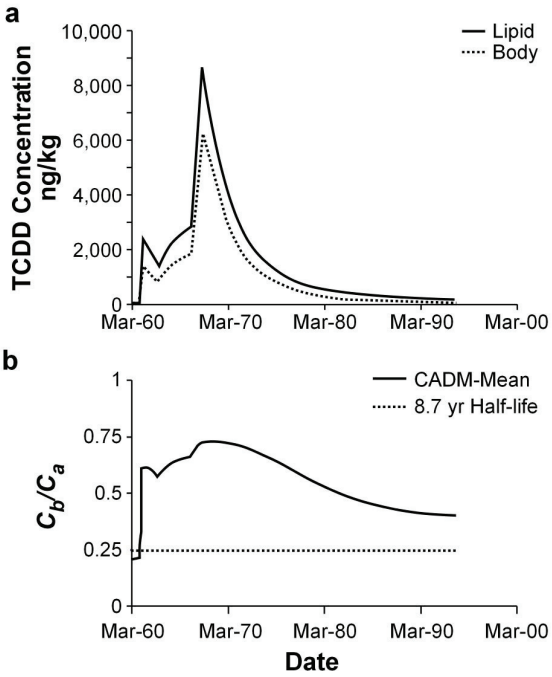


Figure 6: a) Relationship between body concentration and serum lipid concentrations from the CADM with mean parameter set for the same individual as illustrated in Figure 5. b) Ratio of body concentration C_b to adipose/lipid concentration C_a for the CADM-Mean and 8.7-yr half-life first-order model. The conventional assumption that all TCDD resides in lipid and that body burden can be estimated by the product of the lipid concentration and lipid body fraction ignores TCDD sequestered in liver and results in an underestimate of true body concentration, which attains a significant fraction of the lipid concentration at high body burdens due to the amount of TCDD sequestered in the liver.

The impact of the kinetic model choices can be seen when looking at the modeled lifetime serum lipid TCDD concentration profiles generated from different model cases. Figure 5a presents the exposure index-vs.-time profile for one individual

from the NIOSH cohort, and Figure 5b shows the resulting modeled serum lipid concentrations from the first-order model (8.7-yr half-life) and from the CADM-Mean.

The modeled body and adipose concentrations using the CADM-Mean for this same individual are presented in Figure 6a. Body concentration estimates based on conventional first-order elimination modeling and measured serum lipid or adipose concentrations of TCDD assume that all compound resides in fat and that fat tissue accounts for about 25% of body mass (Crump *et al.*, 2003; USEPA, 2000). Under this assumption, the body concentration in ng/kg is calculated to be approximately 25% of the observed or modeled lipid concentration in ppt. However, the CADM accounts for hepatic sequestration of TCDD due to induction of hepatic binding proteins (CYP1A2) at elevated body concentrations (a phenomenon that is universally observed in laboratory animal studies and in limited paired human liver and adipose sample data from persons highly exposed to a related polychlorinated dibenzofuran compound [Carrier *et al.*, 1995b; DeVito *et al.*, 1998; Santostefano *et al.*, 1996]). This sequestration results in modeled body concentrations that are much higher than would be predicted based on the measured lipid concentrations and the assumption of distribution solely in lipid tissue. Figure 6b illustrates the predicted ratio of body concentration to adipose concentration (C_b/C_a) over time for this individual for both the first-order model and the CADM-Mean.

Full-cohort dose estimates

The median serum lipid AUC values by cohort septile for the first-order and CADM models (each with two parameter sets for elimination rate) under a range of regression-model assumptions are presented in Table 3 and compared with the median serum lipid AUC values derived by Steenland *et al.* (2001). The results of the AUC dose analysis by Steenland *et al.* (2001) were not fully reported in that paper. A subsequent cancer risk meta-analysis by Crump *et al.* (2003) reported median AUC values by septile from a personal communication from Steenland. However, these values were based on an analysis that imposed a 15-year "lag" on the dose estimates. That is, the estimates reported in Crump *et al.* (2003) omitted the most recent 15 years of AUC prior to the date of death or end of follow-up. Based on descriptions in Steenland *et al.* (2001), those estimates also likely omitted persons whose occupational exposure was "lagged out" (occurred within 15 years of death or end of follow-up) and likely also omitted 94 individuals whose age at death or end of follow-up occurred at an age younger than the youngest cancer decedent, according to the authors. Table 3 presents the median AUC estimates based on the Steenland *et al.* (2001) analysis (as reported by Crump *et al.*, 2003) along with estimates from the current modeling of median serum lipid TCDD AUC based on the same elimination model (first-order, 8.7-year half-life) with no exposure lag for all 3,538 individuals in the cohort.

These estimates can then be compared with the estimates from the first-order model with a half-life of 7.1 years and with the results from the CADM with both parameter sets.

In this analysis, the modeled serum lipid concentrations at the time of last exposure and at end of follow-up resulting from the central estimate parameter set for the first-order model with an 8.7-yr half-life were approximately 10% lower than the corresponding modeling results reported in Steenland *et al.* (2001). Similarly, attempts to replicate the median AUC values used in Crump *et al.* (2003, Table 1) for the NIOSH cohort under the assumption of a 15-year lag in exposure resulted in AUC estimates that were approximately 10% to 12% lower than those used by Crump *et al.* (2003) (see Table 3, columns 1 and 2). We were unable to resolve this discrepancy in results but it may be due in part to the exclusions from the full cohort made by Steenland *et al.* (2001) for the Cox regression analysis (and summarized above).

The unlagged median serum lipid AUC estimates for the lowest septile varied by approximately a factor of 2 among all models. However, estimates for the remaining septiles varied by approximately a factor of 1.5 to 2 among the first-order models, and by a factor of about 5 among all models for the highest-exposed septile. This degree of variation in the modeled dose estimates demonstrates that such estimates are subject to substantial uncertainty.

We assessed the impact of omitting the age-dependency from the concentration-dependent model. The estimates of AUC generally increased in models not incorporating the age-dependent behavior compared to those with the age-dependence (results not shown).

Table 3: Median AUCs (ppt-yrs) by septile for selected model cases from Table 2 with regression on untransformed or ln-transformed data, and regression intercept β_0 or background dose β_2

Model Case	8.7-yr HL[a]	8.7-yr HL[b]	8.7-yr HL	7.1-yr HL	7.1-yr HL	CADM-LCI	CADM-Mean
Lagged?	Yes	Yes	No	No	No	No	No
Ln-transformed?	No	No	No	No	Yes	Yes	Yes
Intercept or background?	No	No	No	No	Yes, intercept β_0	Yes, background β_2	Yes, background β_2
Septile[c]							
1	260	210	290	300	690	545	640
2	400	390	450	470	1,000	1,400	2,600
3	850	700	850	1,000	1,700	3,700	6,700
4	1,900	1,700	2,200	2,900	3,800	8,000	16,000
5	4,400	4,100	5,800	7,500	9,200	15,000	37,000
6	12,000	11,000	19,000	26,000	30,000	37,000	100,000
7	60,000	52,000	85,000	110,000	130,000	160,000	480,000

[a] Values as reported in Crump *et al.* (2003)
[b] Values as calculated in the modeling in this analysis.
[c] Septiles defined as described by Steenland *et al.* (2001): all 3,538 individuals in the cohort were sorted in rank order of increasing AUC, then divided into septiles that contained roughly equal numbers of total decedents. Because the different models result in different rank ordering of individuals, septiles do not contain the same individuals across models.

Discussion

The exposure estimates derived for this cohort vary widely, even among the first-order fixed half-life model results. The modeled exposure estimates are affected not only by choice of elimination model and parameter set, but also by choices in the regression procedure (regression on untransformed vs. ln-transformed data; incorporation of a regression intercept or background dose term). The marked sensitivity to such choices of the dose estimates for the occupationally exposed cohorts has not been noted in previous publications regarding this cohort or others (Flesch-Janys *et al.*, 1998; Ott & Zober, 1996; Steenland *et al.*, 2001).

Our exposure reconstruction effort resulted in only moderate agreement between measured and modeled serum TCDD levels regardless of the model assumptions used, with modeled values often discrepant by an order of magnitude from corresponding measured values. However, the use of the CADM in conjunction with regression on ln-transformed values resulted in reduced residual variance and heteroskedasticity and better R^2 fits to the measured serum lipid data compared to those from the first-order models (Table 2). The remaining discrepancies between measured and modeled values likely result from numerous uncertainties and limitations inherent in the exposure reconstruction process.

For example, the JEM constructed by NIOSH researchers necessarily relied on limited sampling data over time, and on subjective judgments on contact time, contact factor, and relative exposure potential for jobs at 12 different manufacturing facilities over a period of decades (including numerous process changes) (Piacitelli *et al.*, 2000). The parameter for contact factor assigned by Piacitelli *et al.* (2000) varied among jobs by 150-fold (from 0.01 to 1.5), and the total exposure score assigned to individual jobs varied by a factor of more than 1,000,000. Other sources of uncertainty include the kinetic models (none of the models can account for inter-individual variation in elimination efficiency, which can have profound effects over a lifetime), as well as a lack of data on body-weight changes and other individual characteristics that can influence substantially the kinetics of TCDD elimination over time.

Furthermore, the dose-rate regressions presented here and in Steenland *et al.* (2001) for this cohort are based solely on data for a small subcohort of individuals with measured serum lipid TCDD concentrations sampled in 1987/88. These individuals were drawn from a single plant out of the 12 originally included in the NIOSH cohort (only eight plants were included in the exposure reconstruction effort by NIOSH). Thus, the results of the dose-rate regression for these individuals may or may not be representative of the exposures of cohort members from other plants. This is an additional source of uncertainty beyond those associated with the elimination and regression model choices. The degree of uncertainty to which this factor gives rise in the full-cohort exposure estimates cannot be quantified.

Because the septile divisions were based on rank ordering of individuals, and the rank ordering changed among the different models and regression assumptions, the septile categories do not include the same individuals across models. Thus, an accurate evaluation of cancer dose-response in this population based on these revised exposure estimates cannot be made using the previous evaluations of mortality patterns (rate ratio or SMR) of Steenland *et al.* (1999, 2001). In future work we will address alternative assessments of the possible relation between exposure and cancer response based on the range of exposure reconstructions (serum lipid AUC and alternative dose metrics) presented here.

The AUC estimates derived using a first-order model of elimination with an 8.7-year elimination half-life are the lowest of all of the estimates derived using plausible elimination models and regression procedures, often by several-fold. Use of the CADM results in increased AUC estimates over those from the first-order model with either parameter set for the higher exposure septiles. Thus, previous assessments of occupational exposures based on back-calculations over decades that did not incorporate the concentration dependence of the elimination rate likely underestimated occupational exposures by a significant amount.

Serum lipid TCDD AUC estimates have been reported here for ease of comparison with previous dose reconstruction efforts for this and other similar cohorts. Risk assessment evaluations for TCDD have typically relied on a conversion of such measures to average serum lipid TCDD concentrations, and further, to average body burdens (Crump, 2003; USEPA, 2000). However, as discussed above and illustrated in Figure 6, the hepatic sequestration of TCDD, if occurring to the degree observed in animal studies and as suggested by the concentration dependence of elimination behavior discussed previously (Aylward *et al.*, 2005), significantly affects such extrapolations. When hepatic sequestration of TCDD is taken into account, the estimated average body burden of TCDD associated with a given serum lipid AUC (and therefore the intakes required to achieve this body burden) is much higher than would be predicted by the conventional assumption of simple lipid partitioning. This increases the likelihood that the potential cancer potency of TCDD in humans has been overestimated when such estimates have been based on exposure reconstructions using first-order elimination models and simple assumptions about body distribution of TCDD.

The concentration dependence of elimination demonstrated in laboratory animals and Seveso subjects and modeled with the CADM has little impact on elimination at background exposure levels and low body concentrations. In this low-dose regime, the assumption of constant first-order elimination behavior at the rates previously estimated (half-lives in the range of 7 to 9 years) is reasonable. However, incorporating concentration-dependent elimination is critical to the estimation of body burdens through back-calculation over decades in occupational cohorts with serum lipid levels substantially above background. Any estimate of cancer dose-response for TCDD in humans based on data from the occupationally exposed cohorts must account for concentration-dependent kinetics. Furthermore, such cancer potency estimates should explicitly acknowledge the large degree of inherent uncertainty due to the limitations in the occupational exposure scores, inter-individual variability in elimination efficiency, variation in background exposures over time, individual variations in body weight and fat over time, and uncertainties in dose-rate estimates linked to regression-model assumptions.

The most recent published meta-analysis of the cancer dose-response data for human populations that includes the NIOSH data (Crump *et al.*, 2003) relies on dose estimates for this cohort that are less than half of those derived using a first-order model with a 7.1-year half-life, and are more than five times lower than the dose estimates derived from the concentration-dependent model used here for the more highly exposed individuals. This degree of variability and uncertainty in the dose estimates can have a profound impact

on resulting dose-response estimates and such uncertainty should be acknowledged explicitly and quantified to the degree possible.

Acknowledgments
Funding for this project was provided by the Research Foundation for Health and Environmental Effects.

References

Abraham, K., Krowke, R., & Neubert, D. (1988). Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. 1. Dose-dependent tissue distribution and induction of hepatic ethoxyresorufin O-deethylase in rats following a single injection. *Arch Toxicol, 62*(5), 359-368.

Aylward, L. L., Brunet, R. C., Carrier, G., Hays, S. M., Cushing, C. A., Needham, L. L., Patterson, D. G., Gerthoux, P. M., Brambilla, G., & Mocarelli, P. (2005). Concentration-dependent TCDD elimination kinetics in humans: Toxicokinetic modeling for moderately to highly exposed adults from Seveso, Italy, and Vienna, Austria, and impact on dose estimates for the NIOSH cohort. *J Expo Anal Environ Epidemiol*. *15*, 51-65.

Aylward, L. L., & Hays, S. M. (2002). Temporal trends in human TCDD body burden: Decreases over three decades and implications for exposure levels. *J Expo Anal Environ Epidemiol, 12*, 319-328.

Carrier, G., Brunet, R. C., & Brodeur, J. (1995a). Modeling of the toxicokinetics of polychlorinated dibenzo-p-dioxins and dibenzofurans in mammalians, including humans. I. Nonlinear distribution of PCDD/PCDF body burden between liver and adipose tissues. *Toxicol Appl Pharmacol, 131*(2), 253-266.

Carrier, G., Brunet, R. C., & Brodeur, J. (1995b). Modeling of the toxicokinetics of polychlorinated dibenzo-p-dioxins and dibenzofurans in mammalians, including humans. II. Kinetics of absorption and disposition of PCDDs/PCDFs. *Toxicol Appl Pharmacol, 131*(2), 267-276.

Crump, K. S., Canady, R., & Kogevinas, M. (2003). Meta-analysis of dioxin cancer dose response for three occupational cohorts. *Environ Health Perspect, 111*(5), 681-687.

DeVito, M. J., Ross, D. G., Dupuy, A. E., Jr., Ferrario, J., McDaniel, D., & Birnbaum, L. S. (1998). Dose-response relationships for disposition and hepatic sequestration of polyhalogenated dibenzo-p-dioxins, dibenzofurans, and biphenyls following subchronic treatment in mice. *Toxicol Sci, 46*(2), 223-234.

Diliberto, J. J., DeVito, M. J., Ross, D. G., & Birnbaum, L. S. (2001). Subchronic Exposure of [3H]- 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in female B6C3F1 mice: relationship of steady-state levels to disposition and metabolism. *Toxicol Sci, 61*(2), 241-255.

Dorne, J. L. C. M., Walton, K., & Renwick, A. G. (2001). Uncertainty factors for chemical risk assessment: human variability in the pharmacokinetics of CYP1A2 probe substrates. *Food Chem Toxicol, 39*, 681-696.

Fingerhut, M. A., Halperin, W. E., Marlow, D. A., Piacitelli, L. A., Honchar, P. A., Sweeney, M. H., Greife, A. L., Dill, P. A., Steenland, K., & Suruda, A. J. (1991). Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p- dioxin. *N Engl J Med, 324*(4), 212-218.

Flesch-Janys, D., Steindorf, K., Gurn, P., & Becher, H. (1998). Estimation of the cumulated exposure to polychlorinated dibenzo-p-dioxins/furans and standardized mortality ratio analysis of cancer mortality by dose in an occupationally exposed cohort. *Environ Health Perspect, 106 Suppl 2*, 655-662.

Geusau, A., Schmaldienst, S., Derfler, K., Papke, O., & Abraham, K. (2002). Severe 2,3,7,8-tetrachlorodibenzo- p-dioxin (TCDD) intoxication: kinetics and trials to enhance elimination in two patients. *Arch Toxicol, 76*(5-6), 316-325.

Lorber, M. (2002). A pharmacokinetic model for estimating exposure of Americans to dioxin-like compounds in the past, present, and future. *Sci. Tot. Environ., 288*, 81-95.

Michalek, J. E., Pirkle, J. L., Needham, L. L., Patterson, D. G., Caudill, S. P., Tripathi, R. C., & Mocarelli, P. (2002). Pharmacokinetics of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Seveso adults and veterans of operation Ranch Hand. *J Expo Anal Environ Epidemiol, 12*(1), 44-53.

Ott, M. G., & Zober, A. (1996). Cause specific mortality and cancer incidence among employees exposed to 2,3,7,8-TCDD after a 1953 reactor accident. *Occup Environ Med, 53*(9), 606-612.

Piacitelli, L., Marlow, D., Fingerhut, M., Steenland, K., & Sweeney, M. H. (2000). A retrospective job exposure matrix for estimating exposure to 2,3,7, 8-tetrachlorodibenzo-p-dioxin. *Am J Ind Med, 38*(1), 28-39.

Pinsky, P. F., & Lorber, M. N. (1998). A model to evaluate past exposure to 2,3,7,8-TCDD. *J Expo Anal Environ Epidemiol, 8*(2), 187-206.

Santostefano, M. J., Johnson, K. L., Whisnant, N. A., Richardson, V. M., DeVito, M. J., Diliberto, J. J., & Birnbaum, L. S. (1996). Subcellular localization of TCDD differs between the liver, lungs, and kidneys after acute and subchronic exposure: species/dose comparisons and possible mechanism. *Fundam Appl Toxicol, 34*(2), 265-275.

Steenland, K., Deddens, J., & Piacitelli, L. (2001). Risk assessment for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) based on an epidemiologic study. *Am J Epidemiol, 154*(5), 451-458.

Steenland, K., Piacitelli, L., Deddens, J., Fingerhut, M., & Chang, L. I. (1999). Cancer, heart disease, and diabetes in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J Natl Cancer Inst, 91*(9), 779-786.

United States Environmental Protection Agency (USEPA). (2000). *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin and Related Compounds. Draft Final* (No. EPA/600/P-

00/001Be). Washington, D.C.: National Center for Environmental Assessment, U.S. Environmental Protection Agency.

Chapter 8: TCDD Exposure-Response Analysis and Risk Assessment

Cheng, H., L. L. Aylward, C. Beall, T. B. Starr, R. C. Brunet, G. Carrier, E. Delzell. 2006. Risk Analysis 26(4): 1059-1071.

The definitive version is available at www.blackwell-synergy.com.

Abstract

We examined the relation between cancer mortality and time-dependent cumulative exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) estimated from a concentration- and age-dependent kinetic model of elimination, and we estimated incremental cancer risks at age 75. Data from the National Institute for Occupational Safety and Health study of 3,538 workers with occupational exposure to TCDD were analyzed using standardized mortality ratios and Cox regression procedures. Analyses adjusted for potential confounding by age, year of birth, and race and considered exposure lag periods of 0, 10, or 15 years. Other potential confounders including smoking and other occupational exposures were evaluated indirectly. To explore the influence of extreme values of cumulative TCDD ppt-years, we restricted the analysis to observations with exposure below the 95th percentile or used logarithmic (ln) transformed exposure values. We applied penalized smoothing splines to examine variation in the exposure-response relation across the exposure range. TCDD was not statistically significantly associated with cancer mortality using the full data set, regardless of the lag period. When we restricted the analysis to observations with exposure below the 95th percentile, TCDD was associated positively with cancer mortality, particularly when a 15-year lag was applied (untransformed exposure data: regression coefficient (β) = 3.3×10^{-6}, standard error (s.e.) = 1.4×10^{-6}, $p < 0.05$; ln-transformed exposure data: $\beta = 8.1 \times 10^{-2}$, s.e. = 2.9×10^{-2}, $p < .05$). The estimated incremental lifetime risk of mortality at age 75 from all cancers was about 6 to more than 10 times lower than previous estimates derived from this cohort using exposure models that did not consider the age and concentration dependence of TCDD elimination.

Introduction

The International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP) have classified 2,3,7,8-tetrachlorodibenzo-pdioxin (TCDD) as a human carcinogen (IARC, 1997; NTP, 2002). However, both of these decisions were based on controversial mechanistic arguments (Cole et al., 2003), "sufficient" evidence of carcinogenicity in animals, and only "limited" evidence for carcinogenicity in humans (IARC, 1997).

The IARC classification relied partly on epidemiologic studies that had reported small excesses of mortality from all cancers combined among workers occupationally exposed to TCDD. For the subgroup of chemical manufacturing workers most heavily exposed and with the longest time since first exposure to TCDD, IARC calculated an all-cancers summary standardized mortality ratio (SMR) of 1.4 (95% confidence interval (CI) 1.2–1.6) (IARC, 1997). The IARC

review and two subsequent articles reported positive exposure-response relations for all cancers in three of the manufacturing studies (Ott&Zober, 1996; Flesch-Janys et al., 1998; Steenland et al., 1999), although in one study (Ott & Zober, 1996) the positive trend was limited to smokers.

Men living in an area near Seveso, Italy, contaminated by TCDD during an accidental environmental release, and followed up for 20 years after the accident, had cancer mortality rate ratios (RRs) of 1.1 (95%CI 1.0–1.3) for the total time period from first exposure to the end of followup and of 1.3 (95% CI 1.0–1.7) during the period 15–19 years after the accident (Bertazzi et al., 2001). Women living in similarly contaminated areas had cancer mortality RRs of 0.9 (95% CI 0.7–1.1) for the total time period from first exposure to the end of followup of 0.8 (95% CI 0.6–1.2) during the period 15–19 years after the accident (Bertazzi et al., 2001).

Akhtar et al. (2004) investigated cancer incidence among U.S. Air Force veterans who handled Agent Orange and were potentially exposed to TCDD in Southeast Asia (the "Ranch Hand veterans"). Overall, the Ranch Hand veterans had a standardized incidence ratio (SIR) for all cancers of 1.08 (95% CI 0.91–1.26) and a SMR of 0.68 (95% CI 0.50– 0.91). This nonsignificant increase in cancer incidence was due mainly to excesses of prostate cancer (SIR 1.46; 95% CI 1.04–2.00) and melanoma of the skin (SIR 2.33; 95% CI 1.40–3.65) among white Ranch Hand veterans. The interpretation of these results was unclear. There was no persuasive evidence of a TCDD exposure-response relationship for all cancers, melanoma, or prostate cancer, and Akhtar et al. indicated that repeated medical surveillance may have been responsible for the apparent increases. Ketchum and Michalek (2005) recently reported aRR of 0.6 (95% CI 0.2–1.6) for cancer mortality among RanchHandveterans compared to veterans who were in units in Southeast Asia that did not use Agent Orange.

Several investigators have explored whether or not an exposure-response relation exists between TCDD and cancer and have developed quantitative risk assessments (Becher et al., 1998; Crump et al., 2003; Steenland&Deddens, 2003). Questions remain about the strength of the evidence for the carcinogenicity of TCDD in humans, about the most plausible dose metric(s) to use in evaluating the potential dose-response relation between TCDD exposure and human cancer mortality (Starr, 2003; Aylward et al., 2005a, 2005b; Aylward & Hays, 2002), and about possible confounding by other exposures (Cole et al., 2003; Paustenbach, 2002). The current study used data from the National Institute for Occupational Safety and Health (NIOSH) retrospective cohort mortality study of workers with occupational exposure to TCDD (Steenland et al., 1999). The main purposes were (1) to explore potential relations between exposure to TCDD and cancer mortality using new estimates of dose derived from a model that estimates the lipid-based concentration of

TCDD as a function of exposure intensity and age (Aylward et al., 2005b); and (2) to conduct a risk assessment with the new dose estimates, using an approach similar to that applied by NIOSH and the U.S. Environmental Protection Agency (USEPA). We also assessed the internal consistency of the relation between TCDD and cancer mortality by plant and examined indirectly the possibility of confounding by other exposures.

Methods

Study Population

We obtained demographic, vital status, cause of death, and work history data from the NIOSH study on 3,538 men who were employed in the production of chemicals potentially contaminated with TCDD at eight plants in the United States and who had sufficient data to estimate their exposure to TCDD (Steenland et al., 1999). Work histories consisted of the start date, end date, department code, and operation code for each job held by a subject at each plant. Vital status followup for the cohort extended from 1942 through 1993.

Exposure

We linked the work histories to a job-exposure matrix (JEM) provided by NIOSH to obtain aTCDD exposure score (Piacitelli et al., 2000). To each job in a subject's work history, the JEM assigned a quantitative TCDD exposure score that was specific to plant, department, operation, and time period. Each such score was the product of the estimated concentration of TCDD (μg/g) in process materials used in the job, the estimated proportion of the day in which the worker was engaged in the job, and a semiquantitative factor derived from an assessment of the job-specific exposure acquired through dermal contact and inhalation of airborne particulates. Linkage of a subject's work history with the JEM allowed the computation of time-dependent cumulative exposure scores.

We converted the time-dependent cumulative exposure scores into estimated cumulative serum lipid concentrations (in parts per trillion (ppt)-years, also termed area-under-the-concentration-curve (AUC)), that depend on the history of TCDD exposure and the kinetics of TCDD elimination from the body (Aylward et al., 2005a, 2005b).We calculated two different sets of estimates of cumulative serum lipid concentrations. One set used a first-order elimination model with a constant 8.7-year half-life (Michalek et al., 1996). This approach was used previously by the NIOSHinvestigators as well as others, employing constant half-lives ranging from 7.1 years to 8.7 years (Crump et al., 2003; Flesch-Janys et al., 1998; Steenland & Deddens, 2003).The second set of

exposure estimates, described in detail elsewhere (Aylward et al., 2005b), used a concentration- and age-dependent model (CADM) with two first-order elimination processes operating independently on TCDD in hepatic and adipose tissues, and in which the distribution of TCDDbetween liver and adipose tissue is a nonlinear function of body concentration. The CADM modeling provided a calibrating dose per unit of exposure score of 156 ng and a background exposure rate estimate of 0.01 ng/kg-month (Aylward et al., 2005b). Unless specifically indicated otherwise, TCDD exposure variables mentioned in the "Results" section of this article are CADM based.

SMR Analysis

We used SMRs to compare the mortality experience of the TCDD-exposed workers to that of the general population of the United States (Marsh et al., 1998). These analyses sought to replicate the results of Steenland et al. (1999) for cancer mortality by septile of cumulative TCDD exposure score. In addition, to examine the internal consistency of the relation between
TCDD and cancer, we conducted SMR analyses for each plant, as well as for all plants combined.

Cox Regression Analysis

We conducted exposure-response analyses using extended Cox models (Eisen et al., 2004; Therneau & Grambsch, 2000), with age as the time variable in all models. One set of Cox regressions used penalized smoothing spline functions of CADM-based TCDD ppt-years. This approach avoids parametric assumptions about the form of the exposure-response curve and, in particular, accommodates different exposureresponse slopes at "local" levels throughout the full exposure range, with the segments of this range demarcated by knots. Our objectives in using penalized smoothing splines were to determine how the exposure-response relation between TCDD exposure and cancer mortality varied across the exposure range and to provide an informal, visual assessment of whether or not a simple parametric form of the exposure-response relation could provide a reasonable fit to the data.

The hazard from a penalized Cox model for the ith subject in the jth stratum (case and risk set) can be expressed as

$$\lambda_{j,i}(t) = \lambda_{j,0}(t) \exp(Z_i(t)\beta_z + W_i\beta_w + f(X_i(t), \beta_f)) \quad (1)$$

where f (Xi(t), β f) is a smoothing spline of timedependent TCDD ppt-years (Xi(t)), where Zi(t) represents the other time-dependent covariates (potential confounders), where Wi represents fixed covariates, and where βs are vectors of coefficients corresponding to each group of covariates. The spline is

determined by the number and position of knots and then smoothed by a mathematical procedure described by Therneau and Grambsch (2000). We used S-Plus 6 (Insightful Corporation, 2001) to fit penalized Cox models, with the default number of knots (a whole number rounded from (2.5 × df)) equally placed across the range of X. The procedure uses Akaike's Information Criterion (AIC) to select the optimal number of df for smoothing. AIC is a measure of goodness of model fit based on the deviance with a penalty for overfitting.
We also analyzed the exposure-response relation using a classical (unsmoothed) Cox proportional hazards model:

$$\lambda j,i\ (t) = \lambda j,0(t)\ \exp(Zi\ (t)\beta z + Wi\beta w + Xi\ (t)\beta) \qquad (2)$$

where Xi(t) is the untransformed or ln-transformed TCDD ppt-years and β is the ln(hazard ratio) of cancer mortality for an increment of 1 ppt-year TCDD in linear or ln scale.

The Cox regression analyses included 3,455 workers and excluded 83 workers who died before the youngest age at death of any cancer decedent (N =80) or whose entire follow-up period occurred between cancer decedent ages (N = 3). The risk set for each cancer decedent included all other subjects who lived to be at least the same age as that of the index cancer case at the time of his death and who were under followup by that age.We conducted analyses using unlagged exposure or exposure lagged by 10 or 15 years. The latter analyses ignored exposure accumulated during the 10- or 15-year period prior to each index age, based on the presumption that the most recent periods of exposure were not causally related to the probability of cancer mortality.

We conducted two sets of sensitivity analyses. First, to consider the possibility that exposure misclassification was particularly severe at the extremes of the exposure range (Stayner et al., 2003), we removed all observations with exposures within the lower and upper 1, 2.5, or 5th percentiles of the distribution of TCDDppt-years, or removed observations within just the upper 1, 2.5, or 5th percentile of TCDD ppt-years. Both approaches yielded similar results, and we report data only from the analyses after removal of "extreme" high exposure values. Second, we estimated the effect of exposure on cancer mortality using ln-transformed TCDD ppt-years. Both of these sensitivity analyses emphasized the exposure-response relation in the relatively low portion of the exposure range.

For graphical depictions of exposure-response data, ln(hazard ratio) was calibrated and set equal to zero at the mean of the exposure variable (Therneau & Grambsch, 2000; Insightful Corporation, 2001). For example, when TCDD exposure was included in a model as a linear term, ln(hazard ratio) was set to be zero at the mean of TCDD ppt-years, so that the

estimated ln(hazard ratio) is the hazard relative to that associated with the mean TCDD ppmyears (i.e., ln(hazard ratio) = $\beta(X - \overline{X})$). This calibration does not change the shape of the fitted curve. Graphical presentations also display two flattened histograms, or data rugs, along the X-axis for TCDD ppt-years. These depict the density of observations in various regions of the exposure range, the lower histogram representing observations and the upper histogram representing cancer deaths.

We evaluated the proportionality assumption of the Cox proportional hazards models by visual inspection of plots of cumulative sums of Schoenfeld residuals and with a chi-square test for the whole model and individual variables, as described by Therneau and Grambsch (2000). In addition, cross-product interaction terms between TCDD ppt-years and age (untransformed and ln-transformed age) were included in the models to determine if effect estimates changed over age.

Confounding and Other Issues

In all the regression analyses, we adjusted for the potential confounding effects of race with a dichotomous indicator variable (white = 1 and nonwhite = 0) and year of birth with three indicator variables for quartiles of year of birth based on the distribution of all cancer decedents (<1913, 1913–<1919, 1920–<1926, 1926+), with the earliest year of birth category serving as the referent. We included race in models because it is associated with cancer rates, and we included year of birth to control for possible birth cohort effects. Controlling for plant or for duration of employment had little impact on the Cox regression coefficients for TCDD, and there was no evidence of interaction between TCDD and plant, age, or any other variable. Thus, analyses presented here did not control for plant or duration of employment, and final models did not include interaction terms.

To evaluate indirectly whether the association between TCDD and cancer was distorted by smoking, we examined separate models of the relation between TCDD and lung cancer, all smoking-related cancers, and all other cancers. Smoking-related cancers included cancers of the oral cavity, pharynx, larynx, esophagus, lung, and bladder. The assumption was that, if TCDD was associated only with smoking related cancers, the association could be due to confounding
by smoking.

To consider indirectly the possibility that the association between TCDD and cancer was due, not to TCDD, but to other occupational exposures or that the effect of TCDD was modified by other exposures, we repeated our analyses, removing data from one plant at a time. If removal of a particular plant

resulted in the loss of a positive association between TCDD and cancer, the apparent association in the full data could be dependent on other occupational exposures specific to that particular plant. On the other hand, strengthening of a positive association after the removal of a particular plant could suggest a role of co-exposure to agents other than TCDD, not present at the excluded plant. We used untransformed and ln-transformedTCDDppt-years, lagged 15 years, in these analyses, as well as in analyses of smoking related cancers and other cancers.

Risk Assessment

The Cox regression coefficients relate cumulative exposure expressed as serum lipid TCDD AUC in ppt-years to the estimated cancer mortality RR. For the modeling on linear exposure units, the relationship has the form:

$$RR = e^{\beta * AUC} \qquad (3)$$

where β (ppt-years)$^{-1}$ is the Cox regression coefficient. The RR is the ratio between the age-specific all-cancer mortality rate, R_{tot}, at a given increment of AUC and the corresponding background rate (i.e., absent TCDD exposure), R_0. The total mortality rate is the sum of the background rate and the incremental mortality rate, R_D, associated with the additional exposure. Thus, $RR = R_{tot}/R_0 = (R_D + R_0)/R_0$ and $R_D = R_0(RR - 1)$. We estimated the cumulative incremental risk of all-cancer mortality (up to age 75) associated with a constant 5 ppt serum lipid concentration of TCDD.

Results

Replication of Previously Reported Results

We closely replicated the main analyses conducted by Steenland *et al*., obtaining SMRs and RRs similar to those reported previously for septile of cumulative exposure score for all cancers, lung cancer, and smoking-related cancers (Steenland *et al.*, 1999) and for septile of TCDD ppt-years using an 8.7-year half-life elimination model, lagged 15 years (Steenland *et al.*, 2001) (data not displayed in a table). Steenland *et al*. (2001) reported a Cox regression coefficient of 9.7×10^{-2} (standard error, s.e. = 3.2×10^{-2}) for ln-transformed TCDD ppt-years (8.7-year half-life model, lagged 15 years) as a continuous variable in a model that controlled for year of birth and race. We obtained a coefficient of 9.3×10^{-2} (s.e. = 3.2×10^{-2}) from an analogous analysis.

We were not able to replicate some specific results of Steenland and colleagues (Steenland & Deddens, 2003; Steenland *et al*., 2001; Steenland *et*

al., 1999). For example, Steenland *et al*. (1999, Fig. 1, plant 11) reported a median cumulative exposure score for one of the plants of approximately 2.0, whereas we calculated the median score as 3.3 for the same plant. Our plant-specific Cox regression coefficients for ln(TCDD ppt-years) (8.7-year half-life-based, lagged 15 years) were not the same as the coefficients reported by Steenland *et al*. (2001). The risks sets for our Cox regression analyses included 3,455 workers rather than the 3,444 workers reported by Steenland *et al*. (2001).

Comparison of TCDD Exposure Indices

Table I summarizes the distribution of all subjects (n = 3,538), all decedents (n = 927), and cancer decedents (n = 256) by indices of TCDD exposure. On average, workers were exposed to TCDD for 2.7 years, decedents were exposed for 3.9 years, and cancer decedents for 4.8 years. Mean values of TCDD exposure score and ppt-years were similar for cancer decedents and all decedents. In comparison to the mean TCDD ppt-years estimated with an 8.7-year fixed half-life model and a 15-year lag, mean TCDD ppt-years estimated using the CADM model and a 15-year lag were 4.5–5.2 times higher. These two exposure estimates were highly correlated, with a Pearson correlation coefficient of 0.97 ($p < 0.0001$) and a Spearman rank correlation coefficient of 0.98 ($p < 0.0001$). Mean and median values of cumulative exposure varied considerably among the eight plants. Subjects and cancer decedents from Plants 8 and 9 had the highest mean values of CADM-based TCDD ppt-years, lagged 15 years (Fig. 1), whereas subjects and cancer decedents from Plants 8 and 10 had the highest median exposure values, and those from Plant 9 had the third lowest median value.

Table I: Mean (*SD*) Cumulative Exposure to TCDD for All Subjects, All Decedents, and All Cancer Decedents, Overall

	All Subjects (*n* = 3,538)	**All Decedents (*n* = 927)**	**All Cancer Decedents (*n* = 256)**
Duration of exposure, years	2.69	3.87	4.75
	(4.41)	(5.03)	(5.49)
Cumulative exposure score, *unlagged*, score-years	9,892 (59,888)	19,972 (94,224)	18,788 (70,228)
Cumulative exposure based on CADM model, *unlagged*, ppt-years	111,786 (755,645)	239,700 (1,289,855)	208,669 (833,768)
Cumulative exposure based on CADM model, *lagged 15 years*, ppt-years	84,057 (564,951)	144,991 (869,050)	126,348 (458,454)
Cumulative exposure based on 8.7-year half-life model, *lagged 15 years*, ppt-years	18,829 (116,446)	27,680 (148,985)	26,494 (92,352)

Note: Cumulative exposure as of end of followup or death based on 3,538 workers.

SMR Analyses

Compared to the general population of the United States, the NIOSH study group had a 17% increase in mortality from all cancers, a 22% increase in mortality from smoking-related cancers, and a 12% increase in mortality from other cancers (Table II). For all cancers, lung cancer, and smoking- related cancers, SMRs were statistically significantly elevated only at Plant 10. Nonmalignant respiratory disease also was statistically significantly elevated at Plant 10. At Plant 8, where an excess of bladder cancer has been attributed to exposure to 4- aminobiphenyl (Collins *et al*., 1993; Collins *et al*., 1999), only smoking-related cancers (including bladder cancer) were statistically significantly elevated. Plants 3, 4, 7, and 8 either did not have an excess of all cancers (Plants 4 and 7) or had a cancer excess that was limited to smoking-related cancers (Plants 3 and 8).

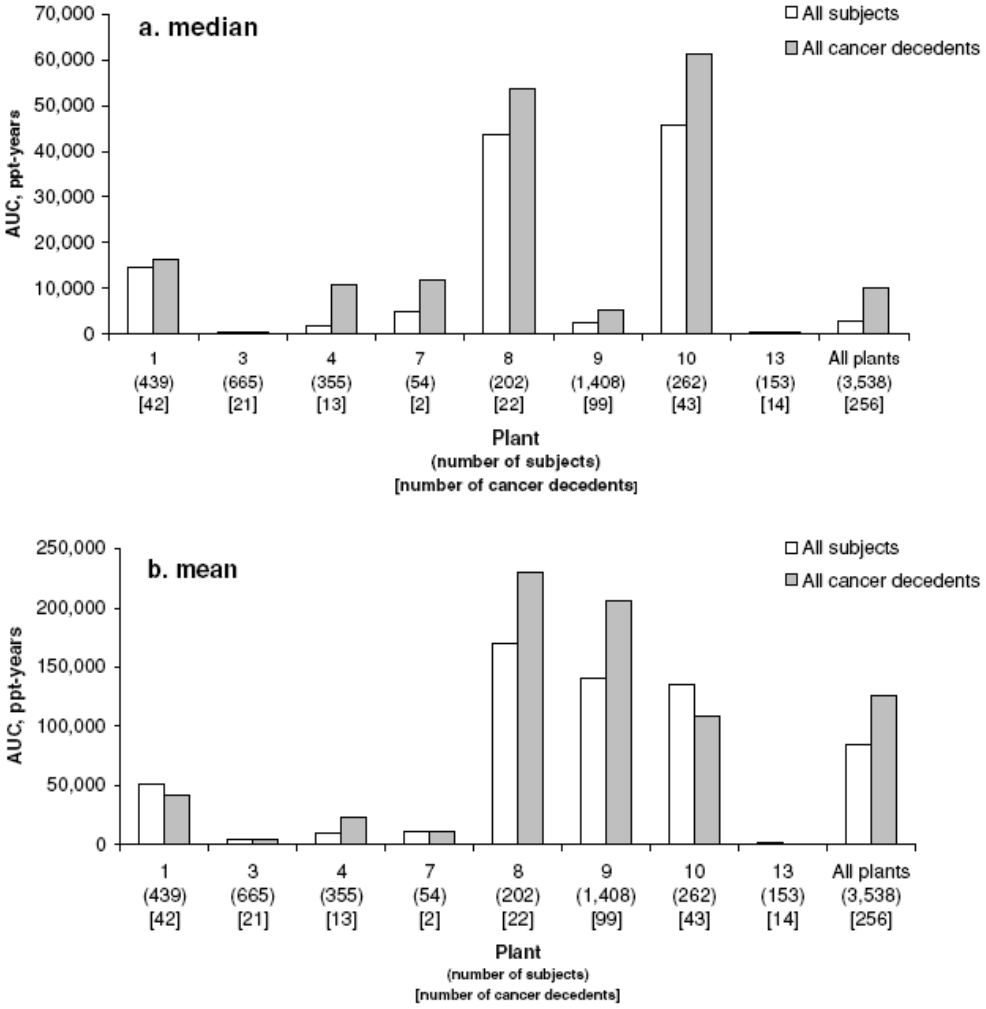


Figure 1: Average TCDD ppt-years and number of observations and cancer cases by plant with 15-year lagged exposure: (a) median TCDD ppt-years; (b) mean TCDD ppt-years.

Table II: Observed and Expected Numbers of Deaths, Standardized Mortality Ratio, and 95% Confidence Interval for All Cancer, Lung Cancer, Smoking-Related Cancer, All Other Cancer, and Nonmalignant Respiratory Disease for the Eight Plants Included in the Exposure-Level Analysis

	Cause of Death									
	All Cancer		Lung Cancer		Smoking-Related Cancer*		All Other Cancer		NMRD	
Plant	O/E	SMR (CI)	O/E	SMR (CI)	O/E	SMR (CI)	O/E	SMR (CI)	O/E	SMR (CI)
1	42/38	111 (80-150)	12/14	89 (46-155)	17/17	99 (58-159)	25/21	121 (78-179)	10/11	94 (45-172)
3	21/18	116 (72-178)	9/6.0	150 (69-285)	12/7.6	157 (81-275)	9/10	86 (40-164)	5/5.4	93 (30-217)
4	13/15	90 (48-153)	5/4.8	105 (34-245)	6/6.2	97 (36-211)	7/8.3	84 (34-173)	1/4.5	22 (1-124)
7	2/3.3	62 (8-223)	2/1.2	171 (21-617)	2/1.5	133 (16-481)	0/1.7	0 (0-212)	0/0.8	0 (0-479)
8†	22/18	125 (78-189)	9/6.5	139 (64-264)	18/8.1	224 (133-353)	4/9.6	42 (11-107)	9/4.7	191 (87-363)
9‡	99/98	101 (82-124)	25/35	72 (46-106)	35/44	80 (56-111)	64/54	119 (92-152)	13/27	49 (26-83)
10¶	43/23	187 (135-252)	20/8.5	235(144-364)	24/11	225 (144-335)	19/12	154 (93-240)	12/6.0	200 (103-349)
13§	14/7.8	180 (98-301)	5/3.0	166 (54-388)	6/3.8	157 (58-342)	8/4.3	185 (80-364)	2/1.9	106 (13-384)
Total£	256/220	117 (103-132)	87/78	111 (89-137)	120/99	122 (101-145)	136/121	112 (94-133)	52/61	86 (64-112)

O = observed number of deaths; E = expected number of deaths; SMR = standardized mortality ratio; CI = confidence interval; NMRD = nonmalignant respiratory disease.

*Includes cancer of the oral cavity and pharynx, esophagus, larynx, lung, and bladder.

†Plant 8 also had an excess of bladder cancer based on 7 observed/0.41 expected, SMR = 1692, CI = 680–3487.

‡Plant 9 also had an excess of prostate cancer based on 15 observed/6.5 expected, SMR = 229, CI = 128–378.

¶Plant 10 also had an excess of larynx cancer based on 4 observed/0.33 expected, SMR = 1218, CI = 332–3119.

§Plant 13 had an excess of cancers of digestive organs other than esophagus based on 7 observed/1.65 expected, SMR = 424, CI = 170–874.

£The excess of smoking-related cancer was due to excesses of larynx and bladder cancers, in addition to cancer of the lung.

Cox Regression with Penalized Splines

Figs. 2a and 2c depict the ln(hazard ratio) for all-cancer mortality across the full range of untransformed TCDD ppt-years, unlagged and lagged 15 years, respectively. These figures indicate a positive exposure-response relation throughout most of the exposure range, up to about 1.4×10^6 ppt-years for unlagged exposure and up to about 4.5×10^6 ppt-years for 15-year lagged exposure. Only 2.2% and 0.2% of observations, respectively, had TCDD levels above these two values. Figs. 2b (unlagged exposure) and 2d (15-year lagged exposure) depict the ln(hazard ratio) for all cancer mortality after removal of observations with TCDD ppt-years falling in the upper 5% of the exposure distribution, corresponding to unlagged exposures above 5.4×10^5 ppt-years and to 15-year lagged exposures above 2.5×10^5 ppt-years. The restricted data on unlagged TCDD ppt-years included 240 of the 256 total cancer decedents and 3,351 of the 3,455 subjects included in the full data. The restricted data on 15-year lagged TCDD ppt-years included 235 cancer decedents and 3,282 subjects. Many of the subjects eliminated by removing the upper 5% of observations came from Plant 9 (66% and 50%, respectively, for the unlagged and 15-year lagged exposure variables).

The earliest TCDD exposures among subjects in the study occurred at Plant 9, where trichlorophenol production began in 1942. Most of the production processes at Plant 9 involved potential TCDD exposure, with the exception of aniline processes in one building.The p-splines displayed in Figs. 2b and 2d are reasonably consistent with linear exposure-response relations.

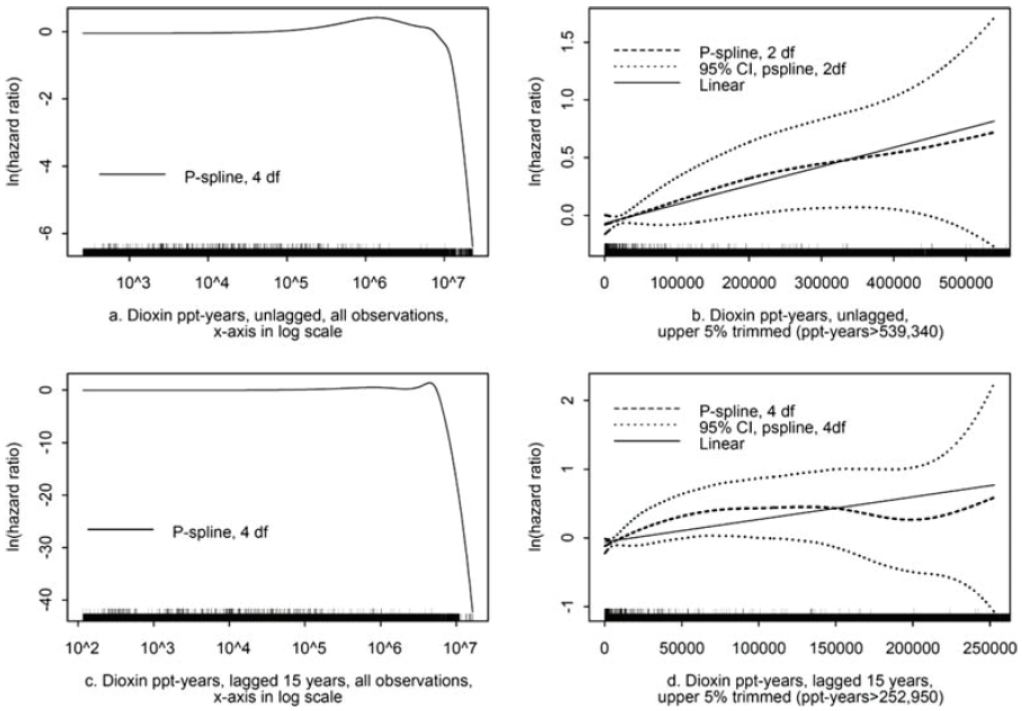


Figure 2: Penalized splines for TCDD ppt-years and ln(hazard ratio) of cancer mortality, with all observations and with upper 5% of observations trimmed. Histograms (rugs) represent observations (lower rug) and cases (upper rug) at corresponding TCDD ppt-years. When the upper 5% of the observations are trimmed (Figs. 2b and d), only the linear portion of the spline was significant.

Classical Cox Regression

Table III summarizes results of using a linear or ln-linear relation between TCDD ppt-years and cancer mortality hazard. Unlagged TCDD ppt-years were not statistically significantly associated with cancer mortality in analyses that used the full data on untransformed or ln-transformed TCDD or that used data on untransformed TCDD from which the upper 1% of observations were removed. A statistically significant positive association with untransformed, unlagged TCCD ppt-years arose only after exclusion of the upper 2.5% or 5% of observations. With a 15-year lag period, TCDD ppt-years was statistically significantly associated with cancer mortality only in analyses that used untransformed TCDD ppt-years with removal of the upper 5% of observations or that used the full data on ln-transformed TCDD. We obtained similar results using a 10-year lag period (data not presented). Analyses that were restricted to data above the 95th percentile indicated an inverse dose-response trend that was not statistically significant. The Cox regression coefficient (β) was -2.0×10^{-8} (s.e. = 1.2×10^{-7}, $p = 0.87$) for untransformed, 15-year lagged TCDD ppt-years. The association between TCDD ppt-years and cancer mortality was similar for smoking-related cancers (ln-transformation and a 15-year lag: $\beta = 8.4 \times 10^{-2}$ (s.e. = 4.2×10^{-2}) and for other cancers ($\beta = 7.8 \times 10^{-2}$, s.e. = 4.0×10^{-2}). Removing one Plant at a time from analyses of TCDD ppt-years (ln transformed, lagged 15 years) had little impact. The regression coefficients for TCDD ranged from 5.4×10^{-2} after excluding Plant 10 to 9.8×10^{-2} after excluding Plant 9 or Plant 13, and all coefficients were statistically significant or borderline statistically significant. Regression coefficients for TCDD after removing one plant at a time from analyses of untransformed TCDD ppt-years (lagged 15 years) ranged from 1.1×10^{-8} after excluding Plant 4 to 3.9×10^{-8} after excluding Plant 9, and no coefficient was statistically significant.

Table III: Summary of Results of Cox Regression Models for the Relation Between TCDD ppt-Years (CADM Based) and All Cancer

Exposure Lag Period, TCDD Exposure Variable, and Model*	Coefficient Estimate for TCDD (β)	Standard Error of β
TCDD ppt-years, unlagged		
Full data, untransformed†	-8.9×10^{-9}	5.8×10^{-8}
Full data, ln-transformed	5.3×10^{-2}	3.1×10^{-2}
Excluding observations with ppt-years in the upper 1% range (2,409,588 and higher) of the exposures	3.6×10^{-7}	2.0×10^{-7}
Excluding observations with ppt-years in the upper 2.5% range (1,106,145 and higher) of the exposures	1.0×10^{-6}	3.2×10^{-7} §
Excluding observations with ppt-years in the upper 5% range (539,340 and higher) of the exposures	1.6×10^{-6}	6.2×10^{-7} §
TCDD ppt-years, lagged 15 years		
Full data, untransformed‡	1.7×10^{-8}	9.1×10^{-8}
Full data, ln-transformed	8.1×10^{-2}	2.9×10^{-2} §
Excluding observations with ppt-years in the upper 1% range (1,432,507 and higher) of the exposures	6.4×10^{-7}	3.2×10^{-7}
Excluding observations with ppt-years in the upper 2.5% range (661,664 and higher) of the exposures	6.4×10^{-7}	7.1×10^{-7}
Excluding observations with ppt-years in the upper 5% range (252,950 and higher) of the exposures	3.3×10^{-6}	1.4×10^{-6} §

*All models included year of birth and race; race: white or nonwhite; year of birth: quartiles of year of birth based on distribution of cancer decedents, with the first category as the referent.
†Range: 130–22,932,289 ppt-years.
‡Range: 6–17,284,554 ppt-years.
§$p < 0.05$.

Risk Assessment

Table IV presents estimates of incremental cancer risk associated with a lifetime average serum lipid TCDD concentration of 5 ppt based on the Cox regression using untransformed exposure estimates in TCDD ppt-years. These calculations rely on two estimates of background cancer risk, R_0, for males at age 75: 12.4%, as used by Steenland *et al*. (2001), and 11.2%, as estimated for all males in the 1999–2001 Surveillance Epidemiology and End Result data set (Statistical Research and Applications Branch, 2004). The incremental risks based on the revised exposure reconstruction (incorporating concentration and age-dependent elimination kinetics) are approximately 6- to

more than 10-fold lower than those resulting from the previous reconstruction that relied on first-order elimination kinetics with a constant 8.7-year half-life (Steenland *et al*., 2001). Risk estimates for low background exposures are not presented for the ln-transformed Cox regressions because they yielded biologically implausible results. While the use of ln-transformed exposure reduces the impact of the large variability in exposure estimates on the dose-response assessment, it has the effect of greatly emphasizing the impact of small changes in exposure estimates at the low end of the exposure range. For example, the regression model for ln-transformed exposure predicts an implausibly large 20% decrease in general population all-cancer mortality due to the reductions in average general population TCDD body burdens in the United States over the past 30 years. The implausibility of this functional form of the dose-response relationship for use in evaluating background exposure estimates was noted by the USEPA (2003).

Table IV: Estimated Incremental Risk Associated with an Average Lifetime Serum Lipid Concentration of TCDD of 5 ppt for Two Estimates of Background Cancer Mortality Risk, R_0, in Males at Age 75

Model	β (ppt-year)$^{-1}$	Incremental Risk[a] R_0= 0.124	Incremental Risk[a] R_0 = 0.112
Previous analysis			
Piecewise linear[b]	1.5×10^{-5}	7.0×10^{-4}	6.3×10^{-4}
Current analysis			
Unlagged exposure			
Piecewise linear[c]	1.4×10^{-6}	6.5×10^{-5}	5.9×10^{-5}
Linear, lower 95% of obs.	1.6×10^{-6}	7.4×10^{-5}	6.7×10^{-5}
Linear, full data	-8.9×10^{-9} *	<0	<0
Lagged exposure (15 years)			
Linear, lower 95% of obs.	3.3×10^{-6}	1.2×10^{-4}	1.1×10^{-4}
Linear, full data	1.7×10^{-8} *	6.3×10^{-7}	5.7×10^{-7}

[a] From Equation (2), assuming 5 ppt serum lipid TCDD concentration for 75 years (unlagged) or 60 years (taking into account a 15-year lag).
[b] Cox regression coefficient from the Steenland *et al*. (2001) analysis as reported in the USEPA (2003) Draft Dioxin Reassessment, 2003, Part III, pp. 5–34, males, no lag. The value of this coefficient for the piecewise linear modeling was not explicitly reported by Steenland *et al*. (2001).
[c] Piecewise linear with cutpoint set at maximum likelihood for model fit, 452,000 ppt-years.
* Not statistically significantly different from 0.

Discussion

The analysis of this study is based on the serum lipid TCDD estimated from a concentration- and age-dependent elimination model.We have presented the rationale for this two-compartment model elsewhere (Aylward *et al*., 2005b). Recent studies have demonstrated that the elimination of TCDD in humans occurs at a fast rate when body concentrations are relatively high and at a slow rate in older age (Aylward *et al*., 2005a, 2005b). For the data used in our analysis, the CADM model yielded improved fits to the serum sampling data compared to those obtained with first-order models. The use of the CADM model to derive cumulative TCDD serum lipid levels yielded exposure estimates about five times higher than use of the age-dependent only, 8.7-year half-life model for more highly exposed members of the cohort, while exposure estimates from the two approaches were closer together for subjects with lower exposure scores. Nevertheless, the estimates from the CADM and agedependent only models were highly correlated, and the shapes of the exposure-response relation were similar using these two exposure variables.

The current investigation found that a positive association between TCDD exposure and cancer mortality was absent or weak unless a lag period of 10–15 years or ln-transformation or restrictions are applied to the exposure data.The strengthening of the association with the application of a 10- to 15-year lag period suggests several possibilities.

- "Recent" exposure was particularly poorly estimated. This seems implausible given that all of the exposure estimates are backextrapolations from recent serum estimates.
- Recent cumulative TCDD exposure does not contribute biologically to, and may even confound, the association of previous cumulative TCDD exposure with all-cancer mortality. It is reasonable to assume that the initiation and promotion phases for the cancers experienced by the subjects in the study were completed some years before death, thus making the most recent exposure period etiologically irrelevant. This could account, at least in part, for the strengthened association that resulted from use of a 10–15-year lag period. Alternatively, TCDD could be acting primarily as an initiator rather than a promoter of cancer, but this is inconsistent with the principal finding from experimental studies that TCDD is not a genotoxic chemical (Dragan & Schrenk, 2000).
- Sustained high-level exposures are more likely to contribute to cancer development than those acquired only recently. Application of a 10–15-year lag period produced the greatest reductions in estimated exposure for persons with only recent occupational exposure. Thus, application of the lag period has the effect of emphasizing those individuals with sustained periods (greater than 10–15 years prior) of elevated internal

exposure. This impact of a lag period is consistent with the theoretical and experimental basis for tumor promotion, in which the promoting agent must be present for a sustained period to accelerate tumor development (Pitot & Sirica, 1980).

Selection of an appropriate lag period should consider biological plausibility, which is likely to be related to such factors as the age of the person exposed, the intensity, duration, and timing of exposure (Langholz *et al*., 1999), and the carcinogenic properties of the agent. The absence of an association between cancer mortality and TCDD exposure without employment of a 10–15-year lag suggests that exposures acquired during the lag period did not contribute materially to cancer mortality, irrespective ofTCDD's potential role as a cancer-promoting agent.

We observed that the positive association between TCDD and cancer was markedly stronger when the upper 2.5–5% of the observations in the study were excluded. This strengthening could be due to exposure misclassification being most severe at the high end of the exposure range or to statistical instability resulting from sparse data in the high end of the exposure range, as suggested by Stayner *et al*. (2003).

Without removing observations from the analysis, modeling with ln-tranformed TCDD is one alternative approach to reduce the leverage of extremely high exposure values. However, ln transformation also overweights exposures at the low end of the exposure range. In this study, ln-transformed TCDD (lagged 15 years) was statistically significant (β = 8.1 × 10^{-2}, s.e.=2.9×10^{-2}) (the ln-transformed analysis was not significant using unlagged exposure estimates). Other occupational studies have observed a similar pattern of effect estimates obtained with untransformed splines and ln-transformed data (Eisen *et al*., 2004; Steenland & Deddens, 2003). However, the ln-transformed model produces implausibly hazard ratio estimates at background exposure levels. For example, threefold increases in TCDD exposure, from 1 to 3 ppt-years, and from 1,000 to 3,000 ppt-years, both result in an approximate 10% increase in the estimated RR using the ln-transformed, and 15-year exposure estimates (lagged Cox regression coefficient = 0.081).

We did not find clear evidence of confounding by smoking, as the association with TCDD exposure was present even for cancers not related to smoking, or by occupational exposures other than TCDD, as the association with TCDD persisted in analyses that eliminated one plant at a time. Nonetheless, individuals in this study were exposed to multiple agents, and TCDD was certainly not responsible for all of the excess cancer deaths that were observed. For example, some of the bladder cancers at Plant 8 were attributable to 4-aminobiphenyl exposure (Collins *et al*., 1993; Collins *et al*., 1999). At least two deaths from mesothelioma, indicating asbestos exposure,

also occurred in this cohort (Fingerhut *et al*., 1991). The elevations in deaths from nonmalignant respiratory disease in Plants 8 and 10 suggest that smoking may have contributed to the observed excess lung and total cancer mortality at these plants. However, we do not know if smoking confounded the dose-response relation between TCDD and cancer mortality, or if so, the net direction of such confounding.

The estimates of incremental cancer risk presented in Table IV can be compared to estimates prepared by USEPA (2003) based on all-cancer mortality dose-response assessments of three occupational cohorts (Becher *et al*., 1998; Ott & Zober, 1996; Steenland *et al*., 2001) that relied on first-order elimination kinetics for exposure reconstruction.TheUSEPA (2003) estimated cancer slope factors from each study with first-order elimination kinetics at background exposure levels, a typical proportion of body fat, and an intake absorption fraction. These include an estimate of 1,500,000 $(mg/kg\text{-}day)^{-1}$ from the NIOSH cohort using Steenland *et al*.'s (2001) piecewise linear Cox regression coefficient. With the same assumptions, the Cox regression coefficients from this analysis (using the CADM exposure reconstruction) yield cancer potency estimates ranging from approximately 10,000 to 240,000 $(mg/kg\text{-}day)^{-1}$. Because the other cohort assessments included in the USEPA (2003) analysis also relied upon first-order constant half-life back-extrapolations to estimate exposure, application of concentration- and age-dependent elimination kinetics could have an effect of similar magnitude on the risk estimates obtained from updated analyses of these cohorts as well.

Current serum lipid concentrations of TCDD and related chlorinated dioxin and furan compounds (expressed in TCDD toxicity equivalents (TEQ)) in the general U.S. population are highly age-dependent, with older individuals displaying much higher concentrations than younger individuals (Patterson *et al*., 2004). This is thought to be a consequence of the substantial decreases in TCDD concentrations in the general environment and food supply over the past 30 years (Hays & Aylward, 2003; Lorber, 2002). For young adults (ages 45 and below), current serum lipid TCDD concentrations average between 1 and 2 ppt, while current average TEQ concentrations range between approximately 6 and 12 ppt. For individuals older than 60 years, current average serum lipid TCDD and TEQ concentrations are higher, at approximately 4 and 36 ppt, respectively (Patterson *et al*., 2004). For all age groups, serum lipid concentrations appear to decline continuously (Lorber, 2002; Patterson *et al*., 2004).

Application of the incremental risk estimates from Table IV to general population serum lipid TCDD levels suggests that the lifetime cancer mortality risk that may be attributable to background TCDD exposures is below 1 in 10,000. If the Cox regression results are applied to serum lipid TEQ concentrations, background risks for younger individuals in the population fall

in the same range, but older individuals might have incremental risks as much as 10 times higher. However, the relative potency estimates (toxic equivalency factors (TEFs)) used to summarize dioxin and furan concentrations as TEQ were intended for application with intake rates, not tissue or body concentrations (Van den Berg *et al*., 1998). Studies using subchronic exposure regimens have demonstrated that the intake-based TEFs overpredict the tumor promotion potency of some dioxin and furan congeners when they are used inappropriately with tissue concentrations (Waern *et al*., 1991), and recent bioassays from the NTP provide additional data that also support this discrepancy (2004a, 2004b, 2004c; Budinsky *et al*., 2006; Gray *et al*., 2006).

This analysis reveals some of the substantial uncertainty that resides in quantitative risk rstimates derived from occupational cohort mortality data. Use of concentration-dependent elimination kinetics results in a decrease of 6- to more than 10-fold in estimated risk compared to estimates reported previously by Steenland *et al*. (2001). However, even within the present analysis, a range of estimated risks spanning more than two orders of magnitude can be obtained using various plausible assumptions. This uncertainty does not include the additional contributions from interindividual differences in elimination kinetics, uncertainties due to uncontrolled confounding, or the additional variability that could be expected from inclusion of data from other occupational cohorts. Such variability and uncertainty needs to be acknowledged when quantitative assessments of potential human cancer risks at background exposure levels are conducted.

Acknowledgments
Funding for this project was provided by the Research Foundation for Health and Environmental Effects.

References

Akhtar, F. Z., Garabrant, D. H., Ketchum, N. S., & Michalek, J. E. (2004). Cancer in US AirForce veterans of the Vietnam War. *Journal of Occupational and Environmental Medicine*, *46*(2), 123–136.

Aylward, L. L., Brunet, R. C., Carrier, G., Hays, S. M., Cushing, C. A., Needham, L. L., Patterson, D. G., Jr., Gerthoux, P. M., Brambilla, P., & Mocarelli, P. (2005a). Concentration dependent TCDD elimination kinetics in humans: Toxicokinetic modeling for moderately to highly exposed adults from Seveso, Italy, and Vienna, Austria, and impact on dose estimates for the NIOSH cohort. *Journal of Exposure Analysis and Environmental Epidemiology*, *15*(1), 51–65.

Aylward, L., Brunet, R., Starr, T. B., Carrier, G., Delzell, E., Cheng, H., & Beall, C. (2005b). Exposure reconstruction for the TCDD-exposed NIOSH

cohort using a concentration- and age-dependent model of elimination. *Risk Analysis*, *25*(4), 945– 956.

Aylward, L. L., & Hays, S. M. (2002). Temporal trends in human TCDD body burden: Decreases over three decades and implications for exposure levels. *Journal of Exposure Analysis and Environmental Epidemiology*, *12*(5), 319–328.

Becher, H., Steindorf, K., & Flesch-Janys, D. (1998). Quantitative cancer risk assessment for dioxins using an occupational cohort. *Environmental Health Perspectives*, *106*(Suppl. 2), 663–670.

Bertazzi,P. A., Consonni,D., Bachetti, S., Rubagotti, M., Baccarelli, A., Zocchetti, C., & Pesatori, A. C. (2001). Health effects of dioxin exposure: A 20-year mortality study. *American Journal of Epidemiology*, *153*(11), 1031–1044.

Budinsky, R. A., Paustenbach, D., Fontaine, D., Landenberger, B., & Starr, T. B. (2006). Recommended relative potency factors for 2,3,4,7,8 pentachlorodibenzofuran: The impact of different dose metrics. *Toxicological Sciences*, *91*(1), 275–285.

Cole, P., Trichopoulos, D., Pastides, H., Starr, T., & Mandel, J. S. (2003). Dioxin and cancer: A critical review. *Regulatory Toxicology and Pharmacology*, *38*(3), 378–388.

Collins, J. J., Strauss, M. E., Levinskas, G. J., & Conner, P. R. (1993).The mortality experience of workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin in a trichlorophenol process accident. *Epidemiology*, *4*(1), 7–13.

Collins, J. J., Strauss, M. E., & Riordan, S. G. (1999). Mortalities of workers at the Nitro Plant with exposure to 2-mercaptobenzothialzole. *Occupational and Environmental Medicine*, *56*(10), 667–671.

Crump, K. S., Canady, R., & Kogevinas, M. (2003). Meta-analysis of dioxin cancer dose response for three occupational cohorts. *Environmental Health Perspectives*, *111*(5), 681–687.

Dragan, Y. P., & Schrenk, D. (2000). Animal studies addressing the carcinogenicity of TCDD (or related compounds) with an emphasis on tumour promotion. *Food Additives and Contaminants*, *17*(4), 289–302.

Eisen, E. A., Agalliu, I., Thurston, S. W., Coull, B. A., & Checkoway, H. (2004). Smoothing in occupational cohort studies: An illustration based on penalised splines. *Occupational and Environmental Medicine*, *61*(10), 854–860.

Fingerhut, M. A., Halperin, W. E., Marlow, D. A., Piacitelli, L. A., Honchar, P. A., Sweeney, M. H., Greife, A. L., Dill, P. A., Steenland, K., & Suruda, A. J. (1991). Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *New England Journal of Medicine*, *324*(4), 212–218.

Flesch-Janys, D., Steindorf, K., Gurn, P., & Becher, H. (1998). Estimation of the cumulated exposure to polychlorinated dibenzop-dioxins/furans and standardized mortality ratio analysis of cancer mortality by dose in an

occupationally exposed cohort. *Environmental Health Perspectives*, *106*(Suppl. 2), 655– 662.
Gray, M. N., Aylward, L. L., & Keenan, R. E. (2006). Relative cancer potencies of selected dioxin-like compounds on a body burden basis. Comparison to current toxic equivalency factors (TEFs). *Journal of Toxicology and Environmental Health. Part A*, *69*(10), 907–917.
Hays, S. M., & Aylward, L. L. (2003). Dioxin risks in perspective: Past, present, and future. *Regulatory Toxicology and Pharmacology*, *37*(2), 202–217.
IARC. (1997). Polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans. In *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* (Vol. 69). Lyon, France: IARC Press.
Insightful Corporation. (2001). *S-Plus 6 for Windows Guide to Statistics*. Seattle: Insightful Corp.
Ketchum, N. S., & Michalek, J. E. (2005). Postservice mortality of Air Force veterans occupationally exposed to herbicides during the Vietnam War: 20-year followup results. *Military Medicine*, *170*(5), 406–413.
Langholz, B., Thomas, D., Xiang, A., & Stram, D. (1999). Latency analysis in epidemiologic studies of occupational exposures: Application to the Colorado Plateau uranium miners cohort. *American Journal of Industrial Medicine*, *35*(3), 246–256.
Lorber, M. (2002). A pharmacokinetic model for estimating exposure of Americans to dioxin-like compounds in the past, present, and future. *Science of the Total Environment*, *288*(1–2), 81–95.
Marsh, G. M., Youk, A. O., Stone, R. A., Sefcik, S., & Alcorn, C. (1998). OCMAP-PLUS: A program for the comprehensive analysis of occupational cohort data. *Journal of Occupational and Environmental Medicine*, *40*(4), 351–362.
Michalek, J. E., Pirkle, J. L., Caudill, S. P., Tripathi, R. C., Patterson, D. G., Jr., & Needham, L. L. (1996). Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 10-year followup. *Journal ofToxicology and Environmental Health*, *47*(3), 209–220.
National Toxicology Program (NTP). (2004a). *NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2,3,7,8-Tetrachlorodibenzo-[rho]-dioxin (TCDD) (CAS no. 1746-01-6) in Female Harlan Sprague-Dawley Rats (Gavage Studies)*. Research Triangle Park, NC: National Toxicology Program, U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health.
National Toxicology Program (NTP). (2004b). *NTP Technical Report on the Toxicology and Carcinogenesis Studies of 3,3',4,4', 5-Pentachlorobiphenyl (PCB 126) (CAS no. 57465-28-8) in Female Harlan Sprague-Dawley Rats (Gavage Studies)*. Research Triangle Park, NC: National Toxicology Program,U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health.

National Toxicology Program (NTP). (2004c). *NTP Technical Report on the Toxicology and Carcinogenesis Studies of a Mixture of 2,3,7,8-Tetrachlorodibenzo-[rho]-dioxin (TCDD) (CAS no. 1746-01-6), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (CAS no. 57117-31-4), and 3,3',4,4',5-Pentachlorobiphenyl(PCB126) (CAS no. 57465-28-8) in Female Harlan Sprague-Dawley Rats (Gavage Studies)*. Research Triangle Park, NC: National Toxicology Program, U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health.

National Toxicology Program (NTP). (2002). *Report on Carcinogens, Tenth Edition: Carcinogen Profiles 2002*, 10th ed. Research Triangle Park, NC: National Toxicology Program, U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health.

Ott, M. G., & Zober, A. (1996). Cause specific mortality and cancer incidence among employees exposed to 2,3,7,8-TCDD after a 1953 reactor accident. *Occupational and Environmental Medicine*, *53*(9), 606–612.

Patterson, D. G., Canady, R., Wong, L.-Y., Lee, R., Turner, W., Caudill, S., Needham, L. L., & Henderson, A. (2004). Age specific dioxin TEQ reference range. *Organohalogen Compounds*, *66*, 2878–2883.

Paustenbach, D. J. (2002). The U.S. EPA Science Advisory Board Evaluation (2001) of the EPA dioxin reassessment. *Regulatory Toxicology and Pharmacology*, *36*(2), 211–219.

Piacitelli, L., Marlow, D., Fingerhut, M., Steenland, K., & Sweeney, M. H. (2000). A retrospective job exposure matrix for estimating exposure to 2,3,7,8-tetrachlorodibenzo-pdioxin. *American Journal of Industrial Medicine*, *38*(1), 28–39.

Pitot, H. C., & Sirica, A. E. (1980). The stages of initiation and promotion in hepatocarinogenesis. *Biochimica et Biophysica Acta*, *605*(2), 191–215.

Starr, T. B. (2003). Commentary on issues raised by Crump *et al*.'s meta-analysis of three occupational cohorts exposed to dioxin. *Environmental Health Perspectives*, *111*(12), 1443–1447.

Statistical Research and Applications Branch. (2004). *Probability of Developing or Dying of Cancer Software, Version 5.2*.Available at http://srab.cancer.gov/devcan.

Stayner, L., Steenland, K., Dosemeci, M., & Hertz-Picciotto, I. (2003). Attenuation of exposure-response curves in occupational cohort studies at high exposure levels. *Scandinavian Journal of Work, Environment & Health*, *29*(4), 317–324.

Steenland, K., & Deddens, J. (2003). Dioxin: Exposure-response analyses and risk assessment. *Industrial Health*, *41*(3), 175–180.

Steenland, K., Deddens, J., & Piacitelli, L. (2001). Risk assessment for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) based on an epidemiologic study. *American Journal of Epidemiology*, *154*(5), 451–458.

Steenland, K., Piacitelli, L., Deddens, J., Fingerhut, M., & Chang, L. I. (1999). Cancer, heart disease, and diabetes in workers exposed to 2,3,7,8-

tetrachlorodibenzo-p-dioxin. *Journal of the National Cancer Institute*, *91*(9), 779–786.

Therneau, T. M., & Grambsch, P. M. (2000). *Modeling Survival Data: Extending the Cox Model*. New York: Springer.

U.S. Environmental Protection Agency (USEPA). (2003). *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin and Related Compounds*. Available at http://www.epa.gov/ncea/pdfs/dioxin/nas-review.

Van den Berg, M., Birnbaum, L., Bosveld, A. T., Brunstrom, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., Van Leeuwen, F. X. R., Liem, A. K. D., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F.,&Zacharewski, T. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environmental Health Perspectives*, *106*(12), 775–792.

Waern, F., Flodstrom, S., Busk, L., Kronevi, T., Nordgren, I., & Ahlborg, U. G. (1991). Relative liver tumour promoting activity and toxicity of some polychlorinated dibenzo-p-dioxin and dibenzofuran-congeners in female Sprague-Dawley rats. *Pharmacology & Toxicology*, *69*(6), 405–458.

Chapter 9: A Margin of Exposure Approach to Assessment of Non-Cancer Risks of Dioxins Based on Human Exposure and Response Data

Aylward, L.L., J.E. Goodman, G. Charnley, L.R. Rhomberg. 2008. Environ. Health Persp. 116:1344-1351.

Abstract

Background: Risk assessment of human environmental exposure to polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and other dioxin-like compounds is complicated by several factors including limitations in measuring intakes due to the low concentrations of these compounds in foods and the environment and interspecies differences in pharmacokinetics and responses. **Objectives:** We examine the feasibility of relying directly on human studies of exposure and potential responses to PCDD/Fs and related compounds in terms of measured lipid-adjusted concentrations to assess margin of exposure (MOE) in a quantitative, benchmark dose-based framework using representative exposure and selected response data sets. **Methods:** We characterize estimated central tendency and upper bound general US population lipid-adjusted concentrations of PCDD/Fs from the 1970s and early 2000s based on available data sets. Estimates of benchmark concentrations for three example responses of interest (induction of cytochrome P4501A2 activity, dental anomalies, and neonatal thyroid hormone alterations) were derived based on selected human studies. **Results:** The exposure data sets indicate that current serum lipid concentrations in young adults are approximately six- to seven-fold lower than 1970s-era concentrations. Estimated MOEs for each endpoint based on current serum lipid concentrations range from less than 10 for neonatal thyroid hormone concentrations to more than 100 for dental anomalies - approximately six-fold greater than would have existed during the 1970s. **Conclusions:** Human studies of dioxin exposure and outcomes can be used in a benchmark dose framework for quantitative assessments of margin of exposure. Incomplete exposure characterization can complicate the use of such studies in a benchmark dose framework.

Introduction

Exposure to dioxins and related compounds has been declining in the US for over two decades in response to regulatory and other actions taken to reduce their generation and emissions to the environment (reviewed in Hays and Aylward 2003). Estimates of quantifiable emissions of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) and of dioxin-like polychlorinated biphenyls (PCBs) declined by a factor of ten between 1987 and 2000, with the greatest reduction achieved through the control of municipal waste incineration (U.S. EPA 2006). Overall emissions declines have been paralleled by several-fold reductions in estimates of dietary intake (Figure 1) and serum lipid adjusted concentrations in the general population (reviewed in Hays and Aylward 2003; Lorber 2002).

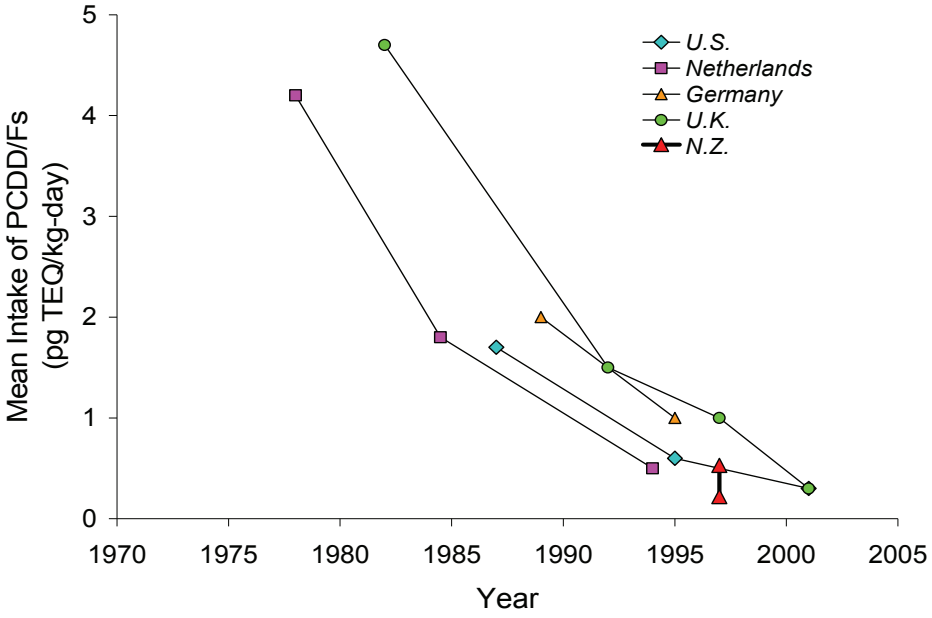


Figure 1: Estimated dietary intakes of PCDD/Fs in the US (U.S. EPA 2000; U.S. FDA 2008), the Netherlands (Liem et al. 2000), the United Kingdom (UKFSA 2003), Germany (Furst and Wilmers, 1997), and New Zealand (Smith and Lopipero 2001). Figure adapted from Hays and Aylward (2003). New Zealand data represent a range of estimates based on central and upper-end intake and concentration estimates.

The concomitant reduction in human health risks that have accompanied the declines in PCDD/F/PCBs exposure can be characterized by comparing past and present margins of exposure (MOEs) for different effects. MOEs are determined by dividing a "point of departure" (POD), derived from dose-response data, by relevant human exposure data. As exposure declines, MOEs become larger. MOEs do not reflect judgments about safety and are not themselves an indication of the likelihood of a risk. Interpreting MOEs in the context of risk assessment and risk management takes into account such factors as the slope of the dose-response relationship in the observable range, type of effect, mode of action, nature and extent of associated uncertainties, and human variation in susceptibility to the response of concern (Risk Commission 1997). In its recent draft risk assessment of dioxins the US Environmental Protection Agency (U.S. EPA) relied on a margin-of-exposure approach to evaluate potential risks from PCDD/Fs (U.S. EPA 2003) based principally on animal data. U.S. EPA has so far chosen not to establish a reference dose or other exposure limit for PCDD/Fs and no acceptable daily intake has been established by the US Food and Drug Administration.

Meanwhile, the UK Food Standards Agency (UKFSA), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the European Commission's Scientific Committee on Food (ECSCF) have established tolerable daily, weekly, or monthly intakes for combined exposures to PCDD/Fs based on the most sensitive endpoint observed in laboratory animals, developmental reproductive effects in male rats exposed prenatally to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (ECSCF 2001; JECFA 2001; UK COT 2001). These efforts derived from earlier efforts by the WHO (1998) which pioneered the use of "body burden" for risk assessment of dioxins. While health-protective, those limits are derived from an animal model with questionable relevance to human health both qualitatively and quantitatively (Charnley and Kimbrough 2006; Connor and Aylward 2006). U.S. EPA's most recent draft risk assessment of PCDD/Fs relies primarily on laboratory animal data to calculate a series of MOEs for different effects (U.S. EPA 2003), raising important interspecies dose metric and toxicity extrapolation issues.

In the recent evaluations, both the ECSCF and the WHO/FAO JECFA noted that human datasets were insufficient for quantitative risk assessment. However, since these evaluations in 2001, a substantial body of literature examining human responses to dioxins using measurements of serum lipid concentration as the marker of exposure has developed. This paper explores the use of human data sets on exposure and non-cancer endpoints in the context of a margin of exposure approach to risk assessment for PCDD/Fs and TEQ-contributing PCB compounds. Exposure and response data in this effort are based on the most commonly used metric in such studies: serum lipid concentration of TCDD toxic equivalents (TEQs) as estimated using the WHO toxic equivalency factors (TEFs) (van den Berg et al. 1998; van den Berg et al. 2006). Reliance on circulating serum lipid concentrations avoids issues associated with estimating "body burden" (estimates of body burden are highly influenced by assumptions regarding body fat content and degree of liver sequestration) and provides an exposure metric that can be assessed directly (rather than calculated using assumptions) and is of high biological relevance to a variety of potential target tissue responses.

Exposure data are derived from several sources: analysis of adipose tissue samples collected from young adults of service age during the Vietnam era (including Vietnam veterans) during the 1970s (Kang et al. 1990); analysis of blood samples collected from a representative sample of the US population from the National Health and Nutrition Evaluation Survey (NHANES) conducted in 2001-2002; and analysis of blood samples collected from residents of Michigan studied as part of an exposure evaluation conducted by the University of Michigan in 2005 (UMDES 2008). Exposures are compared among age groups and among birth cohorts. Using lipid-adjusted total dioxin

serum toxic equivalency (TEQ) values as the dose metric allowed us to compare internal doses based entirely on human data.

Example data sets on non-cancer endpoints were chosen to demonstrate various approaches to using epidemiological data in a quantitative risk assessment and on the basis of biological plausibility and interest in the data sets. Endpoints include induction of CYP1A2 enzyme among highly exposed adults who accidentally ate contaminated rice oil in Taiwan (the Yucheng cohort) (Lambert et al. 2006); the occurrence of developmental dental defects among children highly exposed to TCDD as a result of the 1976 reactor vessel explosion at a chemical plant in Seveso, Italy (Alaluusua et al. 2004); and changes in thyroid hormone measures in infants exposed to background levels of PCDDs and PCBs in the Netherlands in the late 1980s and early 1990s (Koopman-Esseboom et al. 1994). In each case we define a point of departure (POD) using an appropriate benchmark dose (BMD) approach (U.S. EPA 2000), expressing dose as serum lipid TEQ. Current MOEs for those effects – the margins between the benchmark doses and measures of serum lipid TEQ in the general US population – are estimated and the change in MOE compared to 1970s exposures is discussed.

The objective of this paper is to a) provide an assessment of the degree of change in lipid-adjusted TEQ concentrations over the past three decades, and b) demonstrate the use of example human data sets in a margin of exposure framework for assessing non-cancer risks of dioxins and related compounds. A comprehensive assessment of the weight of the evidence for the selected endpoints and within the full body of available human data was not conducted and was outside the scope of this effort. However, implementation of this MOE approach in a comprehensive risk assessment for dioxins would include a weight of evidence assessment as a critical component.

Methods

Exposure characterization

For this analysis, three data sets were evaluated to estimate serum lipid concentrations of PCDDs and PCDFs. U.S. EPA conducted the National Human Adipose Tissue Survey (NHATS) from 1970 to 1987 to monitor chemicals in adipose tissue in a statistically representative sample of US residents. Seventeen PCDD/Fs were measured in a subset of NHATS tissue sampled between 1971 and 1982 from 36 Vietnam veterans, 79 non-Vietnam veterans, and 80 civilian men who were born between 1936 and 1954 and were between 20 and 45 years of age at the time of sampling (this subset was not necessarily statistically representative) (Kang et al. 1990). No differences in any congener concentrations were found among these groups, so the entire

study group of 195 was used in this effort to characterize adipose tissue lipid-adjusted concentrations of PCDD and PCDF compounds during the 1970s. No PCB compounds were measured in this study. The lipid-adjusted adipose tissue concentrations measured in this study are assumed to reflect the lipid-adjusted concentrations in serum in these individuals (Patterson et al. 1988).

To determine how congener levels have changed over time, the results from the 1971-1982 samples analyzed by Kang et al. (1990) were compared with recent data from two data sets. The first dataset comprises measurements from serum collected in 2001-2002 and analyzed for 17 PCDD/Fs from a subsample of participants in NHANES. That study used a complex, multistage, probability sampling design to select participants representative of the civilian, non-institutionalized US population. Using analytical guidelines for these data provided by the National Center for Health Statistics and the NHANES Program, we analyzed TCDD and TEQ concentrations in people who were 20 to 45 years of age in 2001-2002 (to compare against the similarly aged group in the 1970-1987 NHATS survey) or were born between 1936 and 1954 (47 to 66 years of age, i.e., of the same birth cohort as included in the study by Kang et al. 1990). The assessment of percentiles was conducted in Stata 9.0 (Stata Corporation, College Station, TX). For the NHANES data, the assessment employed the subsample-specific sampling weights provided in the NHANES datasets.

The second dataset of more recent TEQ determinations comprises measurements taken in 2005 as part of the University of Michigan Dioxin Exposure Study (UMDES). The study was focused on assessing potential relationships between environmental dioxin exposures in the area of Midland and Saginaw counties, Michigan, where elevated concentrations of PCDD/F compounds have been identified in soils. The UMDES included a two-stage random sample of the population of Jackson and Calhoun Counties in Michigan (n=251); these two counties constituted an external referent population in the UMDES study, believed to be without unusual exposure to PCDD/Fs. This dataset provides a key advantage over the NHANES dataset owing to the use of far larger serum volumes with the resulting increase in analytical sensitivity. The individual data were not available for analysis, but population weighted summary data and statistics were taken from the study web site (UMDES 2008).

We calculated TEQ values for individual subjects in each study using the 1998 WHO toxic equivalency factors (TEFs) for individual congeners (van den Berg et al. 1998) for PCDD/Fs and constructed box plots to describe interindividual variability in serum PCDD/F TEQ within each data set using Stata 9.0. The 1998 TEF values were used because the response datasets evaluated here relied upon those TEF values (or an earlier version) and the original data were not available for recalculation.

Dose-response characterization

The general approach used was to assess a quantitative relationship between the endpoint of interest and measured serum lipid adjusted TEQ concentrations, and then to estimate a dose associated with a consistent benchmark response level across studies. For data sets addressing continuous variables, the benchmark response level was set at 10% extra risk of exceeding the "normal" range (as discussed, for example, in Gaylor and Aylward 2004). In this case the limits of the normal range are identified as the 2.5^{th} and 97.5^{th} percentiles in the general population, which corresponds to the typical delineation of clinical reference ranges (Siparsky and Accurso 2007). For data sets addressing quantal endpoints, the benchmark response level was likewise set to 10% extra risk of the event. These benchmark doses (BMD_{10}s) can then be used as the basis of an assessment of margin of exposure and changes in the margin of exposure over time in the general US population.

Three endpoints, each one based on data from a different study, were selected for this exploratory analysis. The selection of these studies or endpoints does not represent a conclusion that a causal association between the exposure and the response has been established. Endpoints were selected to be carried forward for quantitative analysis based on the biological relevance and plausibility of the endpoint examined, previous interest in the study and population, and as examples of various types of data that are found in the epidemiological literature.

CYP1A2 activity. Lambert et al. (2006) followed a group of people highly exposed to PCBs and PCDFs due to accidental ingestion of contaminated rice oil in Taiwan, the Yucheng cohort. A total of 174 Yucheng and 134 control subjects were studied in an effort to determine the effectiveness of using induction of the cytochrome P450 1 (CYP1) family of enzymes as a biomarker of exposure and effect in that cohort. Because CYP1A2 activity cannot be measured directly in humans, the caffeine breath test (CBT), a marker for CYP1A2 activity, was conducted. 3-N-demethylation of caffeine is catalyzed by CYP1A2, so measurement of the proportion of exhaled ^{13}C-labeled caffeine metabolites within a given time period (1 hour) following a known dose of ^{13}C-labeled caffeine serves as a measure of that enzyme's activity (Landi et al. 1999). Increasing enzyme activity (as reflected by increased caffeine metabolism rate) with increasing serum TEQ may represent aryl hydrocarbon receptor (AhR)-mediated induction of CYP1A2. These data constitute an evaluation of TEQ levels that can alter AhR-mediated gene expression in a human population as expressed by an early biochemical response that is known to be directly linked to AhR activitation. Serum measurements included

17 PCDD/Fs and PCBs 77, 81, 126, 169, 105, 118, 156, 157, 167, and 189. Total dioxin TEQ was calculated based on the 1998 WHO TEFs (van den Berg et al. 1998). Lambert et al. (2006) presented a linear regression of % of ^{13}C-labeled caffeine dose metabolized in an hour versus ppt serum TEQ. A BMD_{10} for CYP1A2 induction was developed using this regression together with reported information on CBT variation among the controls.

Developmental defects of tooth enamel. Alaluusua et al. (2004) examined developmental defects of tooth enamel among subjects who were < 5 years old (representing the age window during which development of permanent teeth occurs) at the time of exposure to TCDD following the explosion of a trichlorophenol production reactor in Seveso, Italy in 1976. The endpoint is of interest because a number of animal studies have demonstrated dental defects in rats exposed during the developmental period (reviewed in Alaluusua and Lukinmaa 2006) and because the Seveso population presents one of the few situations in which well-characterized exposure to TCDD has occurred in a non-occupational population. Dental examinations conducted twenty-five years after the accident were reported for 36 individuals who lived within the "ABR" zone—the area exposed during the accident—and 39 individuals who lived outside the ABR zone. The authors reported TCDD levels (but not levels of other dioxin congeners) for those subjects based on serum that had been collected and frozen in 1976, and presented a categorical analysis by level of measured serum lipid TCDD in four exposure categories: non-ABR zone (background exposure), 31-226 ng/kg TCDD, 238-592 ng/kg TCDD, and 700-26,000 ng/kg TCDD. No individual measurements of other TEQ-contributing congeners were made in the Seveso serum samples and no serum TCDD or TEQ measurements were available for the reference individuals. Therefore, we used the concentrations of 17 PCDD/F compounds and nine TEQ-contributing PCB congeners measured in pooled serum samples for children aged 1-12 collected during the 1970s period from outside the Seveso area as reported by Eskenazi et al. (2004). We assumed that Seveso children had similar serum levels of these non-TCDD congeners at the time of the accident and therefore estimated non-TCDD TEQ concentrations for Seveso residents as well as average total TEQ for the non-ABR reference individuals based on the data from Eskenazi et al. (2004). Because exposures are likely to be lognormally distributed, we took the anti-log of the average of the log of the minimum and maximum of each categorical exposure range to represent a central estimate of exposure within each category. Benchmark dose modeling was conducted to estimate the serum lipid TEQ concentration corresponding to a 10 percent extra risk of dental defects using U.S. EPA Benchmark Dose Software (version 1.4.1b with a variety of dichotomous models, with slopes restricted to be non-negative in order to allow for the possibility of non-zero defect levels at zero exposure).

Thyroid hormone concentrations in infants. Koopman-Esseboom et al. (1994) examined thyroid hormone levels in 78 two-week-old infants from the general population in Rotterdam between 1990 and 1992 and related them to TEQ in their mothers' milk (which was considered to represent lipid-adjusted TEQ of the mother and therefore a marker for in utero exposure levels). The mother-infant pairs were divided into two exposure groups: low (maternal milk TEQ less than or equal to the median, 72.43 pg TEQ/g lipid) and high (maternal milk TEQ >72.43 pg TEQ/g lipid), and the mean and standard deviation of infant free thyroxine (FT_4) concentrations were reported for each group. Based on the reported mean, median, and standard deviation of maternal TEQ concentration, and assuming an overall lognormal distribution of TEQ concentrations in maternal milk, we estimated the mean milk TEQ concentrations in the lower and upper exposure groups and assumed a simple linear relationship between maternal milk TEQ and infant FT_4 concentrations. We assume that the population variation in FT_4 at any particular TEQ level is normally distributed with a degree of variability around its mean that is independent of TEQ and is equal to the average of the reported standard deviations of FT_4 within each of the two exposure groups ("low" and "high"). The effect of TEQ on mean FT_4 is calculated by the regression line between the "high" and "low" exposure groups. Maternal milk lipid-adjusted TEQ concentrations were assumed to reflect maternal serum TEQ concentrations, an assumption that appears to be approximately correct, although some differential partitioning between breast and serum lipids occurs for higher chlorinated congeners (Wittsiepe et al. 2007). This differential partitioning likely has a limited effect on total TEQ in human serum and milk due to the relatively low contribution of higher chlorinated congeners.

Characterization of changes in margin of exposure

The margin of exposure in a population is a unitless ratio between the point of departure and the estimate of dose or exposure in that population:

$$MOE = \frac{POD}{Dose} \qquad [1]$$

For this effort, both the POD and current exposure estimates are presented in terms of lipid-adjusted TEQ (WHO 1998 TEFs) concentration, and an estimate of the current MOE, as well as discussion of change in MOE from the 1970s era, are presented.

Results

Exposure

The adipose tissue samples analyzed by Kang et al. (1990) do not represent either a specific sampling year or specific age group, but can generally be described as representing 1970s TEQ concentrations in males born between 1936 and 1954 (with a general age range at that time from 20 to 45 years). Descriptive statistics on the distribution of lipid-adjusted TEQ concentrations (using the WHO 1998 TEF values) are presented in Table 1 and Figure 2. No information on detection limits was provided, and non-detects were included in the calculations as "0". Thus, the calculated TEQ concentrations may underestimate actual concentrations for this age group during this time period.

The results of the analysis of the NHANES dataset for PCDD/F TEQ are also presented in Table 1. Data were analyzed for two groups: a) Adults aged 20 to 45 at the time of the 2001-2002 sampling effort, and b) adults who were born between 1936 and 1954. These two groups correspond to the age range and birth cohort, respectively, included in the Kang et al. (1990) dataset. Because the NHANES dataset relied on very small serum sampling volumes, limits of detection were quite high for many congeners. Ferriby et al. (2007) demonstrated that, particularly for individuals with relatively lower concentrations (for example, younger individuals), the choice of how to replace non-detected concentrations in TEQ calculations could affect estimated TEQs by 50 to 100 percent. We used two approaches, estimating percentiles under the assumption that non-detectable concentrations were zero (as used for the Kang et al. dataset, for which no information on detection limits was available) and assuming that non-detects were equivalent to the limit of detection divided by $\sqrt{2}$. For the younger age groups, choice of replacement for non-detectable concentrations results in nearly a 100 percent increase in the estimate of current median serum lipid concentrations. Upper bound estimates are less sensitive to the assumption.

Table 1: Median and 95th percentile of measured lipid-adjusted PCDD/F TEQ concentrations from sampling conducted in the US in the 1970s and from 2001 to 2005.

		WHO_{1998} TEQ, PCDD/F Median (95th %ile)	
	Sampling year(s)	**ND=0**	**ND=LOD/√2**
Birth cohort, 1936-1954			
Kang et al. (1990)	1971-1982	60.2	
NHATS		(151.1)	
NHANES	2001-2002	12.4 (43.5)	17.2 (44.5)
UMDES Referents, birth year <1940	2005		25.7 (46.7)
UMDES Referents, birth years 1940-1960	2005		17.6 (30.1)
Adults aged 20-45			
Kang et al. (1990)	1971-1982	60.2	
NHATS		(151.1)	
NHANES, ages 20-45	2001-2002	5.9 (22.4)	11.7 (26.7)
UMDES Referents, ages 18-29	2005		7.0 (9.7)
UMDES Referents, ages 30-44	2005		12.2 (20.2)

The data from the UMDES study reference population (Jackson and Calhoun counties, sampling in 2005) are available only in summary form for specific age groups (18-29, 30-44, 45-59, and 60+ years). These age breakdowns allow a fairly direct comparison for the 20 to 45 age group, but are less useful for describing the concentrations in individuals born between 1936 and 1954 (ages 51 to 69 in 2005), particularly due to the substantial age-related changes in serum dioxin concentrations in persons over 40 (Ferriby et al. 2007). Summary statistics for the relevant overlapping age groups from the UMDES study are also presented in Table 1. In this study, substantial serum volumes were available for all analyses, so TEQ estimates are not affected substantially by the presence of non-detectable concentrations.

Figure 2 presents boxplots comparing the 1970s data with the current data sets for persons aged 20 to 45 during both time periods (Figure 2A) and for persons born during the 1936 to 1954 period over time (Figure 2B). Within individuals born between 1936 and 1954, both median and 95th percentile PCDD/F TEQ concentrations between the 1970s-era sampling and the NHANES 2001-2002 sampling years decreased by approximately 75%. Serum lipid TEQ

concentrations in persons aged 20 to 45 in the 2001-2002 NHANES dataset decreased by approximately 85% compared to the 1970s-era sampling, a difference reflected in both the medians and 95th percentiles. These comparisons do not include PCB contributors to TEQ, but a comparison of wet weight serum PCB concentrations observed in studies conducted in the 1970s (Kreiss 1985) with results from the NHANES 2001-2002 data sets (Nichols et al. 2007) suggests a similar or larger magnitude of decline in serum PCB concentrations over the same time period, although no statistically representative datasets are available to specifically assess changes in TEQ-contributing PCB congener concentrations over that time period.

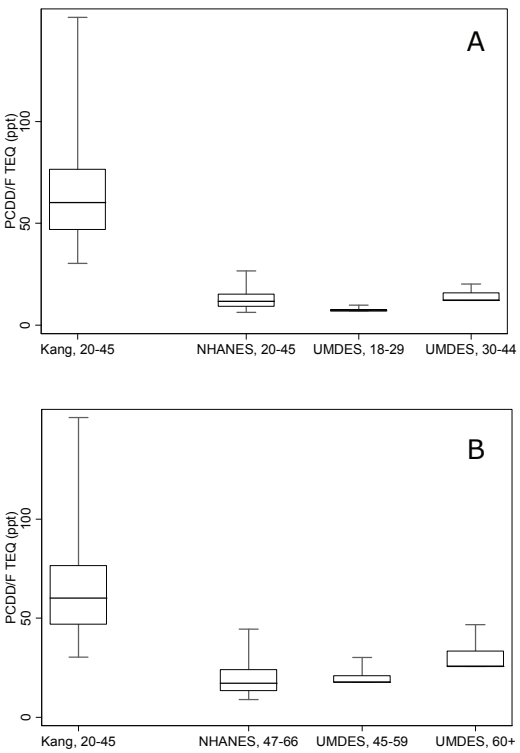


Figure 2: Box plots illustrating the distribution of measured PCDD/F TEQ (WHO 1998 system) concentrations in samples from individuals aged 20 to 45 (A) or born between 1936 and 1954 (B) in the general population in the US. Boxes represent the interquartile range (25th to 75th percentiles; median indicated by horizontal line) and whiskers extend to the 5th and 95th percentiles. Key: Kang: lipid adjusted adipose tissue concentrations from NHATS samples collected between 1971 and 1982; NHANES: lipid adjusted serum concentrations from the NHANES 2001-2002 survey assuming non-detects are present at LOD/√2 ; UMDES: lipid adjusted serum concentrations from samples collected in 2005 from residents of referent counties in Michigan from the University of Michigan Dioxin Exposure Study; note, 5th and 25th percentiles were not reported from this study, and so are not included in the boxplots here. Numbers indicate age range of included individuals.

The decrease in serum lipid TEQ observed among persons born between 1936 and 1954 indicates that ambient exposures have declined substantially since the 1970s. Because the elimination of PCDD/F compounds occurs with half-lives on the order of 5 to 10 years, declines in serum lipid TEQ will continue for many years following decreases in exposure levels (Aylward and Hays 2002; Lorber 2002). As a result, serum TEQ concentrations in the population in the future will likely decline further, and evaluations of MOE for the purposes of assessing potential risks from current intake rates should be based on measured concentrations in younger individuals with the least impact from historically higher exposure levels. As discussed above, the UMDES study appears to provide the most reliable dataset for characterizing current serum concentrations in young adults, owing to the very low detection limits attained in this study. The median and 95th percentile total TEQ (according to the WHO 1998 TEF scheme, including PCB contributors to TEQ) reported for the UMDES study referent population aged 18 to 29 based on 2005 sampling are 9.2 and 13.3 ppt TEQ, respectively. These concentrations are used below to characterize current MOE in the young adult population of reproductive age in the US, a population of high interest for risk assessment of dioxins.

Response benchmark characterization

CYP1A2 induction. Lambert et al. (2006) presented a linear regression of % of ^{13}C-labeled caffeine dose metabolized in an hour versus ppt serum TEQ (WHO 1998 TEQ) with a reported slope of 0.0029 and an intercept of 1.17 % of dose metabolized. An estimate of the pooled (male and female) weighted average and standard deviation of CBT in the 134 controls can be derived from data presented in the paper (1.18% ± 1.20) of the CBT in male and female controls. If the distribution of CBT is normal, then the 97.5th percentile of the amount metabolized in one hour is approximately 3.5%; this appears to be generally consistent with the data presented in graphical form by Lambert et al. (2006).

Assuming that this degree of inter-individual variability applies at any specific dioxin serum level (as appears reasonable from examination of Lambert et al. Figure 1), then a BMD_{10} can be calculated as the serum TEQ necessary to shift the distribution such that an additional 10% of the population has a CBT exceeding 3.5%. Using the regression equation calculated by Lambert et al. and the assumption of normal variability in the CBT outcome around its mean value yields an estimate of serum TEQ BMD_{10} of approximately 340 ng/kg lipid. Assumption of lognormal distribution of values around the mean results in a BMD_{10} estimate of approximately 400 ppt TEQ.

Dental defects. Table 2 presents the prevalence of dental aberrations, the measured TCDD and estimated serum lipid TEQ ranges, and estimated

midpoint TEQ concentrations, by category from Alaluusua et al. (2004). The results of the benchmark dose modeling for the dataset are presented in Table 3. The log-logistic model produced the best fit to the data (i.e., the lowest AIC value), and three of the models resulted in slightly higher BMD_{10} estimates.

Table 2: Developmental defects of enamel in individuals who were children (< age 5) at the time of the Seveso accident, referents and Seveso residents by tertile (from Alaluusua et al. 2004)

Exposure group (n)	Range of serum TCDD ng/kg lipid	Estimated median TCDD concentration ng/kg lipid[a]	Estimated TEQ ng/kg lipid[b]	Prevalence (%)
Non-ABR subjects (39)	NM	40.5	116.6	10 (26)
Seveso -1st tertile (10)	31-226	83.7	159.8	1 (10)
-2nd tertile (11)	238-592	375.4	451.5	5 (45)
-3rd tertile (15)	700-26,000	4266.1	4342.2	9 (60)

NM -- Not measured

[a] Assumes lognormal distribution of serum TCDD measurements; see Methods.

[b] Estimated based on data from Eskenazi et al. (2004). Average non-TCDD TEQ (16 PCDD/F and 9 PCB compounds) measured in two pooled serum samples from children aged 1 to 12 from outside Seveso collected during the Seveso time period was 76.1 ng TEQ/kg lipid; this value was added to estimated median TCDD values for each Seveso exposure group. Total TEQ (including background TCDD) averaged 116.6 ng/kg TEQ in the two pooled samples; this value was used as the TEQ concentration for the non-ABR referents.

Table 3: BMD_{10} Modeling results for dental defects data from Alaluusua et al. 2004

Model	BMD_{10}	$BMDL_{10}$	AIC
Gamma (power ≥ 1)	666.2	330.8	93.1582
Log-Logistic (slope ≥ 1)	445.3	140.1	92.9532
Multistage (1-degree, beta ≥ 0)	666.2	330.8	93.1582
Probit (slope ≥ 1)	1296.9	636.3	93.6437
Weibull (power ≥ 1)	666.2	330.8	93.1582

Thyroid hormone concentrations. For the dose-response analysis for FT_4 from Koopman-Esseboom et al. (1994) we estimated mean exposures for both exposure groups. Koopman-Esseboom et al. (1994) reported maternal milk TEQ concentrations using the 1993 WHO TEF values. However, the changes in the WHO TEF values between 1993 and 1998 result in offsetting changes in estimates of TEQ (approximately a 15% increase in estimates of PCDD/F TEQ, and a similar decrease in PCB TEQ estimates at the mean, due to the changes in TEF values between the two versions of the TEF system). Thus, the TEQ concentrations reported in that study are used directly in this analysis, on the grounds that the WHO-1998 TEQ, if it could be calculated from the reported data, would be expected to be similar. Because the mother-infant pairs were drawn from the local population without prior knowledge of exposure, we assumed a lognormal distribution. Using the arithmetic mean (74.86 pg TEQ/kg lipid) and standard deviation (26.19) for milk TEQ reported for the overall cohort we calculated a geometric mean (70.66 pg TEQ/kg lipid) and geometric standard deviation (1.405). This estimated geometric mean corresponds well with the reported median of 72.43 pg TEQ/kg lipid and the lognormal assumption predicts the observed range of the milk TEQ values well. Assuming this lognormal distribution for the overall cohort, mean TEQs for the low- and high-exposure groups were estimated to be 56.3 pg TEQ/g lipid and 97 pg TEQ/g lipid, respectively, as illustrated in Figure 3.

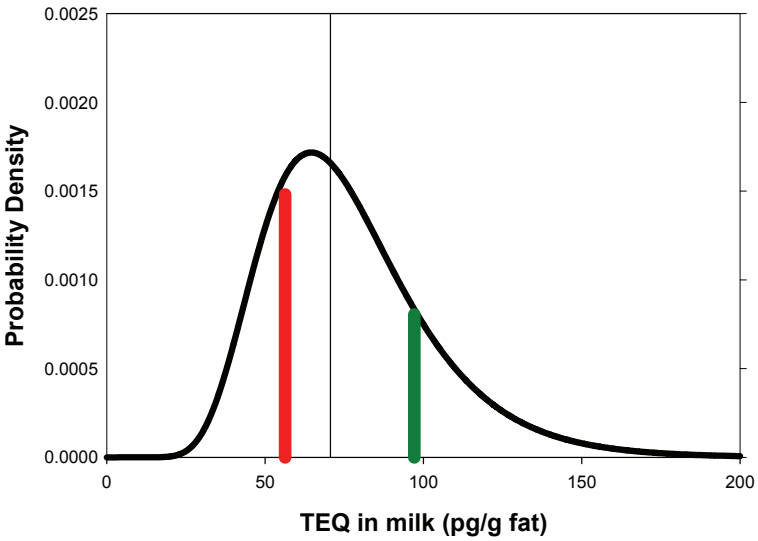


Figure 3: Estimated probability distribution of lipid-adjusted human milk concentrations from Koopman-Esseboom et al. (1994) based on summary statistics provided in the publication (see text for discussion).

Assuming that the relationship between FT_4 and milk TEQ is linear, the two calculated mean exposure levels and corresponding means of the measured FT_4 concentrations for the low- and high-exposure groups define a regression line; increasing levels of TEQ were associated with decreasing levels of FT_4. The slope of the regression line is -0.0392 pmol/L per pg TEQ/g lipid in milk and the intercept is 26.8 pmol/L.

For a given TEQ there will be natural variation in FT_4 levels among infants. Standard deviations for measured FT_4 concentrations were given by Koopman-Esseboom et al. (1994) as 3.5 pmol/L for the low-exposure group and 3.3 pmol/L for the high-exposure group. The BMD_{10} is defined in this case as the TEQ exposure that would change the mean FT_4 such that an additional 10% of the variable population drops below the FT_4 level that constitutes the 2.5th percentile in an unexposed population (with zero exposure). To do this, we assume that the population variation in FT_4 at each exposure level is normally distributed with a mean calculated by the regression line and a standard deviation calculated as the average of the standard deviations for the two measured data points, namely (3.5+3.3)/2=3.4 pmol/L. We assume further that the variability around the regression is independent of TEQ.

At an exposure level of zero TEQ the mean FT_4 is estimated to be 26.8 pmol/L (the regression-line intercept) and, using the observed standard deviation of 3.4 pmol/L (mean of the two provided standard deviations), the 2.5th percentile FT_4 level is 20.1 pmol/L. This value is consistent with published neonatal thyroid hormone reference ranges (Hubner et al. 2002). The BMD_{10} is then the TEQ exposure at which an extra 10% of the population has FT_4 concentrations $<=$20.1 pmol/L, which occurs when the mean FT_4 concentration is 24.0 pmol/L and corresponds to a BMD_{10} of approximately 70 pg TEQ/g lipid in milk.

MOE characterization

The BMD_{10} values calculated for each endpoint are presented in Table 4. These values are best estimates from the specific studies and datasets using the assumptions outlined in this manuscript, and are provided as examples of potential quantitative approaches to a variety of epidemiological data sets that are available. Table 4 also provides an overview of the estimated margins of exposure for each of the endpoints based on estimates of current serum lipid TEQ concentration (including PCDD/F and PCB compounds) in young adults at the median and at the 95^{th} percentile. MOEs for the same endpoints in the 1970s would have been approximately six to seven times lower, based upon the comparative exposure data summarized above.

Table 4: Summary of modeled BMD_{10} values for three endpoints and estimated MOEs at the median and upper bound of current lipid-adjusted TEQ concentrations in young adults of reproductive age in the US for three endpoints based on example data sets.

		MOE[a]	
Endpoint	**BMD_{10} ng TEQ/kg lipid**	**At median current US TEQ (9.2 ppt)**	**At 95%ile current US TEQ (13.3 ppt)**
CYP1A2 activity	340	35	25
Dental defects	450-1300[b]	50-140	30-95
Neonatal FT_4 changes	70	8	5

[a] Compared to current median and 95%ile exposures as estimated by UMDES reference population aged 18-29, 29 PCDD/F and PCB congeners, 1998 WHO TEQ. Median: 9.2 ppt. 95%ile: 13.3 ppt.
[b] Range of BMD_{10} estimates from different benchmark dose models.

Discussion

Consideration of Target MOE

The MOE estimates presented in Table 4 can be compared to a "target" minimal MOE related to the usual application of uncertainty factors in the derivation of reference doses, minimal risk levels, and other benchmarks for general population environmental exposures. Table 5 outlines the typical uncertainty factors applied, and provides a discussion of the applicability of such factors to a risk assessment based on human data using an internal measure of biologically relevant exposures. Selection of a target MOE requires a judgment regarding the biological relevance or adversity of the modeled benchmark response, so that larger or smaller components of the "LOAEL to NOAEL" uncertainty factor might be selected for a given endpoint or selected benchmark response level (e.g., a 10% increase in the proportion of the population exhibiting increased enzyme activity might be considered a less adverse benchmark than a 10% increase in the proportion exhibiting decreased FT_4 concentrations). Because both the dose-response assessment and the exposure assessments for this evaluation are based upon biologically relevant internal dose metrics (circulating serum TEQ concentration), the uncertainty factor component typically applied for intra-species toxicokinetic differences is replaced by the actual sampling data in the population, which explicitly reflects the variations in toxicokinetics among individuals. That is, individuals who are pharmacokinetically "sensitive" (presumably, those who eliminate the compounds more slowly) directly reflect this sensitivity in the

measured serum lipid concentrations, which will be higher than those in the less-sensitive members of the population.

The current MOEs estimated for CYP1A2 and for dental defects are generally above the range of target minimal MOEs, suggesting that these endpoints may not be of concern at current background serum concentrations. However, the current MOEs for thyroid hormone alterations in infants based on the Koopman-Esseboom et al. (1994) data and current US exposure data are at the lower end of the range of target MOE values, although there is a clear separation between current US concentrations in young adults of reproductive age and the BMD_{10} for this endpoint. That is, the current upper bound TEQ concentration in young adults of reproductive age in the US (approximately 14 ppt TEQ) is approximately five-fold lower than the BMD_{10}. Recent studies of this endpoint provide conflicting results: Maervoet et al. (2007), in a study of 140 Flemish infants, report an association between maternal serum TEQ and umbilical cord FT_4 of similar magnitude, while Wilhelm et al. (in press) report no relation between maternal milk or blood TEQ and cord blood thyroid hormone concentrations in a smaller study of German infants. Other researchers have also found conflicting results (reviewed in Giacomini et al. 2006 and Maervoet et al. 2007). The high degree of correlation between serum TEQ and non-TEQ-contributing organochlorine compounds complicates the identification of causal agents and relationships. Other factors not typically controlled for in such studies, such as gestational age and serum lipid concentration, may also confound the relationships observed (reviewed in Maervoet et al. 2007).

The BMD_{10} estimates presented here in terms of serum lipid TEQ can be compared with the adipose tissue concentrations reported in key animal studies previously used to derive the JECFA, UKCOT, and ECSCF tolerable daily intakes. Maternal wet-weight adipose tissue concentrations in the key studies at the LOAELs or NOAELs can be estimated as ranging from approximately 65 to 200 ppt TCDD (Faqi et al. 1998; Hurst et al. 2000). If these wet-weight concentrations are adjusted assuming 80% lipid content of adipose tissue, these correspond to approximately 80 to 250 ppt TEQ, comparable to the range of benchmark doses derived here (Table 4).

Table 5: Evaluation of typical uncertainty factor components in the context of identification of target minimal MOE for risk assessments based on human studies employing serum lipid TEQ concentrations as the exposure metric.

Uncertainty Factor Component	Typical Value	Applicable?	Target Value
LOAEL to NOAEL	up to 10	Data-dependent. Depending upon the benchmark chosen, the point of departure may be regarded as more similar to a NOAEL or a minimal or frank LOAEL.	Varies by endpoint 1-10
Interspecies	10	No – risk assessment based on human data	1
Intraspecies – PK	3	No – interindividual variations in pharmacokinetics are directly reflected in measured serum lipid TEQ concentrations. Pharmacokinetically "sensitive" individuals will manifest higher measured concentrations, so the sensitivity is reflected directly in the exposure assessment.	1
Intraspecies – PD	3	Yes, although if the study used as the basis of the BMD determination includes the sensitive subpopulation, this value may be reduced.	1-3
Target MOE:			1-30

The assessment of current exposures based on measured serum lipid concentration has several strengths over conventional exposure assessments based on intake. Such measurements aggregate exposure from all exposure routes and media, which individually may be difficult to quantify due to low concentrations. Reliance on measured serum concentrations also reduces uncertainties in risk assessment regarding potential interindividual pharmacokinetic differences, because such differences are directly reflected in the measured concentrations. Finally, circulating serum lipid concentration is likely to be a good surrogate for critical target tissue exposure concentrations in a range of organs and tissues due to the lipophilic nature of dioxin-like compounds.

When interpreting these results, some limitations that are inherent in the use of measured TEQ concentrations for exposure quantification should be borne in mind. The TEF system was developed based on relative potencies on the basis of intake, not tissue concentration. Thus, differential toxicokinetics among

congeners can lead to distortions in the estimate of total toxic equivalency when TEQ is assessed on tissue concentration basis rather than an intake basis. Such distortions might be of limited concern when the dose-response assessment is conducted on populations with similar distributions of congeners as the current background population. However, for two of the data sets used here, the Lambert et al. (2006) data on CYP1A2 activity and the Seveso data on dental defects, the TEQ tissue concentrations resulted from unusual exposures to congeners or mixtures that are not reflective of background exposure patterns, and thus extrapolation of responses observed in those populations to background exposures on a serum TEQ concentration basis may pose additional uncertainties.

Interpretation of results from epidemiological studies must proceed cautiously because of the complexities inherent in such studies: cross-sectional epidemiological studies are not analogous to toxicological experiments, and data from single studies cannot be interpreted in isolation. Numerous factors as outlined by Hill (1965) including consistency of evidence across studies, biological plausibility, and accounting for potential confounding, must be assessed. The three examples presented here represent data sets on endpoints with varying degrees of consistency in the epidemiological literature. Induction of CYP1A2 activity in humans has been observed previously in individuals with highly elevated exposures (reviewed in Connor and Aylward 2006), and the magnitude of dose-response reported in the Lambert et al. (2006) study is consistent with previous reports. The pattern of evidence related to potential impacts of dioxin-like compounds on infant thyroid hormone levels is less consistent, as discussed above. The dental anomalies reported by Alaluusua et al. (2004) in Seveso children are biologically plausible based on results from studies in rodents, and data from the Seveso study and other studies support a conclusion that exposures above current background exposures would be required to induce such effects (Alaluusua and Lukinmaa 2006).

The data sets used to characterize dose-response in this exercise are typical of many epidemiological studies in that data are not generally presented in a framework that allows direct application of quantitative risk assessment methodologies. Several common epidemiological methods used in such studies pose challenges in the risk assessment context.

When exposures and responses are presented in categorical analyses (for example, in the analyses by Koopman-Esseboom et al. 1994, and Alaluusua et al. 2004), evaluation of dose-response and identification of benchmark doses requires numerous assumptions regarding representative exposures in each category. For example, in the assessment of the dental defect data for Seveso, we estimated the median TCDD concentration in each exposure category based on an assumption of an overall lognormal distribution of

exposures. However, other assumptions could have been used and the estimated BMD values would change accordingly.

In continuous analyses, full regression results are often not reported, making it difficult to estimate benchmark doses at appropriate values of important covariates. In addition, such regressions typically impose a shape on the fitted dose-response curve, including an assumption of linearity that extends to the lowest exposure levels. Because the slope of a linear regression is often dominated by responses at the higher end of exposures, the possibility of a zero slope at lower exposures (in effect, the existence of a threshold for the response) is masked in the regression.

Characterization of the inter-individual variability in the response parameter of interest, including description of the distribution type (normal, lognormal) and the magnitude of typical variability, is often lacking. In particular, information sufficient to evaluate interindividual variability in a parameter in the absence of the influence of the exposure of interest is generally not provided.

In many epidemiological studies, results are reported as odds ratios. Conversion of relationships reported in this way into continuous functions that can be used to estimate benchmark doses can be difficult or impossible, depending upon what other information is provided. Similarly, epidemiological studies often report results using analyses based on logarithmically transformed exposure measures. Such results are difficult to extrapolate beyond the range of the experimental data with any confidence. For example, such dose-response functions imply that a change in exposure from 0.1 to 0.3 ppt TEQ might be associated with the same magnitude of response as an increase in exposure from 100 to 300 ppt, which may not be biologically plausible. When attempting to estimate background responses, such functions may not be appropriate for extrapolation from higher exposures.

A comprehensive risk assessment effort based on the available human data sets would require a systematic assessment of the consistency and weight of the evidence and human relevance of each endpoint before quantitative analysis of the data sets was conducted. Quantitative analysis could then proceed on endpoints with strong or moderate levels of evidence to provide a full quantitative context for interpretation of exposure data.

The analysis presented here demonstrates that young adults in the US population have current serum TEQ concentrations six-fold or more lower than adults of the same age in the 1970s. Thus, the margins of exposure for all potential effects of dioxin-like compounds have increased by a factor of six over the past three decades. Because a substantial body of epidemiological studies of potential health impacts of dioxins relying upon measured serum TEQ as the exposure metric now exists, such exposure data can be used

directly in the risk assessment arena to assess margins of exposure for responses of interest. Some uncertainties arise in using such data in a risk assessment context because of limitations in reporting exposure or response parameters in such studies. However, application of risk assessment methodologies to this body of epidemiological data avoids uncertainties inherent in interspecies extrapolation and in accounting for pharmacokinetic variability that may result in differential impacts of a given estimated intake dose.

References

Alaluusua S, Calderara P, Gerthoux PM, Lukinmaa PL, Kovero O, Needham L, et al. 2004. Developmental dental aberrations after the dioxin accident in Seveso. Environ Health Perspect 112(13):1313-1318.

Alaluusua S, Lukinmaa PL. 2006. Developmental dental toxicity of dioxin and related compounds--a review. Int Dent J 56(6):323-331.

Aylward LL, Hays SM. 2002. Temporal trends in human TCDD body burden: Decreases over three decades and implications for exposure levels. J Expo Anal Environ Epidemiol 12:319-328.

Charnley G, Kimbrough RD. 2006. Overview of exposure, toxicity, and risks to children from current levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds in the USA. Food Chem Toxicol 44(5):601-615.

Connor KT, Aylward LL. 2006. Human response to dioxin: aryl hydrocarbon receptor (AhR) molecular structure, function, and dose-response data for enzyme induction indicate an impaired human AhR. J Toxicol Environ Health B Crit Rev 9(2):147-171.

Eskenazi B, Mocarelli P, Warner M, Needham L, Patterson DG, Samuels S, et al. 2004. Relationship of serum TCDD concentrations and age at exposure of female residents of Seveso, Italy. Environ Health Perspect 112(1):22-27.

ECSCF (European Commission Scientific Committee on Foods), 2001. Opinion of the Scientific Committee on Food on the risk assessment of dioxins and dioxin-like PCBs in food. Update based on new scientific information available since the adoption of the SCF opinion of 22nd November 2000. Rep. CS/CNTM/DIOXIN/20 final. Available: http://europa.eu.int/comm/food/fs/sc/scf/out90_en.pdf [accessed 15 January 2008]. Brussels, Belgium: European Commisssion.

Faqi AS, Dalsenter PR, Merker HJ, Chahoud I. 1998. Reproductive toxicity and tissue concentrations of low doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin in male offspring rats exposed throughout pregnancy and lactation. Toxicol Appl Pharmacol 150(2):383-392.

Ferriby LL, Knutsen JS, Harris M, Nony P, Unice KM, Paustenbach DJ, et al. 2007. Evaluation of PCDD/F and dioxin-like PCB serum concentration data from the 2001-2002 National Health and Nutrition Examination

Survey of United States citizens. J Exp Sci Environ Epidemiol 17(4):358-371.
Furst P, Wilmers K. 1997. Decline of human PCDD/F intake via food between 1989 and 1996. Organohalogen Compounds 33:116-121.
Gaylor DW, Aylward LL. 2004. An evaluation of benchmark dose methodology for non-cancer continuous-data health effects in animals due to exposures to dioxin (TCDD). Regul Toxicol Pharmacol 40:9-17.
Giacomini SM, Hou L, Bertazzi PA, Baccarelli A. 2006. Dioxin effects on neonatal and infant thyroid function: routes of perinatal exposure, mechanisms of action and evidence from epidemiology studies. Int Arch Occup Environ Health 79(5):396-404.
Hays SM, Aylward LL. 2003. Dioxin risks in perspective: past, present, and future. Regul Toxicol Pharmacol 37(2):202-217.
Hill AB. 1965. The environment disease: Association or causation. Proceed Royal Soc Med 58:295-300.
Hubner U, Englisch C, Werkmann H, Butz H, Georgs T, Zabransky S, et al. 2002. Continuous age-dependent reference ranges for thyroid hormones in neonates, infants, children and adolescents established using the ADVIA Centaur Analyzer. Clin Chem Lab Med 40(10):1040-1047.
Hurst CH, DeVito MJ, Birnbaum LS. 2000. Tissue disposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in maternal and developing long-evans rats following subchronic exposure. Toxicol Sci 57(2):275-283.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2001. Fifty-seventh meeting Rome, 5-14 June 2001. Summary and conclusions. Available: http://www.who.int/ipcs/food/jecfa/summaries/en/summary_57.pdf [accessed 15 January 2008].
Kang HK, Watanabe KK, Breen J, Remmers JA, Stanley J, Flicker M. 1990. Dioxins and Dibenzofurans in Adipose Tissue of U.S. Vietnam Veterans and Controls. NTIS PB-91167585.
Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van der Paauw CG, Tuinstra LG, et al. 1994. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res 36(4):468-473.
Kreiss K. 1985. Studies on populations exposed to polychlorinated biphenyls. Environ Health Perspect 60:193-199.
Lambert GH, Needham LL, Turner W, Lai TJ, Patterson DG, Jr., Guo YL. 2006. Induced CYP1A2 activity as a phenotypic biomarker in humans highly exposed to certain PCBs/PCDFs. Environ Sci Technol 40(19):6176-6180.
Landi MT, Sinha R, Lang NP, Kadlubar FF. 1999. Human cytochrome P4501A2. IARC Sci Publ 148:173-195.
Liem AK, Furst P, Rappe C. 2000. Exposure of populations to dioxins and related compounds. Food Addit Contam 17(4):241-259.

Lorber M. 2002. A pharmacokinetic model for estimating exposure of Americans to dioxin-like compounds in the past, present, and future. Sci Tot Environ 288:81-95.

Maervoet J, Vermeir G, Covaci A, Van Larebeke N, Koppen G, Schoeters G, et al. 2007. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. Environ Health Perspect 115(12):1780-1786.

Nichols BR, Hentz KL, Aylward L, Hays SM, Lamb JC. 2007. Age-specific reference ranges for polychlorinated biphenyls (PCB) based on the NHANES 2001-2002 survey. J Toxicol Environ Health A 70(21):1873-1877.

Patterson DG, Jr., Needham LL, Pirkle JL, Roberts DW, Bagby J, Garrett WA, et al. 1988. Correlation between serum and adipose tissue levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in 50 persons from Missouri. Arch Environ Contam Toxicol 17(2):139-143.

Risk Commission. 1997. U.S. Commission on Risk Assessment and Risk Management. Final Report, Vol 1. Framework for Environmental Health Risk Management. GPO #055-000-00567-2. Washington, DC:U.S. Commission on Risk Assessment and Risk Management.

Siparsky G, Accurso FJ. 2007. Chapter 43. Chemistry & hematology reference intervals. In Current Pediatric Diagnosis and Treatment, 18th Edition (Hay WW, Levin MJ, Sondheimer JM, Deterding RR, eds). New York:McGraw Hill, Available: http://www.accessmedicine.com/content.aspx?aid=2356943 [accessed 16 May 2008].

Smith AH, Lopipero P. 2001. Evaluation of the Toxicity of Dioxins and Dioxin-like PCBs: A Health Risk Appraisal for the New Zealand Population. A report to the Ministry for the Environment, Wellington, New Zealand. Available: http://www.mfe.govt.nz/issues/waste/ocreports.htm [accessed 15 January 2008].

UMDES (University of Michigan Dioxin Exposure Study), 2008. . Available: http://www.sph.umich.edu/dioxin/index.html [accessed 15 January 2008].

UKCOT (United Kingdom Committee on Toxicology), 2001. Statement on the tolerable daily intake for dioxins and dioxin-like polychlorinated biphenyls. Available: http://www.food.gov.uk/science/ouradvisors/toxicity/statements/cotstatements2001/dioxinsstate [accessed 15 January 2008].

U.K. FSA (United Kingdom Food Standards Agency), 2003. Dioxins and dioxin-like PCBs in the UK diet: 2001 total diet study samples. Report 38/03.

U.S. EPA (United States Environmental Protection Agency), 2000. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin and Related Compounds. Draft Final EPA/600/P-00/001Be. Washington, D.C.: National Center for Environmental Assessment, U.S. Environmental Protection Agency.

U.S. EPA (United States Environmental Protection Agency), 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin and Related Compounds. NAS Review Draft. Washington, D.C.: National Center for Environmental Assessment, U.S. Environmental Protection Agency.

U.S. EPA (United States Environmental Protection Agency), 2006. An Inventory of Sources and Environmental Releases of Dioxin-Like Compounds in the United States for the Years 1987, 1995, and 2000. National Center for Environmental Assessment, Office of Research and Development. EPA/600/P-03/002F.

U.S. FDA (United States Food and Drug Administration), 2008. PCDD/PCDF Exposure Estimates from TDS Samples Collected in 2001-2004. U.S. Food and Drug Administration Center for Food Safety and Nutrition. Available: http://www.cfsan.fda.gov/~lrd/dioxee.html [accessed 15 January 2008].

Van den Berg M, Birnbaum L, Bosveld AT, Brunstrom B, Cook P, Feeley M, et al. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 106(12):775-792.

Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, et al. 2006. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 93(2):223-241.

Wilhelm M, Wittsiepe J, Lemm F, Ranft U, Kramer U, Furst P, et al. In press. The Duisburg birth cohort study: Influence of the prenatal exposure to PCDD/Fs and dioxin-like PCBs on thyroid hormone status in newborns and neurodevelopment of infants until the age of 24 months. Mutat Res.

Wittsiepe J, Furst P, Schrey P, Lemm F, Kraft M, Eberwein G, et al. 2007. PCDD/F and dioxin-like PCB in human blood and milk from German mothers. Chemosphere 67(9):S286-294.

WHO (World Health Organization). 1998. Executive summary. Assessment of the health risks of dioxins: re-evaluation of the Tolerable Daily Intake (TDI). WHO Consultation, May 25-29, 1998, Geneva, Switzerland. Available: http://www.who.int/ipcs/publications/en/exe-sum-final.pdf [accessed 15 May 2008].

Chapter 10: Discussion and Conclusions

The research presented in this dissertation addresses a variety of issues related to the application of biokinetics in the risk assessment of TCDD and related compounds. As discussed in the introduction, risk assessment for dioxins includes a significant number of issues that are impacted by biokinetic considerations, including notable interspecies differences in biokinetic behavior, substantial inter-congener differences in distribution and elimination, and differences in distribution between high and low dose exposure regimens. The persistence of TCDD has made it possible to detect evidence of elevated exposures in humans long after external exposure has ceased, as well as providing a basis for attempting to reconstruct historical exposure levels in occupational cohorts based on serum sampling conducted decades later.

The profound differences between humans and laboratory animals in elimination rates and accumulation potential for TCDD and related compounds led to recognition in the 1990s on the part of the international risk assessment community that some measure of internal dose was far superior to administered dose for interspecies extrapolation and risk assessment for TCDD and related compounds (DeVito et al. 1995; WHO 1998). As an initial approach, "body burden" of TCDD was used for comparison and extrapolation across species. However, this approach can be further refined to incorporate the detailed biokinetic data that are available for TCDD and some related compounds.

The following sections discuss the key steps and decision points for risk assessment for TCDD and related compounds in light of the research presented in this dissertation and other relevant data. Key data gaps or areas of potential research are identified, and general conclusions are presented.

Key Recommendations for Risk Assessment

Key issues for risk assessment of TCDD include selection of toxic endpoint of most concern, selection of relevant dose metric for inter- and intra-species extrapolations, appropriate methods for extrapolating from toxicity data for TCDD (which are plentiful) to predicted toxicity for non-TCDD congeners (for which there are far less data), appropriate approaches for high to low dose extrapolation, and evaluation of approaches for integration of available human epidemiological data into the risk assessment process. Biokinetic characteristics of TCDD and related compounds impact each of these issues. In particular, the notable hepatic sequestration observed in laboratory rodents (and for which there is both direct and indirect evidence in humans) affects

both the distribution and elimination of TCDD and related compounds, and may also influence the target organ toxicity observed. Differences in hepatic sequestration across congeners, between species, between high and low dose exposure regimens, and between acute and chronic dosing regimens may significantly affect all of the key extrapolations that must occur in risk assessment.

Selection of Data Sets

Selection of appropriate endpoints and data sets for dose-response modeling should consider several factors. For studies conducted in animals, dosing regimen is important due to impacts on distribution due to both dose range and dosing mode (bolus vs. dietary). The impact of bolus dosing on distribution is discussed in Chapter 3 and has been recognized in previous international risk assessments for dioxins (JECFA 2001). Because human environmental exposures and tissue concentrations are essentially pseudo-steady state in nature, the ideal study design will incorporate at least subchronic (if not chronic) dosing, will include 3 or more dose levels, and will include dose levels that result in tissue concentrations not orders of magnitude higher than those observed due to human environmental exposures. Finally, measured tissue concentrations will increase options for dose metric selection. Table 10-1 presents several studies meeting these criteria, although others are likely to be available. Only endpoints demonstrating a statistically significant response at some dose level should be modeled.

Human studies may also provide a basis for risk assessment for non-cancer endpoints. As discussed above, numerous epidemiological studies with dose-response assessment based on measured serum lipid-adjusted concentrations are now available. These include studies of dozens of women's health and reproductive outcomes, studies of biochemical indices in infants, and others (see Table 10-2).

Table 1: Candidate animal studies for dose-response evaluation for non-cancer endpoints. This is a partial list of studies incorporating subchronic or chronic dosing regimens and measures of internal dose; additional relevant studies are likely to be available.

	Tissue Concentrations?		Organ System or Toxicity Type								
Study	*Hepatic*	*Adipose*	*Immune*	*Cardiovascular*	*Hepatic*	*Neurological*	*Endocrine*	*Reproductive/ Developmental*	*Minimum TCDD Adipose or Serum lipid, ppt wet wt*	*Lipid Adjusted, ppt (assumes 80% lipid in adipose)*
Van Birgelen et al. 1995	✓	✓			✓		✓		620	775
Bell et al. 2007a, b	✓	✓				✓		✓	400	500
NTP 2006	✓	✓		✓	✓		✓		370	462.5
Smialowicz et al. 2008	*	*	✓						180	225
DeVito et al. 1997, 1998	✓	✓			✓				180	225

* Can be estimated from parallel studies

Table 2: Available studies of human populations assessing potential effects of TCDD and related compounds using biomonitoring data as the exposure metric

Alaluusua, S., Calderara, P., Gerthoux, P. M., Lukinmaa, P. L., Kovero, O., Needham, L., Patterson, D. G., Jr., Tuomisto, J., and Mocarelli, P. 2004. Developmental dental aberrations after the dioxin accident in Seveso. Environ Health Perspect 112: 1313-8.

Baccarelli, A., C. Hirt, A. C. Pesatori, D. Consonni, D. G. Patterson, Jr., P. A. Bertazzi, G. Dolken, and M. T. Landi 2006. t(14;18) translocations in lymphocytes of healthy dioxin-exposed individuals from Seveso, Italy. Carcinogenesis 27: 2001-7.

Baccarelli, A., Giacomini, S.M., Corbetta, C., Landi, M.T., Bonzini, M., Consonni, D., Grillo, P., Patterson, D.G., Pesatori, A.C., Bertazzi, P.A. 2008. Neonatal Thyroid Function in Seveso 25 Years after Maternal Exposure to Dioxin. PLOS Medicine 5(7):e161.

Baccarelli, A., Pesatori, A. C., Consonni, D., Mocarelli, P., Patterson, D. G., Jr., Caporaso, N. E., Bertazzi, P. A., and Landi, M. T. 2005. Health status and plasma dioxin levels in chloracne cases 20 years after the Seveso, Italy accident. Br J Dermatol 152: 459-65.

Calvert GM, Sweeney MH, Deddens J, Wall DK. 1999. Evaluation of diabetes mellitus, serum glucose, and thyroid function among United States workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Occup Environ Med 56(4):270-276.

Cao Y, Winneke G, Wilhelm M, Wittsiepe J, Lemm F, Furst P, Ranft U, Imohl M, Kraft M, Oesch-Bartlomowicz B, Kramer U. 2008. Environmental exposure to dioxins and polychlorinated biphenyls reduce levels of gonadal hormones in newborns: results from the Duisburg cohort study. Int J Hyg Environ Health 211(1-2):30-39.

Eskenazi, B., M. Warner, P. Mocarelli, S. Samuels, L.L. Needham, D.G. Patterson, Jr., S. Lippman, P. Vercellini, P.M. Gerthoux, P. Brambilla, and D. Olive. 2002b. Serum dioxin concentrations and menstrual cycle characteristics. Am J Epidemiol. 156(4):383-392.

Eskenazi, B., M. Warner, S. Samuels, J. Young, P. M. Gerthoux, L. Needham, D. Patterson, D. Olive, N. Gavoni, P. Vercellini, and P. Mocarelli 2007. Serum dioxin concentrations and risk of uterine leiomyoma in the Seveso Women's Health Study. Am J Epidemiol 166: 79-87.

Eskenazi, B., P. Mocarelli, M. Warner, S. Samuels, P. Vercellini, D. Olive, L.L. Needham, D.G. Patterson, Jr., P. Brambilla, N. Gavoni, S. Casalini, S. Panazza, W. Turner, and P.M. Gerthoux. 2002a. Serum dioxin concentrations and endometriosis: a cohort study in

Seveso, Italy. Environ Health Perspect. 110(7):629-634.
Eskenazi, B., P. Mocarelli, M. Warner, W.Y. Chee, P.M. Gerthoux, S. Samuels, L.L. Needham, and D.G. Patterson, Jr. 2003. Maternal serum dioxin levels and birth outcomes in women of Seveso, Italy. Environ Health Perspect. 111(7):947-953.
Eskenazi, B., Warner, M., Marks, A. R., Samuels, S., Gerthoux, P. M., Vercellini, P., Olive, D. L., Needham, L., Patterson, D., Jr., and Mocarelli, P. 2005. Serum dioxin concentrations and age at menopause. Environ Health Perspect 113: 858-62.
Eskenazi, B., Warner, M., Samuels, S., Young, J., Gerthoux, P. M., Needham, L., Patterson, D., Olive, D., Gavoni, N., Vercellini, P., and Mocarelli, P. 2007. Serum Dioxin Concentrations and Risk of Uterine Leiomyoma in the Seveso Women's Health Study. Am J Epidemiol 166: 79-87.
Giacomini SM, Hou L, Bertazzi PA, Baccarelli A. 2006. Dioxin effects on neonatal and infant thyroid function: routes of perinatal exposure, mechanisms of action and evidence from epidemiology studies. Int Arch Occup Environ Health 79(5):396-404.
Halperin W, Kalow W, Sweeney MH, Tang BK, Fingerhut M, Timpkins B, Wille K. 1995. Induction of P-450 in workers exposed to dioxin. Occup Environ Med 52(2):86-91.
Halperin W, Vogt R, Sweeney MH, Shopp G, Fingerhut M, Petersen M. 1998. Immunological markers among workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Occup Environ Med 55(11):742-749.
Kaneko H, Matsui E, Shinoda S, Kawamoto N, Nakamura Y, Uehara R, Matsuura N, Morita M, Tada H, Kondo N. 2006. Effects of dioxins on the quantitative levels of immune components in infants. Toxicol Ind Health 22(3):131-136.
Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van der Paauw CG, Tuinstra LG, Brouwer A, Sauer PJ. 1994. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res 36(4):468-473.
Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer PJ. 1996. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics 97(5):700-706.
Landi, M. T., D. Consonni, D. G. Patterson, Jr., L. L. Needham, G. Lucier, P. Brambilla, M. A. Cazzaniga, P. Mocarelli, A. C. Pesatori, P. A. Bertazzi, and N. E. Caporaso 1998. 2,3,7,8-Tetrachlorodibenzo-p-dioxin plasma levels in Seveso 20 years after the accident. Environ Health Perspect 106: 273-7.
Matsuura N, Uchiyama T, Tada H, Nakamura Y, Kondo N, Morita M, Fukushi M. 2001. Effects of dioxins and polychlorinated biphenyls (PCBs) on thyroid function in infants born in Japan--the second report from research on environmental health. Chemosphere 45(8):1167-1171.
Mocarelli, P., Gerthoux, P. M., Ferrari, E., Patterson, D. G., Jr., Kieszak, S. M., Brambilla, P., Vincoli, N., Signorini, S., Tramacere, P., Carreri, V., Sampson, E. J., Turner, W. E., and Needham, L. L. 2000. Paternal concentrations of dioxin and sex ratio of offspring. Lancet 355: 1858-63.
Neubert, R; Maskow, L; Webb, J; et al. (1993) Chlorinated dibenzo-p-dioxins and dibenzofurans and the human immune system. 1. Blood cell receptors in volunteers with moderately increased body burdens. Life Sci 53:1995-2006.
Neubert, R; Maskow, L; Delgado, I; et al. (1995) Chlorinated dibenzo-p-dioxins and dibenzofurans and the human immune system. 2. In vitro proliferation of lymphocytes from workers with quantified moderately-increased body burdens. Life Sci 56:421-436.
Patandin S, Koopman-Esseboom C, de Ridder MA, Weisglas-Kuperus N, Sauer PJ. 1998. Effects of environmental exposure to polychlorinated biphenyls and dioxins on birth size and growth in Dutch children. Pediatr Res 44(4):538-545.
Rogan, W. J., B. C. Gladen, Y. L. Guo, and C. C. Hsu 1999. Sex ratio after exposure to dioxin-like chemicals in Taiwan. Lancet 353: 206-7.
Ryan, J. J., Z. Amirova, and G. Carrier 2002. Sex ratios of children of Russian pesticide producers exposed to dioxin. Environ Health Perspect 110: A699-701.
Schnorr, T. M., C. C. Lawson, E. A. Whelan, D. A. Dankovic, J. A. Deddens, L. A. Piacitelli, J. Reefhuis, M. H. Sweeney, L. B. Connally, and M. A. Fingerhut 2001. Spontaneous

abortion, sex ratio, and paternal occupational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ Health Perspect 109: 1127-32.
Smith, A. H., and P. Lopipero 2001. Invited commentary: how do the Seveso findings affect conclusions concerning TCDD as a human carcinogen? Am J Epidemiol 153: 1045-7; discussion 1048-9.
ten Tusscher GW, Steerenberg PA, van Loveren H, Vos JG, von dem Borne AE, Westra M, van der Slikke JW, Olie K, Pluim HJ, Koppe JG. 2003. Persistent hematologic and immunologic disturbances in 8-year-old Dutch children associated with perinatal dioxin exposure. Environ Health Perspect 111(12):1519-1523.
Warner, M., B. Eskenazi, D. L. Olive, S. Samuels, S. Quick-Miles, P. Vercellini, P. M. Gerthoux, L. Needham, D. G. Patterson, and P. Mocarelli 2007. Serum dioxin concentrations and quality of ovarian function in women of Seveso. Environ Health Perspect 115: 336-40.
Warner, M., B. Eskenazi, P. Mocarelli, P. M. Gerthoux, S. Samuels, L. Needham, D. Patterson, and P. Brambilla 2002. Serum dioxin concentrations and breast cancer risk in the Seveso Women's Health Study. Environ Health Perspect 110: 625-8.
Warner, M., Eskenazi, B., Olive, D. L., Samuels, S., Quick-Miles, S., Vercellini, P., Gerthoux, P. M., Needham, L., Patterson, D. G., and Mocarelli, P. 2007. Serum dioxin concentrations and quality of ovarian function in women of Seveso. Environ Health Perspect 115: 336-40.
Warner, M., S. Samuels, P. Mocarelli, P.M. Gerthoux, L. Needham, D.G. Patterson, Jr., and B. Eskenazi. 2004. Serum dioxin concentrations and age at menarche. Environ Health Perspect. 112(13):1289-1292.
Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, Hooijkaas H. 2000. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. Environ Health Perspect 108(12):1203-1207.
Weisglas-Kuperus N, Sas TC, Koopman-Esseboom C, van der Zwan CW, De Ridder MA, Beishuizen A, Hooijkaas H, Sauer PJ. 1995. Immunologic effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. Pediatr Res 38(3):404-410.
Weisglas-Kuperus N, Vreugdenhil HJ, Mulder PG. 2004. Immunological effects of environmental exposure to polychlorinated biphenyls and dioxins in Dutch school children. Toxicol Lett 149(1-3):281-285.
Weisglas-Kuperus N. 1998. Neurodevelopmental, immunological and endocrinological indices of perinatal human exposure to PCBs and dioxins. Chemosphere 37(9-12):1845-1853.
Wilhelm M, Wittsiepe J, Lemm F, Ranft U, Kramer U, Furst P, Roseler SC, Greshake M, Imohl M, Eberwein G, Rauchfuss K, Kraft M, Winneke G. 2008. The Duisburg birth cohort study: influence of the prenatal exposure to PCDD/Fs and dioxin-like PCBs on thyroid hormone status in newborns and neurodevelopment of infants until the age of 24 months. Mutat Res 659(1-2):83-92.
Yoshimura, T., S. Kaneko, and H. Hayabuchi 2001. Sex ratio in offspring of those affected by dioxin and dioxin-like compounds: the Yusho, Seveso, and Yucheng incidents. Occup Environ Med 58: 540-1.

Selection of Toxic Endpoints and Relevant Dose Metrics

Risk assessments for TCDD and related compounds have focused on both cancer and non-cancer endpoints, and the selection of the endpoint of interest for risk assessment affects the interpretation and application of biokinetic considerations in the risk assessment process. The non-cancer endpoints that have attracted the most scrutiny for risk assessment have generally been subtle developmental effects. These effects have been identified as among

the most sensitive endpoints observed in laboratory animal studies, and are of concern for the general population. Cancer endpoints of interest include liver tumors in laboratory rodent studies as well as the observation that in human occupationally exposed populations there appears to be a tendency to a non-specific elevation in total cancer mortality in at least some TCDD-exposed cohorts. Notably, liver tumors, while clearly a sentinel response in laboratory rodents, do not seem to occur with any increased frequency in these cohorts. The significant hepatic enzyme induction (which results in hepatic sequestration) that occurs in laboratory rodents under the conditions of animal bioassays may be involved both mechanistically (the enzyme induction may play a mechanistic role in perturbing normal tissue homeostasis) and, through hepatic sequestration of TCDD, in increasing the target tissue dose.

The choice of endpoint for risk assessment affects the dose metric of most interest for both interspecies extrapolation and for high to low dose extrapolation. For hepatic toxicity and tumor responses observed in laboratory animals, as noted above, the induction of hepatic enzymes (notably CYP1A2) and accompanying hepatic sequestration of TCDD may interact to result in increased tumor development. One key issue related to the potential impact of the CYP1A2-bound TCDD in rodent liver has not been resolved. Is the observed CYP1A2 binding irreversible? If so, does that binding render the bound TCDD inactive and unavailable for producing toxic responses? Or do TCDD and CYP1A2 protein exist in a reversible binding equilibrium, in which free and bound TCDD is present and in which at least some proportion of the increased hepatic TCDD concentration is available for producing toxic responses? This question has not been thoroughly investigated, but could be investigated using CYP1A2 knockout mice. The appropriate internal dose metric for investigating the hepatic toxicity of TCDD is likely to be an estimate of the "free" TCDD concentration, but at the current time, no clear procedures or data allows a reliable model or estimation for this parameter.

For risk assessment of potential human cancer responses, hepatic concentration probably is not of interest, particularly with respect to the non-site-specific elevations in overall cancer mortality observed in some of the occupational cohort studies. However, the free hepatic concentration associated with tumor response may be informative for the impact of available compound to non-hepatic tissue responses. For these responses, a dose metric of interest may correspond to a cumulative estimate of circulating serum lipid TCDD. This metric proportionally reflects available compound for all organs, with distribution to each organ likely to occur based on the lipid content of that organ. The research described in Chapter 2 of this dissertation used the metric of area under the curve (AUC) of serum lipid concentration to compare carcinogenic dose-response in humans and rats. This analysis found that, using a simple biokinetic approach to reconstruct estimated AUC for the human occupational cohort data available at that time, the occupational

epidemiology suggested a lower responsiveness in humans than laboratory rodents.

In contrast to the case of carcinogenesis, risk assessment for potential developmental effects of TCDD and related compounds should focus on the available dose to the fetus during critical developmental windows. The review presented in Chapter 3 addresses a number of biokinetic issues that affect that available dose in laboratory animals, selection of relevant dose metrics for this endpoint, extrapolations from high to low dose, extrapolation across congeners, and finally, extrapolation from laboratory rodents to humans. Key conclusions from that review include the observation that the congeners that contribute most to human body burdens are highly sequestered in the liver of maternal animals under the experimental conditions typically used to investigate developmental effects of TCDD and related compounds. More importantly, based on the limited available data, several of the non-TCDD congeners of interest for human environmental exposure are far more highly sequestered than TCDD (Chen et al. 2001). This suggests that a far lower proportion of administered compound is available for these congeners to produce non-hepatic responses than for TCDD. However, the studies used as the basis for derivation of tolerable daily intakes (JECFA 2001; ECSCF 2001) administered only TCDD in the experimental protocol. The TEF values assigned to non-TCDD congeners have, in general, been derived from estimates of relative potency for hepatic-mediated effects. Thus, application of these TEFs to risk assessment for extra-hepatic effects (such as fetal developmental effects) might misestimate the relative potency of these compounds for such effects.

One approach for estimating compound available to the fetus may be to use the circulating maternal lipid concentration as a surrogate dose measure. Data from two sets of experiments in rats demonstrate that that under the condition of subchronic exposures most relevant to human environmental exposures, fetal levels of TCDD are directly proportional to maternal lipid concentration across a wide range of concentrations and dose levels (Figure 10-1a). This is in contrast to use of maternal body burden as the relevant dose metric. Fetal concentration decreases relative to maternal body burden at higher doses, as hepatic sequestration causes the retained maternal TCDD to shift from circulating lipid (available to the fetus) to maternal liver (probably unavailable to the fetus) (Figure 10-1b). These data sets also illustrate the increased distribution to the fetus that occurs when dosing is administered to the maternal animal as a bolus rather than in a subchronic mode. At the same maternal "body burden" or lipid concentration, acute bolus dosing results in far greater distribution to the fetus. This probably occurs because under bolus dosing conditions, the hepatic sequestration resulting from induction of CYP1A2 protein is incomplete, allowing a greater proportion of the administered compound to reach the fetus.

Finally, for studies demonstrating developmental effects, the impact of lactational transfer to the offspring must be considered. In addition, differences and similarities between humans and rodents in lactational exposure patterns in relationship to infant biokinetics, developmental stages, and other factors should also be considered. This is an area that deserves further research and analysis. Potential species differences in these areas may suggest, where possible, that risk assessments rely primarily on studies of human populations rather than extrapolation from rodent studies.

Some of the advantages and disadvantages of various potential dose metrics for risk assessment are summarized in Table 10-3. Lipid-adjusted serum or adipose tissue concentration provides a directly relevant, directly measurable metric that reflects inter- and intra-species differences in biokinetics and distribution for non-hepatic tissues (including the fetus) and toxicity endpoints and reflects integrated exposure over time. Lipid adjusted concentration also allows direct comparison to human biomonitoring data to allow an assessment of margin of exposure from an identified benchmark exposure (see Chapter 9 for a discussion and demonstration of this approach). Hepatic concentration may be an appropriate dose metric for hepatic endpoints, but, as discussed above, the impact of CYP1A2 binding on available compound must be considered.

Table 3: Attributes of candidate dose metrics for dose-response assessment.

Dose Metric	Reflects inter- and intra-species differences in elimination rate	Reflects inter- and intra-species differences in fat content/volume of distribution	Directly measurable in animals	Directly measurable in humans	Reflects tissue concentrations in non-hepatic tissues	Reflects tissue concentrations in hepatic tissue
Administered dose			✓	with difficulty		
Body concentration	✓					
Serum or adipose lipid-adjusted concentration	✓	✓	✓	✓	✓	
Hepatic tissue concentration	✓		✓			✓

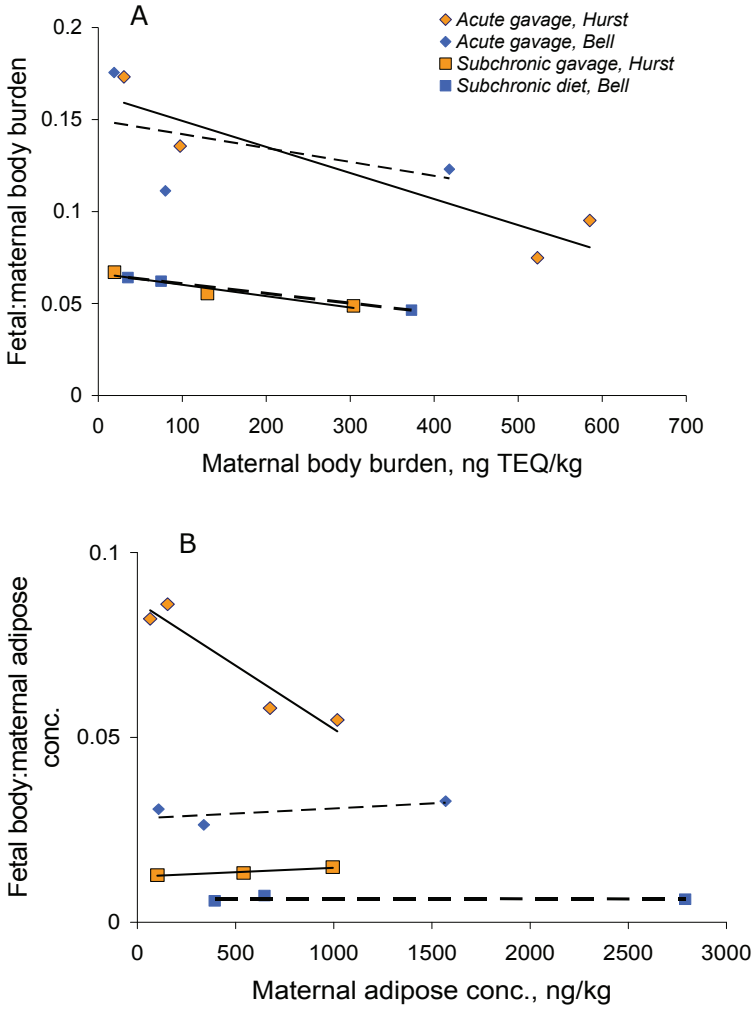


Figure 1: Ratio of fetal conc. to maternal a) "body burden" or b) adipose conc. for TCDD following acute bolus or subchronic dosing. Distribution to the fetus is highly dependent on dosing mode (and also differs by congener). Data from Hurst et al. 2000a,b; Bell et al. 2007c. See also Aylward et al. 2005 and Chen et al. 2001.

Selection of Response Metrics

Quantitative risk assessment requires identification and characterization of a point of departure - a starting point – for extrapolations between species and from high to low dose. While risk assessments have often been based on identified "no adverse effect levels" (NOAELs) or "lowest adverse effect levels" (LOAELs), benchmark dose modeling, when sufficient data are available, provides a more statistically robust and informative method for identification of a point of departure (Allen et al. 1994; Crump 1984, 1995; Gaylor and Slikker 1990). Selection of benchmark response metrics and levels should consider the following:

Comparability of effect level across endpoints. A benchmark response defined in terms of increased population risk of an abnormal response allows evaluation of comparable population risks across endpoints both continuous and quantal (Crump 1995; Allen et al. 1994). For continuous endpoints, this requires:

- Identification of control population variability and the "normal" range
- Identification of a benchmark risk level (for example, 1 or 10% extra risk of an abnormal response) and corresponding required shift in population mean

This approach is illustrated in Figure 10-2 and reviewed in more detail in Gaylor and Aylward (2004).

Degree of adversity. A 1% extra risk of abnormal enzyme activity does not carry the same level of adversity as a 1% extra risk of cleft palate. Benchmark response levels for biochemical changes may be higher than for frankly toxic responses, and/or the degree of adversity should be considered in the selection of uncertainty factors. Historically, LOAEL and NOAEL levels in animal toxicology studies typically correspond to benchmark response levels in the range of 5 to 10% (Allen et al. 1994).

Visual/statistical evaluation of BMD results. Such evaluations ensure that the benchmark dose (BMD) estimates are meaningful and are not the result of data or modeling artifacts. The BMDs should also be compared to the observed dose range in the study used. BMDs below the observed dose range may suggest selection of alternative benchmark response levels or selection of a study using lower doses.

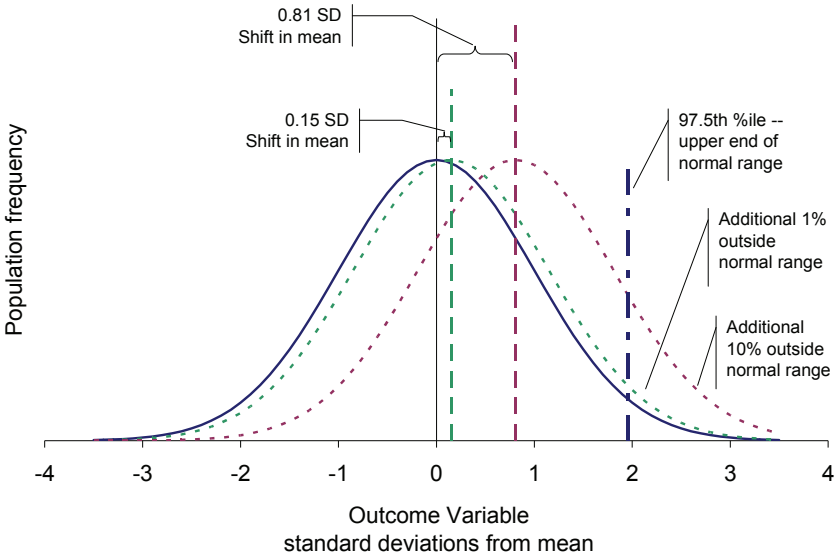


Figure 2: Illustration of the application of benchmark dose modeling based on population risk levels for continuous endpoints. After characterizing the range of the variable in the control population, shifts in the mean associated with benchmark increased risks of abnormality can be identified. The benchmark dose required to produce the target shift in the mean can then be estimated. This illustration and approach depends upon an assumption of a normally-distributed variable in the general population.

Appropriate Methods for Extrapolation Across Congeners

As noted above, the TEF approach has been developed for the purposes of extrapolation from toxicity data developed for TCDD to estimating potential risks from mixtures of dioxin-like compounds. The TEF estimates of relative congener potency were explicitly derived based on potency comparisons on an external dose basis. However, the system is routinely applied to measures of internal dose. Because of the differential accumulation, distribution, and elimination rates among congeners, application of the current WHO TEFs to tissue concentration measures may distort the estimate of relative potencies of other congeners or an observed mixture, and this fact has been explicitly recognized in the consensus documents presenting the TEFs (van den Berg et al. 2006). This issue is clearly demonstrated in the analyses presented in Chapter 4 of this dissertation, in which the dose-response for hepatic tumor responses observed in the recent NTP bioassays of TCDD, 4-PeCDF, and PCB 126 (as well as the mixture of the three compounds) were analyzed using body burden as the dose metric. Body burden in this case was estimated as the sum of the total compound distributed in the liver, adipose tissue, blood, and lung (in practice, only liver and adipose contributed significantly to the body burden estimates). When dose-response was analyzed on a body burden basis, both 4-PeCDF and PCB 126 were significantly less potent compared to TCDD than predicted on the basis of the external dose TEFs. The issue may be of even greater importance for non-hepatic responses, because the hepatic sequestration displayed by many of the dioxin and furan compounds is far greater than that displayed by TCDD (DeVito et al. 1998; Chen et al. 2001). A systematic evaluation of the relative potency on a tissue concentration basis of various congeners of interest for human environmental exposure would be of interest as a future research project.

Methods for High to Low Dose Extrapolation

If an internal dose metric such as hepatic tissue concentration or circulating serum lipid concentration is selected as most relevant for dose-response assessment and risk assessment, biokinetic modeling or measured tissue concentration data are required for the process. In particular, the significant dose-dependent differences in distribution and elimination observed for TCDD and related compounds must be accounted for. Chapter 5 presents an implementation of the Carrier et al. (1995a, 1995b) biokinetic model for Sprague-Dawley rats for TCDD, 4-PeCDF, and PCB 126 using the tissue concentration datasets provided by the recent NTP bioassays of these congeners (NTP 2006a, 2006b, 2006c). This physiologically based model reflects the dose-dependent changes in hepatic sequestration and resulting

changes in elimination rate through a simple mathematical construction, and allows for estimation of relevant internal dose measures in rats for use in dose-response or risk assessment.

Dose-response analysis based on human studies of highly-exposed occupational populations also requires extrapolation from high to low doses. In particular, quantitative cancer risk estimates have been made based on occupational mortality data and application of simple biokinetic models to back-calculate serum lipid concentrations in these populations (Steenland et al. 2001). Such back-extrapolations require a biokinetic model of the elimination of TCDD, as well as assumptions regarding exposure rate characteristics during employment. However, the original efforts in this area did not incorporate any evaluation of potential concentration-dependence of elimination rates in humans. Such behavior could substantially affect the estimated exposure levels associated with increased cancer mortality, and thus, the resulting estimates of cancer risks associated with incremental background exposures.

Chapter 6 presents the implementation and parameterization of the Carrier et al. (1995a, 1995b) model for humans for TCDD based on newly available serial sampling data sets from adults exposed to TCDD during the Seveso accident in Italy in 1976 and from three adults exposed to high levels of TCDD from an unknown source in Austria (Abraham et al. 2002; Geusau et al. 2002). The data sets from Seveso included both women and men, and included 3 to 10 measurements of TCDD concentrations in serum over a period of more than 16 years, and both datasets demonstrated clear evidence of non-linearity in exposure rates as well as relatively rapid elimination at the higher measured serum levels (half-lives under 3 years). The original Carrier et al. (1995) model structure resulted in prediction of extremely slow elimination of TCDD at background exposure levels (half-lives in excess of 15 years). Available data from persons in the general population showing significant decreasing temporal trends in serum TCDD concentrations suggested that these extremely long half-lives were incorrect. At the same time, data available from Schlummer et al. (1998) and Moser and McLachlan (2002) appeared to demonstrate a substantial passive elimination pathway in the intestinal tract that was related to circulating serum lipid concentrations. This pathway was incorporated and parameterized based on these data to improve the overall performance of the model at lower exposure levels. One important conclusion from this work is that the concentration-dependent model improves the ability to model the available serial sampling data sets. However, a second, perhaps more important, conclusion from this work is that the available datasets demonstrated a great deal of interindividual variability in elimination behavior, and that application of any model over extended periods of time to back-extrapolate serum lipid concentrations of TCDD led to highly uncertain results.

Chapter 7 demonstrated the effect of the application of the modified Carrier model on exposure estimates for the National Institute of Occupational Safety and Health (NIOSH) 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) manufacturing cohort in the United States compared to estimates derived from application of the simple first-order elimination rate model used by Steenland et al. (2001). The concentration-dependent model predicted substantially greater serum lipid concentrations in the occupational cohorts at the time of employment than predicted using the simple first-order model. When integrated with the available mortality data, these exposure estimates resulted in a reduction in the estimated cancer potency on the order of five-fold compared to the original estimates derived by Steenland et al. (2001) (Chapter 8). Overall, this research demonstrated that there is substantial uncertainty in quantitative risk assessments based on such long-term exposure reconstructions derived from single serum sampling data points collected decades after last occupational exposure. This uncertainty translates directly into uncertainties in the quantitative risk assessment.

Approaches for Integration of Human Epidemiological Data

Extrapolation of potential cancer risks from occupational cohort studies requires some biokinetic reconstructions, however uncertain. However, for non-cancer endpoints, significant numbers of studies are now available that rely on current measures of exposures (generally reported in terms of serum lipid TEQ concentrations) to quantify exposure and assess dose-response patterns. These epidemiological studies include a number of general population studies as well as numerous studies of individuals from Seveso (see, for example, Baccarelli et al. 1998). Table 10-1 provides a listing of nearly forty studies in these categories, and this listing is not exhaustive. The endpoints examined in these studies include potential alterations in endocrine system parameters, reproductive and developmental endpoints, and immune system parameters, all endpoints of *a priori* interest based on the laboratory toxicological data.

Chapter 9 of this dissertation proposes that such studies be formally incorporated into quantitative risk assessment efforts for TCDD and related compounds. This work demonstrates benchmark dose modeling approaches to three candidate studies of human populations and endpoints. The chapter suggests that such analyses be conducted for selected studies and endpoints following a weight of evidence evaluation of the available datasets which identifies those endpoints with the greatest evidence suggesting causal associations. The advantages of such benchmark dose modeling based on biomonitoring data include the ability to directly interpret the results in the context of the most common metric of human exposure (measured serum lipid

concentrations of TCDD) as well as an ability to quantify risks of adverse outcomes in a probabilistic fashion based on the benchmark dose modeling, as recommended in the recent report from the National Research Council of the National Academy of Sciences, *Science and Decisions: Advancing Risk Assessment* (NRC 2008).

Additional Considerations

The focus of the work presented in this dissertation has been the application of biokinetic modeling and data in risk assessment for dioxin-like compounds. However, in addition to biokinetic considerations, potential intrinsic differences in species sensitivity to effects of TCDD and related compounds should also be considered in the risk assessment process. Substantial data suggest that biokinetic differences alone do not account for the observed differences in species sensitivity to dioxin-like compounds. In Chapter 3 of this dissertation, one example is cited: based on datasets on developmental toxicity of TCDD and when assessed on a body burden basis, mice may be approximately 300-fold less sensitive than rats. Similarly, Moriguchi et al. (2003) demonstrated that insertion of the human AhR into the C57 mouse model resulted in a decrease in sensitivity comparable to that seen in the DBA mouse. The human AhR carries the DBA-type mutation which apparently partially inhibits binding between TCDD and the AhR (reviewed in Connor and Aylward 2006). *In vitro* assays have repeatedly demonstrated lower sensitivity of human hepatocytes compared to rat hepatocytes (Silkworth et al. 2005; other datasets as reviewed in Connor and Aylward 2006). A full discussion of the issues associated with interspecies differences in intrinsic sensitivity to dioxins is outside the scope of this dissertation, but this facet must be considered in the risk assessment process.

Considerations in the Selection of Uncertainty Factors or Target Margin of Exposure

Choices made in the selection of dose metrics, data sets, and response metrics should influence the selection and application of uncertainty factors in the derivation of tolerable daily intakes or reference doses. Some of these considerations are summarized in Table 10-4. Selection of a directly relevant and directly measured internal dose metric may result in defacto replacement of biokinetic components of the interspecies or intra-species uncertainty factors.

Table 4: Considerations in the selection of uncertainty factor components for risk assessment of dioxins

UF Component	Considerations
LOAEL to NOAEL	Depends on the selection of the POD. Evaluation of the adversity of the POD should include comparison to historical choices (see Allen et al. 1994, for discussion)
Interspecies–PK	Use of an internal dose metric which reflects biokinetics may be considered to replace this component with actual data on accumulation and distribution
Interspecies–PD	Consider that human AhR carries the "DBA-type" mutation in the ligand binding domain, resulting in ~10-fold lower sensitivity to TCDD compared to most lab rodent species (reviewed in Connor and Aylward 2006). JECFA (2001) and ECSCF (2001) evaluations both concluded that the most sensitive humans are likely to be no more sensitive, and may be less sensitive than the most sensitive animal species. AhR from more than 200 human donors have been cloned and sequenced; none contained the "high affinity" mutation in the ligand binding domain, and few polymorphisms in any region of the AhR have been identified (Harper et al. 2002; Rowlands et al., unpublished data). Values from 0.1 to 1 could be considered.
Intraspecies–PK	Replaced by the use of internal dose metrics to estimate target internal concentrations. Differences in human elimination rate (5 to 12 yrs) and body fat composition (~15 to 40%) can be incorporated into an assessment of the range of external chronic doses likely to lead to the identified target internal tissue concentration.
Intraspecies–PD	Consider whether sensitive subpopulations have been included in the critical study and endpoint evaluation. If the critical study is based on animal data, consider the conclusion from the ECSCF (2001) evaluation that no intraspecies PD factor would be required because the most sensitive human was likely to be no more sensitive than the most sensitive rodent species.

Potential Areas of Additional Research

Based on the discussions and information presented above, there are two major areas of potential research that may provide key relevant data that could impact the risk assessment of dioxin compounds. Both of these areas are directly affected by biokinetic considerations.

CYP1A2 Binding: Impact on Availability of TCDD to Produce Hepatic Toxic Responses and on Relative Potency Estimates Across Congeners

The relevant internal dose metric for hepatic response to TCDD remains to be fully evaluated due to the potential impact of binding to the induced CYP1A2 protein. The hepatic sequestration may distort estimates of concentration-based relative potency *if* the CYP1A2-bound compound is relatively inactive, or unavailable, for causing dioxin-like responses through binding to the Ah receptor. That is, it may be of interest to examine the "free" vs. total (free plus CYP1A2-bound) compound. Very little data are available to evaluate the hypothesis that the CYP1A2-bound compound is not available to produce dioxin-like responses. However, if this hypothesis is correct, relative potency estimates for a compound based on measures of concentration and responses in hepatic tissue (or mediated through hepatic responses) could be distorted from what is actually relevant for human studies in two ways.

- Because of the strong dose-dependency of the induction of CYP1A2 and hepatic sequestration of dioxin-like compounds, the relationship between hepatic tissue concentration and response may be very different at elevated doses than at environmentally relevant concentrations.

- If a compound is more highly sequestered in the liver than the reference compound, and responses are estimated as being related to total hepatic tissue concentration rather than some estimate of "free" tissue concentration, the resulting relative potency estimate may be an underestimate of the actual tissue-based relative potency for non-hepatic responses. The converse is also true: for compounds not displaying substantial hepatic sequestration, relative potency on a tissue concentration basis could be overestimated compared to the reference compound.

Because toxic responses of most interest in human populations are not generally hepatic responses, this potential distortion may be quite significant.

One approach for addressing this issue is an attempt to estimate the "free" concentration of compounds in liver based on an assumption that the concentration of free compound is represented by the lipid-adjusted concentrations measured elsewhere in the body. In this case, the "free" concentration in liver could be estimated as the concentration that would be predicted based on the lipid content of liver and the lipid-adjusted concentrations measured elsewhere (blood or adipose tissue). In other words,

the wet-weight adipose tissue concentration could be adjusted by the relative lipid content of liver compared to adipose tissue to estimate the corresponding wet-weight liver concentration in the absence of any hepatic CYP1A2 binding and sequestration. Hepatic responses could then be examined as a function of this estimated free liver concentration.

This is a complex hypothesis that has not been evaluated against either existing experimental data or through design and execution of experiments designed to test the idea. This hypothesis could be addressed in experiments using CYP1A2 knock-out mice, or perhaps through the use of relative potency estimates based on *in vitro* assays rather than through *in vivo* experiments. The most likely application of relative potency estimates based on tissue concentrations is to evaluate measures of human blood concentrations of dioxins. Because the blood can be thought of conceptually as a circulating culture medium, concentration-response relationships based on culture concentration may be more relevant to non-hepatic responses than relationships estimated based on the (potentially confounded) hepatic concentrations.

Role of Lactational Transfer in Production of Developmental Effects: Interspecies Extrapolation Issues

Despite the numerous studies addressing potential developmental effects of TCDD on animals exposed in utero, key questions regarding the relative role of lactational vs. in utero exposures in producing these effects are unresolved (Bell et al. 2007a, b, c). Additional information on this issue is of importance because human infant exposures through human milk are relatively large on an external dose basis. However, the impact of these external exposures on tissue concentrations in the developing infant is muted by the substantially more rapid elimination of dioxins by infants and dilution of body levels through rapid growth. Finally, as discussed above, the impact of elevated tissue concentrations during infancy in humans may not be the same as in laboratory rodents due to potential interspecies differences in the window of sensitivity for developmental effects. These issues have not been addressed in a structured, thoughtful framework that addresses both biokinetic, temporal, and pharmacodynamic issues of interspecies extrapolation. It may be that sufficient information is not available to conduct a thorough analysis of this issue. In this case, it may be worthwhile to focus again on the available human developmental effects literature, rather than conduct interspecies extrapolations for these endpoints.

Conclusions

The biokinetic properties of TCDD and related compounds affect nearly every facet of the typical risk assessment procedure as applied to these compounds. Qualitative and quantitative differences in the distribution and elimination of TCDD exist between high and low doses, between species, and even between bolus vs. subchronic dosing administration regimens; similarly, differences exist between TCDD and other dioxin, furan, and PCB compounds of interest. These factors should be considered in risk assessments for dioxins. Because of these complexities, preference should be given to studies most easily interpretable in the context of current human exposure tracking and assessment, which is dominated by the use of biomonitoring efforts. Where possible, use of human studies that rely upon biomonitoring data for exposure quantification concurrent with the measurement of outcome of interest may provide the most reliable basis for risk assessment. Where such data are judged to be unavailable or insufficient, animal studies conducted under chronic or subchronic dosing regimens with measured tissue concentrations may provide the most relevant dose-response data. The substantial uncertainties and interindividual variability in human biokinetics suggests that exposure-response assessments relying on extensive back-calculation of serum TCDD levels should be used only with a great deal of caution, perhaps as supportive analyses rather than the main basis for quantitative risk assessment.

References

Abraham K, Geusau A, Tosun Y, Helge H, Bauer S, Brockmoller J. 2002. Severe 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) intoxication: insights into the measurement of hepatic cytochrome P450 1A2 induction. Clin Pharmacol Ther 72(2):163-174.

Allen BC, Kavlock RJ, Kimmel CA, Faustman EM. 1994. Dose-response assessment for developmental toxicity. II. Comparison of generic benchmark dose estimates with no observed adverse effect levels. Fundam Appl Toxicol 23(4):487-495.

Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S. 2007a. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the developing male Wistar(Han) rat. I: No decrease in epididymal sperm count after a single acute dose. Toxicol Sci 99(1):214-223.

Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S.. 2007b. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the developing male Wistar(Han) rat. II: Chronic dosing causes developmental delay. Toxicol Sci 99(1):224-233.

Bell DR, Clode S, Fan MQ, Fernandes A, Foster PM, Jiang T, Loizou G, MacNicoll A, Miller BG, Rose M, Tran L, White S. 2007c. Relationships between tissue levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), mRNAs, and toxicity in the developing male Wistar(Han) rat. Toxicol Sci 99(2):591-604.

Carrier G, Brunet RC, Brodeur J. 1995. Modeling of the toxicokinetics of polychlorinated dibenzo-p-dioxins and dibenzofurans in mammalians, including humans. II. Kinetics of absorption and disposition of PCDDs/PCDFs. Toxicol Appl Pharmacol 131(2):267-276.

Carrier G, Brunet RC, Brodeur J. 1995. Modeling of the toxicokinetics of polychlorinated dibenzo-p-dioxins and dibenzofurans in mammalians, including humans. I. Nonlinear distribution of PCDD/PCDF body burden between liver and adipose tissues. Toxicol Appl Pharmacol 131(2):253-266.

Chen CY, Hamm JT, Hass JR, Birnbaum LS. 2001. Disposition of polychlorinated dibenzo-p-dioxins, dibenzofurans, and non-ortho polychlorinated biphenyls in pregnant long evans rats and the transfer to offspring. Toxicol Appl Pharmacol 173(2):65-88.

Chen CY, Hamm JT, Hass JR, Birnbaum LS. 2001. Disposition of polychlorinated dibenzo-p-dioxins, dibenzofurans, and non-ortho polychlorinated biphenyls in pregnant long evans rats and the transfer to offspring. Toxicol Appl Pharmacol 173(2):65-88.

Connor KT, Aylward LL. 2006. Human response to dioxin: aryl hydrocarbon receptor (AhR) molecular structure, function, and dose-response data for enzyme induction indicate an impaired human AhR. J Toxicol Environ Health B Crit Rev 9(2):147-171.

Crump K. 1984. A new method for determining allowable daily intakes. Fund Appl Toxicol 4:854-871.

Crump K. 1995. Calcualtion of benchmark doses from continuous data. Risk Anal 15:79-89.

DeVito MJ, Birnbaum LS, Farland WH, Gasiewicz TA. 1995. Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. Environ Health Perspect 103(9):820-831.

DeVito MJ, Diliberto JJ, Ross DG, Menache MG, Birnbaum LS. 1997. Dose-response relationships for polyhalogenated dioxins and dibenzofurans following subchronic treatment in mice. I. CYP1A1 and CYP1A2 enzyme activity in liver, lung, and skin. Toxicol Appl Pharmacol 147(2):267-280.

DeVito MJ, Ross DG, Dupuy AE, Jr., Ferrario J, McDaniel D, Birnbaum LS. 1998. Dose-response relationships for disposition and hepatic sequestration of polyhalogenated dibenzo-p-dioxins, dibenzofurans, and biphenyls following subchronic treatment in mice. Toxicol Sci 46(2):223-234.

European Commission Scientific Committee on Foods (ECSCF). 2001. Opinion of the Scientific Committee on Food on the risk assessment of dioxins and dioxin-like PCBs in food. Update based on new scientific information available since the adoption of the SCF opinion of 22nd November 2000. Rep. CS/CNTM/DIOXIN/20 final. http://europa.eu.int/comm/food/fs/sc/scf/out90_en.pdf CS/CNTM/DIOXIN/20 final. Brussels, Belgium: European Commisssion.

Gaylor DW and Slikker W. 1990. Risk assessment for neurotoxic effects. Neuro Toxicology 11:211-218.

Gaylor DW, Aylward LL. 2004. An evaluation of benchmark dose methodology for non-cancer continuous-data health effects in animals due to exposures to dioxin (TCDD). Regul Toxicol Pharmacol 40:9-17.

Geusau A, Schmaldienst S, Derfler K, Papke O, Abraham K. 2002. Severe 2,3,7,8-tetrachlorodibenzo- p-dioxin (TCDD) intoxication: kinetics and trials to enhance elimination in two patients. Arch Toxicol 76(5-6):316-325.

Harper PA, Wong JY, Lam MS, Okey AB. 2002. Polymorphisms in the human AH receptor. Chem Biol Interact 141(1-2):161-187.

Hurst CH, DeVito MJ, Birnbaum LS. 2000. Tissue disposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in maternal and developing long-evans rats following subchronic exposure. Toxicol Sci 57(2):275-283.

Hurst CH, DeVito MJ, Setzer RW, Birnbaum LS. 2000. Acute administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in pregnant Long Evans rats: association of measured tissue concentrations with developmental effects. Toxicol Sci 53(2):411-420.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2001. Fifty-seventh meeting Rome, 5-14 June 2001. Summary and conclusions. http://www.who.int/ipcs/food/jecfa/summaries/en/summary_57.pdf.

Leung HW, Kerger B, Paustenbach DJ, Ryan JJ, Masuda Y. 2005. Age- and concentration-dependent elimination half lives of chlorinated dibenzofurans in Yusho and Yucheng patients. Organohalogen Compounds(67):1726-1729.

Leung HW, Kerger BD, Paustenbach DJ. 2006. Elimination half-lives of selected polychlorinated dibenzodioxins and dibenzofurans in breast-fed human infants. J Toxicol Environ Health A 69(6):437-443.

Moriguchi T, Motohashi H, Hosoya T, Nakajima O, Takahashi S, Ohsako S, Aoki Y, Nishimura N, Tohyama C, Fujii-Kuriyama Y, Yamamoto M. 2003. Distinct response to dioxin in an arylhydrocarbon receptor (AHR)-humanized mouse. Proc Natl Acad Sci U S A 100(10):5652-5657.

Moser GA, McLachlan MS. 2002. Modeling digestive tract absorption and desorption of lipophilic organic contaminants in humans. Environ Sci Technol 36(15):3318-3325.

National Research Council. 2008. Science and Decisions: Advancing Risk Assessment. Committee on Improving Risk Analysis Approaches Used by the U.S. EPA. Board on Environmental Studies and Toxicology,

Division on Earth and Life Studies. National Academy Press, Washington, DC. www.nap.edu.

National Toxicology Program (NTP). 2006. NTP Toxicology and Carcinogenesis Studies of 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4) in Female Harlan Sprague-Dawley Rats (Gavage Studies). Natl Toxicol Program Tech Rep Ser(525):1-198.

National Toxicology Program (NTP). 2006. NTP toxicology and carcinogenesis studies of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in female Harlan Sprague-Dawley rats (Gavage Studies). Natl Toxicol Program Tech Rep Ser(520):4-246.

National Toxicology Program (NTP). 2006. NTP technical report on the toxicology and carcinogenesis studies of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (CAS No. 1746-01-6) in female Harlan Sprague-Dawley rats (Gavage Studies). Natl Toxicol Program Tech Rep Ser(521):4-232.

Rowlands, J.C. Personal communication, February 10, 2009.

Schlummer M, Moser GA, McLachlan MS. 1998. Digestive tract absorption of PCDD/Fs, PCBs, and HCB in humans: mass balances and mechanistic considerations. Toxicol Appl Pharmacol 152(1):128-137.

Silkworth JB, Koganti A, Illouz K, Possolo A, Zhao M, Hamilton SB. 2005. Comparison of TCDD and PCB CYP1A Induction Sensitivities in Fresh Hepatocytes from Human Donors, Sprague-Dawley Rats, and Rhesus Monkeys and HepG2 Cells. Toxicol Sci. 87(2):508-19.

Smialowicz RJ, DeVito MJ, Williams WC, Birnbaum LS. 2008. Relative potency based on hepatic enzyme induction predicts immunosuppressive effects of a mixture of PCDDS/PCDFS and PCBS. Toxicol Appl Pharmacol 227(3):477-484.

Steenland K, Deddens J, Piacitelli L. 2001. Risk assessment for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) based on an epidemiologic study. Am J Epidemiol 154(5):451-458.

van Birgelen AP, DeVito MJ, Akins JM, Ross DG, Diliberto JJ, Birnbaum LS. 1996. Relative potencies of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls derived from hepatic porphyrin accumulation in mice. Toxicol Appl Pharmacol 138(1):98-109.

van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Peterson RE. 2006. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 93(2):223-241.

WHO (World Health Organization). 1998. WHO (World Health Organization). 1998. Executive summary. Assessment of the health risks of dioxins: re-evaluation of the Tolerable Daily Intake (TDI). WHO Consultation, May 25-29, 1998, Geneva, Switzerland. Available: http://www.who.int/ipcs/publications/en/exe-sum-final.pdf [accessed May 15, 2008].

Summary

The Application of Internal Dose Measures, Biokinetics, and Biomonitoring Data in the Risk Assessment of Dioxin-Like Compounds

The biokinetic properties of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related coplanar polychlorinated dioxin (PCDD), furan (PCDF), and biphenyl (PCB) compounds have played an integral role in the identification, experimental evaluation, and risk assessment of potential health effects of these compounds dating from the earliest occupational evaluations of manufacturing workers engaged in the production of chlorinated phenoxyacetic acid compounds. Because these compounds are highly persistent in human tissues, the detection and quantification of exposure using biomarkers such as adipose or blood lipid concentrations is possible in both populations with elevated exposure potential (such as occupational cohorts) and in persons in the general population exposed to trace levels of these compounds accumulated in the food chain. Availability of these biomarkers has also led to the estimation of half-lives of elimination for TCDD and many related compounds in humans through the analysis of serial biological samples in individuals. In turn, such estimates have resulted in efforts to back-extrapolate estimated internal doses in individuals exposed in specific occupational or accidental incidents years or decades prior to collection of a biological sample.

In parallel, studies of biokinetic properties of TCDD and related compounds in laboratory animals have informed the design and interpretation of toxicological studies. In the risk assessment context, extrapolations across congeners, from high to low dose, and from animals to humans are commonly performed. Because TCDD has historically been the most-studied compound of the class, generalizations based on data on TCDD have been applied, sometimes inaccurately, across the whole class of congeners. In addition, experimental data in animals and data from human studies has revealed concentration-dependent behavior for the elimination of TCDD and other congeners. Finally, physiological differences in humans compared to laboratory rodents can also influence the biokinetics of these compounds. However, these inter-congener, across dose, and inter-species differences have not always been integrated into the risk assessments performed for dioxins.

This dissertation presents research and analysis pertinent to the integration of biokinetic understanding and biomonitoring data into the risk assessment of

potential health effects of TCDD and related compounds. The first several chapters address topics related to the use of biokinetic data and internal dose evaluations in risk assessments of dioxin-like compounds based on toxicological data.

Chapter 2 presents an early analysis using cumulative internal dose estimates derived from simple biokinetic models applied to occupational and accidentally exposed cohorts to compare the apparent dose-response for carcinogenesis from occupational cohorts to that from animal bioassay data. This analysis extended the developing concept of "body burden" as a dose metric to estimation of time-varying serum lipid concentrations in occupational cohorts and proposed a variety of considerations for selection of dose metrics and interspecies comparisons of dioxin toxicity. Interestingly, regardless of the dose metric chosen, the cancer rate in humans in the NIOSH cohort, if due to TCDD, is almost completely insensitive to dose. Our analysis indicates that human exposure to background levels of TCDD (about 5 ppt serum lipid concentration) should not pose an incremental cancer risk.

Chapter 3 discusses a variety of biokinetic and dose metric issues in the context of risk assessment for potential developmental effects of dioxins. This chapter explores in some detail the impact of inter-congener differences on the extrapolation of data on the reproductive and developmental toxicity of TCDD to other congeners and to human exposures. Based on the evaluation presented here, the use of body burden as a dose metric does not account for or eliminate the substantial differences in sensitivity to dioxin observed across species or between different strains of the same species and, thus, does not eliminate the need to consider the relative sensitivity of humans compared to laboratory animal models in risk assessments.

Chapter 4 uses the tissue concentration data from the recent National Toxicology Program (NTP) long term carcinogenesis bioassays of TCDD, 4-PeCDF, PCB 126, and the TEQ mixture of the three (NTP 2006a, 2006b, 2006c, 2006d) to examine the relative carcinogenic potency of the three compounds on an internal dose basis. The relative potency estimates resulting from this analysis are compared to the Toxic Equivalency Factors (TEFs) used for dioxin risk assessment on an intake dose basis. On a body burden basis, PCB 126 and 4-PeCDF were 2- to 3-fold and 10- to 12-fold less potent than predicted based on the WHO TEFs, respectively, while the TEQ mixture was approximately 3- to 5-fold less
potent than predicted by the TEFs. The current WHO TEF values, which were derived from data on noncancer endpoints evaluated on an administered dose basis, overpredict the carcinogenic potency of these compounds on a body-burden basis compared to TCDD.

Chapter 5 implements the Carrier et al. concentration-dependent biokinetic model framework for three dioxin-like compounds using the NTP bioassay tissue concentration data. This analysis demonstrates similarities and differences in the distribution and elimination behavior of these compounds and provides modeling tools that can be useful in estimation of internal doses in other laboratory animal studies that do not include a tissue analysis component. These differences in kinetic and distribution behavior have important implications for dose-response assessment under conditions of chronic intake. They suggest that the TEQ approach can lead to important mis-estimation of body burden, distribution, and elimination behavior under chronic administration conditions.

The next section of the dissertation includes chapters that address the incorporation of biokinetics, internal dose concepts, and biomonitoring data in the evaluation and use of epidemiological data for risk assessment of environmental exposures to dioxins.

Chapter 6 presents the modification and application of the Carrier concentration-dependent biokinetic model for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) based on the rich datasets from Seveso and the Austrian poisoning patients (Abraham et al. 2002; Geusau et al. 2002). In this effort, recent data demonstrating passive elimination mechanisms for dioxins were incorporated into the Carrier et al. (1995) model structure, and the overall TCDD model parameterized based on the detailed serial sampling datasets from highly exposed humans. Application of the model to serum sampling data from the cohort of US herbicide-manufacturing workers assembled by the National Institute of Occupational Safety and Health (NIOSH)indicates that previous estimates of peak serum lipid TCDD concentrations in dioxin-exposed manufacturing workers, based on first-order back extrapolations with half-lives of 7–9 years, may have underestimated the maximum concentrations in these workers and other occupational cohorts by several-fold to an order of magnitude or more. Such dose estimates, based on a single sampling point decades after last exposure, are highly variable and dependent on a variety of assumptions and factors that cannot be fully determined, including inter-individual variations in elimination efficiency. Dose estimates for these cohorts should be re-evaluated in light of the demonstration of concentration-dependent elimination kinetics for TCDD, and the large degree of uncertainty in back-calculated dose estimates should be explicitly incorporated in quantitative estimates of TCDD's carcinogenic potency based on such data.

Chapter 7 evaluates the impact of use of the concentration-dependent model compared to a simple first-order model on the cumulative TCDD exposure estimates for the National Institute of Occupational Safety and Health (NIOSH) 2,4,5-T manufacturing cohort. Use of a concentration- and age-dependent

model of elimination results in increases of up to five-fold in AUC estimates for the more highly exposed members of the cohort compared to estimates obtained using the first-order model with 8.7-year half-life. This degree of variation in the internal dose estimates for this cohort would affect substantially the cancer potency estimates derived from the mortality data from this cohort. Such variability and uncertainty in the reconstructed internal dose estimates for this cohort, depending on elimination model, parameter set, and regression model, have not been described previously and are critical components in evaluating the dose-response data from the occupationally exposed populations.

Chapter 8 illustrates the potential impact of the alternative biokinetic approach on the assessment of cancer mortality patterns and potency estimates derived from the NIOSH cohort. The full mortality dataset from the NIOSH study was re-analyzed using the exposure reconstructions resulting from the concentration-dependent model. Cancer potency estimates were compared to previous analyses derived from use of a simple first-order kinetic model. The estimated incremental lifetime risk of mortality at age 75 from all cancers was about 6 to more than 10 times lower than previous estimates derived from this cohort using exposure models that did not consider the age and concentration dependence of TCDD elimination.

Chapter 9 illustrates the use of benchmark dose modeling with selected biomonitoring-based epidemiological data sets to derive a quantitative, margin-of-exposure (MOE) framework for assessing human health risks of dioxins at environmental exposure levels. Numerous issues inherent in exposure quantification, dose-response assessment, and interpretation of the results in the context of background exposures are addressed. The exposure data sets indicate that current serum lipid concentrations in young adults in the United States are approximately 6- to 7-fold lower than 1970s-era concentrations. Estimated MOEs for each end point based on current serum lipid concentrations range from < 10 for neonatal thyroid hormone concentrations to > 100 for dental anomalies—approximately 6-fold greater than would have existed during the 1970s. Human studies of dioxin exposure and outcomes can be used in a benchmark dose framework for quantitative assessments of MOE. Incomplete exposure characterization can complicate the use of such studies in a benchmark dose framework.

Finally, Chapter 10 presents a discussion of the major findings of Chapters 2 through 9 in the context of recommendations for scientifically sound methods for risk assessment of dioxins that account for the detailed biokinetic understanding available for these compounds. Key elements include identification of toxic endpoint of interest and the corresponding relevant dose metric, and based on this identification, understanding of the impact of hepatic sequestration on that dose metric. Based on this understanding, the dose-

dependency and across congener factors influencing likely risk at relevant human exposure levels can be addressed. Chapter 10 also identifies some of the remaining data needs for appropriate integration of biokinetics and internal dose concepts in risk assessment for dioxins. These include development of understanding and estimation of the relative potency of various key congeners on an internal dose basis, understanding of the impact of hepatic sequestration on available compounds for hepatic and non-hepatic toxic responses, and factors influencing the role of lactational transfer and infant biokinetics of dioxins on the potential for developmental effects in humans from dioxin exposures.

Samenvatting

De toepassing van interne blootstellingsmetingen, biokinetiek en biomonotoring gegevens bij de risicobeoordeling van dioxine-achtige verbindingen

De biokinetische[1] eigenschappen van 2,3,7,8-tetrachloordibenzo-p-dioxine (TCDD) en toxicologisch verwante gechloreerde dioxines (PCDDs), dibenzofuranen (PCDFs) en bifenylen (PCB's) hebben een integrale rol gespeeld bij de identificatie, experimentele evaluaties en risicoschatting van de mogelijke gezondheidseffecten van deze stoffen. De eerste gezondheidskundige aspecten van deze stoffen werden reeds onderzocht in het kader van beroepsmatig blootgestelde werknemers bij de productie bestrijdingsmiddelen, die gechloreerde fenoxyazijnzuren bevatten, waaronder het bekende 2,4,5-T. Omdat deze stoffen slecht worden afgebroken in het menselijk lichaam is de detectie en kwantificering hiervan mogelijk in bijvoorbeeld bloed, vet en moedermelk. Deze meetmethoden zijn de afgelopen decennia sterk in kwaliteit toegenomen. Als gevolg hiervan is het mogelijk om in humane populaties, die zijn blootgesteld via de arbeidssituatie of de voedselketen, nauwkeurig interne concentraties van deze stoffen te bepalen. Als gevolg van deze ontwikkelingen in de analytische chemie en het gebruik van meerdere metingen over een langere tijdsperiode bij dezelfde personen kunnen relatief nauwkeurige biokinetische parameters worden vastgesteld, bijvoorbeeld de eliminatiesnelheid van TCDD en verwante stoffen. Met behulp van deze biokinetische gegevens kan dan retrospectief worden uitgerekend wat de historische blootstelling van een individue geweest moet zijn, veelal jaren tot decennia voordat de feitelijke chemische analyses plaatsvonden.

Tegelijkertijd heeft laboratoriumonderzoek naar biokinetische eigenschappen van TCDD en verwante stoffen in proefdieren er toe geleid dat toxicologische studies zodanig ontworpen konden worden, dat met specifieke weefselverdeling en trage eliminatie uit het lichaam rekening kon worden gehouden. Bij de risicobeoordeling worden regelmatig extrapolaties van een hoge naar een lage dosis uitgevoerd, terwijl daarnaast de relevantie van de resultaten van deze dierproeven voor de mens moet worden ingeschat.

[1] *Opgemerkt wordt hier dat het begrip farmacokinetiek, toxicokinetiek en biokinetiek uitwisselbaar in het proefschrift gebruikt kunnen worden. De betreffende terminologie heeft in de afgelopen decennia meerdere veranderingen ondergaan. Afhankelijk van de auteur of het tijdschrift heeft hierbij één van deze termen over de afgelopen jaren de voorkeur genoten.*

Historisch gezien zijn de meeste toxicologische experimenten uitgevoerd met TCDD en zijn in eerste instantie dus veel generalisaties bij de risicoschatting gemaakt op grond van deze verbinding. Meer recent onderzoek heeft echter aangetoond, dat de biokinetiek van veel andere PCDDs, PCDFs en PCBs niet zonder meer gelijkgesteld kan worden aan die van TCDD. Daarnaast is gebleken dat het biokinetisch gedrag van deze dioxine-achtige stoffen eveneens concentratie-afhankelijk kan zijn. Daarnaast spelen fysiologische verschillen tussen mens en dier eveneens een belangrijk rol bij de interpretatie van proefdierstudies voor de humane risicoschatting. In het licht van bovenstaande argumenten moet geconstateerd worden, dat bovengenoemde aspecten in het verleden niet altijd even adequaat zijn toegepast bij de risicoschatting voor dioxine-achtige stoffen.

In dit proefschrift wordt onderzoek beschreven, waarbij de integratie van biokinetische kennis en gegevens over biomonitoring bij mensen gebruikt kan worden om de mogelijke schadelijke effecten van dioxine-achtige stoffen te bepalen. In de eerste hoofdstukken wordt met name onderzocht, hoe het gebruik van biokinetische data en de interne dosis bij de mens gebruikt kunnen worden voor de risicoschatting, waarbij wordt uitgegaan van de resultaten van toxicologische laboratoriumstudies.

In hoofstuk 2 wordt een eerste analyse gedaan op grond van de geschatte cumulatieve interne dosis van TCDD, waarbij gebruik gemaakt wordt van een éénvoudig biokinetisch model. Dit wordt toegepast op beroepsmatige of per ongeluk hoog blootgestelde werknemers. De resultaten hiervan worden vergeleken met de mogelijke dosis-effect relaties die gevonden zijn in carcinogeniteitsonderzoek met proefdieren. In deze studie wordt het concept van lichaamsbelasting ("body burden") als dosismetrie-parameter verder uitgewerkt. Met behulp van deze benadering kan een schatting gemaakt worden van de tijdsafhankelijke concentraties in serum in deze groepen werknemers. Daarnaast worden een aantal overwegingen op het gebied van dosismetrie en soortverschillen in toxiciteit van dioxine-achtige stoffen nader uitgewerkt en beschouwd. De resultaten tonen aan dat onafhankelijk van het type dosismetrie, de kankerincidentie door TCDD bij beroepsmatige blootstelling vrijwel geheel dosis-onafhankelijk is. Daarnaast wijzen deze berekeningen uit dat bij een achtergrondblootstelling van circa 5 ppt TCDD in serum lipide geen toename van kanker bij de mens te verwachten is.

In hoofdstuk 3 worden verschillende aspecten op het gebied van de dosismetrie en biokinetiek nader uitgewerkt en bediscussieerd in relatie tot de mogelijke effecten van dioxines op het ontwikkelende organisme. Verder wordt in dit hoofdstuk de invloed van verschillen in de biokinetiek tussen verschillende congeneren nader onderzocht en de invloed hiervan op de reproductie en ontwikkelingstoxicologie. Tot slot wordt geconcludeerd dat het

gebruik van lichaamsbelasting (body burden) als dosismetrie-parameter niet voldoende de verschillen verklaart in gevoeligheid tussen mens en dier.

In hoofdstuk 4 worden meetgegevens gebruikt uit de recente meerjarige carcinogeniteits-studies, die zijn gedaan in het kader van het National Toxicology Program (NTP) in de VS. Hierbij zijn de weefselconcentraties van TCDD, 2,3,4,7,8-PnCDF en 3,3',4,4',5-PCB (PCB126) en een mengsel van deze drie stoffen gebruikt. Met behulp van deze experimentele gegevens zijn de relatieve kankerverwekkende eigenschappen van deze drie stoffen berekend, waarbij de interne dosis als uitgangspunt diende. Deze relatieve potenties voor carcinogeniteit zijn daarna vergeleken met het Toxische Equivalentie Factoren (TEF) concept, dat wereldwijd algemeen gebruikt wordt. Dit huidige TEF concept is echter gebaseerd op toegediende dosis. Uit de resultaten van dit onderzoek kan geconcludeerd worden als TEFs voor dioxine-achtige verbindingen gebaseerd zouden zijn op de interne dosis, deze waarden tot één ordegrootte kunnen afwijken van die van de Wereldgezondheids Organisatie (WHO). Daarnaast lijken deze WHO TEF waarden, voornamelijk gebaseerd op niet carcinogene eindpunten, een overschatting te geven voor de carcinogeniteit van deze dioxine-achtige stoffen.

In hoofdstuk 5 wordt gebruik gemaakt van het zogenaamde "Carrier" model, waarbij er van uitgegaan wordt dat de biokinetiek van TCDD en verwante verbindingen concentratie-(dosis-) afhankelijk is. De berekeningen met de NTP-gegevens volgens dit model voor bovengenoemde drie verbindingen toont aan, dat er zowel verschillen als overeenkomsten zijn met een meer eenvoudige dosis-onafhankelije biokinetische benadering. De resultaten illustreren tevens, dat deze methode in de toekomst gebruikt kan worden voor het berekenen van de interne dosis uit proefdierstudies, waarbij geen weefselanalyses hebben plaatsgevonden. Gebaseerd op de resultaten van dit onderzoek, kan eveneens geconcludeerd worden dat het gebruik van het huidige WHO TEF systeem kan leiden tot een verkeerde schatting van lichaamsbelasting, weefselverdeling en eliminatie bij chronische blootstelling .

Het tweede deel van het proefschrift bevat een aantal studies, die nader in gaan op de toepassing van biokinetische modellen, het interne dosis concept en biomonitorgegevens bij de evaluatie van epidemiologische data.

In hoofdstuk 6 wordt een aanpassing en toepassing gepresenteerd van het "Carrier" concentratie afhankelijk biokinetische model voor TCDD. Hierbij is gebruik gemaakt van de chemisch-analytische data die verkregen zijn bij het ongeluk in Seveso (Italie) en de moedwillige vergiftigingen met TCDD in Oostenrijk. Bij dit onderzoek werden ook recente gegevens omtrent de passieve eliminatie van TCDD verwerkt en toegepast op mensen, die aan een hoge éénmalige dosis zijn blootgesteld. De resultaten van deze berekeningen tonen aan dat het gebruik van een concentratie-onafhankelijk één-

compartiment model een onderschatting kan veroorzaken voor de initiële lichaamsconcentraties van de besmette personen. Voorgesteld wordt deze berekeningen opnieuw uit te voeren om een beter zicht te krijgen omtrent de onzekerheden, die voor de carcinogeniteit van TCDD bij de mens een rol kunnen spelen.

In hoofdstuk 7 vindt opnieuw een vergelijking plaats tussen het concentratie-afhankelijk biokinetische model van Carrier en een éénvoudig eerste-orde biokinetiek model. Uitgangspunt hierbij is nu echter de cumulatieve blootstelling van werknemers in een 2,4,5-T herbicide fabriek, zoals die eerder geschat is door het National Institute of Occupational Safety and Health (NIOSH) in de VS. Via het dosis afhankelijke "Carrier" model is berekend dat de historisch blootstelling mogelijk en maximaal vijfmaal hoger is geweest dan in eerder onderzoek is berekend. Een dergelijk verschil heeft een belangrijke invloed op de eerdere geschatte kankerverwekkende potentie van TCDD, die tot stand gekomen is met meer éénvoudige biokinetische modellen.

In hoofdstuk 8 wordt nader beschreven, wat de mogelijke invloed is van de bovenstaande alternatieve biokinetische benadering op het inschatten van de potentie en mortaliteit door kanker voor TCDD in het eerder genoemde NIOSH cohort. Hierbij is de volledige databank van deze NIOSH studie opnieuw gebruikt en geanalyseerd. Hierbij werd uitgegaan van de geschatte historische blootstellingsgegevens, die volgens het concentratie-(dosis-)afhankelijke biokinetische model berekend waren. Opnieuw zijn deze uitkomsten vergeleken met eerdere gegevens, die verkregen waren via een éénvoudig eerste order biokinetiek model. Deze vergelijkende berekeningen tonen aan dat het risico op ontstaan van kanker door TCDD in deze groep werknemers circa 6 tot 10 maal lager was, dan eerder was berekend met modellen die geen rekening hielden met leeftijd en dosis-afhankelijkheid.

In hoofdstuk 9 wordt gebruikt gemaakt van een "benchmark" model en gegevens die verkregen zijn uit epidemiologisch studies. Met behulp hiervan werd een kwantitatieve blootstellingsmarge (Margin of Exposure) afgeleid voor gezondheidschadelijke risico's voor de mens met een achtergrond-blootstelling aan dioxine-achtige stoffen. In relatie hiermee worden diverse aspecten op het gebied van blootstelling, dosis-effect relaties en interpretaties van verkregen resultaten bediscussieerd. Deze studie toont aan dat de concentraties van dioxine-achtige stoffen op dit moment circa 6 tot 7 maal lager zijn dan in de zeventiger jaren. Uitgaande van deze "benchmark" benadering wordt geschat dat de blootstellingsmarge met de berekende drempelwaarde minder dan een factor 10 is voor effecten op de schildklier bij de pasgeborene tot meer dan twee orde groottes voor gebitsafwijkingen.

Tot slot worden in hoofdstuk 10 de belangrijkste uitkomsten uit voorgaande hoofdstukken nader bediscussieerd. Een gerichte aanbeveling wordt gedaan

voor verdere wetenschappelijk onderbouwing van de risicoschatting voor dioxine-achtige verbindingen. Hierbij wordt aangegeven dat een gedegen kennis omtrent de biokinetiek van deze stoffen noodzakelijk is om deze risico's betrouwbaar te bepalen. Een belangrijke factor hierbij is het vaststellen van een voor de mens relevant toxicologisch eindpunt met bijbehorende dosismetrie. Daarnaast speelt de congeneer-specifieke en concentratie-afhankelijke verdeling in de lever een belangrijke rol bij een wetenschappelijk verantwoorde extrapolatie van proefdier naar mens. Wanneer deze biokinetische gegevens op congeneer-specifieke basis verwerkt worden in de humane risicoschatting, kunnen in de toekomst de risico's van deze stoffen bij een achtergrondblootstelling betrouwbaarder berekend worden. In deze discussie worden verder een aantal tekortkomingen geidentificeerd, die moeten worden opgelost om een juiste integratie van biokinetiek en het interne-dosis concept te verkrijgen. Bij deze tekortkomingen in de huidige kennis kan oa. gedacht worden aan congeneer-specifieke potentie op basis van interne dosis (concentratie), het belang voor de mens van andere dan toxische effecten op de lever (bijv. bij de reproductie en ontwikkeling), de rol van overdracht via de placenta en moedermelk en de specifieke biokinetische processen die hierbij een rol spelen voor moeder en kind.

Curriculum Vitae

Lesa L. Aylward was born in Rockford, Illinois, on August 16, 1962. She was raised in Arizona, and attended the Massachusetss Institute of Technology where she earned Bachelor's and Master's degrees in the Department of Materials Science and Engineering. During graduate school, she became interested in the intersection of science and policy presented by the practice of chemical risk assessment. She joined Karch & Associates, Inc., in Washington, D.C., where she received additional training in risk assessment, toxicology, and physiologically based biokinetic modeling.

Ms. Aylward has worked in chemical risk assessment consulting for more than 20 years at several firms. She now holds the position of Principal at Summit Toxicology, LLP. She specializes in applying biokinetic approaches to toxicology, exposure, and risk assessment, including interpretation of biomonitoring data for assessing human health risks from a variety of chemicals. Ms. Aylward has published extensively on biokinetics and biomonitoring for dioxins and other persistent organochlorines. Her recent work has focused on developing approaches for integrating biokinetic data with existing chemical risk assessments to provide tools for screening-level evaluation of population biomonitoring data.

Selected Recent Publications

LaKind, J.S., D.Q. Naiman, S.M. Hays, L.L. Aylward, B.C. Blount. In press. Public health interpretation of trihalomethane blood levels in the United States: NHANES 1999-2004. J. Exp. Sci. Environ. Epidem.

Aylward, L.L., K.M. Bodner, J.J Collins, M. Wilken, D. McBride, C.J. Burns, S.M. Hays, N. Humphry. In press. TCDD exposure estimation for workers at a New Zealand 2,4,5-T manufacturing facility based on serum sampling data. J. Exp. Sci. Environ. Epidem.

Garabrant, D.H., L.L. Aylward, S. Berent, Q. Chen, C. Timchalk, C.J. Burns, S.M. Hays, and J.W. Albers. In press. Cholinesterase inhibition in chlorpyrifos workers: Characterization of biomarkers of exposure and response in relation to urinary TCPy. J. Exp. Sci. Environ. Epidemiol.

Moore, J.M., J.L. Newsted, M. Hecker, M.J. Zwiernik, S.D. Fitzgerald, D.P. Kay, X. Zhang, E.G. Higley, L.L. Aylward, K.J. Beckett, R.A. Budinsky, S.J. Bursian, and J.P. Giesy. 2009. Hepatic P450 enzyme activity, tissue morphology and

histology of mink (Mustela vison) exposed to polychlorinated dibenzofurans (PCDFs). Arch. Environ. Contam. Toxicol. 57:416–425.

Hays, S.M. and L.L. Aylward. 2009. Using biomonitoring equivalents to interpret human biomonitoring data in a public health risk context. J. Appl. Toxicol. 29(4):275-288.

LaKind, J.S., S.M. Hays, L.L. Aylward, and D.Q. Naiman. 2009. Perspective on serum dioxin levels in the United States: An evaluation of the NHANES data. J. Exp. Sci. Environ. Epidemiol. 19:435-441.

Budinsky, R.A., J.C. Rowlands, S. Casteel, G. Fent, C.A. Cushing, J. Newsted, M.V. Ruby, and L.L. Aylward. 2008. A pilot study of oral bioavailability of dioxins and furans from contaminated soils: Impact of differential hepatic enzyme activity and species differences. Chemosphere 70:1774-1786.

Butterworth, B.E., L.L. Aylward, S.M. Hays. 2007. A mechanism-based cancer risk assessment for 1,4-dichlorobenzene. Regul. Toxicol. Pharmacol. 49:138-48.

Nichols, B.R., K.L. Hentz, L. Aylward, S.M. Hays, J.C. Lamb. 2007. Age-specific reference ranges for polychlorinated biphenyls (PCB) based on the NHANES 2001-2002 survey. J. Toxicol Environ Health A. 70:1873-7.

Connor, K.C. and L.L. Aylward. 2006. Human response to dioxin: Aryl hydrocarbon receptor (AhR) molecular structure, function, and dose-response data for enzyme induction indicate an impaired human AhR. J. Toxicol. Environ. Health Part B: Critical Reviews. 9:147-171.

Hays, S.M., and L.L. Aylward. 2003. Dioxin risks in perspective: past, present, and future. Regul Toxicol Pharmacol. 37(2):202–217.

Aylward, L.L. and S.M. Hays. 2002. Temporal trends in human TCDD body burden: Decreases over three decades and implications for exposure levels. J. Exp. Anal. Environ. Epidemiol. 12:319–328.

Acknowledgements

The work presented in this thesis is the result of more than two decades of learning, growth, and collaboration. As such, it is not the work of one person but rather the outgrowth of many sources of support, knowledge, collaboration, and interaction.

Several people have contributed materially to the completion of this thesis. I would like to thank Ingrid Dahmen, who has provided invaluable practical support in navigating the specific requirements of the thesis preparation, submission, and defense process and who provided the necessary translation services. I would also like to thank Sylvia van den Eshof for her assistance in this process. Finally, I thank Martin van den Berg for suggesting and encouraging this endeavor and for his role as Promotor for this thesis.

Several mentors encouraged me and provided support in my development and career. I would like to thank Nate Karch, who provided me with my start in the fascinating realm of chemical risk assessment in general and dioxin in particular, and who generously supported my professional development and growth. I also owe a debt of gratitude to Dennis Paustenbach, who encouraged me to publish my work and who initiated and fostered my collaborations with Sean Hays.

I have had the opportunity to collaborate with many extremely talented people from whom I learned a great deal. In particular, I want to thank Paolo Mocarelli for his generous collaboration. His foresight, wisdom, and dedication have enriched our knowledge immeasurably, and I appreciate his trust and friendship. I would also like to thank Robert Brunet and Gaétan Carrier for their enthusiastic participation in our work together, and Elizabeth Delzell for her patient, rigorous, and disciplined approach to our analyses. Many other people have contributed to the work presented in this thesis, including, but not limited to, Jim Lamb, Lorenz Rhomberg, Julie Goodman, Russell Keenan, Mike Gray, Gail Charnley, Hong Cheng, Colleen Cushing, and Tom Starr, and I learned from and enjoyed working with each of them.

I have had the good fortune to have my work sponsored by several individuals who have been keenly interested in the science underlying and resulting from my work. In particular, I would like to thank David Fischer and Ann Mason for their support and interest.

My family is the web and the fabric of my life, providing the essential grounding and context without which my work would be impossible or unimportant. I thank my parents for supporting me and providing a wonderful

example of lives that are full and active and focused on family. My husband, Steve Warner, has been my partner and friend in our life together and has trusted me to find my path. My children, Thomas and Emily, have been a constant source of wonder, joy, and amazement. As I watch them head off into the world I know that no matter how fascinating the science and my work are, the tapestry that we are weaving in our lives is infinitely more satisfying.

I am also supported by smart and loving friends who have enriched my professional and personal life. In particular, I love sharing with Lisa Yost and Yvette Lowney the joys, tribulations, challenges, and humor of our lives as scientists, professionals, parents, and women.

Finally, I thank my friend and long-time collaborator and colleague, Sean Hays. His vision, energy, and enthusiasm inspire and encourage me to see beyond the immediate and to believe in the possibilities and promise of our ideas and our work. It is no coincidence that the body of work contained in this thesis commences with our initial collaboration: our work together has always been the most enjoyable, creative, and rewarding of my career, and I look forward to many more years of partnership and shared endeavors.