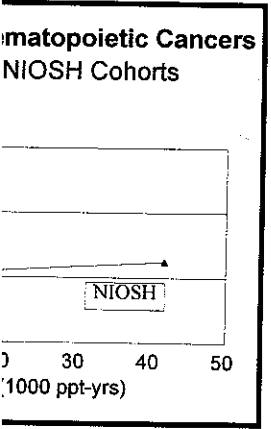
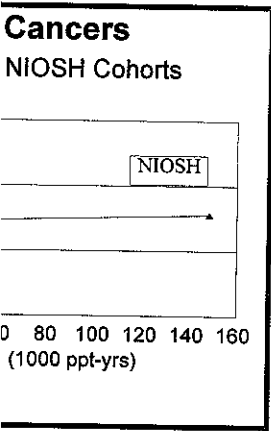


NIOSH³⁾ and male
 C: Rectal cancer
 and
 <1 year and ≥1 year
 cancers were observed



Estimating Minimally Effective Inducing Doses (ED01s) of TCDD in Livers of Rats and Humans Using Physiologically Based Pharmacokinetic Modeling

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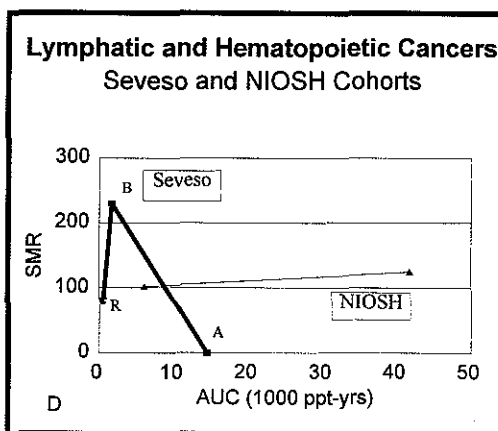
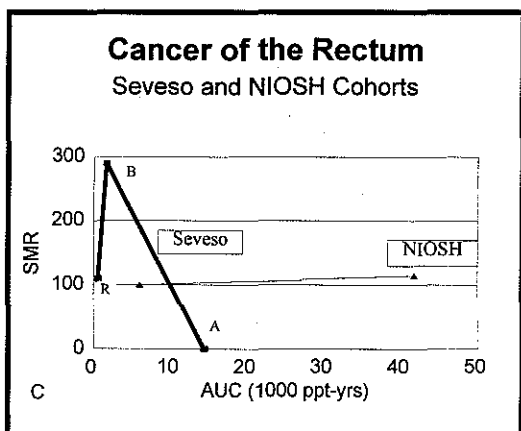
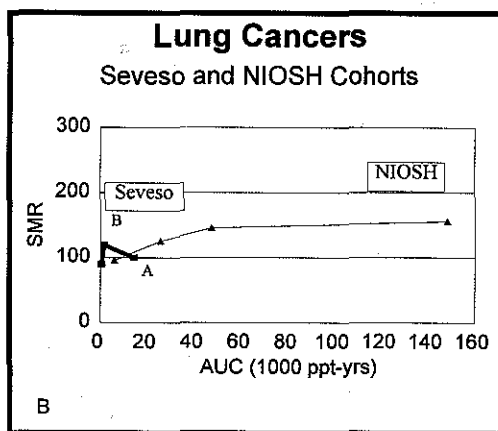
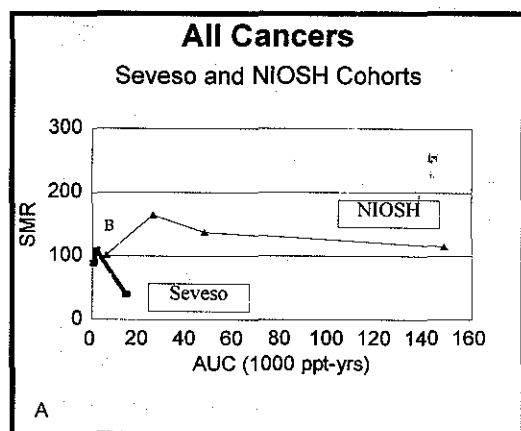
Abstract: TCDD causes diverse biological effects in test animals and the doses causing these effects vary significantly. Protein induction in the liver is among the most sensitive of these effects. We have applied a physiologically based pharmacokinetic (PB-PK) model of TCDD related protein induction along with a geometric model of the liver acinus to estimate the average daily TCDD intake required to give a 1% increase (ED01) in CYP1A1 concentrations in rats and in humans. The calculations were for a 0.3 kg rat with 8% body fat or for an 80 kg human with 25% body fat. ED01s under these conditions were 1450 and 16 picograms/kg body weight /day for rat and human, respectively. As confidence increases in the values of induction parameters in the human PB-PK/protein induction model, predictions of ED01s in humans might be used as the starting point for margin of exposure risk assessments with TCDD.

Introduction: TCDD and related compounds cause induction of several cytochrome P450 (CYP450) family isozymes in liver and other tissues. In the liver, induction of CYP1A1 and CYP1A2 is not uniform throughout the entire organ.^{1,2} For instance, at low doses, induction is centrilobular and there is a sharp boundary between areas of the liver that are fully induced and areas of the liver where there is no appreciable induction. As dose increases the proportion of induced cells in the liver increases and the region showing induction fills out from the centrilobular toward the periportal area. The sharp boundary between fully induced and non-induced cells is indicative of highly non-linear processes in the induction of these enzymes by TCDD.³ Recently, quantitative models of regional induction have been developed to describe both total induction in liver and the regional distribution of induced protein within the acinus.^{4,5}

Cancer risk assessments with TCDD have generally been based on average daily intakes with standard defaults for interspecies (body-weight) and low dose (linearized multistage) extrapolation. The upper-bound estimate of the 1 in a 1,000,000 cancer risk for humans calculated from this approach is 6 femtograms/kg body weight/day. These calculations have largely ignored information about interspecies differences in elimination rates of TCDD. With the increases in information about the species differences in TCDD metabolism and about the significance of regional distribution for low-dose extrapolation, it is possible to improve current estimates of the expected extent of biological responses at low doses. In addition, with the publication of the proposed US EPA cancer risk assessment guidelines,⁶ intermediate effects, such

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Figure 2: Cancer mortality SMRs versus serum lipid TCDD AUC for the NIOSH³⁾ and male Seveso²⁾ populations. A: All cancer mortality. B: Lung cancer mortality. C: Rectal cancer mortality. D: Lymphatic and hematopoietic cancer mortality. For rectal and lymphatic/hematopoietic mortality, cancer rates were available only for the <1 year and ≥1 year exposure duration groups from the NIOSH cohort. No cases of these two cancers were observed in the Zone A Seveso subcohort, but less than 1 of each was expected.



Estimating Minimally Rats and Humans U

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Hart/ChemRisk, 1135 Atlar

Abstract: TCDD causes effects vary significantly. We have applied related protein induction al daily TCDD intake required in humans. The calculation 25% body fat. ED01s und for rat and human, respecti the human PB-PK/protein i starting point for margin of

Introduction: TCDD an (CYP450) family isozymes CYP1A2 is not uniform th centrilobular and there is a areas of the liver where th induced cells in the liver centrilobular toward the pe induced cells is indicative TCDD.³ Recently, quanti both total induction in liver

Cancer risk assessments w standard defaults for in extrapolation. The upper calculated from this appro largely ignored information increases in information a significance of regional dist estimates of the expected publication of the proposed

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as protein induction, could be used to derive acceptable daily intakes for TCDD and related compounds. In this report, a PB-PK/protein induction model for TCDD is used to estimate daily TCDD intake rates that would be expected to produce a 1% increase in hepatic levels of CYP1A1 in rats and humans.

Model Characteristics: This geometric model of the liver, based on general ideas of hepatic zonation proposed earlier by Lamers et al.⁷, divides the hexagonal acinus into 5-subcompartments (Figure 1). The first is concentric with the portal triad, the second periportal compartment is interconnected throughout the liver, and the last three are concentric around the central vein. Radiating from the periportal to the centrilobular regions these zones are numbered sequentially, 1 to 5. The surface areas and associated volumes for each of these 5 zones were calculated to be 13.5, 25.2, 33.9, 20.3, and 6.8 % of the total liver,⁴ respectively.

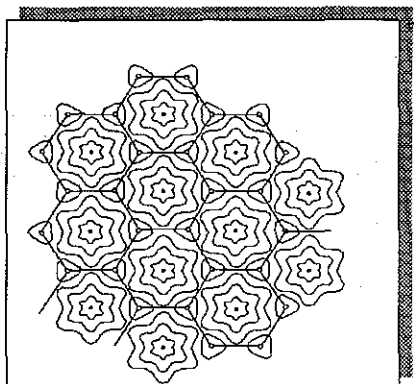


Figure 1: Geometric Representations of Several Liver Acini: Each hexagonal acinar structure is centered on a central vein while the corners of the hexagon represent portal triads. Numbering (1-5) proceeds from the periportal region outward to the central vein. The 5 subcompartments are organized as concentric zones, except zone 2 which fenestrates throughout the entire liver. Zone 5, representing 6.8% of the total liver, is the first concentric zone around the central vein.

The PB-PK model for TCDD has fat, liver, richly perfused, and poorly perfused tissue compartments. The liver itself is subdivided into the five zones defined in the geometric model and these zones are connected in series. Blood perfuses the subcompartments sequentially beginning with zone 1 and exiting from zone 5 to join the mixed venous blood pool. In the liver, protein induction occurs due to presumed binding of TCDD-Ah receptor complexes with dioxin response elements (DRE's) on DNA. Each subcompartment has parameters for the binding affinity between TCDD and the Ah receptor (K_b) and between the Ah-TCDD complex and DNA promoter sites for the CYP1A1 and CYP1A2 genes (K_d 's). The enhanced transcription of CYP1A1 (the calculation of which is shown in Figure 2) was assumed to be proportional to DRE occupancy by the Ah-TCDD complex. The shape parameter (n) was adjusted to achieve a sharp boundary between induced and non-induced areas in the liver. Because TCDD binds strongly to CYP1A2; a protein that is also induced by TCDD, the model also has to calculate CYP1A2 concentrations and the binding of TCDD to this protein. The TCDD-CYP1A2 dissociation constant was 7.5 nM.

Based on previous PB-PK modeling with TCDD kinetics and protein induction in the rat,⁵ the binding affinity of the Ah receptor for TCDD was estimated as 0.2 nM in each compartment. The sharp demarcation between induced and non-induced areas was achieved by varying the binding affinities of the Ah-TCDD complex for the DREs in each of the five liver subcompartments and

using large n values in the model for the Ah-TCDD complex. K_d values of 0.6, 0.20, and 0.067 nM were used in the model to data on CYP1A1 induction in a rat study² and in studies of CYP1A1

inducible synt

$k_o ; k(ind)$

$$d[Pr]/dt = k_o +$$

Figure 2: The Hill equation for protein synthesis, $d[Pr]/dt$, is dependent on the concentration of the Ah-TCDD complex. K_d15 is the concentration of the Ah-TCDD complex that induces 15% of the maximum CYP1A1-related Di

To create curves relating $d[Pr]/dt$ to the PB-PK model was run until steady state was reached. To facilitate these calculations, the steady state models were used. The steady state models use fully equilibrated with all tissue compartments, but is well suited for long term kinetics, but is well suited for long term calculations were done for a long time. All model parameters associated with the binding were held constant.

Results: The rate constant for protein synthesis, k_o , was varied from previous work⁵. For the human PB-PK model was run with a range of k_o values. The rate constant was then varied from 0.01 to 10 years. The value of k_{fc} required

Predicted dose response curves for rats are shown in Figure 3. The number of Ah receptor sites was estimated to be 1450 and 1000 respectively. These estimates were based on body weight during adulthood. The response increased with increasing or decreasing body weight. The purpose of the

using large n values in the Hill equations. Values used for $Kd1i$, that is the dissociation constant for the Ah-TCDD complex with the CYP1A1 promoter in compartments $i = 1-5$, were 5.4, 1.8, 0.6, 0.20, and 0.067 nM, respectively. These estimates had been obtained by calibrating the model to data on CYP1A1 induction in rats dosed for 13-weeks as part of an initiation-promotion study² and in studies of CYP1A1 m-RNA induction by TCDD after single, acute doses.⁸

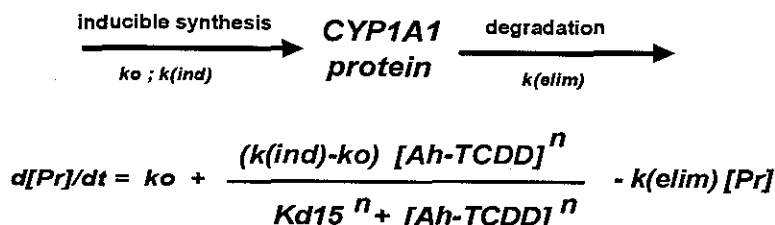


Figure 2: The Hill Equation Used for Enzyme Induction. The basal rate of protein synthesis, ko , is augmented to a maximally induced rate, $(k(ind)-ko)$, depending on the presumed occupancy of DNA promoter sites for the Ah-TCDD complex. $Kd15$ is the binding affinity of the Ah-TCDD complex for specific CYP1A1-related Dioxin Responsive Element (DRE) binding sites in Zone 5.

To create curves relating daily intake rate with proportion induction over a wide dose range, a PB-PK model was run until steady state for intake rates ranging from 0.0001 to 10000 ng/kg/day. To facilitate these calculations, the original model was simplified following work of Carrier et al.⁹ The steady state models use a single input equation and assume that the body burden of TCDD is fully equilibrated with all tissues. This type of model would be inappropriate for examining short term kinetics, but is well suited to evaluation of steady-state conditions as modeled here. The calculations were done for a 0.3 kg rat with 8% body fat or for a 80 kg human with 25% body fat. All model parameters associated with protein induction and Ah-TCDD and Ah-TCDD-DRE binding were held constant between rats and humans.

Results: The rate constant for elimination of TCDD in rats (k_{fc} , 3/hr) was estimated from previous work⁵. For the human simulations, the rate constant was determined by simulation. The PB-PK model was run with constant intake for 30 years; then the intake rate was set to zero. The rate constant was then varied to provide a half-life for whole body elimination of TCDD of 7.5 years. The value of k_{fc} required to give this half-life was 0.25/hr.

Predicted dose response curves for induction in the centrilobular region, zone 5, in humans and rats are shown in Figure 3. The ED01 values for total hepatic induction were estimated in these species to be 1450 and 16 picograms TCDD/kg body weight/day for the rat and human, respectively. These estimates are for the steady state exposures in rats and humans at constant body weight during adulthood. These calculations can be extended to other growth conditions with increasing or decreasing fat compartments associated with aging, prolonged illness or severe dieting. The purpose of these initial calculations was simply to illustrate the daily TCDD intake

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rate required to give similar induction responses in these two species. Since the binding affinities, partition coefficients and mode of action for induction were assumed to be constant across species for these calculations, the differences between the two species are primarily due to lower metabolic clearance in humans.

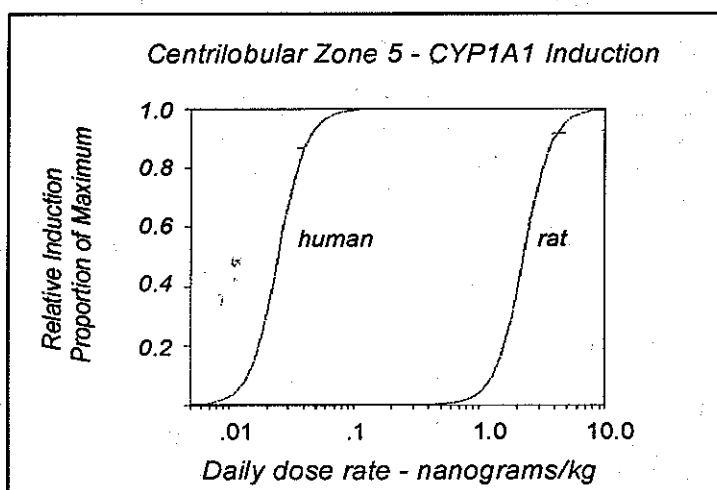


Figure 3: Estimated Induction in the Centrilobular Region (Zone 5) of the Geometric Model of the Liver Acinus. Since zone 5, the area with induction at the lowest dose, represents 6.8% of the liver, 15% induction in this zone is equal to 1% induction in the entire liver (i.e., it is equal to the ED01).

Because protein induction is highly non-linear, the ED01s obtained are extremely sensitive to several of the induction parameters. The primary factors affecting the predicted values were K_{d15} (the dissociation constant for the Ah-TCDD complex with DNA sites in zone 5), K_b (the dissociation constant for the Ah-TCDD complex), V_l (liver volume), n_1 (the Hill coefficient for induction of CYP1A1) and k_{fc} (the rate constant for hepatic TCDD metabolism). The normalized sensitivity coefficients for many of these parameters were significantly greater than 1. For instance, a 2-fold increase in k_{fc} decreased the proportion induction in zone 5 at a dose of 1.5 ng/kg/day from 0.166 to 0.0137, a change of greater than a factor of 10. In contrast, the volume and partition coefficient for the fat compartment had virtually no effect on these curves.

Discussion: Dose metrics are those measures of tissue dose that are most directly related to the biological activity of a xenobiotic. Administered dose may be directly related to a tissue dose metric for compounds with purely linear kinetics, but administered dose cannot provide a mechanistic understanding of the relationship of observed effects to biochemical or macromolecular interactions. Aylward et al.¹⁰ recently evaluated various dose metrics for TCDD in order to compare relative sensitivities of rodents and humans with respect to observed or potential carcinogenicity of TCDD in these species. These authors favored using measures of internal tissue concentration maintained over time and specifically argued for measures of dose related to total or time-averaged area under the blood TCDD concentration curve. These internal

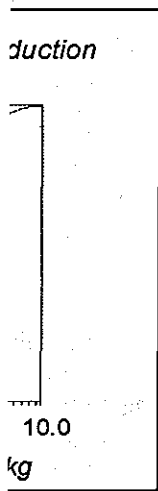
dose metrics account for effect compared across species and biological effects over time.

The next step in deriving details of the molecular interaction of the receptor, and the differences in the assessments with TCDD receptor dose (ng/kg/day), TCDD receptor complex (nM), the number of DREs by the Ah-TCDD complex, and the number of proteins for adverse health effects. These steps up through the induction occurs quickly. The occupancy of DREs (Figure 3) by the TCDD complex for DNA sites in zone 5 concentration in the liver hepatocytes are either full. Zone 5 are very sensitive to PK/gene induction model. A variety of available data are used in this process. While these models they included similar induction curves, these various modeling efforts are predictions for the rat ED01.

In contrast, predictions in humans and the assumptions for humans are similar to the estimates; these parameters are available. Because of current data, these can be considered approximate characteristics and Ah-TCDD

The present approach comparing internal dose metrics for humans about 100-fold, are due to differences in the distribution after TCDD treatment. Instead, these characteristics to a new set of maximum induction in humans converted to a 'TCDD-receptor' phenotype with a set of parameters that takes place in a concerted manner subsequent toxic effects, de-

Since the binding affinities, to be constant across species are primarily due to lower



n (Zone 5) of the with induction at the zone is equal to 1%

are extremely sensitive to the predicted values were (A sites in zone 5), K_b (the n₁ (the Hill coefficient for metabolism). The normalized n₁ is generally greater than 1. For zone 5 at a dose of 1.5 10. In contrast, the volume on these curves.

most directly related to the related to a tissue dose ed dose cannot provide a ffects to biochemical or ous dose metrics for TCDD with respect to observed or favored using measures of rgued for measures of dose ration curve. These internal

dose metrics account for the duration and concentration of TCDD within target organs. The effect compared across species, cancer, is a complex biological process that requires ongoing biological effects over time to influence the probability of the tumorigenic response.

The next step in deriving dose metrics for interspecies comparisons is to include the biological details of the molecular interactions of TCDD with cellular macromolecules, primarily the Ah receptor, and the differences in these interactions across species. Mechanistically-based assessments with TCDD require greater understanding of the relationships between administered dose (ng/kg/day), TCDD dose achieved in target tissues (nM), concentrations of Ah-TCDD receptor complex (nM), the relative extent of activation of TCDD responsive genes by occupancy of DREs by the Ah-TCDD complex, and the consequences, if any, of the induction of these proteins for adverse health effects of TCDD. Our PB-PK/protein induction model includes all these steps up through the dose-response of protein induction. Unlike tumor promotion, protein induction occurs quickly. The dose metric for induction in these calculations is the fractional occupancy of DREs (Figure 2). This dose metric depends on the affinity constants of the Ah-TCDD complex for DNA sites, the affinity of TCDD for the Ah receptor, and the Ah receptor concentration in the liver. Because of the highly non-linear behavior of induction, where hepatocytes are either fully induced or non-induced, the estimates of proportional induction in zone 5 are very sensitive to the induction characteristics included in the model. The rat PB-PK/gene induction model and associated parameters have been well established by modeling a variety of available data and at least two groups^{11,12} had already developed PB-PK models for this process. While these earlier models did not account for regional heterogeneity of induction, they included similar induction equations. Because of the extensive data base and the successes of these various modeling efforts in describing total and regional induction, the confidence in the predictions for the rat ED01 based on this regional induction model should be high.

In contrast, predictions in humans are based on direct observations of reduced clearance of TCDD in humans and the assumption that binding affinities and tissue capacities for protein induction in humans are similar to the rat. This latter assumption is critical to the accuracy of the ED01 estimates; these parameters will have to be more carefully evaluated before definitive results are available. Because of current uncertainty in these values, the human ED01 estimated here should be considered approximate and may well change as more information is obtained on Ah receptor characteristics and Ah-TCDD-DNA binding properties with human proteins.

The present approach compares daily intake rates in rats and humans required to produce equal internal dose metrics for protein induction. With TCDD the difference in values between species, about 100-fold, are due to the longer human half-life. The heterogeneity of hepatic CYP1A1^{1,2} distribution after TCDD treatment and the high n-value are signs that the this induction does not occur smoothly. Instead, induction appears to entail a switching of the cell from one set of characteristics to a new set over a very narrow range of dose.⁴ Thus, estimates of the proportion of maximum induction in the liver are the same as estimating the proportion of cells that have converted to a 'TCDD-responding' phenotype. The activation of these cells to a different phenotype with a set of fully induced proteins suggests that extensive cellular reprogramming takes place in a concerted step. This step, while it may or may not be directly involved in subsequent toxic effects, does appear to be prerequisite for the toxicity seen at higher doses.

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Since protein induction/cell activation in liver is a sensitive effect of TCDD exposure and is believed to be an obligatory prerequisite for toxicity, estimates of the human ED01s for protein induction (about 16 picogram TCDD /kg body weight /day in this present work; see Figure 3) eventually could be suitable for use as a starting point in margin of exposure risk assessments for TCDD. The lack of genotoxicity of TCDD and the evidence for a promotional mode of action for TCDD carcinogenicity support a non-linear extrapolation and the ED01 for a sensitive precursor effect could serve as a conservative starting point for such an analysis. The high slope value for induction also means that the response should fall off very rapidly as daily intakes are further reduced below the ED01.

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Examination of *In Vitro* Compound

John R. Petrusis and Nigel J. Guelph, Guelph, Ontario, C

Abstract

The activation of Sp... binding to an oligonucleo... electrophoretic gel mobility (TCDD), various DLCs (P... mixtures of these DLCs w... binding experiments demon... concentration of 10 nM. In... the EC₅₀ relative to 10 nM... by TCDD. Analysis of bina... interactive effects *in vivo*... activation/DRE binding. W... TCDD (1 nM) and an exce... concentration of TCDD was... caused effects that were an... greater than the EC₅₀ of th...

Introduction

The Ah receptor is a... of the toxic responses to... referenced to the most pote... Most other DLCs have lov... intense effects both *in vi*... interactive effects that are... DLCs. Many of these st... following maternal exposur... examined^{3,4,5}. These inve... 1,3,4, effects caused by mix...

The present work... mixtures is being examine...