

Supplemental Technical Comments on Pharmacokinetic Modeling and the Derivation of Reference Doses in the Mode of Action Analysis in

EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments

Comments to the EPA Science Advisory Board Dioxin Review Panel

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Prepared on behalf of:

Chlorine Chemistry Division
American Chemistry Council
1300 Wilson Boulevard
Arlington, VA 22209

Prepared by:

Lesla L. Aylward, Ph.D.
Principal
Summit Toxicology, LLP
6343 Carolyn Drive
Falls Church, VA USA



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Executive Summary

The following comments present technical issues and considerations relevant to EPA's Draft *Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments* ("Draft Reanalysis"). These comments are a supplement to technical comments that I submitted in July after an initial review of the Draft Reanalysis and address three areas main topics.

Quantitative Issues in Toxicokinetic Modeling

Three important quantitative issues exist related to the use of the Emond PBPK model in deriving human candidate RfD estimates based on animal datasets. First, the shape of the dose-response curve for induction of cytochrome P450 in liver incorporated in the model needs to be updated to reflect available *in vivo* data. Second, the value assigned to the human fat: blood partition coefficient significantly underpredicts the central tendency demonstrated in three available datasets. This underprediction will result in underpredictions of candidate RfD values. Finally, the Emond PBPK model underpredicts measured concentrations in rat fat and liver tissues in key datasets, and this under-estimation could further impact the estimated RfDs.

Interspecies Toxicodynamic Uncertainty Factor Component (UF_{A-TD})

The decision in the Draft Reanalysis to use a default factor for relative interspecies toxicodynamic sensitivity is contrary to a robust body of data indicating that for early key events in the MOA analysis (including activation of the AhR in liver), human cells are significantly less sensitive than rodent cells. Many data sets demonstrate that human liver cells require concentrations 10-fold or more higher than rat liver cells to activate the AhR and produce key early responses such as CYP1A1 induction, which is an early biomarker event in the EPA MOA analysis. This difference in sensitivity is well documented and well understood, and is directly traceable to a known, well-conserved mutation in the human AhR that results in reduced ligand-binding affinity for TCDD. Based on this information, application of a UF_{A-TD} greater than one cannot be supported for this and other early hepatic events postulated to occur in response to AhR activation, and a value *less* than one could be justified in the MOA modeling for these endpoints.

Non-Reproducible Values in MOA Candidate RfD Derivation

In our review of the MOA analysis, we were unable to reproduce the underlying values and analyses that led to most of the candidate RfDs presented in Table 5-21, an annotated version of which is included in Appendix A of these comments. EPA staff confirmed that the numbers presented in Table 5-21 were generally not correct, and they presented a corrected version of the table in San Antonio at the Dioxin 2010 meeting.

While we appreciate that EPA has now corrected the values in Table 5-21, this episode raises larger questions of the general reliability of the multitude of quantitative analyses presented in this document. Given the importance of the analysis and results, we think that the quantitative content of the document requires additional quality control efforts before finalization.

Quantitative Issues Associated with the Emond PBPK Model

Three important quantitative issues exist related to the use of the Emond PBPK model in deriving human candidate RfD estimates based on animal datasets. First, the shape of the dose-response curve for induction of cytochrome P450 in liver incorporated in the model needs to be updated to reflect available *in vivo* data. Second, the value assigned to the human fat:blood partition coefficient significantly underpredicts the central tendency demonstrated in three available datasets. This underprediction will result in underpredictions of candidate RfD values. Finally, the Emond PBPK model underpredicts measured concentrations in rat fat and liver tissues in key datasets, and this under-estimation could further impact the estimated RfDs.

CYP1A2 Induction Dose-Response Function

The Emond PBPK model relies upon certain parameter values that were first postulated about 13 years ago by Wang et al. (1997) in their initial publication of an NIEHS PBPK model for dioxin. Some of the parameters carried forward without critical evaluation include the Hill coefficient for CYP1A2 induction of 0.6 and a generic fat:blood partition coefficient of 100. At the last meeting there was discussion of the need to identify data to inform the Hill coefficient for CYP1A2 induction. Walker et al. (1999) provides a subchronic, *in vivo* dataset with measured tissue concentrations and CYP1A2 mRNA with a fitted Hill model with coefficient of 0.94. These data should be used to update and revise the Emond model to reflect the appropriate dose response for this function, which influences low-dose behavior of the model.

Human Fat:Blood Partition Coefficient

The procedure employed by EPA for toxicokinetic extrapolation between species for identification of illustrative MOA RfDs relies upon estimating an intake dose associated with a target whole blood concentration in humans using the Emond PBPK model. The Emond model incorporates a fat:blood partition coefficient (PF) of 100 for humans. The PF value was apparently arbitrarily set at 100, perhaps because of use of this value in early versions of an NIEHS PBPK model for TCDD (see, for example, Wang et al. 1997). However, significant theoretical work has been done regarding the appropriate value of PF for highly lipophilic organic chemicals such as TCDD (Haddad et al. 2000) based on the relative lipid content of blood and fat. Haddad et al. (2000) suggest that for highly lipophilic compounds, the value of PF in humans should be approximately 200 based on the typical ratio of lipid content of human fat and blood, and experimental data for a wide range of such compounds generally confirm this value.

Human data are available to evaluate the appropriate value for this parameter for TCDD. Figure 1 presents the distribution of observed partition coefficients for TCDD between fat and whole blood or serum in three studies. Median values between approximately 150 and 200 were observed. The predicted fat:blood tissue concentration ratio (as demonstrated in the surface

tables in Appendix C4 of the Draft Reanalysis document) is at or below below the 25th percentile for both datasets

The impact of the underestimate of PF is as follows. For a given intake dose rate, if fat or liver tissue concentrations are accurately modeled (and fat concentrations or lipid-adjusted serum concentrations have generally been the data used to parameterize and validate this and other models in humans), the concentration in whole blood will be **overestimated** by a factor of up to 2 at the median.

Conversely, if a target whole blood concentration in humans is used to identify a corresponding human intake dose (as in the MOA RfD procedure used by EPA), the intake dose associated with that target whole blood concentration will be **underestimated** by approximately two-fold. This would have the direct effect of resulting in a two-fold underestimate of the corresponding RfDs as well.

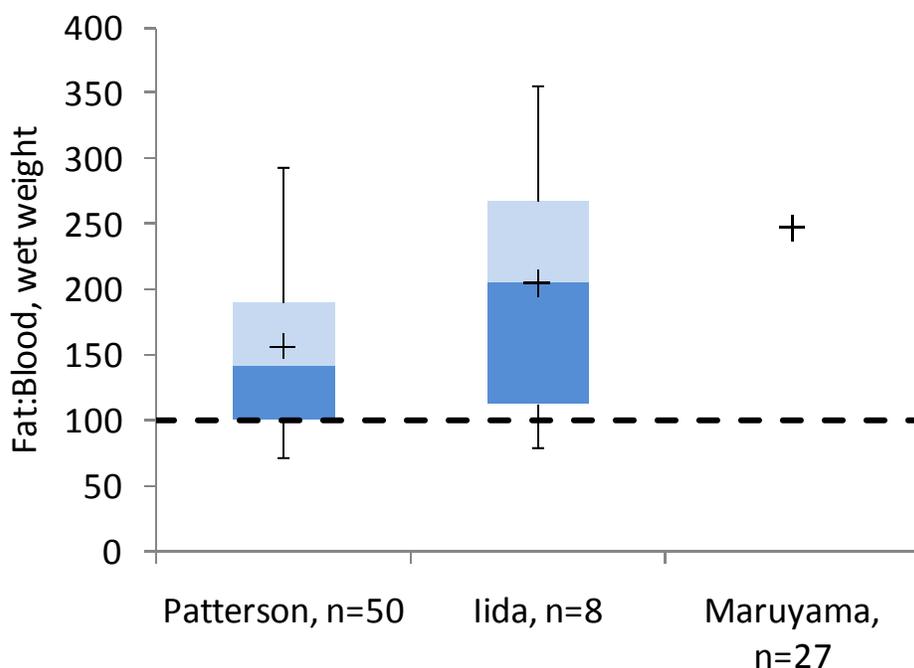


Figure 1: Distribution of ratios of wet weight concentrations of TCDD in fat and serum (Patterson et al. 1988) or whole blood (Iida et al. 1999; Maruyama et al. 2002). Boxes represent the interquartile range; whiskers the 5th and 95th %iles; means are indicated by the + symbol. No distribution data were available from Maruyama et al. (2002); only the central tendency based on 27 paired samples from autopsy was available. Dotted line indicates ratio of fat: blood concentrations predicted by Emond PBPK model (see Appendix C4 of the Draft Reanalysis document).

While this degree of imprecision is common in PBPK models, the level of effort and attention involved in dioxin risk assessment, and the direct impact of this issue on estimated MOA RfDs, warrants use of all of the available data to improve the risk assessment estimates to the degree possible. Use of a data-derived central tendency value for the fat: blood partition coefficient is warranted in lieu of the use of a “conservative” value because the EPA is applying

an uncertainty factor component for intraspecies toxicokinetic variation in the derivation of these RfD values.

Underprediction of Tissue Concentrations in a Key Study

The National Toxicology Program (NTP) 2006 bioassay of TCDD is arguably the most complete, robust, and relevant dataset for assessment of elements of mode of action and dose-response for tumor development in laboratory rodents. Among many other components, this study includes repeated tissue concentration measurements in liver and adipose tissue over the course of the bioassay (lung and blood concentrations were also measured, but were often below the limit of quantitation in the assay). Although many figures demonstrating the performance of the Emond PBPK model were presented in the document, no comparison of the model performance with the NTP tissue concentration data was presented.

Figures 2 below present the measured adipose and liver tissue concentrations, respectively, at four time points from the NTP bioassay, along with the modeled tissue concentrations from the Emond PBPK model as reported in Appendix C of the Draft Reanalysis document. The modeled tissue concentrations are consistently below the measured tissue concentrations from the NTP bioassay, often by a factor of two.

The consequence of this underestimation is unclear, given the reliance on whole blood as a dose metric for dose-response modeling and for interspecies extrapolation. Because blood concentrations are rarely measured (or require limits of quantitation lower than those achieved in studies such as the NTP study), a similar evaluation of the accuracy of modeled blood concentrations cannot be made.

This degree of correspondence between measured and modeled concentrations, within approximately a factor of two, is generally considered to be acceptable in the context of PBPK modeling. However, this example raises two key questions:

- Why is whole blood concentration the appropriate dose metric for dose-response modeling for key events in the liver leading to liver tumors? The relationship between concentrations in the tissue of interest, liver, and whole blood varies widely due to the dose-dependent induction of CYP1A2 protein and resulting hepatic sequestration. Use of whole blood concentration is particularly problematic in light of the very limited whole blood data available for model validation and the issues associated with PF described above.
- Why use modeled tissue concentrations for dose-response assessment when measured tissue concentrations in the tissue of interest (liver) are available from nearly all of the key studies used in the MOA analysis?

Of the four studies used for dose-response modeling in the liver tumor MOA analysis, NTP (2006) and Vanden Heuvel et al. (1994) report hepatic tissue concentrations at all dose groups included in the studies. The Hassoun et al. (2000) data were obtained in tissues taken from the 2006 NTP cancer bioassay for which tissue TCDD concentrations were available. Van Birgelen et al. (1995) also report hepatic tissue concentrations for the hepatic retinol and retinyl palmitate endpoints considered in the lung tumor MOA analysis. The use of modeled whole

blood concentrations, rather than measured target tissue concentrations, for dose-response assessment introduces an unneeded level of reliance on an unvalidated aspect of the PBPK model. Target hepatic tissue concentrations can be derived based on dose-response modeling that employs measured tissue concentrations. The PBPK model can then be used to estimate human intake doses associated with a target hepatic tissue concentration from rat studies just as easily (and probably more reliably) than for those associated with whole blood concentration. These comments provide reference to key datasets that should be used to inform and refine the PBPK model and other quantitative approaches and assumptions used by EPA in the quantitative dose-response and risk assessment analyses conducted in the Draft Reanalysis document.

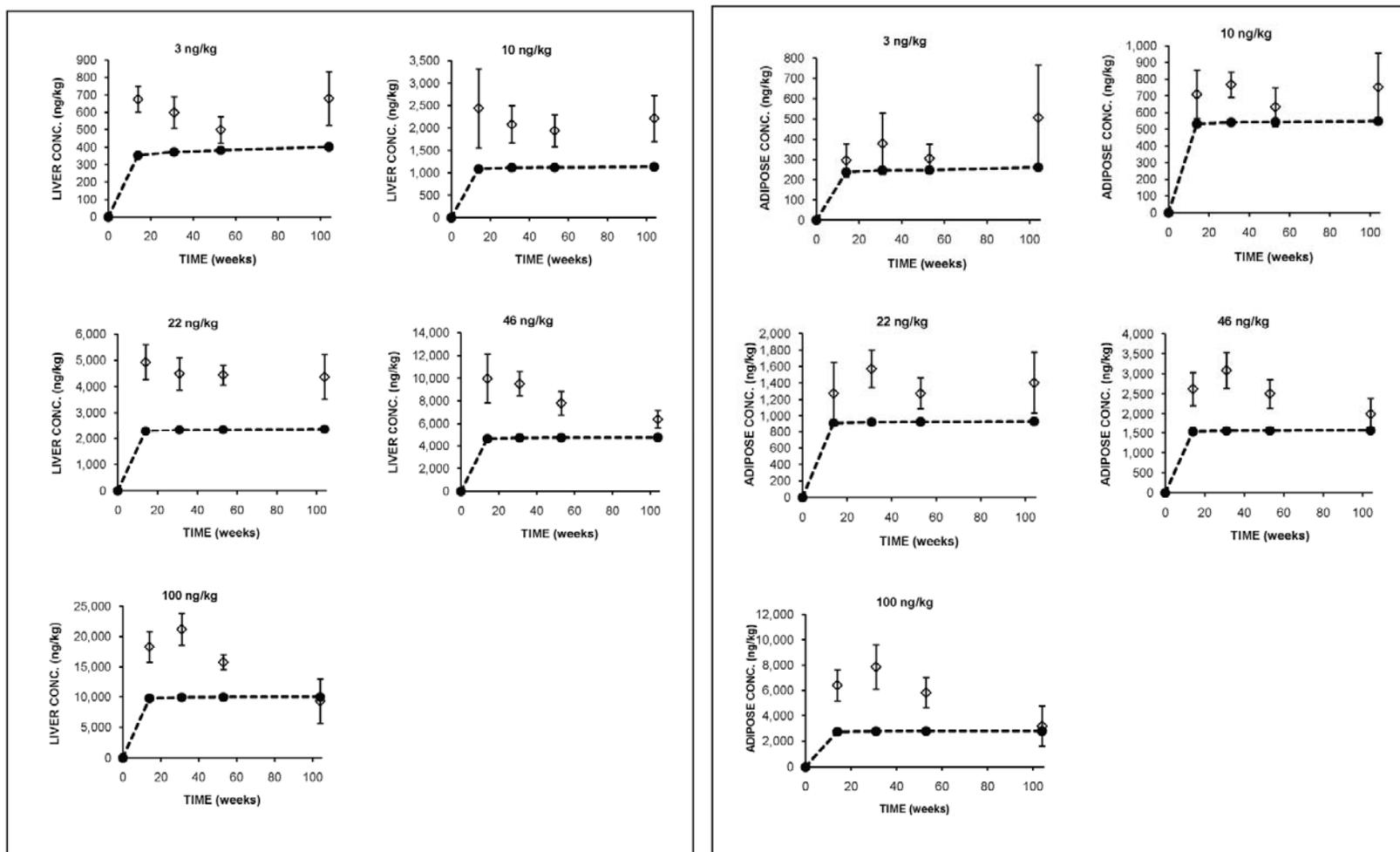


Figure 2: Comparison of measured liver and adipose tissue concentrations (first and second panel, respectively) from the NTP (2006) bioassay at four time points (open diamonds, mean +/- 1 S.D. at each time point) to modeled concentrations from the Emond PBPK model (filled circles, connected by dashed lines), as reported in Appendix C.

Relative Sensitivity of Rats and Humans: Interspecies Toxicodynamic Uncertainty Factor Component (UF_{A-TD})

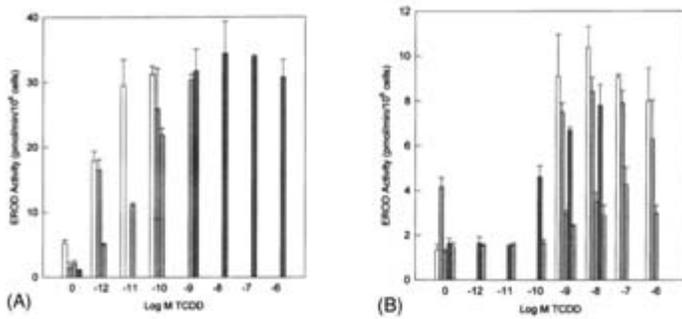
The body of evidence for the toxicodynamic extrapolation factor suggests that humans are less sensitive to TCDD activation of the AhR than either rodents or non-human primates, generally by a factor of 10 or more. Figure 3 shows the plots from three studies papers in which AhR activation was measured in primary hepatocytes in humans and in laboratory species. In all cases, the laboratory species were more sensitive than humans. These results are consistent with numerous earlier studies that assessed comparative responses in transformed human and rat cell lines (reviewed in Connor and Aylward 2006).

The difference in sensitivity is due in part to a mutation in the structure of the AhR ligand-binding domain, which results in a bulky substitution in the binding pocket and reduced ligand binding affinity (reviewed in Connor and Aylward 2006). Numerous studies have demonstrated the consistently reduced ligand-binding affinity of human AhR compared to most rodent AhRs as well as the impact on functional responses (reviewed in Connor and Aylward 2006; Silkworth et al. 2005; Flaveny et al. 2010). EPA cites data on ligand-binding affinity to demonstrate that human AhRs assessed to date show a range of ligand-binding affinity, and thus may vary in sensitivity to TCDD. However, evidence on relative human to animal sensitivity to biomarkers of AhR activation and early key events such as CYP1A1 induction is consistent in indicating a reduced sensitivity of human cells to TCDD compared to rodents, and recent work has addressed concerns about possible polymorphisms in the human AhR. Rowlands et al. (2010) sequenced 108 human AHR genes to identify single nucleotide polymorphisms (SNPs) from six ethnic populations that included Japanese, Chinese, European/Caucasian, African American, South East Asian, and Hispanic. With these new sequences, more than 200 human AHR gene sequences have been analyzed for SNPs and only 10 SNPs in the exonic sequences have been identified.

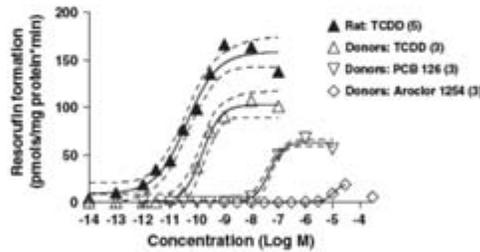
The results indicate a very limited presence of polymorphisms in the human AHR gene. The potential functional impact of six of the SNPs identified in Rowlands et al (2010) and other previous studies was investigated by Celius and Matthews (2010). The investigators reported that none of the AHR variants displayed an altered ability to modulate TCDD-dependent gene expression suggesting that the effects of these SNP changes might have minimal functional impact in the human population. Collectively, the SNP studies indicate that the human AHR is similar across the human population and the presence of subpopulations of TCDD sensitive humans has so far not been supported by the AHR genetic data.

Human hepatocytes consistently display response benchmarks indicating AhR activation at concentrations 10-fold or more higher than the corresponding benchmarks in rat primary hepatocytes. Thus, at a minimum, estimation of RfD values for responses such as hepatic enzyme induction and related downstream events should employ a data-derived

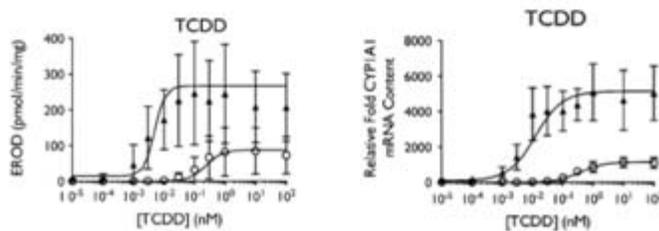
interspecies toxicodynamic uncertainty factor component (UF_{A-TD}) no larger than 1 (and, more appropriately, less than 1) rather than the default of 3. Given the assumption that all responses (not just hepatic responses) to TCDD are mediated through activation of the AhR, this UF_{A-TD} should probably be applied to all interspecies extrapolations.



Comparison of Dose Responses of Rat (A) and Human (B) EROD Activity in primary hepatocytes from Xu et al. (2000)



Comparison of Dose Responses of Rat (filled triangles) and Human (unfilled triangles) EROD Activity in primary hepatocytes from Silkworth et al. (2005)



Comparison of Dose Responses of Rat (filled triangles) and Human (unfilled circles) CYP1A1 mRNA expression and EROD Activity in primary hepatocytes from Budinsky et al. (2010)

Figure 3. Plots from three studies that show the relative sensitivity of rats and humans. Human hepatocytes consistently require 10-fold or more increased concentrations of TCDD to exhibit AhR activation and responses.

Lack of Reproducibility of Values in Table 5-21

Table 5-21 of the EPA Reanalysis document presents the results of the effort to derive illustrative RfDs related to the MOA analysis. However, of the 11 RfDs presented, only 3 could be reproduced by following the EPA-described procedure and using the data and analyses presented in the Appendices to the EPA document. However, one of those three was based on a benchmark response level that was inconsistent with the stated criterion listed by EPA. Finally, numerous issues related to the validity or accuracy of the underlying identification of points of departure and conversions from whole blood concentrations to human equivalent intake doses were also identified. Each of these issues is documented in Appendix A to this set of comments.

This lack of reproducibility in Table 5-21 was confirmed by EPA staff. Given the data- and model-intensive nature of the quantitative analyses presented in this document, this lack of reproducibility raises troubling questions regarding the reliability of the reported results in the whole document. Many of the modeling results in the document could be checked by external reviewers in the same way that Table 5-21 was checked (albeit, with a significant input of time and effort). However, many others would require significantly more effort. For example, the PBPK model output tables presented in Appendix C.4 are critical to the inter- and intra-species extrapolations described in the document, but checking these would require multiple runs of the Emond PBPK model in ACSL, a significant undertaking. This effort would also be required in order to check the results of PBPK modeling presented for the animal and human studies considered in derivation of RfDs. Finally, some of the analyses presented in the document are not described in sufficient detail to allow them to be reproduced with any amount of effort. These include the Bayesian analysis conducted in order to combine tumor types (described on pp. 5-40 to 5-41). Some of these results simply must be taken on faith, and that is more difficult to do when pervasive errors are found in a table such as 5-21.

These issues suggest that additional quality control efforts are needed on the Draft Reanalysis document prior to finalization.

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Appendix A: Annotated Table 5-21

The following is an annotated version of Table 5-21 which presents the illustrative RfDs derived by EPA for a mode of action-based evaluation of rodent liver and lung tumors. Annotation codes are described below. The table presents comments related to the ability of a reviewer to reproduce the reported RfD values based on information presented in the Draft Reanalysis and its appendices. Only 3 of the 11 RfDs can be reproduced, and of these, one relies upon benchmark dose modeling that uses incorrectly input standard deviations, so the value does not correspond to EPA's stated benchmark response target. Cells and columns in light green were added to provide intermediate numbers not originally reported in Table 5-21 to assist in the clarity of the review. Figure A1 below presents the process used in the attempt to reproduce the numbers in Table 5-21.

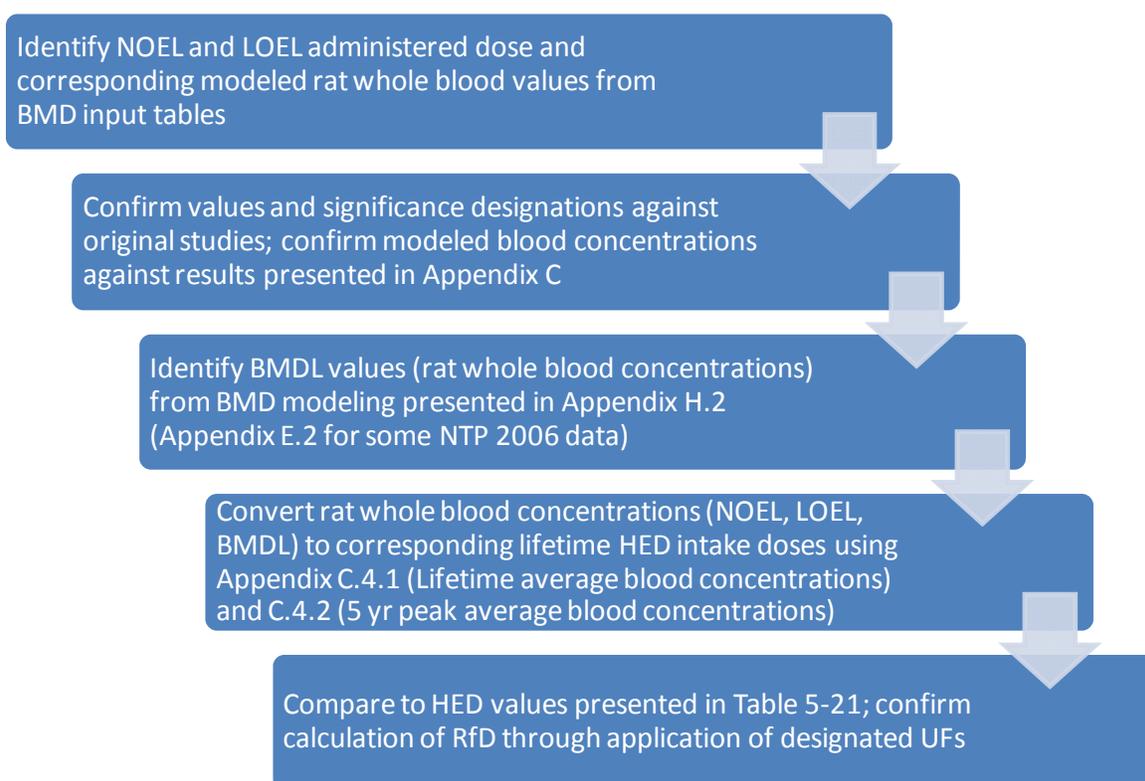


Figure A1: Review process for Table 5-21.

Annotated Table 5-21

Key to Annotations:

Text in black with green highlight: Values that can be reproduced.

Text in Red: Human lifetime intake rates corresponding to whole blood concentrations in columns A, B, and C obtained using tables in Appendix C.4.1 (lifetime average, LT) and Appendix C.4.2 (non-gestational 5 yr average, 5YR).

Text in Purple: Annotations regarding characterization of BMD model fits or other miscellaneous issues.

Text in Blue: Annotations regarding whole blood concentrations at NOAEL, LOAEL, or BMDL and comments related to identification of values as NOELs or LOELs, and transcription of values from original studies.

Text in Green: Values missing from original Table 5-21 but available in the underlying Appendices.

Cells added to clarify intermediate steps.

RfD that cannot be reproduced.

Key event	Endpoint and exposure duration	These columns were added present rat whole blood concentrations corresponding to NO(A)EL, LO(A)EL, and modeled BMDLs			Lifetime Human Equivalent Doses (HED) yielding lifetime (LT) and peak 5-yr (5YR) average blood concentrations (from Appendices C.4.1 and C.4.2, respectively) equal to rat whole blood concentrations from columns A, B, and C			RfD ^b (mg/kg-day)	Study
		NO(A)EL (ng/kg) A	LO(A)EL (ng/kg) B	BMDL ^a (ng/kg) C	NO(A)EL _{HED} (ng/kg-day)	LO(A)EL _{HED} (ng/kg-day)	BMDL _{HED} ^a (ng/kg-day)		
Liver tumors									
Changes in gene expression	CYP1A1 mRNA, 1 day	1E-02 <i>p. H-2</i>	1.1E-01 <i>p. H-2</i>	1.68E-01 <i>p. H-37</i> BMR in BMDS output does not correspond to 1 SD	1.8E-05 5.6E-05 (LT) 2.8E-05 (5YR)	3.4E-04 1.2E-03 (LT) 7.4E-04 (5YR)	2.3E-03 ^c (Appendix H) 2.3E-03 (LT) 1.4E-03 (5YR) Lifetime model apparently used for 1 day rat study	6E-13 ^{d,e}	Vanden Heuvel et al. (1994, 594318)
Changes in gene expression	Benzo(a)pyrene hydroxylase (BPH) activity (CYP1A1), 1 day	6E-02 <i>p. H-1</i>	2E-01 <i>p. H-1</i>	4.9E-01 <i>p. H-18</i>	9.2E-04 5.3E-04 (LT) 3.0E-04 (5YR)	6.0E-03 3.0E-03 (LT) 1.9E-03 (5YR)	4.6E-04 ^{c,d} (Appendix H) 1.1E-02 (LT) 7.7E-03 (5YR)	2E-11 ^{d,e}	Kitchin and Woods (1979, 198750)

	EROD (CYP1A1), 53 weeks Liver	none <i>p. H-2</i>	2.46 <i>p. H-2</i>	9.351E-02 <i>p. H-22</i>	none	1.4E-01 1.3E-01 (LT) 1.0E-01 (5YR)	9.5E-03 ^e (Appendix H) 9.5E-04 (LT) 5.8E-04 (5YR) Footnote “c” indicates poor BMD fit, but Appendix H.2.5 does not support that.	3E-10 ^e	NTP (2006, 197605)
Oxidative stress	DNA single-strand breaks, 90 days	none <i>p. H-1</i>	1.94 <i>p. H-1</i>	9.165E-01 <i>p. H-7</i> SDs transcribed incorrectly from study into BMD Input Table H.1.1, so BMR does not correspond to 1 SD from study	none	3.3E-02 9.1E-02 (LT) 7.0E-02 (5YR)	2.2E-02 ^e (Appendix H) 2.9E-02 (LT) 2.1E-02 (5YR) Footnote “c” indicates poor BMD fit, but Appendix H.2.2 does not support that.	7E-10 ^e Does not correspond to stated BMR	Hassoun et al. (2000, 197431)
	TBARS, 90 days	1.94 <i>p. H-1</i> Incorrectly noted as “LOAEL” in Table H.1.1	4.61 <i>p. H-1</i>	1.737E+00 <i>p. H-14</i> SDs transcribed incorrectly from study into BMD Input Table H.1.1, so BMR does not correspond to 1 SD from study	– 9.1E-02 (LT) 7.0E-02 (5YR)	– 3.4E-01 (LT) 2.7E-01 (5YR)	4.4E-02 (Appendix H) 7.7E-02 (LT) 5.9E-02 (5YR)	2E-09 ^e	Hassoun et al. (2000, 197431)
	Cytochrome C reductase, 90 days	4.61 <i>p. H-1</i>	8.15 <i>p. H-1</i> LOAEL incorrectly identified in Table H.1.1	3.1E+00 <i>p. H-3</i> SDs transcribed incorrectly from study into BMD Input Table H.1.1, so BMR does not correspond to 1 SD from study	– 3.4E-01 (LT) 2.7E-01 (5YR)	– 7.8E-01 (LT) 6.3E-01 (5YR)	8.8E-02 (Appendix H) 1.9E-01 (LT) 1.5E-01 (5YR)	3E-09 ^e	Hassoun et al. (2000, 197431)

Hepatotoxicity	Toxic hepatopathy, 2 years	none <i>p. E-10</i>	2.56 <i>p. E-10</i>	3.06 <i>p. E-195</i>	none	1.4E-01 1.4E-01 (LT) 1.1E-01 (5YR)	1.8E-01 ^c (Appendix E) 1.8E-01 (LT) 1.4E-01 (5YR) Footnote “c” indicates poor BMD fit, but Appendix E.2.39 does not support that.	5E-09 ^f 5E-10 (UF of 30 was apparently applied instead of UF of 300 as indicated by footnote f)	NTP (2006, 197605)
	Hepatocyte hypertrophy, 31 weeks Modeled data are from 2 yr time point	none <i>p. E-10</i> All dose groups are significant.	2.56 <i>p. E-10</i>	7.9E-01 <i>p. E-181</i>	9.3E-02 none	3.3E-01 1.4E-01 (LT) 1.1E-01 (5YR)	8.8E-03 (Appendix E) 2.3E-02 (LT) 1.7E-02 (5YR) Appendix E.2.35 suggests model fit is inadequate so footnote “c” should be applied.	3E-10 ^e	NTP (2006, 197605)
Hepatocellular proliferation	Labeling index, 31 weeks	none <i>p. H-2</i>	2.33 <i>p. H-2</i>	3.13 <i>p. H-33</i>	none	1.4E-01 1.2E-01 (LT) 9.4E-02 (5YR)	6.6E-02 ^c (Appendix H) 1.9E-01 (LT) 1.5E-01 (5YR)	2E-09 ^e	NTP (2006, 197605)
Lung tumors									
Metabolic enzyme induction	EROD (CYP1A1), 53 weeks Lung	none <i>p. H-2</i>	2.46 <i>p. H-2</i>	5.91E-02 <i>p. H-26</i>	none	1.4E-01 1.3E-01 (LT) 1.0E-01 (5YR)	2.9E-04 ^e (Appendix H) 5.2E-04 (LT) 2.9E-04 (5YR) Footnote “c” indicates poor BMD fit, but Appendix H.2 does not support that.	1E-11 ^e	NTP (2006, 197605)

Retinoid homeostasis	Hepatic retinol and retinyl palmitate, 90 days	none <i>p. E-13</i>	7.20 <i>p. E-13</i>	3.36 (retinol) <i>p. E-234</i> 3.65E+01 (retinyl palmitate) <i>p. E-241</i>	none	1.1E+00 6.5E-01 (LT) 5.2E-01 (5YR)	1.7E-01^d (Appendix E) 2.1E-01 (LT) 1.7E-01 (5YR) (retinol) 6.1E+00 (LT) 5.0E+00 (5YR) (retinyl palmitate)	6E-09^e	Van Birgelen et al. (1995, 198052)
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^a BMR for continuous endpoints—1 standard deviation; for quantal endpoints—10%.

^b Bolded NOAEL, LOAEL, or BMDL is selected POD; poorly-fitting BMDLs above the LOAEL not used.

^c Poor BMD model fit or no good model fit.

^d Could be higher depending on the effect of background exposure (see Section 5.3.2.1).

^e UF = 30; UFA = 3; UFH = 10.

^f UF = 300; UFA = 3; UFH = 10; UFL = 10.