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United States Environmental Protection Agency

TOXICOLOGICAL REVIEW OF METHANOL (NONCANCER) APPENDICES

(CAS No. 67-56-1)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

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ACGIH	American Conference of Governmental and Industrial Hygienists
ADH	alcohol dehydrogenase
ADH1	alcohol dehydrogenase-1
ADH3	formaldehyde dehydrogenase-3
AIC	Akaike Information Criterion
ALD	aldehyde dehydrogenase
ALDH2	mitochondrial aldehyde dehydrogenase-2
ALT	alanine aminotransferase
ANOVA	analysis of variance
AP	alkaline phosphatase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve, representing the cumulative product of time and concentration for a substance in the blood
β-NAG	N-acetyl-beta-D-glucosaminidase
Bav	oral bioavailability
BMD	benchmark dose(s)
BMD_{1SD}	BMD for response one standard deviation from control mean
BMDL	95% lower bound confidence limit on BMD (benchmark dose)
$BMDL_{1SD}$	BMDL for response one standard deviation from control mean
BMDS	benchmark dose software
BMR	benchmark response
BSO	butathione sulfoximine
BUN	blood urea nitrogen
BW, bw	body weight
C_1 pool	one carbon pool
C_{max}	peak concentration of a substance in the blood during the exposure period
C-section	Cesarean section
CA	chromosomal aberrations
CAR	conditioned avoidance response
CASRN	Chemical Abstracts Service Registry Number
CAT	catalase
CERHR	Center for the Evaluation of Risks to Human Reproduction at the NTP
CH_3OH	methanol
CHL	Chinese hamster lung (cells)
CI	confidence interval
Cl_S	clearance rate
C_{max}	peak concentration
CNS	central nervous system
CO_2	carbon dioxide
con-A	concanavalin-A
CR	crown-rump length
CSF	Cancer slope factor
C_{ss}	steady-state concentration
CT	computed tomography
C_{VB}	concentration in venous blood
C_{VBbg}	background concentration in venous blood
C_{VBmb}	concentration in venous blood minus constant background
CYP450	cytochrome P450
d, δ, Δ	delta, difference, change
D_2	dopamine receptor
DA	dopamine
DIPE	diisopropyl ether
DMDC	dimethyl dicarbonate
DNA	deoxyribonucleic acid
DNT	developmental neurotoxicity test(ing)
DOPAC	dihydroxyphenyl acetic acid
DPC	days past conception
DTH	delayed-type hypersensitivity
EFSA	European Food Safety Authority
EKG	electrocardiogram
EO	Executive Order
EPA	U.S. Environmental Protection Agency
ERF	European Ramazzini Foundation
EtOH	ethanol
F	fractional bioavailability
F_0	parental generation
F_1	first generation
F_2	second generation
F344	Fisher 344 rat strain
FAD	folic acid deficient
FAS	folic acid sufficient
FD	formate dehydrogenase

FP	folate paired
FR	folate reduced
FRACIN	fraction inhaled
FS	folate sufficient
FSH	follicular stimulating hormone
γ-GT	gamma glutamyl transferase
g	gravity
g, kg, mg, μg	gram, kilogram, milligram, microgram
G6PD	glucose-6-phosphate dehydrogenase
GAP43	growth-associated protein (neuronal growth cone)
GD	gestation day
GFR	glomerular filtration rate
GI	gastrointestinal track
GLM	generalized linear model
GLP	good laboratory practice
GSH	glutathione
HAP	hazardous air pollutant
HCHO	formaldehyde
HCOO	formate
Hct	hematocrit
HEC	human equivalent concentration
HED	human equivalent dose
HEI	Health Effects Institute
HERO	Health and Environmental Research Online (database system)
HH	hereditary hemochromatosis
5-HIAA	5-hydroxyindolacetic acid
HMGSH	S-hydroxymethylglutathione
Hp	haptoglobin
HPA	hypothalamus-pituitary-adrenal (axis)
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substances Databank
HSP70	biomarker of cellular stress
5-HT	serotonin
IL	interleukins
i.p.	intraperitoneal
IPCS	International Programme on Chemical Safety
IQ	intelligence quotient
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
k_1	first-order urinary clearance
k_1C	first-order urinary clearance scaling constant; first order clearance of methanol from the blood to the bladder for urinary elimination
k_{ai}	first order uptake from the intestine
k_{as}	first order methanol oral absorption rate from stomach
k_{bl}	rate constant for urinary excretion from bladder
k_{iv}	respiratory/cardiac depression constant
KLH	keyhole limpet hemocyanin
KLL	alternate first order rate constant
K_m	apparent Michaelis-Menten constant; substrate concentration at half the enzyme maximum velocity (V_{max})
k_{si}	first order transfer between stomach and intestine
L, dL, mL	liter, deciliter, milliliter
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LH	luteinizing hormone
LLF	(maximum) log likelihood function
LMI	leukocyte migration inhibition (assay)
LOAEL	lowest-observed-adverse-effect level
M, mM, μM	molar, millimolar, micromolar
MeOH	methanol
MLE	maximum likelihood estimate
M-M	Michaelis-Menten
MN	micronuclei
MOA	mode of action
4-MP	4-methylpyrazole (fomepizole)
MRI	magnetic resonance imaging
mRNA	messenger RNA
MTBE	methyl tertiary butyl ether
MTX	methotrexate
N_2O/O_2	nitrous oxide
NAD^+	nicotinamide adenine dinucleotide
NADH	reduced form of nicotinamide adenine dinucleotide
NBT	nitroblue tetrazolium (test)
NCEA	National Center for Environmental Assessment
ND	not determined
NEDO	New Energy Development Organization (of Japan)
NIEHS	National Institute of Environmental Health Sciences

NIOSH	National Institute for Occupational Safety and Health
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NP	nonpregnant
NR	not reported
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program at NIEHS
NZW	New Zealand White (rabbit strain)
OR	osmotic resistance
ORD	Office of Research and Development
OSF	oral slope factor
OU	oculus uterque (each eye)
OXA	oxazolone
P, p	probability
PB	blood:air partition coefficient
PBPK	physiologically based pharmacokinetic model
PC	partition coefficient
PEG	polyethylene glycol
PFC	plaque-forming cell
PK	pharmacokinetic
PMN	polymorphonuclear leukocytes
PND	postnatal day
POD	point of departure
ppb, ppm	parts per billion, parts per million
PR	body:blood partition coefficent
PWG	Pathology Working Group of the NTP of NIEHS
Q wave	the initial deflection of the QRS complex
$Q_C C$	cardiac output scaling constant
Q_P	pulmonary (alveolar) ventilation
QRS	portion of electrocardiogram corresponding to the depolarization of ventricular cardiac cells.
R^2	square of the correlation coefficient, a measure of the reliability of a linear relationship.
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
$R_{Øbg}$	zero-order endogenous production rate
ROS	reactive oxygen species
S9	microsomal fraction from liver
SAP	serum alkaline phosphatase
s.c.	subcutaneous
SCE	sister chromatid exchange
S-D	Sprague-Dawley rat strain
SD	standard deviation
S.E.	standard error
SEM	standard error of mean
SGPT	serum glutamate pyruvate transaminase
SHE	Syrian hamster embryo
SOD	superoxide dismutase
SOP	standard operating procedure(s)
t; $T_{½}$, $t_{½}$	time; half-life
T wave	the next deflection in the electrocardiogram after the QRS complex; represents ventricular repolarization
TAME	tertiary amyl methyl ether
TAS	total antioxidant status
Tau	taurine
THF	tetrahydrofolate
TLV	threshold limit value
TNFα	tumor necrosis factor-alpha
TNP-LPS	trinitrophenyl-lipopolysaccharide
TRI	Toxic Release Inventory
U83836E	vitamin E derivative
UF(s)	uncertainty factor(s)
UF_A	UF associated with interspecies (animal to human) extrapolation
UF_D	UF associated with deficiencies in the toxicity database
UF_H	UF associated with variation in sensitivity within the human population
UF_S	UF associated with subchronic to chronic exposure
V_d	volume of distribution
V_{max}	pseudo-maximal velocity of metabolism
V_{maxC}	multiplier for allometric scaling of V_{max}
VDR	visually directed reaching test
VitC	vitamin C
VPR	ventilation perfusion ratio
v/v	volume of solute/volume of solution
VYS	visceral yolk sac
WBC	white blood cell
WOE	weight of evidence
w/v	weight (mass of solute)/volume of solution
χ^2	chi square

APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS, AND DISPOSITION

A.1. External Peer Review Panel Comments

The draft toxicological review of methanol (U.S. EPA, 2011a, c) has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006b). An external peer-review meeting was held July 22, 2011. There were seven external peer reviewers. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. At the workshop, they discussed their responses to each of the charge questions and consensus was not sought. A summary of significant comments made by the external reviewers and EPA's responses to these comments arranged by charge question follow.

A subsequent follow-up peer review was completed in July 2013 to obtain feedback from members of the original 2011 peer review panel on the 2013 revised draft methanol (noncancer) toxicological review and EPA's response to the 2011 peer review comments. The follow-up comments from these peer reviewers and EPA responses are presented in this section, with general comments at the beginning of the section and charge specific comments at the end of each charge question. Two other members of the original 2011 peer review panel submitted written public comments, which are addressed in the public comment section (Section A.2) of this appendix.

The summary of the peer review comments quotes the reviewer comments extensively, but synthesizes and paraphrases in some cases for the sake of clarity and conciseness. Additionally, the reviewers made a number of editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

EPA received comments from the public on the 2011 and 2013 draft toxicological reviews prior to the 2011 peer review and the 2013 follow-up peer review, which were distributed to both peer review panels for their consideration. Public comments are posted to the federal docket at www.regulations.gov; search for docket ID No. EPA-HQ-ORD-2009-0398.[1] A summary of these public comments and EPA's responses are included in Section A.2 of this appendix.

[1] Public comments on the draft methanol (noncancer) Toxicological Review posted to www.regulations.gov can be found at the following URL: http://www.regulations.gov/#!docketDetail;D=EPA-HQ-ORD-2009-0398.

General Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: One reviewer stated that "The revised (May 2013) version of 'Toxicological Review of Methanol (noncancer)' has been improved significantly in comparison to its external peer-review draft (U.S. EPA, 2011a, c) version. It addressed the key recommendations, comments, and suggestions provided in my Post-Meeting Comments of 7/31/2011."

Response: EPA appreciates the affirmation of sufficient revisions to the Toxicological Review in response to previous peer-review comments.

Comment 2: One reviewer commented that "The EPA and the authors of this review of the non-cancer effects of methanol are to be commended for this latest version" and added that "[t]he overall document is much more concise and direct in detailing the key features of the risk assessment that has been conducted." The reviewer stated that "…a number of edits that responded well to the comments of previous reviewers as well as the public…include the utilization of background methanol levels in the PBPK model, the discussion of the relevance of the blood levels resulting from the RfC/RfD numbers in comparison to endogenous methanol levels, as well as a better explanation of various parameters in the PBPK models (such as using only the Sprague-Dawley rat data, not the F344 rat, and adding more human data to the validation)."

Response: EPA appreciates the affirmation of sufficient revisions to the Toxicological Review in response to previous peer-review comments.

Comment 3: One reviewer requested that "a short statement that the CNS damage seen in the acute overdose exposures most likely results from the acidosis and not from methanol per se" be added to the beginning of Appendix C.

Response: A short statement has been added to the beginning of Appendix C, as requested, to indicate that CNS damage seen in the acute overdose exposures most likely results from the acidosis and not from methanol per se.

A.1.1. "Toxicokinetics and PBPK Modeling"

A.1.1.1. Charge A1. Please comment on the scientific soundness of the PBPK model used in this assessment.

Summary of Comments: *In general, four reviewers stated that the PBPK model structure was sound for the purposes of this assessment, a fifth reviewer stated that they noticed no*

obvious flaws but could not comment on a technical level due to a lack of expertise, and two reviewers did not explicitly state whether the PBPK models were sound but provided comments. Several reviewers commented that the models were comprehensively documented and that stated assumptions are justified for the purposes of this assessment. One reviewer commended the Agency for "developing a consistent model framework and sets of species-specific parameters which have been validated across several somewhat diverse data sets." Another reviewer commented that "the Sprague-Dawley (S-D) rat PBPK model is inappropriately parameterized (or insufficiently validated) for the inhalation route" and provided specific suggestions to improve or validate the rat, mouse and human PBPK models developed by EPA for the purposes of this assessment. Specific comments or suggestions made by the reviewers with respect to this charge are described below, along with EPA responses.

Comment 1: One reviewer asked for "clarification of the process for evaluating the usefulness of each model for the assessment and why the nonhuman primate model was not included."

Response: EPA has described a framework (Chiu et al., 2007; U.S. EPA, 2006a) useful to evaluate models for inclusion in an IRIS assessment. This framework includes review of the model purpose, model structure, mathematical representation, parameter estimation, computer implementation, predictive capacity, and statistical analyses. Currently, there is no specific EPA policy or criteria for PBPK model use in IRIS assessments; consequently, the usefulness of a PBPK model for a given species and assessment is a matter of scientific judgment and a number of EPA PBPK experts are involved in making this judgment. Specific criteria used in evaluating methanol models are presented in Section 3.4.1.2.

The ability of the model to fit a wide range of experimental data with a single set of parameters is one of the critical considerations. When a chemical-specific (e.g., methanol) model is able to predict experimental data for a range of doses or exposure conditions, there is confidence that the model can predict chemical-specific (e.g., methanol) pharmacokinetics under exposure conditions for which one does not have data. Confidence that one or more animal species are properly represented by the model increases EPA's confidence that the models can be used to extrapolate test animal exposures to human exposures.

Regarding the nonhuman primate model, EPA had incorrectly stated [in Appendix C, Section C.3 of the draft assessment (U.S. EPA, 2011b)] that external concentrations were used for dose-response modeling for the monkey. However, a nonhuman primate classical PK model (not a PBPK model) was adapted for use in the draft assessment and is used in the final assessment to estimate internal doses (blood methanol C_{max} values) for derivation of internal

dose BMDLs associated with the Burbacher et al. (2004b; 1999b) monkey study. See Appendix D, Section D.4, and Table D-10 in the final assessment.

Comment 2: One reviewer suggested that "the use [of] a bladder compartment is atypical [thus] the EPA should consider recoding the model to include a kidney/renal compartment that considers excretion of methanol by the kidney."

Response: Urine passes through the bladder, which serves as a storage reservoir between urine voids, so it is biologically realistic to include a compartment that represents this part of the elimination pathway. Human urinary data are sufficient to identify a bladder residence-time constant, but similar time-course data are not available for rats; therefore, the compartment only impacts the human PBPK model. While only a small fraction of ingested/absorbed methanol is excreted via the urinary elimination pathway, inclusion of a bladder compartment is significant in that it allows a more precise fit to the human urine time-course data, which show a slight nonlinearity in the human dosimetry.

Additionally, since the kidney glomeruli filter the blood directly, it is biologically realistic to describe renal excretion as elimination directly from the blood compartment at a rate proportional to the methanol concentration in blood. Inclusion of an explicit kidney compartment of the form typically used for PBPK models would be most beneficial if the kidney was a target tissue for toxicity. Development of a model of glomerular filtration for methanol would require more extensive research, time, and resources. Addition of this type of compartment is not expected to significantly impact the PBPK model predictions that are currently well predicted and validated using the model structure applied in this assessment.

Comment 3: One reviewer questioned using a bladder component for only the human model and suggested that the impact of a bladder component on the rodent models should be tested.

Response: The bladder compartment is present in the model code used for both rats and humans. The bladder compartment time constant (k_{bl}) was identified for humans, and urinary excretion rates were plotted and compared with existing human urine data (Figure B-7). Similar urinary excretion data are not available for rats; hence, the bladder time-constant (k_{bl}) cannot be identified for that species. The use of the bladder compartment and k_{bl} has no impact on predicted blood concentrations; and hence, no impact on any of the rat model predictions.

Comment 4: One reviewer suggested that descriptions (e.g., page 3-45 of the draft assessment) of the two divergent models that were considered (Michaelis-Menten or not) are confusing, and should be clarified.

Response: All discussion of this and other considerations in the development and calibration of the human PBPK model have been consolidated into Appendix B, Section B.2.6, and revised for clarification.

Comment 5: With respect to the monkey PK model, one reviewer commented that "the description of the chamber volume [page 3-49 of the draft assessment] should be expanded" to clarify the equipment in question and whether there is any evidence that incomplete mixing occurred.

Response: The monkey PK model description and analysis is now found in Appendix B, Section B.3 of this final assessment. Information regarding the chamber volume adjustment for the PK model was added to this section. Briefly, the chamber volume was fit to the chamber concentration data to allow for a better fit to the "mixing time" in the chamber (mixing time is the time it takes for the chamber concentration to rise or fall after the inlet concentration is turned on or off) and to account for the volume filled by the monkey and other chamber equipment. The "accessible [chamber] volume" reported by Burbacher et al. (1999b) was 1,380 L, and the model fitted volume was 1,220 L. A detailed description of the chamber set-up is included in the original Burbacher et al. (1999b) report and was not included in Appendix B of this assessment.

Since methanol chamber time-course data were available for this study, and model predictions did not match the data when the assumption of perfect mixing was used (chamber volume of 1,380 L), EPA considered it reasonable to use the available data to calibrate the residence- or mixing-time for the model of the chamber concentration. The text in Appendix B, Section B.3 has been modified to indicate why V_{ch} was varied.

Comment 6: With respect to the human PBPK and the monkey PK models, one reviewer stated that EPA "has not clearly articulated why two different fractional absorption values were used based on the same data base (see pages 3-50 (60%) and 3-42 (86.5%) of the draft assessment)."

Response: In the external peer review draft document the derivation of the value of FRACIN used for humans was described in detail in Appendix B, p. B-29. The exact mean absorption measured by Sedivec et al. (1981) was 57.7%, but this was based on total ventilation. However the human PBPK model uses alveolar ventilation, assumed to be 2/3 of total ventilation, with the remaining 1/3 of each breath assumed to not enter the gas exchange area. Therefore, to yield the same net uptake as Sedivec et al. (1981), 57.7% was divided by 2/3 to yield 86.55% for the human model parameter, FRACIN.

In contrast the monkey model used total respiration rather than alveolar ventilation. This detail was included at the bottom of p. 3-49, where the monkey model parameter R_c was defined as "allometric scaling factor for **total** monkey respiration" (emphasis added). However the parameter symbol "F" was used for the monkey "fraction of inhaled" to further distinguish it from the human FRACIN, given that they are applied to different portions of total ventilation. Further, in the description of the monkey parameter "F" on p. 3-50 (immediately after the value of 60% is given), the document noted that 60% was the "(rounded)" value from Sedivec et al. (1981) and went on to state, "F and V_{mk} cannot be uniquely identified, given the model structure, so F was set to the (approximate) human value to obtain a realistic estimate of V_{mk}. For example, if both F and V_{mk} are increased by 50%, then also increasing the fitted V_{max} by 50% would yield identical model fits. Any positive value could be assigned to F and it would not affect the resulting model fits. Given that the airways in a 2-4 kg monkey are much smaller than those in a 70 kg human, it is unlikely that the transport characteristics for which F and FRACIN account are identical in the two species. But a realistic value was considered desirable for the monkey, so the approximate *total ventilation* human value was assumed to be sufficient.

Since the revised monkey model was ultimately not used in deriving the RfC or RfD, the detailed explanation given here was not considered necessary and was not included in the draft document. The human FRACIN has since been revised to 75% using additional data as noted by another peer reviewer and described in detail in Appendix B, Section B.2. The value used for the monkey model was therefore also revised to 50%, maintaining the 2/3 factor vs. human, and a brief description provided. Reference to the Sedivec et al. (1981) paper was removed from this part of the document, since a different data set is used.

Comment 7: Two reviewers noted that the blood methanol levels predicted by the EPA rat PBPK model are much lower than the levels reported for S-D rats on recently located (supplied by industry at the peer review meeting) pages from the NEDO (1987) report and Perkins et al. (1995). One of the reviewers further noted that if the fraction inhaled (FRACIN) model parameter is changed from 20% to a value more consistent with the mouse (66.5%) and human (86.6%) estimates, the 1,000 ppm blood prediction is in agreement with Perkins et al. (1995).

Response: In the draft assessment, inhalation data for F344 rats were used. Since the current noncancer assessment does not use any bioassay data from F344 rats, all PK data (and PBPK modeling results) for F344 rats have now been removed. The PBPK model in the final assessment uses the S-D rat inhalation data from Perkins et al. (1996a) for calibration, yielding a more appropriate fitted value for FRACIN (81%) and model predictions that are more consistent with the NEDO (1987) reported blood levels (See Sections B.2.2, B.2.4, and B.2.5).

Comment 8: One reviewer noted that, with respect to the Burbacher et al. (1999a) monkey data, EPA has not justified why the second trimester group is considered the most representative.

Response: When the data and fits shown in Figure 3-14 were evaluated, EPA noted that overall there appears to be no significant or systematic difference among the NP and pregnant groups. The solid lines, in the figure, are model simulations calibrated to only the 2nd trimester data (details below), but they just as adequately represent average concentrations for the NP and 3rd trimester data. Likewise, a PK model calibrated to the NP PK data adequately predicted the maternal methanol concentrations in the pregnant monkeys (results not shown). Since any maternal:fetal methanol differences are expected to be similar in experimental animals and humans (with the maternal:fetal ratio being close to one due to methanol's high aqueous solubility and relatively limited metabolism by the fetus), the predicted levels for the 2nd trimester maternal blood are used in place of measured or predicted fetal concentrations.

Thus the primary justification for only showing the results for the 2nd trimester is that it does not matter which stage one selects, since there is not a significant difference in either the data or the model fits among the stages. While there is no clear effect of pregnancy on the PK in monkeys, to the extent that there is some trend (for example, if the AUC decreases slightly with the extent of pregnancy), the value of the metric during the 2nd trimester was expected to be in between the values for the 1st and 3rd trimesters, hence closer to an overall average. In short, because the physiological changes induced by pregnancy are at an intermediate stage in the 2nd trimester relative to the 1st and 3rd, PK parameters were expected to also be intermediate and therefore most representative of the average over all of pregnancy. However, had there been clear time-dependence in the PK data, a quantitative analysis could have been used to incorporate that trend.

Comment 9: One reviewer suggested that the K_m values estimated by the rat and human models "don't seem to reflect the true Michaelis values of the metabolic enzymes themselves."

Response: Since methanol is metabolized by multiple enzymes with differing K_m values, at best one would expect the empirical K_m values identified here to represent an average of the enzyme-specific values (weighted by the contribution of each enzyme to total metabolism). Further, it is quite typical to find that in vivo PK data are not well-predicted when K_m values measured in vitro are used in a model, hence K_m values estimated from in vivo data are not expected to be identical to values measured in vitro. The K_m values identified for the revised PBPK models described here are 28 mg/L for rats and 36 mg/L for humans. Pollack and Brouwer (1996) analyzed the kinetics of formaldehyde formation in vitro and estimated K_m = 39.3 mg/L using nonpregnant adult rat liver homogenates and K_m = 35.5 mg/L with GD 20 homogenates:

quite similar to the revised value for the rat used in the final assessment. Mani et al. (1970) measured methanol kinetics with human liver ADH and obtained a K_m of 48 mg/L. This is likewise quite similar to the K_m estimated here with the human PBPK model.

Comment 10: With respect to the human PBPK model, one reviewer noted that useful human kinetic studies (Haffner et al., 1992; Schmutte et al., 1988) were overlooked, and that these studies "are potentially quite valuable in model parameterization because they do not involve the inhalation route."

Response: Previously only inhalation data was included for humans. These studies [(Haffner et al., 1992) and (Schmutte et al., 1988)] provide i.v. and oral data, and they have been added to the PBPK analysis (see Appendix B, Section B.2.6). Specifically, the oral data are now used for model calibration, allowing identification of human oral absorption rate constant and bioavailability. The i.v. data from Haffner et al. (1992) are from only four individuals; these data were used to validate the model by comparing model predictions following an i.v. dose with the experimental data (Figure B-13).

Comment 11: One reviewer recommended that EPA perform sensitivity analyses of the rat and human PBPK modeling results under conditions approximating the BMDL, stating that "at a minimum, EPA should assess whether or not the model they used in the risk assessment can (adequately) simulate the additional human data identified herein and conduct and provide human model sensitivity analyses at the RfC and RfD."

Response: A sensitivity analysis has been conducted and a detailed description of this analysis is included in Appendix B, Section B.2.7. However, such an analysis can only partly inform the question of model adequacy, which is addressed in more detail in the response to Charge A1 Comment 1 above.

Comment 12: With respect to the mouse PBPK model, one reviewer stated that "it seems odd that, for oral dosing, the mouse blood levels are reported to be insensitive to any parameter related to clearance (e.g., metabolism, blood flow to the liver) (pp B-16 and B-18 of the draft review)," and requested clarification in the text regarding the type of oral dose that is being simulated.

Response: Since direct measurements of mouse (CD-1) blood concentrations for bioassay exposures are available (Rogers et al., 1993b) and used for the BMD analysis in this final assessment, the mouse PBPK model is not utilized in the final assessment to estimate an internal dose metric. Therefore the description, analysis, and discussion of the mouse model are not included in the final assessment.

Comment 13: One reviewer commented that "the runtime files that should reproduce Figures B-2 and B-5 yield simulations that are slightly off." The reviewer also commented, regarding Figures B-6, B-7, and B-8, that "these files do not accurately reproduce the figures in the document."

Response: The figures were produced with the background turned on, while the PBPK runtime files had the background turned off. The current version of the PBPK model, available electronically from the EPA HERO database (U.S. EPA, 2012b), includes runtime files which will exactly reproduce the figures in the toxicological review, aside from legend placement, which is dependent on acslX window sizes.

Comment 14: With respect to the mouse PBPK model sensitivity analysis, one reviewer noted that "EPA does not provide files that fully recreate the sensitivity analyses--only those parameters demonstrated in Figures B-6, B-7, and B-8." This reviewer commented that "the sensitivity analysis does not appear to have been comprehensive," and cited FRACIN as an example of a parameter that was not tested, yet seems to be a parameter to which the mouse PBPK model is sensitive.

Response: As stated in the response to Charge A1 Comment 12, the mouse model has been removed from the final assessment; thus no sensitivity analysis is included for the mouse PBPK model.

Comment 15: One reviewer commented that "it is not clear why two saturable metabolic pathways are needed for the Sprague-Dawley rat and only one for the F344 rat."

Response: The liver metabolism in the S-D rat is now described using a single saturable rate equation. As discussed in response to Charge A1 Comment 7, the analyses of F344 rat PK data has been removed from the toxicological review.

Charge A1 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: One reviewer commented that "[w]hile the revised PBPK model used in the derivation of reference toxicity values of methanol still contains many simplifications and shortcuts, it seems to be adequate for chemical-specific risk assessment - the purpose for which EPA developed this model."

Response: The EPA agrees with the reviewer that although the Agency's PBPK model contains necessary, but simplifying assumptions, it is adequate for the purposes of the methanol (noncancer) assessment.

Comment 2: One reviewer suggested that EPA "reconnect the urine clearance to arterial blood" because, while it "seems to approximate realistically the quantitative clearance of methanol…the assumption that the urine equilibrates with mixed venous blood may be inappropriate for some other chemicals."

Response: The reviewer is correct that by not including an explicit kidney compartment, where urinary clearance would be limited by the arterial blood concentration and flow to the kidney, the model does not include the limitation to clearance that occurs because renal flow is only a fraction of total cardiac output. This limitation would be a significant factor if total urinary clearance was a significant fraction of renal blood flow, which likely occurs for some other chemicals. However for the methanol model the clearance rate for this pathway in the rat is only 0.24% of renal blood flow [using a renal flow fraction of 0.141 from Brown et al. (1997)] and in the human is only 0.07% of renal blood flow [using a renal flow fraction of 0.175 from Brown et al. (1997)]. Therefore including an explicit kidney compartment with its own flow rate would have a negligible impact on the methanol model results reported here. These calculations and a statement that the approach should only be used when renal clearance is a small fraction (<10%) of renal blood flow have been added to Appendix B.

A distinction between arterial and venous blood concentration can also occur due to high rates of gas exchange in the lung. For oral (and i.v. exposures) the rate of exhalation is very low and the arterial and venous blood concentrations were virtually indistinguishable for both rats and humans. For inhalation exposures a small difference occurred, but less than 1% for the rat and 4% for humans. Thus, as suggested by the reviewer, the difference is not significant for methanol, hence has little impact on model predictions.

Comment 3: One reviewer encouraged EPA to "[p]lease keep unchanged equation and parameters for methanol metabolism in the PBPK model, but change in the text the explanation of meaning of V_{max} to 'pseudo-maximal velocity of metabolism' and K_M to 'apparent Michaelis-Menten constant of metabolism'" because "the metabolism of methanol is potentially saturable" and, while the Michaelis and Menten terms "V_{max}" and "K_M" are appropriate for use in EPA's PBPK model equation, "…Michaelis and Menten equation describes initial velocity in homogenous enzymatic systems" and "the [EPA] PBPK model describes rate of metabolism, measured over time in the whole organism."

Response: The EPA appreciates the reviewer's support for the proposed model structure and has chosen to retain this description of metabolism as a saturable process in the PBPK model for both rats and humans. The EPA agrees that there is not an exact correspondence between the parameters, V_{max} and K_m, as used and calibrated in the model versus values obtained from initial

rate experiments *in vitro*. Therefore the descriptions of these parameters in the text and glossary have been adjusted as indicated.

A.1.1.2. Charge A2. Please comment on the scientific justification for the subtraction of background levels of methanol from the data in relation to the quantification of noncancer risks.

Summary of Comments: *EPA stated two key assumptions for this approach in the peer review draft: "(1) endogenous levels do not contribute significantly to the adverse effects of methanol or its metabolites; and (2) the exclusion of endogenous levels does not significantly alter PBPK model predictions." Most reviewers were in general agreement with the first assumption, but expressed the need to better characterize background levels of methanol and their relationship to the RfC/D. Three reviewers were concerned that the first assumption, and the subtraction of methanol background levels, gives the impression that endogenous/background methanol levels are not important. With respect to the second assumption, none of the reviewers disagreed with EPA's determination that the exclusion of endogenous/background levels does not significantly alter PBPK model predictions; however, two reviewers advocated the use of a PBPK model that incorporates a background term and one reviewer favored the use of the simpler PBPK model (without background levels). Specific comments or suggestions made by the reviewers with respect to this charge are described below, along with EPA responses.*

Comment 1: Three reviewers expressed concerns over the first assumption, that endogenous/background levels do not contribute significantly to adverse effects. One reviewer stated that EPA was giving the impression that "cumulative exposures from different sources are not important." A second reviewer indicated that the first assumption is not met because "the RfC and RfD correspond to blood methanol concentrations in humans squarely in the range of normal 'background' levels."." The third reviewer asked "If endogenous levels of methanol do not contribute to adverse effects and an exposure does not produce an increase above background levels, how can that exposure lead to an adverse effect?"

Response: The language in the draft assessment may have confused this issue and has been clarified in Section 3.4.3.2. EPA acknowledges that endogenous/background methanol concentrations can be a contributing factor in health effects that are associated with exogenous methanol exposure. As indicated in response to Comment 2 below, for the sake of obtaining more accurate and reliable toxicokinetic estimates, the PBPK models used in the final assessment incorporate background/endogenous concentrations of methanol. Background estimates on which the models were calibrated are described in Appendix B, Sections B.2.3 (rats), B.2.8

(humans) and B.3 (monkeys). However, for BMD modeling of laboratory animal dose-response data, the species-specific background estimates were subtracted from the dose metric predicted under the relevant bioassay conditions. This approach takes into account the impact of endogenous/background levels on the toxicokinetics of methanol, and allows for the derivation of an RfC (or RfD) that is, by definition, a population level estimate (including sensitive populations) of the amount of a substance that a person can inhale (or ingest) every day over the course of a lifetime [above endogenous/background levels] without an appreciable risk of harm.

As pointed out by the 2nd and 3rd reviewers, the relationship between the RfD and RfC and endogenous/background blood levels is an important consideration. Measured blood concentrations of methanol in humans range between 0.25 mg/L and 5.2 mg/L (see Table 3-1). As described in a new Section 5.3.6, PBPK model estimates of maximum blood level increases of 0.44 mg/L and 0.41 mg/L associated with an RfD or RfC, respectively, are within the 0.7 mg/L standard deviation estimated for the average methanol blood levels (1.5 ± 0.7 mg/L) in humans. From this analysis EPA concludes that the estimated increase in blood levels of methanol from exogenous exposures at the level of the RfD or the RfC (or from the RfC + RfD) are distinguishable from natural background variation.

Comment 2: Two reviewers advocated the use of a PBPK model that incorporates a background term and one reviewer favored the use of the simpler PBPK model. One of these reviewers indicated that use of a background term would be "more rigorous and appropriate for use in this assessment." The latter reviewer warned that "Including the background levels in the models necessarily increases the model complexity and like any model enhancement may increase the uncertainty in the final result, especially when as in this case it may be difficult to design a test of its validity."

Response: As described in the response to Comment 1 above, EPA re-calibrated the PBPK models to account for species-specific estimates of background/endogenous production of methanol. For humans the model was tuned to have an average background level of 1.5 mg/L determined from the corresponding human data in Table 3-1; for rats the model was tuned to a background level of 3 mg/L from the corresponding (control) rat data in Table 3-5. These revised PBPK models were used in estimation of internal dose metrics for the derivation of the RfD and RfC. This addition did increase the model complexity by including an additional term (R_{0bg}, a zero-order endogenous/background production rate, see Appendix B, Section B.2.1) for endogenous/background production of methanol; however, this term was estimated using human data for background blood methanol concentrations (Table 3-1). Since the background term was tuned to match average observed background levels in rats and humans for the corresponding models, there should be minimal systematic error or bias due to the incorporation of the

background term; i.e., the average background level is neither under- nor over-predicted by the model. Moreover, adding the term resulted in only minor changes, less than 20%, in model-predicted blood levels at higher exposure levels (i.e., in the range of the bioassays for rats or the HEC and HED values estimated for humans). Hence model predictions are not sensitive to the inclusion of these average background levels vs. no background at all, and so the effect of and uncertainty due to possible small changes (or errors) in the background term will be minimal.

Comment 3: One reviewer stated that "the upper bound on background concentrations of methanol in target tissue should be carefully evaluated" and that "the lack of determination of the upper statistical bound on normal physiological concentrations of methanol in relevant species, including humans, can be considered to be a major deficiency of the reviewed document."

Response: Statistical bounds on normal physiological concentrations of methanol cannot be determined for all tissues and species. The most complete dataset exists for blood levels of methanol in humans. A discussion of endogenous/background levels of methanol and their relationship to the RfC and RfD has been added to Chapter 5 (Section 5.3.6) and elsewhere in the toxicological review. There is a scarcity of data for endogenous/background methanol levels in the general population. Also, the existing data (Table 3-1) is from populations with various (e.g., age, gender, cultural) characteristics that were asked to adhere to a variety of diets, generally restricted of food and drink that contain or convert to methanol. Measured values have been documented as low as 0.25 mg/L and as high as 5.2 mg/L. From the data gathered for this document (Table 3-1), EPA has estimated a mean background methanol level of 1.5 mg/L with an approximated standard deviation of 0.7 mg/L (see Section 5.3.6).

Comment 4: One reviewer noted that, "in the simulations whose results are listed in the Table B-5, a background level of 2 mg/L has been set to model human internal concentration from inhalation (page B-92; line 29) but not from the oral exposure (page B-92; line 55)."

Response: This inconsistency was corrected and the values in Table B-5 have been updated for both inhalation and oral exposures to reflect concentrations above endogenous/background.

Comment 5: One reviewer noted that EPA did not adequately explain the modest differences in HED and HEC predictions from the PBPK models when background levels of methanol were included or excluded.

Response: This comment was made in relation to a discussion of why including background in the PBPK models might not be necessary. That discussion was removed from the assessment because, as described above in response to Charge A2 Comment 2, the final versions of the PBPK models do include background.

Charge A2 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: One reviewer commented that "[i]n responding to Charge Question A2 for the original report, I expressed concern about treating endogenous and exogenous methanol differently toxicologically when both contribute to the internal dose" and that "…this issue has been addressed by including species-specific background/endogenous methanol in the PBPK models." The reviewer added that "[t]his approach makes a great deal more sense, in my opinion, and is consistent with the concept that risk is a function of internal dose, and that both endogenous and exogenous sources contribute to that dose" and that "I consider this change responsive to my comment."

Response: EPA appreciates the affirmation of sufficient revisions to the Toxicological Review in response to previous peer-review comments and has retained the species-specific estimates of background/endogenous methanol in the PBPK models.

A.1.1.3. Charge A3. The PBPK modeling effort assumed similar methanol pharmacokinetics between pregnant and non-pregnant animals. Please comment on the adequacy of the dose-metric extrapolation based on a PBPK model for non-pregnant adults (i.e., no fetal compartment) for predicting risks associated with fetal/neonatal brain concentrations of methanol.

Summary of Comments: *All reviewers agreed that the existing literature supports the assumption of similar pharmacokinetics between pregnant and nonpregnant animals. Specific comments or suggestions made by the reviewers with respect to this charge are described below, along with EPA responses.*

Comment 1: One reviewer stated they understood the rationale for omitting a fetal compartment in the PBPK model, but felt that "for PBPK modeling to be effective, a fetal compartment will ultimately be needed." This reviewer noted that "PBPK modeling is most useful when the proximate form of the toxicant and mode of action are known, which is unfortunately not the case with developmental effects of methanol."

Response: EPA agrees that a PBPK model with a fetal compartment would be ideal, and that more mode of action information, including the identification of the proximate toxicant, would be helpful. However, studies have shown, and reviewers have agreed, that methanol pharmacokinetics between pregnant and nonpregnant animals are similar and, absent additional information, provide a reasonable justification for extrapolation based on a PBPK model for non-pregnant adults. If there are studies to the contrary, or studies that provide insight into fetal metabolism or the embryotoxic moiety of methanol, a fetal compartment may be considered in the future.

Comment 2: One reviewer expressed concern over the model's ability to predict neonatal blood levels, stating that "this issue is important since the critical study used by EPA to derive an RfC involved combined gestational and lactational (inhalational) exposure of neonates" and that "the use of an adult-based PBPK model could under predict potentially 'toxic' blood methanol concentrations."

Response: It is recognized that neonatal blood levels will likely be higher than maternal blood levels of methanol. Therefore, the ratio of blood concentrations between a human infant and its mother is not expected to be significantly greater than the approximate 2-fold difference that has been observed between rat pups and dams. Further, as stated in the final version of Section 5.1.3.2.2, "the health-effects data indicate that most of the effects of concern are due to fetal exposure, with a relatively small influence due to postnatal exposures." For these reasons and because EPA has confidence in the ability of the PBPK model to accurately predict adult blood levels of methanol, the maternal blood methanol levels for the estimation of HECs from the NEDO (1987) study were used as the dose metric.

Charge A3 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: One reviewer stated that "[e]ven though [using methanol concentration in maternal blood as surrogate dose metric for evaluating postnatal changes] seems to be technically acceptable, without the understanding of exact mechanism of action (MOA) the selection of such a surrogate dose metric remains somewhat speculative."

Response: EPA agrees that there is uncertainty regarding the decision to use methanol concentration in maternal blood as a dose surrogate for evaluating postnatal changes. As discussed in Section 4.7, the decision to model blood methanol concentration as opposed to one of its metabolites was primarily based on a determination that (1) the toxic moiety for developmental effects from methanol exposure is not likely to be the formate metabolite and (2) methanol is an adequate dose metric, even if formaldehyde or ROS are determined to have a significant role in the teratogenicity of methanol. The former determination has been endorsed by other organizations (NTP-CERHR, 2004) and is supported by evidence that formate blood levels do not correlate well with the developmental toxicity observed following methanol exposure. The latter determination is based on evidence that (1) methanol can be metabolized to formaldehye *in situ* by multiple organ systems, (2) the high reactivity of formaldehyde would limit its unbound and unaltered transport as free formaldehyde and (3) the hypothesized ROS MOA would require the presence of methanol to alter embryonic catalase activity (see further discussion in Sections 4.7.1, 4.7.3 and 4.7.5).

As described in Section 5.1.3.2.2, the decision to used maternal blood methanol as a surrogate for neonatal blood levels was based on EPA's confidence in the PBPK models to accurately predict maternal and fetal blood levels and an assumption that the ratio of the difference in blood concentrations between a human infant and mother would be similar to and not significantly greater than the difference in blood concentrations between a rat pup and their rat dam. Further, the health-effects data indicate that most of the effects of concern are due to in utero exposure, with a relatively small influence due to postnatal exposures.

Several research studies recommended by the reviewers in response to Charge D3 (below), including "dual labeled material" studies to "confirm fetal exposure" and "resolve whether formaldehyde is involved in the developmental effects following perinatal methanol exposure" and studies "to confirm the low activity of methanol metabolism in fetal tissues," could help to resolve the "principal sources of uncertainty" referred to by the reviewer. However, as indicated in response to the Charge D3 comments, and consistent with the follow-up comments to EPA's Charge D3 responses, EPA is not planning to delay the completion of this assessment pending the completion of future studies.

A.1.1.4. Charge A4. EPA assumes limited methanol metabolism in the fetus because of limited alcohol dehydrogenase (ADH) activity in the human fetus, limited catalase and ADH activity in fetal rodents, and existing pharmacokinetic data that show nearly equal concentrations in maternal blood vs. the fetal compartment. Please comment on the validity of this assumption given the lack of data regarding potential alternate metabolic pathways in the fetus.

Summary of Comments: *All reviewers agreed that this is a reasonable assumption given the limited data available. Specific comments or suggestions made by the reviewers with respect to this charge are described below, along with EPA responses.*

Comment 1: Two reviewers thought that the assumption of limited methanol metabolism in the fetus was valid based on the methanol pharmacokinetic data, but one of the reviewers noted that embryotoxicity from methanol may be influenced by fetal catalase in mice as demonstrated by a recent study (Miller and Wells, 2011). This reviewer further stated that this and another study (Sweeting et al., 2011) suggest that fetal methanol concentrations in rodents may not be a "good predictor of teratogenic responses in different species.".

Response: While these studies provide insights into the fetal metabolism of methanol, it is unknown if fetal catalase is a controlling factor for methanol teratogenicity in mice. Furthermore, a recent in vivo study (Siu et al., 2013) suggests that high catalase activity does not protect against methanol teratogenicity in the strains of mice tested. EPA evaluated these studies

and, as described in Section 5.3.5 "Choice of Species/Gender," concluded that the available evidence related to fetal catalase and methanol's teratogenicity in mice is contradictory and inadequate to suggest that rodent effects should not be used in an assessment of methanol's potential to cause developmental effects in humans. Also, because the critical gestational window for developmental effects could be different for rabbits versus mice, the claim that rabbits are resistant to teratogenic effects of methanol needs to be verified over several gestational days, as has been done for mice.

Comment 2: One reviewer commented that, "the assumption of limited methanol metabolism in the fetus is probably justified based on the existing studies showing low levels of ADH and catalase in fetal tissues" but added that "these studies have technically measured these proteins using indirect measures such as immunoblotting showing protein amounts or activity measures with ethanol as the substrate."

Response: EPA agrees with this comment. An activity measurement using methanol as the substrate would be ideal. However, lacking such studies, it is reasonable to assume low activity of methanol metabolism in fetal tissues from relevant, indirect studies.

Charge A4 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: No additional comments were received regarding this charge question.

Response: EPA has not changed the approach taken regarding fetal metabolism.

A.1.1.5. Charge A5. Please comment on the scientific justification of the extrapolation approach from rats to humans for in-utero and neonatal lactational and inhalation exposures.

Summary of Comments: *Four reviewers agreed that a reasonable approach was taken given the data available, though one of these reviewers reiterated that issues identified in Charge A1 with respect to the rat and human models need to be addressed. A fifth reviewer reiterated comments made in response to Charge A1 regarding the need for "clarification of the process for evaluating the usefulness of each model for the assessment and why the nonhuman primate model was not included" and noted that the use of the NEDO rat studies which included neonatal exposures is "problematic, given the lack of data on lactational and early postnatal inhalation exposure to methanol." A sixth reviewer suggested that the model should be modified to include gestational and lactational components and expressed concern over the use of rodent data for estimating human risk from developmental effects. A seventh reviewer suggested that*

EPA's assumption that rats and humans would have similar maternal/offspring methanol concentration ratios is a significant source of uncertainty.

Response: EPA agrees with the majority of the reviewers that the extrapolation approach employed is justified given the available data. The reviewer concerns regarding the model evaluation process and the perceived lack of a nonhuman primate model are addressed in response to Charge A1 Comment 1. The lack of data on lactational and early postnatal inhalation exposure to methanol is a recognized data gap that led to the current approach. As discussed in response to comments under Charge A3 above, gestational and lactational compartment may be considered in a future assessment, but they are not necessary at this time for the purposes of this toxicological review. Concerns over use of rodent studies stem from the Sweeting et al. (2011) study. The relevance of the Sweeting et al. (2011) to these concerns is discussed in Section 5.3.5 and elsewhere in the toxicological review and in response to Comment 1 of Charge A4 and Comment 1 of Charge D2. As discussed in response to Charge A3 Comment 2 and Section 5.1.3.2.2 of the toxicological review, the uncertainty surrounding the assumption of similar maternal/offspring methanol concentration ratios between rats and humans is recognized, but the ratio of blood concentrations between a human infant and its mother is not expected to be significantly greater than the approximate 2-fold difference that has been observed between rat pups and dams. Clarifications have been added in this regard to Sections 5.1.3.2.2 and 5.3.5.

Charge A5 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: One reviewer stated that EPA "…continue[s] to state in the current assessment, and reiterate in the response to panel comments from the original review, that the ratio of blood methanol concentrations between a human infant and its mother is not expected to be significantly different than the approximately 2-fold difference seen between rat pups and dams." This reviewer noted that "[t]he main point made in that section [Section 5.1.3.2.2] regarding this issue seems to be that this assumption isn't particularly important because most of the effects of methanol occur *in utero"* and added that "[t]o the extent that it matters, the assumption that maternal/offspring methanol concentration ratios are similar in both humans and rats continues to be poorly justified in my opinion."

Response: The comment reflects the rationale provided, suggesting that the issue is not lack of clarity in the rationale, but that there is not a strong justification, as noted by the reviewer. The unfortunate fact is that methanol dosimetry data are not available for rat pups, human infants, lactating rat dams, nor lactating human mothers (particularly, amounts in breast milk). Given the high aqueous solubility of methanol, it may be reasonable to assume that concentrations expressed in breast milk equal those in maternal blood. However dosimetry in the

developing infant would depend on when and to what extent metabolic capacity develops in rat pups versus human infants. So while it would be possible to extrapolate the existing adult models to those life-stages, such extrapolations, for the infant in particular, would be quite speculative and uncertain. In response to this comment, clarification has been added to Section 5.1.3.2.2 to indicate why EPA does not believe that adding additional analyses would substantially reduce the uncertainty and hence improve the justification around this assumption.

A.1.2. Charge B: "Inhalation Reference Concentration (RfC) for Methanol"

A.1.2.1. Charge B1. A chronic RfC for methanol has been derived from a perinatal inhalation study of the effects from exposing rat dams and pups to methanol during gestation and lactation (NEDO, 1987). Reference values from mouse (Rogers et al., 1993b) and monkey (Burbacher et al., 2004b; Burbacher et al., 1999b) developmental studies, were also derived and discussed, but were not chosen for the RfC. Please comment on whether the selection of the principal study has been scientifically justified.

Summary of Comments: *Two reviewers indicated that selection of NEDO (1987) as the principal study was scientifically justified. Two reviewers stated that choice of the NEDO rat study was based on "practical/technical grounds" or "policy" (i.e., use of the lowest RfC), rather than scientific considerations. One reviewer did not explicitly state whether the use of the NEDO rat study was scientifically justified, but stated that selection of the principal study is contingent on the determination of the HEC/HED after the implementation of suggested model revisions (e.g., Charge A1 Comment 7). Two reviewers suggested that the NEDO rat developmental study was not the most appropriate study for RfC derivation. Specific comments or suggestions made by the reviewers with respect to the advantages and limitations of each of the three studies addressed in the charge are described below, along with EPA responses.*

Comment 1: Two reviewers indicated that the selection of NEDO (1987) as the principal study was scientifically justified and noted the following scientific advantages:

- "The nearly continual exposure (20-22 hours per day depending on the study) represents the types of exposures relative to the RfC/RfD (i.e. the daily exposure over the lifetime)."
- Selection of the NEDO study "is in accordance with the usual guidelines recommending use of the study with the best data quality (including, in this case, availability of a validated PBPK model) and greatest sensitivity."

Two reviewers did not think that the NEDO study was the most appropriate choice and noted the following concerns:

- The prior, EPA-sponsored peer review of the NEDO study questioned "procedures used in the NEDO study (in utero and postnatal exposures, litter effects, etc) that make it difficult to evaluate the study for RfC derivation."
- "The discussion on page 5-10 regarding the complications that arise from using the NEDO study where exposure was both gestational and postnatal postulates a number of assumptions that are supported by little or no data."
- "Data on lactational transfer and early postnatal inhalation exposures are limited."
- The neonatal brain weight response has not been replicated in other studies.
- "The analysis provided by the NEDO authors showed a gender difference (effects seen in males but not female rats)."
- "The NEDO study relied on multiple t-tests as opposed to a more appropriate use of an ANOVA to evaluate gender and treatment responses."
- There were no "corroborating clinical or pathological observations of depressed CNS activity noted in the rats in the NEDO study."

The remaining three reviewers did not address the scientific merits of the NEDO study. However, two of these reviewers suggested that its selection was based on it resulting in the lowest RfC, and one stated that selection of the principal study is contingent on the determination of the HEC/HED and suggested that (due to possible error in the PBPK model) the HEC/HED value for the NEDO rat study "could be on the order of 6-fold too low."

Response: In addition to the advantages of the NEDO (1987) developmental rat study noted by several reviewers (e.g., relevant exposure route and duration, validated PBPK model estimates of internal dose, and a sensitive response endpoint), the NEDO study offers other advantages (described further below) such as the identification of an endpoint that (1) is biologically significant, (2) is observed at a sensitive developmental stage, (3) has been replicated in adult rats and (4) is in an organ system for which suggestive pathology has been observed in adult primates that received acute and chronic exposure to methanol via the same exposure route. While reviewer comments on EPA's choice of the NEDO (1987) developmental rat study were mixed, only two of seven reviewers indicated that its selection was not justified. Though EPA recognizes that the NEDO (1987) study has limitations, these limitations do not preclude its use as the principal study for RfC derivation (see Section 5.3.1 and below).

EPA agrees that data on lactational transfer and early postnatal inhalation exposures are limited, and this is largely the reason that maternal blood levels were used as a dose metric in the analysis of the neonatal brain weight endpoint. The related discussion that was on page 5-10 of the draft assessment has been revised to clarify the Agency's justification and address reviewer concerns regarding the use of maternal (versus neonatal) blood levels of methanol as a basis for the benchmark dose analysis of these data. In essence, the ratio of the difference in blood concentrations between a human infant and mother is assumed to be similar to the approximate two-fold difference that has been observed in rats. Further, while rat studies indicate that postnatal exposure to methanol can impact brain weight, fetal exposure has been shown to have the greatest influence on this endpoint. For these reasons and because EPA has confidence in the ability of the PBPK model to accurately predict adult blood levels of methanol, the maternal blood methanol levels for the estimation of HECs from the NEDO (1987) study were used as the dose metric. EPA has added text to Sections 4 and 5 to further clarify and discuss the limitations of NEDO (1987).

NEDO (1987) observed brain weight reductions in the F1 and F2 generations of their two generation study, in the F1 generation of the supplementary developmental study to the two generation study and in a separate teratogenicity study. They also observed potentially adverse histopathology (astrocytes) in the brains of monkeys receiving acute, subchronic and chronic exposure to methanol (see further discussion in Section 4.4.2). While brain weight reduction has not been observed in developmental bioassays of other laboratories, it has been observed in adult rats exposed to methanol (TRL, 1986). Also, brain weight reduction is not an endpoint that has been extensively measured or focused on in other developmental studies of methanol, such as the Rogers et al. (1993b) mouse studies.

EPA agrees that the multiple t-tests applied in the NEDO study are not optimal for the evaluation of the dose-response data from this study. For this reason, EPA did not rely on this information and, instead, relied on the results of the more definitive benchmark dose analysis of this data (Appendix D), as described in Section 5 of the final methanol toxicological review.

With respect to the use of absolute brain weight change without clinical or pathological corroboration, the Agency's neurotoxicity guidelines (U.S. EPA, 1998a) states that a "change in brain weight is considered to be a biologically significant effect," and further states that "it is inappropriate to express brain weight changes as a ratio of body weight and thereby dismiss changes in absolute brain weight" and that "changes in [absolute] brain weight are a more reliable indicator of alteration in brain structure than are measurements of length or width in fresh brain, because there is little historical data in the toxicology literature."

With respect to the basis for EPA's study choice, while it is true that EPA guidelines generally promote use of the more sensitive endpoint, the relative strengths of candidate studies are not to be ignored (U.S. EPA, 2002, 1994). As discussed above and in Chapter 5 of the methanol toxicological review, the NEDO (1987) study limitations were considered, but do not preclude its use for the derivation of a candidate RfC/D. On the other hand, questions concerning the Burbacher et al. (2004b; 1999b) monkey study dose-response are considered serious enough to not use this study for RfC/D derivation, despite the possibility that a lower BMDL POD would have been derived from this study (see Section 5.3.1 and Appendix D).

Comment 2: Regarding the Burbacher et al. (2004b; 1999b) monkey study, four reviewers had no comment on its potential for use as the principal study and two reviewers stated the following reasons why it should not be used as the principal study:

- "The lack of a dose-response function for the major effects."
- No "convincing evidence of an effect, given the inconsistencies in dose-response, multiple comparisons, and the potential for unreliable identification of 'effects' in small studies."

However, one reviewer suggested that it would be a better choice than the NEDO rat study because it "uses the most appropriate species (monkey) and examined a wide range of reproductive and neurotoxicological endpoints and significant pharmacokinetic data," and two reviewers suggested that the following limitations noted in the toxicological review were overstated:

- Inclusion of wild-caught monkeys
- Influence of C-sections on results
- Not being relevant to persons who are folate deficient
- Lack of a dose-response for VDR in the male monkeys

Response: EPA agrees with reviewer comments regarding the significant difficulties of assessing the dose-response data from the Burbacher et al. (2004b; 1999b) monkey study. These concerns are addressed in Section 5.3.1 of the final review. In response to reviewer concerns, EPA's attempt at performing a benchmark dose analysis of the Visually Directed Reaching (VDR) endpoint from this study (described in Appendix D) are no longer presented in Chapter 5 (i.e., in Table 5-4 or 5-6) alongside the benchmark dose analyses of critical effects from the candidate principal mouse and rat studies. With respect to the concerns that limitations in this study were overstated, EPA has taken the following action:

- *Inclusion of wild-caught or feral-born monkeys* – One section of the draft toxicological review inadvertently referred to monkeys from this study as being "wild" and this statement

has been removed. In two sections, they were referred to as "a mixture of feral-born and colony-bred animals." Since the Burbacher et al. (2004b; 1999b) study investigated for and found no effects that were dependent on origin, EPA agrees that this statement is unnecessary and it has been removed from the review.

- *Influence of C-sections on results* – EPA agrees with the reviewers and the toxicological review has been edited to reflect that Cesarean section (C-section) deliveries performed in the methanol exposure groups did not impact the "decreased length of pregnancy" finding (decreased length of pregnancy was observed in vaginally delivered animals).
- *Not being relevant to persons who are folate deficient* - EPA agrees that this statement could be made about most of the methanol studies reviewed. Hence the statement has been removed.
- *Lack of a dose-response for VDR in the male monkeys* – While the ANOVA test in the male monkeys suggests a statistical significant VDR change at 600 ppm ($p = 0.007$), there was no significant difference between responses and/or variances (indicating lack of a dose-response trend) among the dose levels for males only ($p = 0.321$), even when the high dose group is excluded ($p = 0.182$). However, there was a significant dose-response trend for females only ($p = 0.0265$). This is largely because the females had a larger overall sample size across dose groups than males (21 females versus 13 males). Hence, only the VDR response for females only exhibited a dose-response that could be adequately modeled (see Appendix D).

Comment 3: Regarding the Rogers et al. (1993b) mouse study, two reviewers supported the use of this study over the NEDO rat study and noted the following advantages:

- "The study is scientifically sound and robust."
- "Exposures are limited to the prenatal period and the outcomes are clear."
- "The Rodgers (sic) study has undergone independent peer review, documents responses reported by other laboratories, and has quite robust group sizes."

Response: EPA agrees with reviewer comments regarding the advantages of the Rogers et al. (1993b) mouse developmental study. EPA also agrees with reviewer comments (see Charge B1 Comment 1 above) regarding the advantages of the NEDO rat study, including the use of a continuous, nearly full day exposure regimen and the adequacy of the reported response data for dose-response analysis. As a result, EPA decided to treat both the Rogers and NEDO studies as candidate principal studies and derived candidate RfCs and RfDs for the most sensitive endpoint from each study (see Section 5.1.1.2).

Charge B1 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: One reviewer commented that "[i]n the current assessment and in responses to comments, the U.S. EPA has more clearly and thoroughly described what it perceives as the strengths of the NEDO study, making a better case for its selection as the principal study" and that the Burbacher study is now more appropriately described. However, this and another reviewer were not fully satisfied that EPA's use of BMD modeling fully addressed the suggestion to reanalyze the brain weight data using more appropriate tests for statistical significance. Both reviewers commented that without appropriate tests to determine if the NEDO study results represent a statistically significant change, it is not clear that benchmark dose analysis is warranted or reliable. One commenter stated that "A re-analysis using ANOVA would be easily done and should be done, just to show validity of the NEDO data prior to using it for the BMD analysis."

Response: EPA appreciates the affirmation from the reviewers of the revised study characterizations. With respect to tests for statistical significance, EPA believes that trend tests that use all of the dose-response data, in this case summarized continuous responses (means and SDs), are more appropriate indicators of significance then pair-wise testing of the responses within individual dose groups. With respect to whether the BMD modeling needs to be preceded by other tests for statistical significance, EPA generally considers the results of the BMDS model output to be sufficient to determine whether there is a significant increasing or decreasing trend to the dose-response data. In the case of continuous data such as decreasing brain weight, the results of four BMDS test results (described in detail in the BMDS Help manual available from http://epa.gov/ncea/bmds) are considered. Test 1 is used to determine whether there are significant differences among the means and, to some extent the variances, across dose groups. Test 2 directly tests for homogeneity among the response variances. Test 3 is a test to determine whether variances can be modeled as a power function of the mean. Test 4 is used to determine whether the model adequately fits the mean responses. In general, Test 1, Test 4 and either Test 2 or Test 3 must pass for a set of dose-response data to be considered adequate for derivation of a BMD. While none of the individual test results are suitable for determining whether there is a significant overall (upward or downward) trend in the data, the combined test results are deemed to be adequate for this purpose.

Nevertheless, it is true that none of the BMDS tests described above constitute a traditional Analysis of variance (ANOVA) or trend test. Hence, in response to this comment EPA has applied an ANOVA analysis for summarized response data (Larson, 1992) to all twelve of the neonatal rat brain weight responses reported on page 202 of the NEDO (1987) report. A highly

significant decreasing dose-response trend ($p < 0.000001$) was observed for all but the male and female olfactory bulb weights, which were highly insignificant ($p > 0.3$).

A.1.2.2. Charge B2. Reduction of brain weight at 6 weeks postnatally as reported in the NEDO (1987) developmental rat study was selected as the critical effect. Please comment on whether the rationale for the selection of this critical effect has been scientifically justified. Please identify and provide the rationale for any other endpoints (e.g., other reproductive and developmental effects reported in mouse and monkey studies) that should be considered in the selection of the critical effect.

Summary of Comments: *Four reviewers indicated that the use of brain weight change was justified, but one of these and one other reviewer questioned the use of the 6-week time point. Two reviewers suggest using the cervical rib endpoint from the Rogers et al. (1993b) mouse study. One reviewer expressed a preference for endpoints from the Burbacher et al. (2004b; 1999b) monkey study or Rogers et al (1993b) mouse study over the NEDO rat study. Specific comments or suggestions made by the reviewers with respect to this charge are described below, along with EPA responses.*

Comment 1: Two reviewers suggested that the increased incidence of cervical ribs should serve as the critical effect for RfC derivation, with one stating that "the increases in cervical ribs and supernumerary ribs observed in this Rogers et al. (1993b) study could be considered a more scientifically justified critical effect."

Response: EPA agrees that the Rogers et al. (1993b) study is of high quality. In the final assessment, it is considered a candidate principal study. The preference of these two reviewers for the cervical rib endpoint seems to be based in part on perceived problems with the brain weight change endpoint in rats (NEDO, 1987). The reviewer who stated that the cervical rib endpoint "could be considered a more scientifically justified critical effect" pointed out that the NEDO (1987) developmental rat study did not note "abnormal brain histopathology or functional deficits" and a statistical analysis of the brain weight changes was performed that was questioned in a separate peer review of this study that was conducted for EPA (ERG, 2009). As discussed in the response to Charge B1 Comment 1, EPA neurotoxicity guidelines allow for the treatment of absolute brain weight change as an adverse neurological effect regardless of the existence of corroborating histopathological or functional observations, and EPA used benchmark dose analyses in lieu of the statistical test results reported by the authors for this endpoint.

Comment 2: One reviewer commented that there is a "general lack of transparency" regarding the basis for the selection of the critical effect and stated that EPA chose "the one that led to the lowest RfC," without regard to the limitations of the NEDO study, including

inappropriate use of statistical methods as described in a 2009 EPA-sponsored external peer reviewer of the NEDO study (ERG, 2009). Another reviewer also commented that EPA had not acknowledged errors in the NEDO statistical analysis and recommended that EPA conduct its own analysis of variance (ANOVA) to determine "if there were an overall effect on brain weight" and "which time frame and which methanol level are used in the BMD analysis."

Response: The basis for the selection of the candidate principal studies and effects are primarily described in Section 5.1.1, Choice of Principal Study and Critical Effect(s). The critical effects considered for the derivation of an RfC and RfD were chosen because they were reported in studies of adequate quality, are considered relevant to humans, evidence a clear dose-response and are sensitive indicators of alterations in important organ systems. The dose-response data for the effects that meet these criteria (in this case the mouse cervical ribs and rat brain weight effects) were considered for the derivation of the RfC and RfD. If EPA had based its selection on the effect that "led to the lowest RfC" an endpoint in the Burbacher et al. (2004b; 1999b) or NEDO (1987) monkey studies might have been chosen as some of the endpoints in these studies suggested a lower NOAEL or BMDL. However, as described in Sections 4.2.2.3, 4.4.2, and 5.1.1, these monkey studies did not meet all of the criteria necessary for an effect to be considered a critical effect. As discussed in response to Charge B1 Comment 1, the limitations of the NEDO rat developmental study, including the inappropriate use of statistical methods, are not serious enough to preclude its consideration as a candidate principal study. There is no need for the Agency to perform an ANOVA analysis because a benchmark dose analysis was performed in accordance with EPA guidelines (U.S. EPA, 2012a) for all postnatal time frames (3, 6 and 8 weeks).

Comment 3: Two reviewers were concerned that EPA did not consider other postnatal time points besides 6 weeks, with one stating that this approach "weakens the potential statistical power for a response that appears stable over a wide range of time points (3 to 8 weeks)."

Response: A benchmark dose analysis of brain weight reductions in male and female rats was performed for all postnatal time frames (3, 6 and 8 weeks). In accordance with EPA guidelines (U.S. EPA, 2002, 1998a), the most sensitive developmental time point in the most sensitive gender was used as the basis for the RfC/D. In order to achieve an increase in statistical power by combining data together from separate ages, the animals must be exchangeable (required for Bayesian statistics) or represent the same population (i.e., the brain weights from 3-8 week old S-D rats would have to represent the same population; required of frequentist statistics). The data for the more sensitive gender (males) suggests that each age represents a separate subpopulation (3 wks (mean ± standard deviation): 1.45g ± 0.06; 6 wks: 1.78g ± 0.07; 8 wks: 1.99g ± 0.06). Randomly permuting the individuals across the groups would not yield the

same conclusions, proving a lack of exchangeability. Thus, combining these samples together would in all likelihood violate the exchangeability and independent and identically distributed (i.i.d) assumptions required of Bayesian and frequentist methods, respectively.

Comment 4: Two reviewers were concerned over the "lack of histological or functional follow-up for this [brain weight] response."

Response: See response to Charge B1 Comment 1 regarding brain weight.

Comment 5: Two reviewers noted that EPA may need to reevaluate the endpoint selection if modification of the PBPK analysis for S-D rats significantly alters the relative sensitivity (based on HECs) of the rat, mouse and monkey studies.

Response: EPA agrees with this comment. Consideration has been given to whether the modified PBPK model results warrant a change in the critical effect. While the final candidate RfDs and RfCs from S-D rat brain weight response (NEDO, 1987) and the CD-1 mouse cervical rib response (Rogers et al., 1993b) are similar, the PBPK model modifications do change the relative sensitivities such that the mouse study now serves as the basis for the methanol RfD. The RfC is still based on the brain weight changes observed in the rat study.

Charge B2 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: One reviewer commented that "[i]n the current assessment and in responses to comments, the U.S. EPA has more clearly and thoroughly described what it perceives as the strengths of the NEDO study, making a better case for its selection as the principal study."

Response: EPA appreciates the reviewer's affirmation of the revised NEDO study characterization. As indicate in response to the Charge B1 Follow-up Peer Review Comments, EPA agrees that the EPA analysis of the NEDO rat brain weight data would benefit from, and has therefore applied and described in the assessment, a more reliable trend test to confirm that the positive dose-response trend is significant.

A.1.2.3. Charge B3. Benchmark dose modeling of decreased pup brain weight relative to maternal internal methanol doses predicted by the PBPK model was used to derive the point of departure (POD) for the RfC. Has the BMD/PBPK approach been appropriately conducted? Has adequate justification been provided for the selected internal dose metric, i.e., area under the curve (AUC) for methanol, in the blood of dams? Please identify and provide the rationale for any alternative approaches for the determination of the POD, including choice of another dose metric (e.g., methanol metabolized), and discuss whether such approaches are preferred to EPA's approach.

Summary of Comments: *Four reviewers indicated that the BMD analysis was appropriate and appropriately applied. Three reviewers said that this was not their area of expertise. Five reviewers accepted the choice of AUC as the dose metric, but noted limitations in the data available (MOA and empirical information) for making that choice. One reviewer preferred C_{max} over AUC as the dose metric and one reviewer did not comment on the selected dose metric. Specific comments or suggestions made by the reviewers with respect to this charge are described below, along with EPA responses.*

Comment 1: With respect to the selection of AUC as the dose metric for the BMD analyses of the brain weight endpoint from the NEDO developmental study in which rats were exposed gestationally and postnatally, one reviewer stated that "Without understanding of the exact mechanism of action of the chemical, selection of any surrogate dose metric is somehow speculative." A second reviewer commented that "The Agency has not adequately explained its rationale for the use of AUC rather than C_{max} (e.g., see literature related to methanol and 2-methoxyethanol)." A third reviewer noted that justification for the AUC is "tenuous" because "brain weight does not differ between the 3, 6 and 8 week periods."

Response: When performing BMD analyses, it is important to choose a reliably measured or estimated dose metric that has a close relationship to the health effects under consideration. For the BMD analyses of the mouse cervical rib endpoint, which has been shown to result from just one day of gestational exposure, it is assumed that the level of exposure is more important than duration. Internal methanol blood concentrations reported by Rogers et al. (1993b) for the dams of each dose group at day 6 of gestation were assumed to be approximately equivalent to C_{max} levels and were used as the modeled dose metric. For the BMD analyses of the rat brain weight endpoint following gestational and lactational exposure, PBPK model estimates of AUC methanol in blood for the dams of each dose group were used as the modeled dose metric. As described in Section 5.1.2.1, the decision to use AUC as the dose metric for the gestationally and postnatally exposed rats was made because under this exposure regimen, brain weight is susceptible to both the level and duration of exposure. It is true that the results of

NEDO (1987), described in Section 4.4.2 and shown in Table 4-13, indicate that there is not an obvious cumulative effect of ongoing exposure on brain-weight decrements in rats exposed postnatally for 3, 6 and 8 weeks. However, there is a greater brain-weight effect in rats exposed postnatally versus only during organogenesis (GD7-GD17). Further, brain weight reductions have been observed in adult rats that were exposed for 90 days beginning no earlier than 30 days of age (TRL, 1986). That brain weight is susceptible to continued exposure beyond gestation suggests that a dose metric that incorporates a time component would be more appropriate. For this reason, and because it is more typically used in internal-dose-based assessments and better reflects total exposure within a given day, daily AUC (measured for 22 hours exposure/day) was chosen as the most appropriate dose metric for modeling the effects of methanol exposure on brain weights in rats exposed throughout gestation and continuing into the F1 generation.

Comment 2: One reviewer asked why, for the purposes of Table 5-2 and the PBPK estimation of AUC methanol in rat dam blood, the AUC was calculated with a 5 day 22 hr/day simulation.

Response: The full text of the subject footnote is "AUC values were obtained by simulating 22 hr/day exposures for 5 days and calculated for the last 24 hours of that period." Simulations were run for 5 days as this was sufficient to reach "periodicity" when the daily time-course is the same from one day of exposure to the next. From Figure B-13 it can be seen that model predictions for the second day and beyond are essentially identical, but because the blood level does not drop to zero during the 2 hour "off" period, the AUC is higher on the 2nd day and beyond than the first day. More importantly, the AUC was calculated for a single day of simulated exposure, which happened to be the fifth day. With the PK parameters used for those simulations, the same results would have been obtained if the simulation had only been run for 3 days, or for 30 days.

Comment 3: With respect to the alternative hypothesis that formaldehyde is the teratogenic moiety and that increased effects of methanol in GSH-depleted animals are due to decreased formaldehyde elimination, one reviewer noted that GSH depletion does not necessarily imply formaldehyde involvement because "depletion of GSH, as the major cellular antioxidant, will also increase the accumulation of reactive oxygen species (ROS)."

Response: The toxicological review has been revised (Section 4.7) to reflect that the impact of GSH depletion can support both formaldehyde and ROS involvement in the teratogenic effects of methanol. However, this reviewer and another reviewer agreed with the Agency's position that methanol would play a key transport role in either case, with the latter reviewer stating that "even if the metabolism-related formation of ROS or formaldehyde are important contributors to the observed toxic effects, a methanol-based dose metric is applicable

when the downstream metabolic processes such as removal of ROS or formaldehyde are much faster than the rate-limiting oxidation of methanol."

Comment 4: One reviewer commented that "Neither the 5% nor the 10% BMR have any particular *a priori* justification for continuous data: the default assumption in this case is the BMR of 1 standard deviation of the control dataset (as preferred here). In any case the data need to be examined to determine an appropriate BMR representing a minimal detection level or threshold of biologically significant response: this especially applies for continuous data."

Response: The reviewer's comments with respect to the selection of a BMR are correct and consistent with EPA BMD Technical Guidance (U.S. EPA, 2012a). In the case of the methanol toxicological review, all BMR levels considered for RfC or RfD derivation lie well within the range of the dose-response observations. As indicated in the EPA BMD guidance (U.S. EPA, 2012a), a series of papers (Allen et al., 1994a, b; Faustman et al., 1994) suggest that a 5% BMR is appropriate for dichotomous response data from well designed nested developmental studies such as the Rogers et al. (1993b). For continuous response data, EPA guidance (U.S. EPA, 2012a) suggests that "if there is an accepted level of change in the endpoint that is considered to be biologically significant then that amount of change is the BMR." For continuous response data from developmental studies, comparisons with the NOAEL showed that several cutoff values, including a 5% change in mean fetal weight, could be used to give values similar to the NOAEL (Kavlock et al., 1995). If a 5% change in fetal weight is considered biologically significant, it is reasonable to assume that a 5% brain weight change should also be considered biologically significant. However, in a recent report on the statistical power in the analyses of brain weight measures in pesticide neurotoxicity testing, Weichenthal et al. (2010) state that "if toxicological experts ultimately decide that brain weight changes in the range of 5% are physiologically meaningful, a larger [than 10 per dose group] sample size will be needed to consistently achieve reasonable power to detect this magnitude of effect." EPA BMD guidance (U.S. EPA, 2012a) states that "in the absence of any other idea of what level of response to consider adverse, a change in the mean equal to one control SD from the control mean can be used." Because there is no clear biological basis for choosing one over the other, both are considered and deference is given to the BMR that results in the lower RfC or RfD (see Tables 5-4 and 5-6).

Charge B3 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: No additional comments were made regarding this charge question. However, a related comment regarding the use of methanol concentration in maternal blood as

surrogate dose metric for evaluating postnatal changes is addressed under the Charge A3 Follow-up Peer Review Comments in Section A.1.1, "Toxicokinetics and PBPK Modeling."

Response: EPA has responded to the comment regarding use of methanol concentration in maternal blood as surrogate dose metric for evaluating postnatal changes under Charge A3 Follow-up Peer Review Comments in Section A.1.1, "Toxicokinetics and PBPK Modeling."

A.1.2.4. Charge Question B4. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfC. It is assumed that these UFs account for variability in methanol dosimetry among human newborns following gestational and lactational exposure, and for uncertainty regarding the ratio of newborn-dose to maternal-dose in humans. Please comment on these assumptions and on the scientific justification for the selected UFs.

Summary of Comments: *In general, four reviewers indicated that the selected UFs are adequate and consistent with EPA policy and three reviewers did not agree with certain UFs. Of the four reviewers that generally agreed with EPA's proposed UFs, one suggested that some further examination and discussion of the UF_H would be helpful and another noted that a strong argument could be made for eliminating the UF_D. Of the three reviewers that expressed disagreement, all three stated that the 3-fold UF_D was not necessary, and one suggested that the UF_H of 10 is not warranted. With respect to where the UFs are applied, one reviewer supported the Agency's practice of applying UFs to the HEC and one advocated application of UFs to BMDLs (before HEC derivation). Specific comments or suggestions made by the reviewers with respect to this charge are described below, along with EPA responses.*

Comment 1: Regarding the UF_H, one reviewer stated that a full UF_H of 10 is not warranted because "at the level of the proposed RfC and RfD, intraspecies differences in disposition of exogenous methanol in humans will likely have no meaningful impact on the body burden of 'total' methanol." This reviewer recommended that EPA perform a sensitivity analysis of the human PBPK model to identify variability and/or uncertainty in parameters which have an impact on methanol levels predicted. Also, this and another reviewer stated that the UF_H does not need to account for uncertainty regarding the sensitivity of children because the critical study is in neonates, two-generation study exists, and "no particular developmental susceptibility of humans versus test species is expected." Another reviewer questioned whether a UF_H of 10 was "sufficient in the general case" and recommended further examination and discussion to establish "the limits of the data available to inform the decision on the value for UF_H."

Response: A sensitivity analysis of the human PBPK model has been performed (see Appendix B), and the results suggest that parameter variability is not likely to result in methanol

blood level estimates that vary more than 3-fold, the toxicokinetic portion of the 10-fold UF_H. However, one needs to also consider the variation in endogenous/background levels of methanol (Table 3-1), and variation in toxicodynamics, because both may affect the impact of an exogenous methanol exposure. Overall, the extent of human interindividual variation in (endogenous and exogenous) methanol toxicokinetics and toxicodynamics would be very difficult to quantify given the significant uncertainties that exist regarding background levels and methanol's mode of action.

Toxicodynamic variability can only be discussed qualitatively. As discussed in Section 4.9, there are a number of issues that may lead to sensitive human subpopulations. Potentially sensitive subpopulations would include individuals with polymorphisms in the enzymes involved in the metabolism of methanol and individuals with significant folate deficiencies. The effects used to derive the candidate RfCs are observed in a potentially susceptible and sensitive fetal/neonatal subpopulation. However, there is also variability across fetuses and neonates that need to be taken into account. Children vary in their ability to metabolize and eliminate methanol and in their sensitivity to methanol's toxic developmental effects. Consequently, there exists considerable uncertainty pertaining to human population variability in methanol metabolism, which provides justification for the 10-fold intraspecies UF used to derive the RfC and RfD.

Comment 2: Regarding the UF_A, one reviewer stated that "it is surprising that the EPA used the same interspecies UF_A for rodent and nonhuman primate studies – given the fact that significant species difference exist between rodents and humans and less so between monkeys and people (use UF = 1)."

Response: As discussed in response to Charge B1 Comment 2, due to uncertainties in the dose-response data for the monkey studies, EPA has removed the alternative RfC derivation for monkeys from the toxicological review.

Comment 3: Regarding the UF_D, **t**hree reviewers provided the following reasons why a 3-fold UF_D was not needed:

- Methanol has a "very rich toxicology database."
- "There is never enough data to be certain regarding a risk 'assessment' (that is why it is called risk assessment not a risk determination)."
- Conservative assumptions are "always used," including:
    - use of a single SD for BMDL rather than a 4 or 10% changes as commonly used in some noncancer risk assessments (e.g., see page 5-23),"
    - "PBPK assumes the most conservative scenarios,"

- "BMD analysis itself favors the conservative numbers" and
- "when given the choice of alternative BMD numbers such as those obtained from the 3 versus 6 versus 8 week data, the lowest (i.e., most conservative) number is chosen."

- "The key endpoint is developmental toxicity, which has been evaluated in multiple species, including primate, and special endpoints such as neurotoxicity and immunotoxicity have been evaluated."
- "There is no need to have a UF because 'there is uncertainty regarding which test species is most relevant to humans'—the lowest, high-quality point of departure was used."
- "There is also no need to have a UF_D for "dose spacing" because the BMD analysis counters this potential design deficiency.

Response: The database uncertainty factor accounts for the potential to underestimate noncancer hazard as a result of data gaps. EPA agrees that the database for methanol toxicity is quite extensive: there are chronic and developmental toxicity studies in rats, mice, and monkeys, a two-generation reproductive toxicity study in rats, and neurotoxicity and immunotoxicity studies. However, as discussed in Section 5.1.1.1, chronic and developmental studies in monkeys, the species most likely to best represent the potential for developmental effects in humans, were considered inadequate or inferior to the candidate principal rodent studies for the purposes of RfC/D derivation. As discussed in Sections 5.1.3.2.3 and 5.3.6, the lack of a quantifiable monkey study is an important data gap given the potential relevance to humans and the uncertainties raised by existing monkey studies regarding this species sensitivity to reproductive effects (e.g., shortened pregnancies discussed in Section 4.3.2), CNS degeneration (e.g., stellate cell fibrosis described in Section 4.4.2) and delayed neurobehavioral development (e.g., VDR response described in Section 4.4.2) from methanol exposure. In addition, a full developmental neurotoxicity test (DNT) in rodents has not been performed and is warranted given the critical effect of decreased brain weight in rats and the suggestive (but quantitatively inconclusive) DNT results in monkeys. For these reasons, an UF of 3 was applied to account for deficiencies in the database.

Comment 4: Regarding the application of all UFs, one reviewer stated that EPA should "apply the uncertainty factors to the internal dose point of departure, prior to interspecies extrapolation with the pharmacokinetic model to account for non-linearities in external versus internal dose relationships." This reviewer suggested that EPA should discuss their choice of applying UFs to the HEC/D rather than the BMDL. The reviewer estimated that if UFs are applied first to the mouse cervical rib $BMDL_{05}$, then converted to the candidate RfC using the

PBPK model, the candidate RfC would increase by more than 2-fold. Another reviewer noted that the application of UFs to the HEC/D values is the standard procedure and is "preferred to alternative suggestions that the UFs be applied to intermediate measures such as blood concentrations or AUCs."

Response: The first reviewer is correct in that, after modifications were made to the rat PBPK model (see Response to Charge A1 Comment 7), BMDL estimations from both the rat and mouse candidate principal studies are not within the linear range of EPA's PBPK model predictions. EPA has reevaluated the analysis and applied the UFs prior to HEC/D derivation as suggested. This approach results in more scientifically reliable model predictions by lowering the BMDLs to within the more linear, calibrated range of the human PBPK model. Clarifying text has been added to the Sections 5.1.3.2 and 5.2.2.3.

The concern expressed by the second reviewer regarding departure from EPA practice is recognized, given the uncertainty associated with dividing internal dose BMDLs by UFs that are at least partially based on empirical analyses of ratios of NOAELs obtained from external oral exposures (U.S. EPA, 1994; Dourson and Stara, 1983). In the methanol (noncancer) assessment, the general EPA practice of applying the human PBPK model to derive HEC/D values prior to applying UFs (U.S. EPA, 2002, 1994) would result in RfC/Ds lower than if the PBPK model was used to derive HEC/D estimates after dividing the BMDL internal doses by UFs. However, this general practice if applied to methanol would result in greater model uncertainty because the HECs (1,042 to 1,604 mg/m^3) and HEDs (133 to 220 mg/kg-day) estimated from the BMDLs by the revised PBPK model are well above the inhalation concentrations (655 mg/m^3) and oral exposures (50 mg/kg-day) for which there are human data to calibrate the PBPK model (see Appendix B, Section B.2.7, Table B-6).

Charge B4 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: One reviewer commented that "[t]he authors of the document should be commended for breaking off with almost ten-year-old, scientifically indefensible U.S. EPA tradition of applying all uncertainty factors (UF) to the external human equivalent concentration (HEC estimate)." This reviewer stated that "[e]ven if, within the validated range of a PBPK model, the pharmacokinetics of a chemical appears to be almost linear, by the virtue of potentially saturable mechanisms of absorption, metabolism and excretion - it is still prudent to apply UF_A, UF_H, or perhaps in this case UF_D, to the internal dose (paraphrasing Paracelsus: 'this is the **internal** dose that makes a poison')."

Response: EPA appreciates the reviewer's affirmation of the Agency's decision to apply UFs to the internal dose BMDL PODs for methanol. This approach has been retained in this

assessment, but it should be recognized that there are chemical-specific circumstances warranting this approach for the methanol (noncancer) assessment that may not pertain to other chemical assessments.

Comment 2: Two reviewers thought that the lack of a quantifiable primate study was used to justify both the UF_A and UF_D. One of these reviewers agreed with the public comment that "[t]he lack of primate data should only be applied to one or the other, as this 'double counting' adds yet another forced conservatism to the assessment" and suggested that EPA should "…use the incomplete primate data once - to justify only UF_A" and "[c]onsider using lack of information on MOA to justify UF_D." The other reviewer commented that "It seems to me that the uncertainty contributed by limitations in knowledge about species sensitivity (per the monkey studies) has in effect been double counted in the overall UF."

Response: In the revised Section 5.1.3.2.3, EPA has clarified that the UF_D is based on deficiencies in the methanol toxicological database, particularly with respect to the interpretation of the importance and relevance reproductive, developmental neurotoxicity and chronic CNS effects observed in monkeys. The Agency has determined that a 3-fold UF_D is necessary to account for the possibility that a lower RfD/RfC might have been derived if additional data were available. This is consistent with EPA (2002) guidance, which states that:

> "The database UF is intended to account for the potential for deriving an underprotective RfD/RfC as a result of an incomplete characterization of the chemical's toxicity. In addition to identifying toxicity information that is lacking, review of existing data may also suggest that a lower reference value might result if additional data were available. Consequently, in deciding to apply this factor to account for deficiencies in the available data set and in identifying its magnitude, the assessor should consider both the data lacking and the data available for particular organ systems as well as life stages."

Additional studies to inform the MOA for the reproductive, developmental, and neurological effects of methanol would be helpful and, as discussed in Section 5.1.3.2.3, were suggested by peer reviewers of the monkey studies. However, uncertainty regarding the MOA is generally considered for the purposes of determining the UF_A.

Comment 3: Two reviewers questioned the need for a 3-fold UF_D. One of these reviewers commented that the EPA acknowledges that the database for methanol toxicity is 'quite extensive…'," "…the uncertainty contributed by limitations in knowledge about species sensitivity (per the monkey studies) has in effect been double counted in the overall UF," "[t]he absence of a full DNT test is rodents is stated to be important in part because of the critical effect of decreased brain weight in rats, but I still have reservations about the strength of that finding,"

and "…the report is responsive in terms of providing a clearer case for a database uncertainty factor of 3, but I still question whether it is needed." The other reviewer stated that "[t]he existing database on the developmental toxicity of methanol is sufficiently robust as to set the UFd at 1," "…[the monkey studies] do corroborate the principal study and hence they offer robust data that obviates the need for [a] UFd," and "…it isn't logical to consider these studies as being necessary [to corroborate the principal study] and simultaneously insufficient [for deriving and RfC]."

Response: EPA has clarified in the revised Section 5.1.3.2.3 that while the database for methanol toxicity is extensive in terms of the laboratory species and study design coverage, consisting of chronic and developmental toxicity studies in rats, mice, and monkeys, a two-generation reproductive toxicity study in rats, and neurotoxicity and immunotoxicity studies, it leaves considerable uncertainty with respect to the importance and relevance of reproductive, developmental and chronic effects observed in monkeys. As discussed in Section 5.1.1.1, the available monkey studies are considered inadequate or inferior to the candidate principal rodent studies for the purposes of RfC/D derivation. EPA agrees that this deficiency in the dose-response data would not normally warrant a UF_D given the scope of the existing database and the qualitative value of the chronic and developmental monkey studies for hazard identification. However, this deficiency is of particular concern for methanol given (1) metabolic similarities that suggest monkeys should most closely represent the potential for effects in humans (see Section 3.1) and (2) uncertainties regarding the importance and relevance of the monkey effects (see Section 5.1.3.2.3).

The UF_D does not have the same basis as the UF_A, and was not "double counted" in the overall UF. As stated above, consistent with EPA (2002) guidance , the Agency has determined that a 3-fold UF_D is necessary to account for the possibility that a lower RfD/RfC might have been derived if better or additional data were available. EPA guidance places particular emphasis in this regard on database deficiencies in the area of developmental toxicity, the primary focus of the methanol (noncancer) assessment, stating that "[i]f data from the available toxicology studies raise suspicions of developmental toxicity and signal the need for developmental data on specific organ systems (e.g., detailed nervous system, immune system, carcinogenesis, or endocrine system), then the database factor should take into account whether or not these data are available and used in the assessment and their potential to affect the POD for the particular duration RfD or RfC under development." As described in Section 5.1.3.2.3, NTP-CERHR (2004, 2003) and HEI (Burbacher et al., 2004a; 2004b; 1999a; 1999b) peer reviews of the monkey reproductive/developmental studies and an EPA-sponsored peer review (ERG, 2009) of the NEDO (1987) acute and chronic monkey studies were uncertain about the relevance of the

effects observed, but all of the reviews signaled that the observed effects should not be ignored and suggested additional research that might help resolve some of the uncertainty.

The reviewer's concern regarding the strength of the brain weight finding in the NEDO rat study has been addressed in response to Charge B1 Follow-up Peer Review Comment 1. Further, With respect to the developmental neurotoxicity (DNT) from methanol inhalation exposure, Table 5-5 of Section 5.1.3.2.3 indicates that methanol blood levels associated with DNT effects are a 12-fold higher in rodents versus primates. Some of this dissimilarity may be due to differences in species sensitivity, for which the UFA of 3-fold is intended to account, but some of the difference may be due to other factors, including whether appropriate and comparable endpoints were examined and whether appropriate study designs and quality control measures were used. To account for these additional uncertainties, a 3-fold UFD is applied.

Finally, for comparison purposes, EPA has performed an analysis of the alternative RfD and RfC that would have been derived if a UF_D of 1 had been applied instead of a UF_D of 3. Tables A-1 and A-2 correspond to Tables 5-4 and 5-6 of the assessment and demonstrate that a UF_D of 1 would have resulted in an RfC of 60 mg/m^3 and an RfD of 6 mg/kg-day (after rounding to single digit significance). The EPA has decided against this approach because it believes there is ample evidence that additional data from appropriate studies could result in the derivation of a reference values considerably lower than 60 mg/m^3 and a 6 mg/kg-day. With respect to DNT effects, in addition to the 12-fold higher methanol blood LOAELs in rodents versus primates noted above, a BMD analysis of the VDR DNT effect reported by Burbacher et al. (2004b; 1999b) resulted in a methanol blood C_{max} BMDL of 19.59 mg/L, less than half the methanol blood level PODs shown in Table A-1 and A-2 for rodents. Potential chronic neurotoxicity (fibrosis of "responsive stellate cells") were reported by NEDO (1987) at a 100 ppm exposure level that EPA's monkey PK model estimates corresponds to a methanol blood level of 3 mg/L. While Figure 5-4 of the assessment illustrates that a RfD or RfC exposure would not increase the methanol blood levels of anyone with an background methanol blood level below 2.5 mg/L to above 3 mg/L (see discussion in Section 5.3.6), Figure A-1 shows that a 6 mg/kg-day alternative RfD or a 60 mg/m^3 alternative RfC exposure would result in blood levels above 3 m/L for a substantial percentage (~25%) of those who started out with background blood levels below 2.5 mg/L. These analyses suggest that an alternative RfD of 6 mg/kg-day and an alternative RfC of 60 mg/m^3 associated with a UF_D of 1 are potentially under protective with respect to the DNT and/or chronic neurotoxicity of methanol.

Table A-1 Summary of PODs for critical endpoints, application of UFs and conversion to candidate RfCs using PBPK modeling.

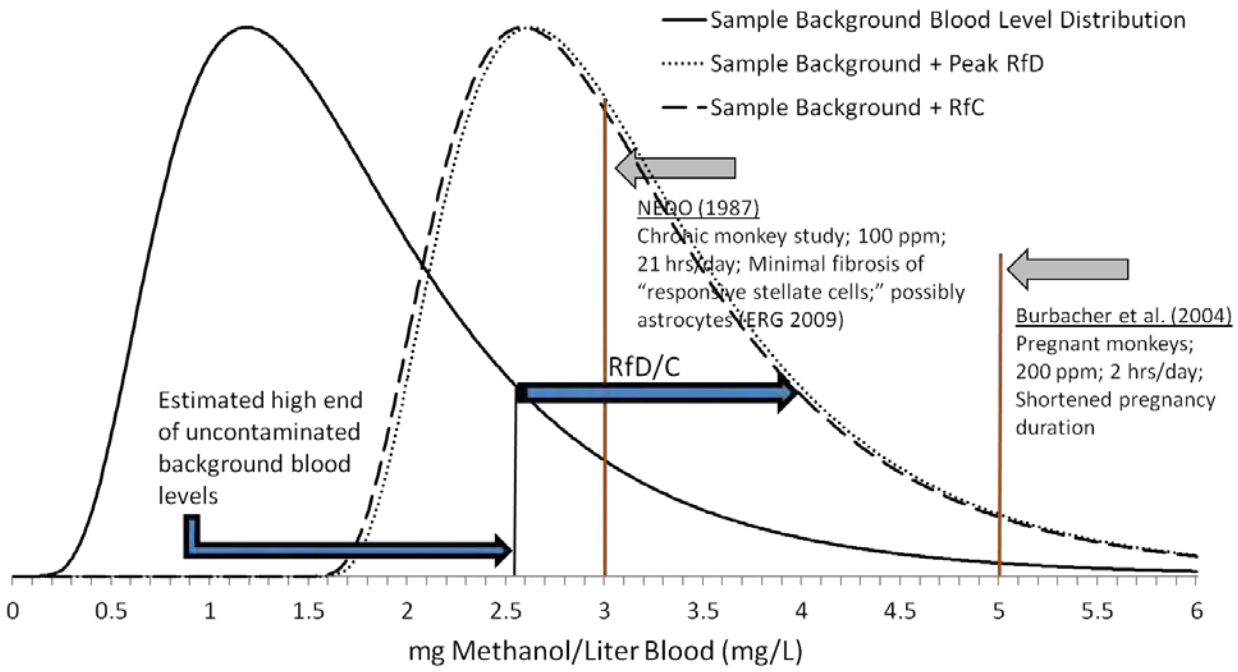
	Rogers et al. (1993b) mouse cervical rib C_{max}		NEDO (1987) rat brain weight AUC	
	10% BMR	**5% BMR**	**5% BMR**	**1 SD BMR**
BMDL = $POD_{internal}$	90.9 mg/L	43.1 mg/L	1,183 mg-hr/L	**858 mg-hr/L**
$RfC_{internal}$ = $POD_{internal}/UFs$[a]	3.03 mg/L	1.44 mg/L	39.4 mg-hr/L	**28.6 mg-hr/L**
RfC (mg/m^3)[b]	134.5	65.7	74.6	**54.6**

[a]UF_A =3; UF_D = 1; UF_H = 10; UF_S = 1; UF_L = 1; product of all UFs = 30.
[b]Each candidate RfC is the inhalation exposure concentration predicted to yield a blood concentration equal to its corresponding $RfC_{internal}$, using the human PBPK model with an background blood concentration of 2.5 mg/L, which corresponds to the estimated maximum background exposure rate of 1,600 mg/day (COT, 2011) in a 70-kg person (see Section 5.3.6); the final RfC is rounded to one significant figure.

Table A-2 Summary of PODs for critical endpoints, application of UFs and conversion to candidate RfDs using PBPK modeling.

	Rogers et al. (1993b) (mouse cervical rib C_{max})		NEDO (1987) (rat brain wt. AUC)	
	10% BMR	**5% BMR**	**5% BMR**	**1 SD BMR**
BMDL = $POD_{internal}$	90.9 mg/L	**43.1 mg/L**	1,183 mg-hr/L	858 mg-hr/L
$RfD_{internal}$ = $POD_{internal}/UFs$[a]	3.03 mg/L	**1.44 mg/L**	39.4 mg-hr/L	28.6mg-hr/L
RfD (mg/kg/day)[b]	12.7	**6.2**	16.4	12.1

[a]UF_A =3; UF_D = 3; UF_H = 10; UF_S = 1; UF_L = 1; product of all UFs = 100; see Section 5.1.3.2 below for details.
[b]Each candidate RfC is the inhalation exposure concentration predicted to yield a blood concentration equal to its corresponding $RfC_{internal}$, using the human PBPK model with an background blood concentration of 2.5 mg/L, which corresponds to the estimated maximum background exposure rate of 1,600 mg/day (COT, 2011) in a 70-kg person (see Section 5.3.6); the final RfC is rounded to one significant figure.



Note: References in this figure refer to the NEDO (1987) and HEI (Burbacher et al., 2004a; 2004b; 1999a; 1999b) reports and the EPA sponsored external peer review (ERG, 2009) of the NEDO (1987) report.

Figure A-1 Relationship of monkey blood levels associated with effects of uncertain adversity with projected impact of daily peak alternative RfC and RfD exposures [derived using aUF_D of 1] on sample background methanol blood levels (mg MeOH/Liter [mg/L] blood) in humans.

A.1.3. Charge C: "Oral Reference Dose (RfD) for Methanol"

A.1.3.1. Charge C1. EPA concluded that the oral RfD should be derived using a route-to-route extrapolation from the more extensive inhalation database given the paucity of oral toxicity data. Please comment on whether the rationale for this approach has been scientifically justified and clearly explained. Please identify and provide the rationale for any alternative approaches for the determining the RfD and discuss whether such approaches are preferred to EPA's approach.

Summary of Comments: *Six reviewers indicated that the approach taken by EPA was appropriate and one reviewer did not comment due to a lack of expertise. Specific comments or suggestions made by the reviewers with respect to this charge are described below, along with EPA responses.*

Comment 1: One reviewer recommended that EPA "provide alternative RfC estimates that would be derived using traditional approaches."

Response: Since this comment was made in response to the oral RfD charge, it is assumed that the reviewer is requesting that EPA provide traditional RfD estimates and not "RfC" estimates. None of the oral studies provided sufficient dose-response data for a dose-response analysis and none of the developmental toxicity studies identified a NOAEL for use in a traditional RfD estimate. The only NOAEL identified was 500 mg/kg-day from the subchronic oral study in adult rats (TRL, 1986). As discussed in Section 5.2.4, the previous IRIS assessment of methanol divided this NOAEL by a 1,000-fold uncertainty factor to obtain an RfD of 0.5 mg/kg-day. This value is lower than the current proposed RfD of 2 mg/kg-day, largely because a 10-fold higher uncertainty factor was employed in the previous assessment.

Comment 2: One reviewer stated that "Human model validation using the oral data of Schmutte et al. (1988) (see Charge D2) could further strengthen confidence in the route-to-route extrapolation."

Response: EPA agrees and as discussed in response to Charge A1 Comment 11, this oral study has been added to EPA's PBPK analysis and used in the validation of the oral human PBPK model.

Charge C1 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: No additional comments were received regarding this charge question.

Response: EPA has retained the route-to-route extrapolation approach in the assessment.

A.1.3.2. Charge C2. A PBPK model was used to derive the RfD via a route-to-route extrapolation, in which the internal-dose POD used for the derivation of the RfC based on data from the NEDO (1987) study was extrapolated to human oral exposure levels using the human PBPK model. Please comment on whether the rationale for this approach has been scientifically justified. Has adequate justification been provided for the selected internal dose metric, i.e., AUC for methanol, in the blood of dams? Is the PBPK model suitable for extrapolation of fetal and neonatal endpoints to human oral exposures? Please provide a detailed explanation.

Summary of Comments: *Five reviewers stated that the approach taken by EPA was appropriate and a sixth reviewer did not comment due to a lack of expertise. A seventh reviewer cited the lack of gestational and lactational components as a weakness in the EPA approach. All*

reviewers either referred to or repeated previous comments on the PBPK model and the RfC derivation approach.

Summary Response: The reviewers did not offer any new comments in response to this charge question that were not covered in response to previous charge questions.

Charge C2 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: No additional comments were received regarding this charge question.

Response: EPA has retained the route-to-route extrapolation approach in the assessment.

A.1.3.3. Charge C3. EPA applied the same UFs to the POD for the derivation of the RfD as for the RfC. Please comment on the rationale for the selection of the UFs.

Summary of Comments: *All but one reviewer agreed with the use of the same UFs for the RfD as for the RfC. One reviewer stated that this was "unexpected" because the database for oral and inhalation are very different.*

Summary Response: The critical effects were systemic, developmental effects that are assumed to be dependent on blood concentrations of methanol. EPA was able to use methanol blood concentrations in its benchmark dose analyses of the critical effects in the candidate principal studies because blood levels were either reported in the study or could be estimated using a validated PBPK model. After application of UFs, a validated human PBPK model was then used to convert the adjusted benchmark dose estimates to an RfD and RfC. For these reasons, EPA was able to derive the oral RfD and inhalation RfC with a similar degree of confidence using the same data set, endpoint, BMD methods and PBPK model.

Charge C3 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: No additional comments were received regarding this charge question.

Response: EPA will continue to apply the same UFs for the RfC and RfD.

A.1.4. Charge D: "General Charge Questions"

A.1.4.1. Charge D1. Is the Toxicological Review logical, clear and concise? Has EPA clearly synthesized the scientific evidence for noncancer and cancer hazards?

Summary of Comments: *In general, reviewers commented that the Toxicological review was logical, comprehensive and clear, but not concise. One reviewer stated that the review "is thorough and well written, and takes care to provide descriptions of the available evidence in a clear, complete, and unbiased form" and "presents a careful and well justified synthesis of these data." However, five of the seven reviewers criticized the repetitive or redundant nature of the review and four reviewers were critical of the review format, with one reviewer stating that "a different format could be much more effective in conveying critical information, interpretations, and decisions regarding available, relevant toxicological literature." Specific major (non-editorial) suggestions made by the reviewers with respect to this charge are described below, along with EPA responses.*

Comment 1: One reviewer suggested that EPA add a "decision tree" to make choices for major decisions more transparent**.**

Response: In light of this concern, an Executive Summary has been added to the beginning of the toxicological review which makes the choices for major decisions more readily apparent and transparent. Exposure-Response arrays for oral and inhalation toxicity studies were also added as Figures 4-1 and 4-2 to better depict the relationship of NOAELs and LOAELs in the overall database of studies.

Comment 2: Two reviewers suggested that EPA make edits consistent with recent NRC (2011) recommendations for the draft EPA formaldehyde assessment to:

a. Reduce text volume, narrative approach, redundancies and inconsistencies,

b. rely more heavily on tables and not repeat individual study descriptions,

c. include "inclusion and exclusion criteria" for cited references, and

d. reduce "extraneous information" contained in Appendices.

Response: In response to this comment EPA has made the following format and text edits to the toxicological review:

a. In response to the suggestion to "reduce text volume, narrative approach, redundancies and inconsistencies," EPA has extensively condensed Section 3.4 (e.g., abbreviating the discussion of model structure and deleting detailed

discussion of model parameter and model calibration in deference to Appendix B), combined Section 4.6 "MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MOA" with Section 4.8 "NONCANCER MOA INFORMATION" into a new Section 4.7, deleted or merged redundant portions of Section 5.3 "UNCERTAINTIES IN THE INHALATION RFC AND ORAL RFD" with Section 5.1.3.2 "Application of UFs," removed portions of Section 5 that were unnecessarily redundant with Appendix D, revised and consolidated portions Section 5 related to the justification of the dose metric (AUC versus Cmax versus total metabolites) employed for the BMD analyses of candidate principal studies and removed Section 6 (in lieu of a new Executive Summary).

b. In response to the suggestion to "rely more heavily on tables and not repeat individual study descriptions," EPA has created new tables for whole embryo studies described in Section "4.3.3 Other Reproductive and Developmental Studies" and the i.p. studies described in Section "4.4.3 Neurotoxicity Studies Employing In Vitro and I.P. Methanol Exposures" and has edited all Sections to reduce unnecessary repetition of individual study descriptions. EPA has also added an exposure-response array (Figure 4-1) to the assessment.

c. In response to the suggestion to include 'inclusion and exclusion criteria' for cited references," EPA has added text to the Preface of the methanol toxicological review that describes how EPA evaluates the quality of studies.

d. In response to the suggestion to "reduce 'extraneous information' contained in Appendices," EPA has removed Appendix D, having determined it to be extraneous due to the stronger biological basis for the choice of a Cmax dose metric described in Section 5.1.2.1, and removed the source code text from Appendix B. The source code text is posted on the EPA HERO database (U.S. EPA, 2012b), as part of a Windows zip file containing a complete package of acslX code necessary to run all of the developed models.

Comment 3: One reviewer suggested that "Table 3-3 should include the Dorman cynomolgus monkey study with a clear indication that it involved lung only exposure of anesthetized monkeys."

Response: Table 3-3 has been revised to include the Dorman cynomolgus monkey study in response to this comment.

Comment 4: One reviewer asked whether EPA considers the Fagan test results from the Burbacher et al. (2004b; 1999b) monkey study to be "biologically significant despite the lack of a statistically significant response?"

Response: There is uncertainty regarding both the biological and statistical significance of the Fagan test results from the Burbacher et al. (2004b; 1999b) monkey study. As explained in Section 4.4.2,

> "Unlike the VDR results discussed previously, results of this test did not appear to be gender specific and were neither statistically significant (ANOVA p = 0.38) nor related to exposure concentration. The findings indicated a cohort effect which appeared to reduce the statistical power of this analysis. The authors' exploratory analysis of differences in outcomes between the 2 cohorts indicated an effect of exposure in the second cohort and not the first cohort due to higher mean performance in controls of cohort 2 (70% + 5% versus 55% ± 4% for cohort 1). In addition, this latter finding could reflect the inherent constraints of this endpoint. If the control group performs at the 60% level and the most impaired subjects perform at approximately the 50% chance level (worse than chance performance would not be expected), the range over which a concentration-response relationship can be expressed is limited."

However, the Fagan test results cannot be ignored and, as described in Section 5.1.1.2.2:

> "Although not statistically significant and not quantifiable, the results of this test need to be considered, in conjunction with VDR test results and brain weight changes noted in the NEDO (1987) rat study, as a possible indication of CNS effects."

Comment 5: One reviewer found Section 3.4.2.4 confusing, and suggested that other models that have been developed with inhaled manganese (Schroeter et al., 2011; Yoon et al., 2011; Yoon et al., 2009a, b) "could form the basis for a gestational and lactational model."

Response: To reduce text volume in response to Charge D1 Comment 2a, Section 3.2.4 has been removed from the toxicological review. It contained an unnecessary discussion of the rat and human isopropanol models described by Gentry et al. (2003; 2002) and Clewell et al. (2001). It was originally included because it was thought to be a possible guide had EPA decided to develop a more complex gestational and lactational model. The reviewer is right in that, had EPA decided to take this approach, other gestational and lactational models, such as the one developed for manganese, could have been considered. However, EPA has determined, and the peer reviewers generally agreed (see "Summary of Comments" under Charges A3 and A5), that such a model was unnecessary for the purposes of the methanol toxicological review.

Comment 6: One reviewer stated that the "the discussion of a two compartment stomach (page 3-28 and elsewhere) for rodents need additional justification (squamous and epithelial portions?)" and questioned whether this structure is "appropriate for people (as indicated on page 3-51)."

Response: EPA agrees with the reviewer and, in response, has simplified the GI absorption model and revised the associated text in the toxicological review. In particular, the GI model for humans has been reduced to a single, first order compartment and rate (see Appendix B, Section B.2.6).

Comment 7: One reviewer commented that the use of "terms that describe model fits as 'quite poor' (e.g., see page 3-40 and elsewhere)" need to be "better clarified (visual inspection, goodness of fit, other?)."

Response: Except where numerical measures of fit are given, all such references to model fit reflect visual inspection. This has been clarified in the toxicological review.

Comment 8: One reviewer requested that EPA "pick one set of units (ppm would be preferred until calculation of the actual RfC value)."

Response: In general, both units are given, with mg/m^3 values provided parenthetically after the ppm values, except for RfC/D and point of departure (e.g., BMDL) values discussed in Section 5.

Comment 9: One reviewer requested a discussion of the use of alcohol dehydrogenase inhibitors as a clinical 'antidote' on "page 4-7 (and possibly elsewhere)."

Response: Explanatory text has been added on page 4-1 and 4-4 to explain that infusion of ADH1 inhibitors such as ethanol or fomepizole (4-methylpyrazole) can serve as treatment for methanol poisoning.

Comment 10: One reviewer asked whether the folate deficiency described on page 4-40 affects methanol concentrations significantly, and "which data support this conclusion?"

Response: Folate is the coenzyme of tetrahydrofolate synthetase, an enzyme that is rate limiting in the removal of formate. However, there is limited evidence regarding how folate deficiency would impact methanol and formaldehyde levels. Hence, the statement on page 4-40 of the draft assessment, that "Folate deficiency would be expected to cause potentially toxic levels of methanol, formaldehyde, and formate to be retained" has been revised in the final assessment (Section 4.3.2), to read" "Folate deficiency would be expected to cause potentially toxic levels of formate to be retained."

Comment 11: One reviewer recommended that EPA remove the Section 4.1 discussion of the CNS effects produced by acute methanol overdosing because it could be perceived as an inappropriate and "biased way to validate the subsequent choice of the NEDO study (decrease in brain weights suggesting a methanol-induced CNS effect)."

Response: Because of the limited usefulness of human case study information to this assessment, this portion of Section 4.1 was moved to a new Appendix C. However, the remainder of Section 4.1 is retained because it contains important information relevant to the acute toxicity of methanol and is one of the only sections in the toxicological review for which human data are available. It is recognized that the CNS effects from acute exposure to methanol are likely the result of a different mode of action than methanol's developmental effects. This is discussed in several places in the toxicological review, particularly Section 4.7 on the MOA for noncancer effects.

Comment 12: One reviewer suggested that EPA needs to improve the synthesis of S-D rat toxicokinetic data for purposes of PBPK model development.

Response: This has been done and Appendix B has been revised accordingly.

Comment 13: One reviewer suggested that EPA correct inconsistencies between the toxicokinetics section of Section 3 and Appendix B.

Response: To avoid redundancy and address inconsistencies, the PBPK discussions in Section 3 have been removed and the reader is referred to Appendix B for technical details.

Comment 14: One reviewer noted that the "clarity of the document is hampered by the lack of a clear synthesis of evidence regarding plausible modes of action for developmental toxicity."

Response: The mode of action discussions previously divided between Section 4.6 and 4.8 have been revised for clarity and consolidated into Section 4.7.

Charge D1 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: One reviewer stated that "[t]he overall document is much more concise and direct in presenting the key features of the risk assessment that has been conducted." Another reviewer stated that "The organization and presentation of the information is greatly improved, creating a much more readable document" and that "[t]he information seems to flow better, redundancies are minimized, and tables are used to more effectively summarize information." Another reviewer stated that "[t]he revised (May, 2013) version of 'Toxicological Review of Methanol

(non-cancer)' has been improved significantly in comparison to its external peer-review draft (2011) version."

Response: EPA appreciates the affirmation of the Agency's revisions to make the methanol (noncancer) toxicological review more clear and concise. The revised format, including the Executive Summary and increased use of tables and appendices, has been retained.

A.1.4.2. Charge D2. Please identify any additional studies that would make a significant impact on the conclusions of the Toxicological Review and should be considered in the assessment of the noncancer health effects of methanol.

Summary of Comments: *Three reviewers identified seven additional studies for EPA to consider (Miller and Wells, 2011; Leavens et al., 2006; Dorman et al., 1995; Bolon et al., 1994; Bolon et al., 1993; Haffner et al., 1992; Schmutte et al., 1988). Specific comments or suggestions made by the reviewers with respect to this charge are described below, along with EPA responses.*

Summary Response: The identified papers were evaluated and are now discussed and referenced in the final assessment. As discussed in response to Charge A1 Comment 10, oral data from two of these studies (Haffner et al., 1992) and (Schmutte et al., 1988)] are now used for model calibration, allowing identification of human oral absorption rate constant and bioavailability. The most informative of the remaining studies may be the in-vitro study of Miller and Wells (2011) which demonstrated that methanol-induced developmental effects are enhanced in mouse embryos with low catalase activity and reduced in mouse embryos with high catalase activity. The authors propose that this observation is related to methanol's impact on the ability of catalase to control the damaging effects of reactive oxygen species (ROS) activity, which would be greater in mouse embryos with low catalase activity. As discussed in Section 5.3.5, there are several problems with this interpretation, including that in vivo results from the same laboratory (Siu et al., 2013) do not support the Miller and Wells (2011) in vitro findings. Further, these observations do not preclude alternative explanations that involve a more direct interaction between methanol and the embryo.

Comment 1: One reviewer suggested that the University of Toronto rabbit studies published by Sweeting and coworkers "were not considered in the EPA's consideration of inter-species differences (i.e., are rat or mice studies appropriate)." Another reviewer commented that the discussion of the University of Toronto studies, especially the "publication regarding the role of ROS in mediating the effects of methanol," needs to be improved and included in the "sections related to choice of POD, critical effect, etc."

Response: EPA has added additional discussion of the University of Toronto (Miller and Wells, 2011; Sweeting et al., 2011) research to the toxicological review. A detailed discussion of the University of Toronto findings and hypotheses regarding species differences and the role of ROS following methanol exposure has been added to Section 5.3 "UNCERTAINTIES IN THE INHALATION RFC AND ORAL RFD" of the toxicological review (see Section 5.3.5 "Choice of Species/Gender"). Miller and Wells (2011) have suggested that developmental studies in rodents may not be suitable for assessing human risk, and Sweeting et al. (2011) have suggested that rabbits would be a more appropriate test species than mice and that rabbits are resistant to methanol teratogenicity. A developmental study in rabbits via an appropriate route of exposure would be of interest, particularly if it involved an investigation of effects over a broad set of gestational days. However, more research is needed before it can be definitively stated that rabbit developmental study would be more relevant to humans than rodent studies and that rabbits are resistant to methanol teratogenicity.

Comment 2: One reviewer stated that "there are also other studies, including work in monkeys, with aspartame that may be supportive (e.g., Reynolds). Since Table 3-2 includes results from aspartame exposure this does not seem to be a clear exclusion criterion."

Response: A review of the aspartame literature is beyond the scope of this toxicological review. The aspartame exposure studies have been removed from Table 3-2.

Comment 3: One reviewer noted that "the ethanol teratology literature has been largely ignored despite some similarities in teratogenic response" and that "this larger literature may help inform the MOA discussions in the draft document and help guide whether formaldehyde should be considered as the proximate teratogen."

Response: A review of the ethanol literature is beyond the scope of this toxicological review.

Comment 4: One reviewer stated that "search terms and databases examined have been poorly defined" and that "there is a lack of inclusion and exclusion criteria" for references.

Response: EPA has added text to the Preface of the methanol toxicological review that describes how literature searches are performed and how studies are evaluated and selected.

Charge D2 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: One reviewer commented that "[i]n relation to this, on pages 4-2 and 4-3 of the main document, the authors indirectly state that formaldehyde and not formate is the likely cause of the ocular toxicity of methanol" and that "[t]his is clearly not true and needs to be revised, as shown by Martin-Amat et al (Methanol poisoning: Ocular toxicity produced by

formate. Toxicol. Appl. Pharmacol. 45: 201-208, 1978; also, McMartin et al, Lack of a role for formaldehyde in methanol poisoning in the monkey. Biochem. Pharmacol. 28: 645-649, 1979)."

Response: Edits have been made to the subject paragraph to emphasize that formate is the likely cause of ocular toxicity and the new reference provided by the reviewer has been cited.

A.1.4.3. Charge D3. Please discuss research likely to substantially increase confidence in the database for *future* assessments of methanol.

Summary of Comments: *Reviewers suggested the following research to increase confidence in the database for a future assessment:*

- *A proper study should be performed "to confirm the low activity of methanol metabolism in fetal tissues."*
- *"Future studies using different animal models from rodents to primates should focus on outcomes related to reproductive function, early sensorimotor development and object memory as well as changes in brain architecture and size.*
- *"Development of a PBPK model that considers gestation and lactational exposure."*
- *Studies that "replicate the findings of the critical study used by NEDO including the inclusion of additional neuropathological and neurobehavioral assessments" and using "NEDO-type" exposures.*
- *"Although additional monkey studies could be considered the Burbacher study is extremely robust and should receive more attention by EPA."*
- *Studies using "dual labeled material to confirm fetal exposure" and "designed to resolve whether formaldehyde is involved in the developmental effects following perinatal methanol exposure."*
- *"Completion of surveys to examine blood methanol concentrations in the U.S. population."*
- *"A study that fully characterizes methanol metabolism [including estimates the K_m and V_{max}] in the intact fetus and the dam using the rat as model… (as opposed to the existing studies that only assess protein levels or activities using ethanol as substrate)."*
- *"Studies of the role of ADH and catalase in the metabolism of methanol by F-344 and Sprague-Dawley rats" to "clarify why there might be two saturable pathways in one strain but only one in the other (as implied by the PBPK model)"*
- *An oral developmental study of methanol sufficient for use in the derivation of an RfD.*

- *"Research to explain the basis for differences in species/strain developmental effects" and determine "the proximate toxicant and mode of action for developmental toxicity."*
- *"Further studies to illuminate the relative sensitivity of rodents and primates to chronic methanol toxicity, especially with regard to developmental and neurotoxicity endpoints."*
- *Studies to elicit better "inhalation kinetic data for Sprague-Dawley rats."*
- *"Monkey studies with longer exposure durations and similar endpoints."*
- *"Additional mode-of-action motivated [including in-vitro] studies"*

Summary Response: EPA agrees that these suggested research studies could enhance the methanol toxicological review. However, EPA is not planning to, and none of the reviewers suggested that EPA should, delay the completion of this assessment pending the completion of any of these future study suggestions.

Charge D3 Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: No additional comments were received regarding this charge question.

Response: EPA completed the methanol (noncancer) toxicological review using available studies.

A.1.4.4. Bonus Charge Question: Please comment on the proposed RfD and RfC values for their intended use in risk assessment. Are these numbers more conservative than they need to be to protect public health? ***Note: During the external review panel meeting an additional charge question was developed by the chair of the panel with input from some panel members. This charge question relates to the RfC/D and their relationship to endogenous background blood levels. While not a part of the EPA Charge to the external review panel, most of the panel members responded to this "Bonus Charge Question" as discussed here.***

Summary of Comments: *The six reviewers that provided comments seem to be in agreement that there needs to be more discussion of the relation of the RfD and RfC to existing endogenous/background blood levels. Five of six reviewers suggested that the RfD and RfC values were more conservative (lower) than necessary. One reviewer pointed out "the RfC and RfD are specifically defined as levels at which the risk assessor can be reasonably confident that adverse effects will not appear" and are "not threshold levels at which effects might start to appear."*

Two reviewers suggested that estimates of the increased blood levels associated with the RfD/C values should be compared with either an upper bound or a standard deviation for existing or normal physiological background levels of methanol. However, another reviewer warned that "in view of the uncertainties as to fetal metabolism, mode of action and contribution of diet and individual metabolic or toxicodynamic differences which are identified in the report it seems very unwise to conclude that high-end [of the distribution of background] exposures which are apparently safe for some individuals are necessarily safe for all."

One reviewer supported the NTP CERHR (2003) opinion that a blood methanol concentration of < 10 mg/L would not be associated with adverse developmental effects. Another reviewer cited the NTP CERHR (2003) report as indicating that "common exposures" are not a concern for developmental toxicity, and suggested that this presents a credibility problem for the proposed RfD and RfC values, which have been likened to common exposures such as a glass of orange juice. Another reviewer expressed concern that the assumption that common exposures or "current background levels" are safe has not been analytically investigated, and suggested that the uncertainty factors applied are needed to reflect these concerns, "which therefore indicates that the proposed values for RfC and RfD are not necessarily unreasonable."

Summary Response: The RfC and RfD have increased by several-fold due to PBPK model revisions made in response to the comments received during external peer review. The final RfD of 2 mg/kg-day and RfC of 20 mg/m^3 are not overly conservative because they (1) are well above the levels associated with common exposures to methanol such as from a glass of orange juice and (2) need to account for uncertainty regarding the sensitivity of primates to the reproductive and developmental neurotoxic effects of methanol.

EPA addressed the recommendation of a reviewer that estimates of the increased blood levels associated with the RfD and RfC be compared with a standard deviation for existing or normal physiological (endogenous blood) background levels of methanol. As described in Section 5.3.6, the methanol blood levels of individuals receiving both an RfC and RfD exposure would increase by a daily maximum of 0.86 mg/L and a daily average of 0.59 mg/L. As shown in Figures 5-3, 5-4 and B-17, these increases are comparable to the 0.7 mg/L standard deviation estimated for the average methanol blood levels (1.5 ± 0.7 mg/L) in humans. Thus, the estimated increase in blood levels of methanol from exogenous exposures at the level of the RfD or the RfC (or from the RfC + RfD) are distinguishable from natural background variation. These RfC and RfD methanol blood level increases are also more than 100-fold higher than the increase that would be associated with a "common exposure" such as from a glass of pasteurized orange juice and about 10-fold higher than the increase that would be associated with exposure to a glass of unpasteurized orange juice (note that this is a relatively rare exposure and FDA requires warning

labels on unpasteurized juice that state "This product has not been pasteurized and therefore may contain harmful bacteria that can cause serious illness in children, the elderly, and persons with weakened immune systems"). Hence there is consistency with NTP CERHR (2003) in this regard.

However, there is uncertainty with respect to the NTP CERHR (2003) statement that methanol blood levels below 10 mg/L would not be associated with adverse developmental effects. As discussed in Sections 5.1.3.2.3 and 5.3.6, there is uncertainty as to whether rodents are as sensitive as monkeys and humans to the reproductive and developmental neurotoxic effects of methanol. The lack of a reliably quantifiable monkey study is an important data gap given the potential relevance to humans and the uncertainties raised by existing monkey studies regarding monkey sensitivity to reproductive effects (e.g., shortened pregnancies discussed in Section 4.3.2), CNS degeneration (e.g., stellate cell fibrosis described in Section 4.4.2) and delayed neurobehavioral development (e.g., VDR response described in Section 4.4.2) from methanol exposure. In the Burbacher et al. (2004b; 1999b) study, statistically significant shortened pregnancy duration was observed in monkeys exposed to 200 ppm and statistically significant VDR delay was observed in male monkey infants exposed to 600 ppm methanol for just 2 hours per day. EPA estimates that these exposures raised the methanol blood levels over background methanol blood levels in these monkeys to peak values of just 3 and 10 mg/L, respectively (see Appendix D, Table D-10), corresponding to total blood levels of 5 and 12 mg/L, respectively. Also, NEDO (1987) observed potential signs of CNS degeneration in histopathology reported for monkeys exposed chronically to 100 ppm for 21 hours per day, which is estimated to be associated with an increase in methanol blood levels over background levels of approximately 1 mg/L (based on EPA monkey model), corresponding to total methanol blood levels of roughly 3 mg/L (assuming an background in these monkeys of 2 mg/L).

Regarding the comment warning that it should not be assumed that "high-end [of the distribution of background] exposures which are apparently safe for some individuals are necessarily safe for all", EPA agrees some individuals may have a high background level of methanol and/or high susceptibility. However, for the purposes of this assessment, EPA assumes that background blood levels of methanol in a human population with normal background variation do not elicit adverse health effects. This greatly simplifies the derivation of an RfD and an RfC which are, by definition, population level estimates (including sensitive populations) of the amount of a substance that a person can inhale (or ingest) every day over the course of a lifetime [above background levels] without an appreciable risk of harm.

As discussed in response to Charge A2 Comment 1, the discussion of the RfC and RfD and their relation to endogenous/background blood levels has been clarified in the revised draft

assessment (see Section 5.3.6). In summary, EPA does not feel that the RfD of 2 mg/kg-day and RfC of 20 mg/m^3 are overly conservative. They are well above the levels associated with common exposures to methanol and they appropriately account for uncertainty regarding the sensitivity of primates to the reproductive and developmental neurotoxic effects of methanol.

Bonus Charge Follow-up Peer Review Comments on 2013 Revised Draft Assessment

Comment 1: One reviewer commented that "[t]he new proposed RfC and RfD are higher values, and the IRIS assessment addresses the issue of comparison of associated blood methanol concentrations with background levels directly" and that "[t]his is a very important addition to the document and helps place the RfC and RfD in perspective."

Response: EPA appreciates the affirmation of sufficient revisions to the Toxicological Review in response to previous peer-review comments.

Comment 2: One reviewer commented that "Nearly all of the studies used to obtain data in humans had restricted dietary intake of foods that might increase methanol levels," and noted that "…this perhaps provides data on methanol blood concentrations that can be expected from endogenous metabolism, but hardly captures the range of blood methanol concentrations in individuals consuming a normal diet." This reviewer also stated that "[i]f the statement in the current assessment that typical blood methanol concentrations are assumed to be without adverse effect (which I support), that presumably applies to the higher blood methanol concentrations that would be expected without dietary restriction." Another reviewer commented that "[t]he endogenous levels in the general population are likely higher than the EPA-derived 1.5 ± 0.7 mg/L (1 SD) (from the special "meta-analaysis" of studies in Table 3-1)" and that "[t]he EPA should search for and include data from studies without dietary restrictions."

Response: In response to these reviewer and public comments on this topic, EPA has revised the methanol (noncancer) assessment, particularly Section 5.3.6, to more clearly reflect the limitations of the data shown in Table 3-1 for estimating population blood levels of methanol (restricted diets, small numbers of studies and individuals, differing results by study), and supplemented its analysis with data and conclusions from the United Kingdom (U.K.) Food Standards Agency "COT Statement on the effects of chronic dietary exposure to methanol" (COT, 2011). In Section 5.3.6, EPA has derived a sample lognormal distribution of methanol blood levels that is consistent with data from study groups in Table 3-1 that did not involve dietary restrictions other than alcohol. EPA compares this sample distribution with data for background (endogenous and dietary) "exposure" rates estimated by the U.K. (COT, 2011). The EPA sample background distribution and U.K. background methanol estimates are consistent with one another in that the methanol blood level predicted by EPA's PBPK model for the U.K.'s

23 mg/kg-day maximum exposure rate estimate and the EPA sample background distribution's mean + 2xSD are similar, 2.5 mg/L and 2.9 mg/L, respectively. EPA recognizes that some individuals may have background methanol blood levels above 2.5 mg/L (~7% according to the sample distribution), but believes that methanol blood level of 2.5 mg/L represents a reasonable approximation of the upper end of the range of background blood levels associated with a diet that includes fruits and vegetables (as discussed in Section 5.3.6).

Comment 3: One reviewer commented that "…for a sizable fraction of the population, exposure at these doses/concentrations would not result in blood methanol concentrations outside the normal range, particularly considering the first point, above [Comment 1]." This reviewer further commented that "…the report represents progress in dealing with the problem of assessing risk from exogenous exposure to endogenous chemicals, but falls short of presenting a compelling case why the toxicity values are not excessively conservative." Another reviewer commented that Figures 5-3 and 5-4 were "persuasive visually" but suggested a "statistical analysis of confidence limits/significance of RfC and/or RfD impact in relation to background/endogenous levels of methanol."Another reviewer commented that RfC/RfD exposures would not be distinguishable from background because "[f]or example, if a person with 0.8 added 0.86, you would be at 1.66 or almost right at the mean level!" This reviewer stated that "Figures 5.3 and 5.4 clearly show how the added levels are NOT distinguishable from background" and that "…in slide #11 of the presentation [given by EPA at the June 26, 2013 follow-up peer review webinar], when the UF_D happened to be set at 1, the two distributions of blood methanol are readily distinguishable."

Response: By definition, RfDs and RfCs are intended to protect "sensitive subgroups" from "appreciable risk." In the case of methanol, sensitive subgroups would include pregnant females and the elderly (toxicodynamic susceptibility) with lifetime methanol blood levels that are at the high end of the range of background methanol blood levels associated with a diet that includes fruits and vegetables (toxicokinetic susceptibility), estimated to be approximately 2.5 mg/L. A determination of whether the daily exogenous exposures at a RfD or RfC are "distinguishable" relative to endogenous methanol blood levels is not a primary consideration. However, in response to this concern, EPA provides an example in Section 5.3.6 which illustrates that the shift in a sample background methanol blood level distribution that would be associated with daily exposures of the entire population to methanol at the RfC or the RfD is estimated to increase the percentage of individuals with peak methanol blood levels at or above 2.5 mg/L from ~7% to ~14%. As discussed in Section 5.3.6, these estimates are not precise and do not account for interindividual variability. However, they suggest that the increase in individuals with higher than 2.5 mg/L methanol blood levels (i.e., higher than the upper range of background

methanol blood levels associated with a diet that includes fruits and vegetables) following a RfD or RfC exposure would not be negligible.

EPA also believes that the RfD and RfC are not "excessively conservative." As stated in response to the Charge B4 Follow-up Peer Review Comment 3, EPA guidance places particular emphasis on database deficiencies in the area of developmental toxicity, the primary focus of the methanol (noncancer) assessment, stating that "[i]f data from the available toxicology studies raise suspicions of developmental toxicity and signal the need for developmental data on specific organ systems (e.g., detailed nervous system, immune system, carcinogenesis, or endocrine system), then the database factor should take into account whether or not these data are available and used in the assessment and their potential to affect the POD for the particular duration RfD or RfC under development." As discussed in Section 5.1.3.2.3, a database uncertainty factor of 3-fold has been applied to account for deficiencies in the methanol database, particularly with respect to deficiencies that do not allow the Agency to full assess methanol's potential to cause developmental neurotoxicity. As discussed in Section 5.3.7, uncertain, but potentially adverse effects have been observed in monkeys at blood levels as low as 3 mg/L. As discussed in Section 5.3.6, a RfC or RfD exposure is expected to raise the methanol blood level of an individual with a high end background blood level of approximately 2.5 mg/L to just below 3 mg/L. A higher exposure would raise background levels above the lowest methanol blood level that has been associated with uncertain, but potentially adverse effects.

A.2. Public Comments

A.2.1. April 18, 2011 to July 6, 2011 Public Comment Period

The following public comments and EPA responses pertain to comments received on the 2011 draft of the methanol (noncancer) toxicological review during the April 18, 2011 to July 6, 2011 public comment period.

Comment 1: "EPA has arbitrarily decided to establish these reference levels to identify risks ONLY for exposure to methanol that increases the body burden of methanol or its metabolites."

Response: As discussed above in response to external peer review Comment 1 of Charge A2 and comments associated with the Bonus Charge Question, the decision to base the RfC/D on exposures that increase the body burden of an individual above their naturally occurring endogenous/background blood levels was not arbitrary. The PBPK models used in the final

assessment incorporate endogenous/background concentrations of methanol; however, for BMD modeling, the PBPK model estimate of background concentration is subtracted from the predicted dose metric under bioassay conditions. This approach for dealing with endogenous/background concentrations of methanol and it metabolites avoids the issue of whether or not individuals experience health effects from endogenous/background concentrations of methanol or its metabolites because only the risk due to exposures above background is thereby evaluated. This greatly simplifies the dose-response assessment for methanol and the derivation of an RfC (or RfD), which is by definition a population level estimate (including sensitive populations) of the amount of a substance that a person can inhale (or ingest) every day over the course of a lifetime [above background levels] without an appreciable risk of harm.

Comment 2: "The recommended reference levels represent a very small addition to the average person's body burden of methanol and by implication suggest that half the population is at risk from their own background level of methanol."

Response: The approach taken by EPA in deriving the RfC and the RfD assumes that endogenous/background blood levels of methanol in a human population with normal background variation do not elicit adverse health effects. There is currently little evidence, epidemiological or otherwise, to challenge this assumption. Given this assumption and lack of evidence to the contrary, if the 2 mg/kg-day RfD or 2×10^{1} mg/m^3 RfC were so low that the resulting (predicted) change in methanol blood levels was only a small fraction of the normal variation in background levels (e.g., 1% of one standard deviation), one could argue that this would be indistinguishable from natural variation and toxicologically irrelevant. Therefore, a comparison of the expected increase in methanol levels in blood resulting from exposure to methanol at the level of the RfC or RfD to the variation in endogenous/background levels of methanol observed in humans is provided in Section 5.3.6 to determine if this might be the case. As shown in Figures 5-3, 5-4 and B 17, the estimated increase in blood levels of methanol resulting from exposure to methanol at the RfC alone, at the RfD alone, or at the RfC + RfD combined is comparable to the variability (represented as one standard deviation) observed in the average estimated methanol blood levels (1.5 ± 0.7 mg/L) in humans (see Table 3-1 and Section 5.3.6). This then demonstrates that the estimated increase in levels of methanol from the RfD or the RfC (or from the RfC + RfD) are distinguishable from natural background variation, but the overall derivation of the RfD and RfC ensures that these increases will not significantly increase adverse health outcomes.

Comment 3: "EPA incorrectly supports its decision to ignore the naturally-occurring background levels of methanol in human blood by citing the results of its PBPK modeling."

Response: As explained above in response to external peer review Comment 2 of Charge A2, EPA has re-calibrated the PBPK models to account for background levels and has used them to derive the revised RfD and RfC. Hence, the justification for not including a background term in the PBPK models has been removed from the toxicological review.

Comment 4: "While the increment of a reference level dose of methanol is a small percentage of the average background blood level in humans, the intake of certain common foods can easily exceed EPA's recommended reference level."

Response: Due to changes in the rat PBPK model, the RfC and RfD are several-fold higher than the previously proposed values and, as explained above in response to comments related to the external peer review Bonus Charge Question, the increase in an individual's methanol blood levels after an exposure equivalent to the final RfC or RfD is expected to be well in excess of the increase that would be associated with a "common exposure" such as from a glass of orange juice.

Comment 5: "Application of a physiologically based pharmacokinetic (PBPK) model to these study data that is inappropriate for modeling exposures to pregnant animals, neonates, and weanling rats, and that is based on a data set that severely underestimates the likely exposures in both studies."

Response: As explained above in response to external peer review Comment 2 of Charge A2, EPA recognizes that neonatal blood levels will likely be higher, approximately 2-fold higher for rats, than maternal blood levels of methanol. However, the ratio of blood concentrations between a human infant and its mother is not expected to be significantly greater than the approximate 2-fold difference that has been observed between rat pups and dams. Further, the health-effects data indicate that most of the effects of concern are due to fetal exposure, with only a small influence due to postnatal exposures. As stated in Section 5.1.3.2.2, for these reasons and because EPA has confidence in the ability of the PBPK model to accurately predict adult blood levels of methanol, the maternal blood methanol levels for the estimation of HECs from the NEDO (1987) study were used as the dose metric.

Comment 6: "Failure to confirm the results of EPA's PBPK modeling against blood methanol concentration data collected in both [Rogers et al. (1993b) and NEDO (1987)] studies."

Response: The mouse PBPK model has now been removed from the assessment and the blood concentration data from Rogers et al. (1993b) used directly for the benchmark dose analysis. For example, the $BMDL_{10}$ for the mouse cervical rib effect based on the blood C_{max} metric has thereby changed from 94.3 mg/L to 90.9 mg/L (both representing concentration increases above background). Data were not available for validating the rat PBPK model

predictions prior to the methanol external peer review. Subsequent to the methanol external peer review, EPA received blood measurements from the NEDO (1987) rat study and has validated model predictions against them (Appendix B, Sections B.2.3, B.2.4 and B.2.5).

Comment 7: "Recent research by Dr. Peter Wells of the University of Toronto, which we detail in these comments, raises serious questions about the use of rodent models for hazard assessment of methanol in humans because rodents and humans metabolize methanol very differently."

Response: As explained above in response to comments made under external peer review Charge D2, a detailed discussion of the University of Toronto findings have been added to Section 5.3 "UNCERTAINTIES IN THE INHALATION RfC AND ORAL RfD" of the toxicological review (see Section "5.3.5 Choice of Species/Gender"). The in-vitro study of Miller and Wells (2011) demonstrated that methanol-induced developmental effects are enhanced in mouse embryos with low catalase activity and reduced in mouse embryos with high catalase activity. The authors propose that this observation is related to methanol's impact on the ability of catalase to control the damaging effects of reactive oxygen species (ROS) activity, which would be greater in mouse embryos with low catalase activity. However, as discussed in Section 5.3.5, there are several problems with this interpretation, including that in vivo results from the same laboratory (Siu et al., 2013) do not support the Miller and Wells (2011) in vitro findings. The University of Toronto studies are informative, but do not demonstrate conclusively that rodent developmental studies are irrelevant to humans.

Comment 8: "Severe reporting deficiencies in the two-generation reproductive study, including a lack of mean and individual animal data in the main study and the absence of details regarding methods and data related to maternal or gestational outcomes in the supplementary study."

Response: As described in Section 5.1.2.2, the supplementary study to the NEDO two generation study provides sufficient dose and response information for a benchmark dose analysis. Uncertainties associated with this study as they relate to the benchmark dose analysis, including the absence of a detailed reporting of methods and maternal or gestational outcomes, are discussed in Section "5.3.1 Choice of Study/Endpoint." Though the methods for this supplementary study are not described, the methods for the parent two-generation study are adequately described and it is reasonable to assume that the supplementary study was performed under the same protocol starting with a number of F0 females appropriate for a one-generation developmental study (see response to public Comment 11 below). While data related to maternal or gestational outcomes in the supplementary study are not given, signs of overt maternal toxicity were not reported in the two-generation study at similar exposure levels and it is

reasonable to assume that they did not occur, and would have been reported had they been observed, in the supplementary study. While this supplementary study no longer forms the basis of the RfD, it does form the basis for the RfC because its limitations are not considered serious enough to preclude its consideration as a candidate principal study and because it documents a clear dose-response for a relevant endpoint for a critical organ system, brain weight reduction, which is consistent with its parent two-generation study and with other teratogenicity (NEDO, 1987) and subchronic (TRL, 1986) study findings with respect to the effect of methanol exposure on brain weight.

Comment 9: "The lack of utility of the NEDO (1987) reproductive study for the purpose of human health risk assessment, as judged by other authoritative bodies."

Response: By "authoritative bodies" the commenter refers specifically to a 2003 report from the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR, 2003) on the reproductive and developmental toxicity of methanol. The methanol toxicological review cites the subsequently peer reviewed and published version of this report (NTP-CERHR, 2004). In this more recent published version of the report, the NTP-CERHR panel states that "a summary of a two-generation rat reproductive toxicity study done by the Japanese NEDO was received, but data were not available in sufficient detail for Expert Panel review." The NEDO summary reviewed by the NTP-CERHR panel did not contain the more detailed supplementary study data, with pup brain weight means and standard deviations that EPA evaluated in its benchmark dose analysis (Appendix D). This information, as well as supplemental methanol blood measurements, was obtained by EPA after the NTP-CERHR panel completed its report. In addition, EPA sponsored an external peer review (ERG, 2009) of the NEDO (1987) report that contained this information, along with two other NEDO chronic rat and mouse studies (NEDO, 1985a, b). This expert peer review panel of five scientists was asked specifically to "Describe the reliability of the subject NEDO studies for consideration in the derivation of EPA IRIS quantitative health benchmarks." With respect to this charge and the two-generation study, including the supplementary study, the main concerns expressed by the peer reviewers were that they "may be useful for RfD derivation if brain weight changes persist when normalized by body weight" and that the authors "should have used ANOVA plus multiple comparison tests to analyze these data." With respect to the former concern, NEDO only reported means and standard deviations for absolute brain weight change and did not report body weight data for the supplementary study. However, body weight data reported for the parent, two-generation study did not indicate a body weight effect in the exposed F1 or F2 generation pups. Further, the absolute brain weights reported by NEDO are an appropriate basis for a dose-response assessment. With respect to the concern over the lack of appropriate statistical testing,

EPA did not rely on the NEDO statistical determinations, but performed its own more definitive benchmark dose analysis of the data (see response to external peer review Comment 1 of Charge B1). Hence, while the NEDO report of the two-generation supplementary study results has limitations, particularly with respect to reporting of methods (see discussion in Section 5.3.1), it was not evaluated by the NTP-CERHR (2004) expert panel, and it was not deemed inadequate, for the purposes of RfC derivation, by a panel of expert peer reviewers (ERG, 2009).

Comment 10: "The use of exposure regimens in both the reproductive study and the 24-month rat study that confound the estimates of exposure."

Response: The stated basis for this comment is the concern over (1) "consumption of [methanol] contaminated feed," (2) "ingestion of methanol during the act of preening," (3) dermal absorption in adult rats, and (4) "increased dermal absorption of neonatal animals [over adults]" because "the epidermal layers of neonatal rats are thinner than those of adult animals, they lack fur for the first week after birth." With respect to the first concern, EPA estimates that data on a rodent breeder diet (labdiet #5013) indicates 10% moisture content. If one assumes that chamber methanol concentrations equilibrate with this moisture content, using the blood:air PC for methanol, and uses a typical pregnancy food consumption of 30 g/day in rats, then the amount of methanol ingested in the chow would be about 3% of that inhaled during a 22 hr/day exposure. This is likely an upper bound since it would take some time for methanol to diffuse into and through a container of chow (equilibration with the chow would take time), and fresh chow is provided each day. Thus the amount ingested by this route is not considered significant and dosimetry calculations have not been adjusted to reflect that possibility.

With respect to the second, third and fourth concerns, methanol is not known to adhere to or be absorbed by rat dermal surfaces in amounts that would significantly impact model predictions. According to Perkins et al. (1996b page 160):

> "The method [flow-through chamber exposure], like all whole-body methods, exposes the animal to the vapor at all dermal surfaces. For the very water-soluble vapors, such as methanol, dermal exposure is not significant; indeed, when taring the chamber by inserting a dead rat versus just opening an empty chamber for the same length of time, no difference in methanol loss was noted except at 20,000 ppm, in which case the steady-state loss was 27% higher with a dead rat than an empty chamber. This higher steady-state loss at 20,000 ppm methanol may be related to physical properties of the compound; at the high vapor level, somewhat more methanol may condense and become adsorbed to the fur. Further experimentation is required to clarify-the significance of this observation."

Since the concentrations used in the NEDO rat studies were well below the 20,000 ppm level at which Perkins et al. (1996b) observed a difference in methanol loss in the chamber, methanol dermal absorption is not expected to significantly impact model predictions of methanol blood levels in the NEDO rat studies [also, EPA presumes that the empty-chamber loss rate, of which the dead rat caused a 27% increase, was fairly small. The actual loss rate was not reported by Perkins et al.(1996b)]. Though the Perkins et al. (1996b) conclusion was based on an adult rat, there is no scientific basis for the belief that neonatal rats would absorb a significantly greater amount of methanol.

Comment 11: "Use of an insufficient number of parental animals in the supplementary reproductive study (from which EPA derives its RfC) to support proper statistical evaluation."

Response: The basis provided by the commenter for this statement is that "EPA and Organisation for Economic Cooperation and Development (OECD) guidelines recommend evaluation of at least 20 litters per group in a two-generation reproduction toxicity test, in order to ensure sufficient statistical power in the study (OECD, 2001; U.S. EPA, 1998b)." The number of F0 parental animals used in the NEDO two-generation study (30 males and 30 females per dose group) was appropriate and in accordance with both EPA and OECD two-generation reproduction toxicity test guidelines. The supplementary study performed by NEDO does not fall under these guidelines because it was not a two-generation reproduction study. According to NEDO (1987 page 201), the purpose of the supplementary study was "to confirm its [decreased brain weight] relationship with the treatment and to know from what period after birth such changes would appear" and, therefore, the test rats were only exposed "from Day 0 of gestation throughout the F1 generation." This type of study and purpose would more appropriately fall under the Agency's developmental neurotoxicity guidelines (U.S. EPA, 1998b), which state that "on postnatal day 11, either 1 male or 1 female pup from each litter (total of 10 males and 10 females per dose group) should be sacrificed" and that "brain weights should be measured in all of these pups." The number of F0 parental animals included per group in the supplemental experiment was not reported. However, the number of pups per dose group was reported and it is reasonable to assume that, consistent with the standard culling protocol used for both the F1 and F2 generations of the two-generation study (NEDO, 1987 pages 185 and 189), each dose group pup came from a different litter (to avoid problems associated with litter correlation). Hence, by examining more than 10 male and 10 female litter-specific pups per dose group at three time points (3, 6 and 8 weeks), the NEDO supplementary study actually went well beyond EPA recommendations for this type of study.

Comment 12: "Use of statistical methods in both the reproductive study and the 24-month rat study that, by today's standards, are considered inadequate."

Response: As mentioned above in response to public Comment 9, EPA did not rely on the NEDO statistical determinations, but performed its own more definitive benchmark dose analysis of the NEDO (1987) rat two-generation an teratogenicity data (see response to external peer review Comment 1 of Charge B1).

Comment 13: "Derivation of an RfC based on absolute brain weight data without considering the significance of other gestational outcome data (including body-weight data) that would put these data in proper context for risk assessment purposes."

Response: As mentioned above in response to public Comment 9, the absolute brain weights reported by NEDO in a supplementary developmental study are an appropriate basis for a dose-response assessment (also see response to external peer review Comment 1 of Charge B1). Other gestational outcome data, including body weight data, were not provided for the supplementary developmental study. However, body weight data reported for the parent, two-generation study did not indicate a body weight effect in the exposed F1 or F2 generation pups. The commenter argued that relative brain weights are important for neonates. While it would have been helpful to have the body weight information for the neonates from the supplementary study, the two-generation data indicate that methanol does not significantly impact pup body weight at the exposure levels of concern. Further, because brain weights are conserved in both neonates and adults, a dose-related reduction in absolute brain weight is an important consideration for both neonates and adults.

Comment 14: "Lack of proper consideration of species differences in sensitivity to developmental toxicity due to methanol exposures in the RfC derivation."

Response: The commenter cites differences in breathing rates, minute volumes and metabolism (i.e., the preference for metabolism via catalase over ADH that is unique to rodents) as factors that are not properly considered. The first two factors are accounted for by the Agency's rat and human PBPK models. The latter factor is considered extensively in the toxicological review (e.g., Section 5.3.5) and is discussed above in response to external peer review Comment 1 of Charge A4, Comment 1 of Charge D2, and public Comment 7. There is currently not enough known about methanol's teratological mode of action to conclude that rodent developmental studies are not relevant to humans.

Comment 15: "Failure of EPA to consider more robust developmental toxicity data in derivation of an RfC value." Specifically, the commenter suggests that the Rogers et al. (1993b) study would be the more appropriate study on which to base an assessment of the developmental toxicity of methanol.

Response: EPA agrees that the Rogers et al. (1993b) study is an appropriate study, and the final RfC and RfD are derived from quantitative analyses of the Rogers et al. (1993b) and NEDO (1987) studies.

Comment 16: "Sweeting et al. demonstrated a large difference in developmental toxicity between mice and rabbits; minor differences in number of stillbirths or postpartum mortality do not equal developmental effects and the EPA's reliance on them is not appropriate."

Response: EPA is not relying on the Sweeting et al. (2011) study results as evidence of teratogenic effects in rabbits, but simply points out that their claim that rabbits are resistant relative to mice to the teratogenic effects of methanol needs to be verified over several gestational days, as has been done for mice, because the critical gestational window for developmental effects could be different for rabbits versus mice. Under different study conditions, the observed increase in postpartum lethality (11% versus 5% in controls) and stillbirths (4% versus 0% in controls) may prove significant given that postpartum lethality ("wasting syndrome") and a shortened gestational period were possible adverse outcomes observed in methanol exposed monkeys (see discussion of Burbacher et al., (2004b; 1999b) in Section 4.3.2).

Comment 17: "The draft assessment states that Sweeting et al. (2011) suggests that low ADH activity in mouse embryos could lead to a '*greater depletion of catalase*.' The assessment further states (line 7,8) 'If ROS accumulation due to this *catalase consumption*…' Sweeting et al. do not postulate a depletion or consumption of catalase."

Response: The text in the final assessment has been clarified.

Comment 18: "The [draft] IRIS Assessment should note here [page 9, paragraph1] that Sweeting et al. postulated that methanol and/or its metabolites may enhance the embryonic production of ROS (by mechanisms that do not involve catalase)."

Response: The text in the final assessment has been clarified.

Comment 19: "The use of the citation from the Tran et al. study to suggest that embryos are in danger of development effects of methanol draws overly broad conclusions from a very limited study."

Response: EPA is not making a broad conclusion regarding the danger of methanol to human fetuses based on the Tran et al. (2007) study. The Tran et al. (2007) study lends uncertainty to the hypothesis presented by others, including Sweeting et al. (2011), that developmental studies in mice are not relevant to humans because human infants do not rely on

catalase to metabolize methanol as do mice. The Tran et al. (2007) study provides limited evidence that catalase may play a role in the metabolism of alcohols in neonates.

Comment 20: "The embryo culture model [used in the Miller and Wells (2011) study] removes the confounding effects of maternal catalase activity, and specifically the maternal peroxidative activity of catalase responsible for metabolizing methanol."

Response: This is offered by the commenter as an explanation for why the in-vivo studies of Siu et al. (2013) did not observe the enhanced embryopathies in aCat (catalase-deficient) mice that were reported in the in-vitro studies of Miller and Wells (2011). As discussed in Section 5.3.5, Miller and Wells (2011) acknowledge that aCat mice in the in-vivo study of Siu et al. (2013) "appeared resistant to methanol teratogenicity." However, they suggest that the in-vivo results were confounded by "maternal factors, including the metabolism of methanol and its formic acid metabolite by maternal catalase (Dorman et al., 1995)," which would presumably reduce the methanol body burden to levels that do not competitively inhibit embryonic catalase antioxidant activity. Alternatively, maternal factors could be protecting the embryo from a more direct interaction with methanol, the compound which this assessment assumes to be the toxic agent.

A.2.2. May 3, 2013 to June 17, 2013 Public Comment Period

The following public comments and EPA responses pertain to comments received from seven individuals/organizations on the 2013 draft of the methanol (noncancer) toxicological review during the May 3, 2013 to June 17, 2013 public comment period.

Comment 1: Compliments/Affirmations:

- "We commend EPA for revising the March 2011 draft toxicological review in order to address previous public comments, peer reviewer concerns and to improve the scientific basis for the derivation of reference values."
- "The May 2013 revised draft assessment addresses the significant concerns that we raised during reviews of the initial draft, and does increase the inhalation reference concentration (RfC) and oral reference dose (RfD)."
- "EPA is to be commended for basing its BMD analyses of the Rogers et al. (1993b) cervical rib malformation data on gestation day 6 blood methanol data that were collected in the very same study, rather than on simulated blood methanol levels that were predicted with an EPA mouse PBPK model."

- "EPA is also to be commended for eliminating the mouse PBPK model altogether from its updated assessment."
- "EPA is to be commended for their responsiveness to concerns raised by the external peer review panel and the public regarding the previous external peer review draft (2011)."
- "I would also like to note that I am very pleased that EPA is applying the uncertainty factors to the internal dose prior to using the human PBPK model for conversion of the internal dose to an acceptable external dose (Section 5.1.3.2, pages 5-15- and 5-16)."
- "…the revised draft assessment demonstrates a clear intent to address the significant concerns that were raised during reviews of the initial draft, and does significantly raise the inhalation reference concentration (RfC) and oral reference dose (RfD)."
- "Given the availability (subsequent to the original draft review) of the toxicokinetic data for S-D rats which are the strain used in the critical experiments for the RfC, it makes sense to use these data as the basis for rat PBPK modeling, and apparently this has been successful in that blood levels reported in the NEDO experiments are matched by the model."
- "It appears to me that it is perfectly reasonable to apply the dose metric conversion after the UFs as has been done in the revised toxicity review. This ensures that the dose metric conversion is made at the actual concentration of interest, i.e. the RfC/RfD. This would also usually be done for a cancer risk assessment, where the dose metric conversion would be done at an observed or estimated concentration or a risk specific level."
- "The change in critical study for the RfD to the Rogers et al. (1993b) is a natural response to the change in relative sensitivities of this and the NEDO studies based on the revised toxicokinetic model. This study is of adequate quality and is reported in detail. Although the data used are from the inhalation study (the oral experiment being much more limited) it has the advantage of having actual blood level measurements which are useable as the internal dose metric, reducing the uncertainties associated with application of the animal PBPK model (although the human PBPK model is still needed to convert to an external oral dose metric for the RfD.)"

- "The Executive Summary is thorough but readable and lays out the basis of the RfC and RfD derivations clearly."
- "The Exposure Response Arrays clearly lay out the comparison between available endpoints which was the basis for selection of the critical studies."
- "The increased use of tabular presentations for study data and comparisons is helpful, generally making the narrative clearer as well as more compact."
- "The move of model details etc. to appendices is also an improvement."
- "The additional materials identified at earlier stages of the review and comment process have been included and incorporated into the report satisfactorily. The most important change obviously is the incorporation of the S-D rat toxicokinetic data and the resulting recalibration of the PBPK model."
- "This additional discussion [of the relation of RfD and RfC to existing endogenous blood levels] addresses the questions raised earlier in the review process and is helpful in clarifying the situation. It is important to note that even without this clarification the RfC and RfD proposals are reasonable."
- "It isn't necessarily incumbent on EPA to show that the predicted exposure increases are 'distinguishable from endogenous background' if a clear hazard is identified at the BMDL exposure level. However, the ability to do so as shown here certainly adds confidence and provides an answer to critics who are naturally disposed to weaken the health protective standards if they can."
- "…the application of the S-D rat toxicokinetic data, which has had the effect of significantly raising the values of both the RfC and RfD and improving the confidence in these values, has significantly eased the task [of demonstrating that RfD and RfC exposures are "distinguishable" from background] by increasing the gap between the endogenous background and the dose-related level at the RfC/RfD."
- "The selection of 1 sd as the BMR [for NEDO brain weight data] is justified in the review since this gives the lower value for the BMDL. However, it is additionally justified as it is based on a generally accepted statistical criterion of what constitutes a clearly observable change in a toxicologically significant parameter."
- "For the Rogers et al. cervical rib incidence data a BMR or 5% is selected and justified based on established U.S. EPA guidance for dichotomous responses in nested-design developmental studies."

- "Many of the comments raised in the previous round of peer review have been addressed as discussed above, or incidentally as a result of the revised basis of the rat PBPK model and selection of Rogers et al. (1993b) as the critical study for the RfD. Other points raised in the earlier peer review discussion appear to have been responded to thoroughly and appropriately."

Response: EPA appreciates these comments and will continue to work towards developing assessments that are responsive to peer reviewer and public comments.

Comment 2: One commenter noted that "EPA's revised draft assessment provides a brief discussion of some potential exposure pathways (e.g., foodstuff or commercial products) but it does not provide information on the specific levels of methanol that humans are likely to be exposed to on a daily basis" and that "[t]he United Kingdom (U.K.) Food Standards Agency has stated that endogenous methanol production ranges from 300 to 600 mg/day and that up to 1,000 mg/day methanol can be consumed in food, particularly fruit and vegetables."

Response: EPA has added the U.K. estimates of endogenous methanol production and consumption in food, particularly fruits and vegetables, to Section 2 of the assessment. Also, discussion of how these estimates compare to EPA's estimate of background blood levels has been added to section 5.3.6. The U.K. report referred to by the commenter (COT, 2011) is now used to support the EPA's estimate of the upper end of the range of methanol blood levels associated with a diet that includes fruits and vegetables.

Comment 3: One commenter noted that "[t]he [ten] studies used to derive the estimated endogenous background methanol blood level involved fasting and or some forms of dietary restrictions." This commenter stated that one of these studies, Woo et al. (2005), involved "… no food intake from the time subjects [18 males] went to sleep the previous night until after the 8:00 a.m. blood sampling" and therefore "… provides data on background blood methanol that may be more representative of endogenous levels with little or no contribution from foods." This commenter further suggested that the RfD would not be toxicologically relevant had EPA used the Woo et al. (2005) study to estimate endogenous methanol blood levels because "the mean incremental blood level of 0.41 mg/L [associated with the RfD] is well within the background level of variation" of 2.62 ± 1.33 mg/L reported by Woo et al. (2005).

Response: In the revised methanol (noncancer) assessment, EPA has clarified that the methanol (noncancer) Toxicological Review "…provides scientific support and rationale for a hazard identification and dose-response assessment of the noncancer effects associated with chronic exposures to exogenous sources of methanol that add to background levels of methanol derived from a diet that includes fruits and vegetables (see further discussion in Section 5.3.6).

Thus, studies that substantially restricted the consumption of fruits and vegetables (Ernstgàrd et al., 2005; Osterloh et al., 1996; Cook et al., 1991; Davoli et al., 1986) are considered inappropriate under this definition. The remaining studies, Batterman and Franzblau (1997), Batterman et al. (1998), Lee et al. (1992), Sarkola and Eriksson (2001), Turner et al.(2006) and Woo et al. (2005), are considered appropriate for the purpose of this analysis as they did not involve substantial fasting (i.e., only two involved fasting, one overnight and one for 4 hours) or dietary restrictions (i.e., only one involved a minimal dietary restriction, no aspartame-containing cereals and no juice on the morning of the test). The analysis of these six studies (see details in Section 5.3.6), after weighting them in accordance with the extent to which they represent the U.S. population (see footnote 61), yields an estimate for the mean and SD for endogenous background methanol blood of 1.3 mg/L and 0.8 mg/L, respectively. As discussed in Section 5.3.6 and in response to Bonus Charge Follow-up Peer Review Comment 1, these estimates are consistent with endogenous methanol production plus dietary exposure ranges reported by the U.K. Food Standards Agency (COT, 2011).

The Woo et al. (2005) alone is not considered an appropriate basis for the estimation of a sample background methanol blood distribution that would be representative of the general U.S. population. The Woo et al. (2005) study subjects were all male with a mean age 23.7 years (range 20 –29 years), the sample size of 18 is small and likely biased somewhat towards subjects who regularly consume alcohol; while the subjects scored low on an alcoholism screening test, they agreed to self-induce an "alcohol hangover state" and all 18 participants "had experienced hangover at least once." Further, since the study was performed in Korea, the subjects are presumed to be Korean, a population prone to having more than one variant of the genes coding for alcohol and aldehyde dehydrogenase (Eng et al., 2007). This suggests marked differences in their alcohol metabolism relative to other ethnicities (see discussion in Section 3.3).

Comment 4: One commenter stated that "EPA's approach to endogenous methanol may set a dangerous precedent" because "…[t]oxic levels of methanol are proposed [by EPA] to be those which fall outside 1 standard deviation [associated with a RfD + RfC exposure] of the population mean of endogenous methanol." Another commenter stated that "…well over one-fifth of the population will have a background level of methanol (without exposure to external methanol) that is above the level deemed safe by EPA (1.5 mg/L average background + 0.4 mg/L RfD exposure) and asked "…how does EPA distinguish between endogenous and exogenous methanol" and "[h]ow can one be "safe" and smaller levels of the other be "unsafe"?"

Response: To address these comments it is helpful to reiterate the definition of an RfD. As stated in the introduction to the methanol (noncancer) toxicological review, "[t]he RfD (expressed in units of milligrams per kilogram per day [mg/kg-day]) is defined as an estimate

(with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime." Thus, the addition of the RfD to the mean of EPA's estimated sample distribution is not meaningful and the RfD is not an estimate of the level above which all are effected. The RfD is the level at or below which there is likely to be no appreciable risk, even to sensitive subgroups. In the case of methanol, sensitive subgroups would include pregnant females (toxicodynamic susceptibility) with relatively high endogenous methanol blood levels (toxicokinetic susceptibility). Thus, it is more appropriate to consider the impact of the RfD on sensitive individuals with methanol blood levels at the high end of the range of background blood levels of methanol associated with a diet that includes fruits and vegetables. As described in Section 5.3.6, EPA has defined the upper end of this background methanol blood levels as 2.5 mg/L. In response to the last question in this comment, smaller exogenous levels can be "unsafe" when they are added to a subgroup's background blood methanol that are already susceptible from a toxicodynamic (e.g., pregnancy) and/or toxicokinetic (e.g., high endogenous methanol blood levels) perspective. This is because, as indicated in the Executive Summary and other sections of the assessment, the RfD is intended to protect sensitive subgroups and the combination of endogenous background levels plus exogenous exposure can lead to toxicity.

Figure 5-4 illustrates how methanol blood level distributions for RfD and RfC exposures to the EPA sample background distribution compares with the blood levels that have been associated with these uncertain, but potentially adverse effects in monkeys. As discussed in the previous section, a RfC or RfD exposure is expected to raise the methanol blood level of an individual with a high end background methanol blood level of 2.5 mg/L to just under 3 mg/L, the lowest methanol blood level that has been associated with these uncertain, but potentially adverse effects.

Comment 5: One commenter noted that "It is quite possible that levels of exposure to some chemicals at background levels are in fact hazardous to human health, and if the technology exists now or at some time in the future to control that exposure, it is incumbent on the Agency to inform the public of the potential risk of these background exposures." This commenter further stated that "In many cases, the question also arises, if the general public is being exposed to this hazardous substance on a routine basis because of background levels, whether we are seeing any adverse health effects resulting from this exposure." Another commenter stated that "…it cannot be assumed automatically that any reported endogenous level is safe…[T]here are a number of examples in the toxicological literature where some individuals, as a result of idiosyncrasies of metabolism, diet, exposure etc., show actual toxicity or at least

substantially increased susceptibility from 'background' exposures." This commenter added that "[t]hese considerations are adequately addressed in the revised toxicity review."

Response: EPA agrees that it is possible that background levels of methanol are hazardous to human health. In the revised assessment, EPA does not state that background levels are without health risk, but states that the "Toxicological Review provides scientific support and rationale for a hazard identification and dose-response assessment of the noncancer effects associated with chronic exposures to exogenous sources of methanol that add to background levels of methanol derived from a diet that includes fruits and vegetables (see further discussion in Section 5.3.6)." In Section 5.3.6 EPA discusses the relationship of the RfD and RfC to background blood levels and derives a sample background distribution of methanol blood levels. EPA sample background distribution and the U.K. (COT, 2011) background methanol estimates are consistent with one another. The upper bound of the combined endogenous and dietary exposures estimated in the U.K. is 23 mg/kg-day. The methanol blood level predicted by EPA's PBPK model for this 23 mg/kg-day maximum exposure rate of 2.5 mg/L is slightly below EPA's sample background distribution estimated mean + 2xSD of 2.9 mg/L. A small percentage (~7%) of the EPA sample background population is predicted to have methanol blood levels above 2.5 mg/L.

Comment 6: One commenter stated that "EPA has indicated that it believes these increases [from a RfC, RfD or RfC + RfD exposure] would be distinguishable from background, but we fail to see how this is possible given that the combined RfC and RfD methanol values are well within the range of background blood methanol levels." Another commenter stated that "[t]his additional discussion [of the relation of RfD and RfC to existing endogenous blood levels] addresses the questions raised earlier in the review process and is helpful in clarifying the situation." This commenter also stated that "[i]t isn't necessarily incumbent on EPA to show that the predicted exposure increases are 'distinguishable from endogenous background' if a clear hazard is identified at the BMDL exposure level."

Response: As indicated in response to the Bonus Charge Follow-up Peer Review Comments and Comment 4 above, the primary consideration in deriving the methanol RfD and RfC is whether they represent daily exogenous exposures that, when added to background levels of methanol associated with a diet that includes fruits and vegetables (resulting in methanol blood levels of up to 2.5 mg/L; see discussion in Section 5.3.6), are not likely to result in an appreciable health risk, even to sensitive subgroups. Consistent with the view of the second commenter above, a determination of whether the daily exogenous exposures at a RfD or RfC are "distinguishable" relative to background methanol blood levels is not a primary consideration for an IRIS risk assessment. However, reviewers of the 2011 draft methanol (noncancer)

toxicological review (U.S. EPA, 2011a, c) asked EPA to investigate this topic and EPA responded by adding Section 5.3.6 to the assessment. In Section 5.3.6, EPA provides an example which illustrates that an RfC or RfD exposure is estimated to increase the percentage of individuals with peak methanol blood levels at or above 2.5 mg/L from ~7% to ~14%. These estimates are not precise and do not account for interindividual variability. However, they suggest that the increase in individuals with higher than 2.5 mg/L methanol blood levels (i.e., higher than the upper range of background methanol blood levels associated with a diet that includes fruits and vegetables) following a RfD or RfC exposure would not be negligible.

Comment 7: One commenter stated that "…EPA has not provided a discussion of the threshold blood methanol level for adverse effects" and noted that "…in 2003 the National Toxicology Program (NTP) issued a monograph which reviewed the potential human reproductive and developmental effects of methanol and found minimal concern that adverse health effects would be associated with <10 mg/L blood methanol concentrations." This commenter suggested that EPA's stated uncertainty with respect to NTP's assumption, "particularly whether rodents are as sensitive as monkeys and humans to the reproductive and developmental effects of methanol exposure…should be further elucidated as well as the threshold blood methanol levels necessary to illicit adverse effects."

Another commenter stated that "EPA's revised draft assessment does not appear to adequately address the plausible adverse health risks associated with levels above background exposures." More specifically, the commenter contends that "[t]he Agency has failed to show how this "measurable" variation from external exposure to [a RfD and/or RfC of] methanol increases any health hazard" and that "…the real question that still needs to be asked which is 'where's the risk?'"

Response: As was discussed in response to the Bonus Charge Follow-up Peer Review and 2013 Public Comments 4 and 6 above, the primary consideration in deriving the methanol RfD and RfC is whether they represent daily exogenous exposures that, when added to background levels of methanol associated with a diet that includes fruits and vegetables (as defined in Section 5.3.6), are not likely to result in an appreciable health risk, even to sensitive subgroups. The RfD and RfC are not estimates of exposures that are health hazards, and the risk associated with exposures above the RfD will vary from individual to individual, with the greatest risk experienced by sensitive subgroups. Nevertheless, the revised assessment includes an expanded discussion of the relationship of the RfD and RfC to methanol blood levels that have been associated with effects in monkeys and humans. This discussion has been moved to a new Section 5.3.7 titled "The Relationship of the RfC and RfD to Methanol Blood Levels In Monkeys Associated with Unquantifiable Effects of Uncertain Adversity." Section 5.3.7

discusses the reasons EPA believes that blood levels of methanol below 10 mg/L, but above 3 mg/L, could pose an uncertain, but potential health risk. EPA's conclusion differs from the NTP-CERHR (2003) conclusion partly because of an evaluation of the methanol blood levels corresponding to effects observed in the Burbacher et al. (2004b; 1999b) reproductive and developmental monkey study using the EPA monkey PK model, and partly because the NTP-CERHR (2003) report focused on the reproductive and developmental effects of methanol, and did not assess the potential for effects from chronic exposure. As discussed in Section 5.3.6, a RfC or RfD exposure is not expected to raise the methanol blood level of an individual with a high end background methanol blood level of 2.5 mg/L to more than 3 mg/L, the lowest methanol blood level that has been associated with uncertain, but potentially adverse effects in monkeys.

Comment 8: Concerning all IRIS chemicals, one commenter stated that "…to date, EPA has not indicated which substances, under review by the IRIS program, will benefit from implementation of the NRC recommendations…[n]or has EPA provided an updated timeline of when it anticipates having each phase completed and fully implemented." The commenter suggests that "EPA should provide this information as soon as possible and expeditiously move forward with fully implementing all of the NRC's recommendations, regardless of the phase" that has been assigned by EPA to each assessment for progressive implementation of the NRC recommendations. Concerning the methanol (noncancer) assessment, the commenter noted that "[t]he revised draft assessment only provides a general overview of the process utilized in EPA's literature search strategy" and suggests that "[i]t would have been more useful for EPA to have included the search terms it used to select appropriate studies for inclusion in the literature search as well as listing the specific inclusion/exclusion criteria." The commenter further suggested that "EPA should be consistent and clear in identifying which elements it will focus on with regard to 'partial implementation' [of the NRC recommendations]" and noted that "…the necessary, major substantive changes that are needed, such as more fully considering an integrated weight of evidence approach, that includes mode of action, as part of IRIS, remain largely unaddressed."

Response: In April 2011, the National Research Council (NRC), in their report Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde (NRC, 2011), made several recommendations to EPA for improving IRIS assessments and the IRIS Program. The NRC's recommendations were focused on Step 1 of the IRIS process, the development of draft assessments. All IRIS assessments currently under review by the IRIS Program will benefit from implementation of the NRC recommendations, and consistent with the advice of the NRC, the IRIS Program is implementing these recommendations using a phased approach and is making the most extensive changes to assessments that are in the earlier stages

of the IRIS process. The methanol (noncancer) assessment is in Phase 1 of implementation of the NRC recommendations. Phase 1 focuses on a subset of the shorter-term recommendations for assessments near the end of the document development process or close to final posting. Consistent with the focus of this phase of implementation of NRC recommendations, the Toxicological Review was edited to be more concise, the rationales for decisions have been made more transparent, and the description of the literature search strategy has been augmented for clarity. Additionally, consistent with direction provided by Congress in The Consolidated Appropriations Act of 2012, the Agency will include documentation in the final methanol (noncancer) IRIS assessment describing how the recommendations of the NRC have been implemented or addressed in the methanol (noncancer) assessment, including an explanation for why certain recommendations were not incorporated.

Comment 9: One commenter noted that "…there does not appear to be a full discussion in the document regarding what study quality criteria were applied to each study in determining its 'acceptable quality.'"

Response: As indicated in Appendix E, because the methanol (noncancer) toxicological review is near the end of the development process and close to final posting, it is a post-peer review, Phase 1 assessment. This means the methanol (noncancer) assessment focuses on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data in assessments. Literature search and study evaluation processes were not substantially revised.

Comment 10: One commenter stated that, for a dose-response analysis of the Rogers et al. (1993b) mouse study, "…average values [see Table 1 in Starr and Festa (2003)] are expected to provide a more accurate and precise characterization of mean blood methanol levels throughout the temporal window of vulnerability for mice from gestation day 6 to 15." The commenter noted that "[t]here were no temporal trends in these data, and EPA has not provided any justification for its selection only of the data from day 6, which were collected from just 3 mice per treatment group." The commenter suggested that "[a]t a minimum, EPA should evaluate what difference it makes in the final results of their BMD computations to employ the full blood methanol data from Rogers et al. (1993b) as opposed to just the data from day 6."

Response: In response to the 2011 peer review comments to streamline the assessment, EPA moved some of the PBPK and dose-response modeling details to Appendices B and D. EPA's rationale for using GD6 blood levels is described in Appendix D, Section D.3. Subsequent to the Rogers et al. (1993b) study, their laboratory narrowed the temporal window of vulnerability for developmental effects in mice to GDs 6 and 7 (Rogers and Mole, 1997; Rogers

et al., 1993a). Therefore the scientific justification for not using measurements of blood concentrations later in gestation is that they are not directly relevant to the endpoint being evaluated. However, EPA agrees that, given that methanol inhalation dosimetry appears to be not significantly affected by the stage of pregnancy, data from the later gestation days could be viewed simply as additional measurements in female CD1 mice. Therefore the BMD modeling results of using weighted concentration averages for all three gestation days measured were compared with EPA's primary approach (using only the GD6 data). The results are not substantially different, and the model fits were not as good as the model fits to the data using the GD6 blood levels. Thus, EPA has decided that the use of the GD6 data as the dose metric is appropriate for this analysis. Text has been added to Section 5.1.2.3 to describe this analysis of the dose metric options.

Comment 11: One commenter noted that "…in Table B-1, only central estimates of these two parameters [V_{maxC} and K_m] are presented for both the rat and human PBPK models."

Response: While the software used for PBPK modeling, acslX, nominally reports measures of certainty in the estimated parameters, these estimates are based on a statistical model which assumes that each data point is an independent measurement from a separate experiment. The model does not account for data in the form of group means (and SDs), nor for repeated measurement sampling as occurs with urine collection over time or repeated blood samples from the same individual. While one could potentially build the correct statistical model as a computational script, that would require the individual subject data rather than summary statistics, and those data are not available for some, likely most of the studies used for parameter estimation. Thus the measures of parameter variance reported by acslX are incorrect and the data needed to correctly estimate the variance are not available. The EPA believes it is preferable to simply not report measures of variance, rather than providing incorrect values.

Comment 12: One commenter stated that "…there is little, if any, data showing that the human metabolism of methanol begins to saturate at blood methanol levels as low as 36 mg/L," that "EPA should make a concerted effort to quantify the uncertainty inherent in its estimates of the human V_{max} and K_m parameters, including the strong positive covariance that is expected to occur between these two parameter estimates" and that "EPA should also implement a purely linear form of their human PBPK model to determine what differences in final RfC values could arise as a consequence of the large degree of residual uncertainty that remains regarding exactly where on the blood methanol scale human metabolism actually does begin to saturate."

Response: It should first be noted that the values of K_m reported previously for monkeys and one human subject by Perkins et al. (1995) were estimated using a classical (non-physiological) model. For one of the studies analyzed by Perkins et al. (1995), Makar et al.

(1968), blood concentrations in the monkey were not measured but were estimated from the total dose and clearance was estimated based on exhaled CO_2 and urinary excretion. For all monkey studies analyzed the initial doses ranged from 1-6 g/kg and for the human subject the initial blood level was estimated to be 1.4 g/L. None of the blood data used by Perkins et al. (1995) to estimate K_m values fell below ~100 mg/L. Thus none of those other data sets were particularly suited to identifying a potential K_m below 100 mg/L, even if that exists. By contrast, the analysis of the much lower, more relevant monkey exposure data from Burbacher et al. (2004b; 2004a) shown in B.3 yielded an estimated K_m of 14.4 mg/L, a value which none of the commenters has called into question. (This result was also obtained with a classical PK model structure.) Therefore, EPA concluded that the prior K_m results from a single published analysis show that the value obtained for humans is likely incorrect. The model as parameterized is clearly consistent with (fits well) the existing human data. The difference between the previously reported values for monkeys and EPA's value for humans is explained by the use of a different data set which covers a much lower range of internal concentrations, in humans.

Comment 13: One commenter noted that EPA's human PBPK model is "seriously flawed" because **"…**predicted human levels are actually markedly larger than the corresponding mouse levels at the effect levels in the Rogers et al. (1993b) study" and "…[f]or many years, the conventional toxicological wisdom regarding interspecies differences in methanol toxicity has been that the human is the least sensitive of the three species (human, rat, mouse), which is followed by the rat, and finally by the mouse, which is consistently the most sensitive, and this hypersensitivity of the mouse is due at least in part to the fact that the mouse's metabolism of methanol saturates at far lower blood methanol levels than do either the rat or human metabolism of methanol."

Response: First, as discussed in response to Comment 7 above and in more detail in Sections 5.1.3.2.3 and 5.3.7 of the methanol (noncancer) toxicological review, there is considerable uncertainty regarding the potential adversity of low level exposures in humans (e.g., resulting in blood concentrations ~10 mg/L). Second, the EPA notes that the "conventional toxicological wisdom" which the commenter states exists for a greater sensitivity to the toxicological effects of methanol of rodents versus human and non-human primates is a hypothesis (i.e., lacks supporting data). While it may have been believed that humans are less sensitive than mice to high exposure levels for certain developmental effects, there are no dose-response data in humans or primates to support this assumption. In fact, there is evidence to indicate the opposite relationship for effects associated with acute (e.g., ocular) and chronic (e.g., CNS) methanol exposures. Third, sensitivity is the result of both pharmacokinetic and pharmacodynamic factors. Hence it is possible that humans experience higher internal doses of

methanol but are less sensitive due to differences in pharmacodynamics. Fourth, the commenter's statement about sensitivity assumes that it is parent methanol that is responsible for methanol's developmental effects. While the EPA has chosen to operate under that assumption for the purpose of this assessment, given the lack of MOA information, if in fact the toxicity is due to methanol metabolites then a low K_m in humans would in fact be consistent with lower sensitivity to high exposure levels. Hence the estimation of K_m = 36 mg/L by the EPA is not inconsistent with that assumption for other dose metrics that might be considered, so it is effectively neutral relative to that assumption.

The EPA's analysis of the human data does indicate that these data do not clearly establish a concentration level at which human metabolism saturates, as reflected by the caveats placed on use of the human model. But the EPA also does not believe that "the fact that the mouse's metabolism of methanol saturates at far lower blood methanol levels than … human metabolism of methanol," is supported by the existing data. The exact value of the K_m in humans is uncertain but the results of EPA's modeling clearly show that the human data are consistent with K_m = 36 mg/L.

Comment 14: Two commenters stated that the lack of usable primate studies has been cited as the justification for retaining a database UF of 3 and an interspecies UF of 10, "thereby using the same source of uncertainty twice" or "double-counting." One of these two commenters also stated that "…the lack of a developmental neurotoxicity study should not trigger a database UF of 3" because "[i]t is unlikely that neurotoxic effects would be seen in a developmental neurotoxicity study at exposures lower than those that caused reduced brain weights in a reproduction study." Another of these two commenters stated that "The UF for database gaps, even though set at 3 rather than 10 is not needed based upon the available data and the choice of the most conservative decision at several points is the derivation of the reference values (See Comment 3 to Charge Question B.4, P. A-26)." Another commenter stated that "the continued use of a database UF of 3 is highly questionable given the well-recognized large data set that already exists for methanol. This commenter noted that "…EPA was able to use not one but two studies with two different toxicological endpoints as a basis for developing a common RfC" and asks "…how much data are enough?" Another commenter stated they were "…not convinced that both a UFD of 3 and UFH of 10 are necessary." Another commenter noted that "…the wide variability of 'endogenous' levels in humans, and the uncertainty in the primate sensitivity data definitely leave grounds for concern. This certainly justifies the inclusion of an additional database uncertainty factor."

Response: The inter-species (animal-human) uncertainty factor, UF_A was not set to 10, but was set to 3, the default value for pharmacodynamic differences between animals and

humans (i.e., allowing that humans may be more sensitive to a given internal dose). The UF_A is 3 instead of 10 because the PBPK model is used to capture inter-species differences in pharmacokinetics. As discussed in response to Charge B4 Follow-up Peer Review Comments 2 and 3, in the revised Section 5.1.3.2.3, EPA has clarified that the UF_D is based on deficiencies in the methanol toxicological database, particularly with respect to the interpretation of the importance and relevance reproductive, developmental neurotoxicity and chronic CNS effects observed in monkeys. Thus, the UF_D does not have the same basis as the UF_A, and was not "double counted."

With respect to the need for an additional developmental neurotoxicity (DNT) study, EPA (2002) guidance places particular emphasis in this regard on database deficiencies in the area of developmental toxicity, the primary focus of the methanol (noncancer) assessment, stating that "[i]f data from the available toxicology studies raise suspicions of developmental toxicity and signal the need for developmental data on specific organ systems (e.g., detailed nervous system, immune system, carcinogenesis, or endocrine system), then the database factor should take into account whether or not these data are available and used in the assessment and their potential to affect the POD for the particular duration RfD or RfC under development." EPA believes that, with respect to the methanol database, the available toxicology studies in monkeys "raise suspicions of developmental toxicity and signal the need for developmental data," particularly with respect to DNT. Table 5-5 of Section 5.1.3.2.3 indicates that methanol blood levels associated with DNT effects are a 12-fold higher in rodents versus primates. Some of this dissimilarity may be due to differences in species sensitivity, for which the UF_A of 3-fold is intended to account, but some of the difference may be due to other factors, including whether appropriate and comparable endpoints were examined and whether appropriate study designs and quality control measures were used. To account for these additional uncertainties, a 3-fold UF_D is applied.

With regard to the sufficiency of the existing database, EPA has clarified in the revised Section 5.1.3.2.3 that while the database for methanol toxicity is extensive in terms of the laboratory species and study design coverage, consisting of chronic and developmental toxicity studies in rats, mice, and monkeys, a two-generation reproductive toxicity study in rats, and neurotoxicity and immunotoxicity studies, it leaves considerable uncertainty with respect to the importance and relevance of reproductive, developmental and chronic effects observed in monkeys.

EPA's response to Comment 15 below addresses the general concern regarding EPA's choice of "…the most conservative decision at several points." It should be noted here, however, that in consideration of the credibility of the available scientific information, EPA did not derive

lower RfD and RfC values using the monkey dose-response data. For instance, as can be seen from Table 5-5, blood levels associated with DNT effects in monkeys were 12-fold lower than blood levels that caused DNT in rats. Thus, a POD derived from the developmental monkey study would have likely been substantially lower than the POD derived from the rat developmental study. Thus, the UF_D is not an additional "conservative decision" but is intended to account for the possibility that deficiencies in the methanol database are causing EPA to derive a RfD/RfC that may not be as health protective as required (i.e., by using the rodent studies instead of the monkey studies). As stated in response to Charge B4 Follow-up Peer Review Comment 2 above, this use of the UF_D is consistent with EPA (2002) guidelines which state that "[t]he database UF is intended to account for the potential for deriving an under protective RfD/RfC as a result of an incomplete characterization of the chemical's toxicity."

EPA agrees with the commenter that stated that "…the wide variability of 'endogenous' levels in humans, and the uncertainty in the primate sensitivity data definitely leave grounds for concern." As stated above, the UF_D accounts for deficiencies in the database that limit the Agency's ability to interpret the overall findings, particularly the primate studies, and derive sufficiently protective RfD and RfC values.

Comment 15: One commenter stated that "…EPA has unnecessarily relied upon automatic use of the choice that will result in the lowest reference value that is scientifically defensible." Another commenter recommended that "…whenever the Agency calculates a reference concentration below background, it should cause the Agency to pause and ask whether the proposed reference concentration calculation is being driven by the best available science or by assumptions about uncertainty and by the choice of a particular model that may be too conservative in this particular case." This commenter noted that "[a]lthough EPA has developed and published methodology for performing Benchmark Dose analysis (BMD), the choice of which of the ten or so models to use [for derivation of the RfC POD] is not based on any understanding of mode of action" but "…is the one that gives the lowest BMD value without indication of bad fit to the data. According to this commenter, "[t]he Agency ignored models that resulted in a 10-fold higher BMD, including the linear model" but "…in other venues, EPA has been asserting that all toxicological risk is linear with dose." This commenter further stated that "[t]he lower the set values, the greater the cost of regulations and clean-ups for manufacturers and consumers, but the benefit to human health must still be determined" and "[t]hus, RfC and RfD values ought to be set at the highest reasonable health-protective values, not the lowest "justifiable" or "measureable" values, and ought to be driven by science, not by arbitrary Agency-made rules."

Response: Consistent with EPA guidelines for the development of RfDs and RfCs (U.S. EPA, 2002, 1994), use of the best available, sound science has been a key focus of the methanol assessment. The credibility of the available science was an important consideration at every step of the RfD/RfC derivation process. EPA's commitment to use the best scientific and most credible toxicological approach available resulted in several choices that do not represent "the lowest reference value that is scientifically defensible," including the use of:

- *rodent studies* instead of the more uncertain, but potentially more sensitive monkey studies,
- *developmental endpoints* instead of more sensitive endpoints of uncertain adversity such as the "reduction in the size of thyroid follicles" and "transient reduction in plasma testosterone levels" endpoints illustrated in Figure 4-2,
- *PBPK modeling to derive the HEC* in lieu of the 3-fold toxicokinetic portion of the UF_A, which would have resulted in a 10–fold lower RfC (BMDL from NEDO rat developmental study of 670 $mg/m^3 \div 300 \cong 2$ mg/m^3 versus 2×10^1 mg/m^3)
- *PBPK modeling to derive the HED* from the Rogers et al. (1993b) inhalation study in lieu of using the oral subchronic study (TRL, 1986) that was used to derive the old, 4-fold lower RfD of 0.5 mg/kg-day (see Section 5.2.3),
- *BMD modeling* in lieu of a NOAEL approach which would have resulted in a 2-fold reduction in the RfC from 2×10^1 mg/m^3 to 1×10^1 mg/m^3 (using a POD of 547 mg-hr/L AUC at the 500 ppm NOAEL of the NEDO (1987) rat developmental study [see Appendix D, Table D-1] instead of the 858 mg-hr/L BMDL [see Table B-4]);
- *BMDL/UFs for HEC and HED derivations (i.e., applying UFs to internal dose BMDLs)* instead of using the BMDLs directly for the HEC and HED derivations, which would have resulted in ~2-fold lower, but less reliable (i.e., less scientifically credible) reference values (see discussion Section 5.1.3.2).

With respect to the suggestion that the Agency has calculated "a reference concentration below background," as described in numerous places in the methanol (noncancer) toxicological review, including the Executive Summary, the RfD and RfC are exposures above background blood levels of methanol associated with a diet that includes fruits and vegetables, which are estimated to be below approximately 2.5 mg/L (see Section 5.3.6). As discussed in the response to the Bonus Charge Follow-up Peer Review Comments and Comments 4 and 6 above, the primary consideration in deriving the methanol RfD and RfC is whether they represent daily

exogenous exposures that, when added to background levels of methanol associated with a diet that includes fruits and vegetables, are not likely to result in an appreciable health risk, even to sensitive subgroups. Consistent with the view of the second commenter above, a determination of whether the daily exogenous exposures at a RfD or RfC are "distinguishable" relative to background methanol blood levels is not a primary consideration for an IRIS risk assessment but has been accounted for in this assessment.

With respect to the Agency's choice of the Hill BMD model for derivation of the RfC POD, the Hill model was the proper choice in accordance with established BMD technical guidance (U.S. EPA, 2012a). However, it also provided a substantially better fit overall (as indicated by a 4-fold higher p-value for model fit to the response means) and in the area of the BMD (as indicated by an 8-fold higher scaled residual for model fit at the dose group closest to the BMD) over other models, including the linear model (See Appendix D, Table D-2). Further, as mentioned in the 5th bullet above, had BMD modeling not been performed the RfC would have been reduced by 2-fold. Finally, it is assumed that the "other venues" the commenter is referring to involve the analysis of dichotomous cancer dose-response data using EPA's multistage cancer model, which is not the same, and bears little relation to, the linear model EPA uses to evaluate continuous noncancer data.

In summary, the methanol (noncancer) reference values that have been established are consistent with EPA guidelines for BMD analysis and the development of RfDs and RfCs (U.S. EPA, 2012a, 2002, 1994) and are supported by the best available, sound science. They represent estimates of exposures over background levels of methanol that are not arbitrary and are not necessarily the lowest "justifiable" or "measureable" values, but are derived by applying scientifically sound methods to the best available science.

Comment 16 – How to take into account endogenous levels of compounds is a key issue that goes beyond methanol: One commenter stated that "[t]he question of how to take into account endogenous levels of compounds for which regulatory exposure values are being developed is an issue that goes far beyond methanol" and "…needs to be discussed in a much larger arena than methanol or any one chemical alone." Another commenter stated that "[b]ecause methanol is not the only substance where there are natural levels of the substance in the human body, absent exogenous exposure, this larger issue of how to conduct hazard assessments of such chemicals needs to be addressed squarely by the Agency, and the methanol non-cancer assessment would be the place to start."

Response: The Agency is considering the cross-cutting issues associated with chemicals for which there are natural, endogenous levels in the human body.

Comment 17 – EPA has not made its PBPK model publically available: One commenter stated that, as of June 12, 2013, they were unable to locate the PBPK model source code files, which EPA states will be "available electronically on the IRIS website [www.epa.gov/iris}" in one place (page A-9) and "will be posted on the EPA IRIS website, along with the final methanol (noncancer) assessment" (Page A-32).

Response: An error was made in the link for the EPA model code on the pages noted by the commenter. The correct link to the EPA HERO database record for the model code is provided here in the following citation, (U.S. EPA, 2012b), and was available in the citation on page B-34 and in the reference section of the 2011 draft Appendices. The correct link is given throughout the Appendices of the current version of the methanol (noncancer) toxicological review.

Comment 18 – EPA implemented nonphysiological urinary clearance in its PBPK model: One commenter stated that they "…find the approach of modeling clearance as occurring from mixed venous blood to be lacking with respect to physiological realism" and that "[t]he more appropriate location for urinary clearance would be from the fraction of arterial blood flow directed to the kidney (Corley RA, Bartels MJ, Carney EW, Weitz KK, Soelberg JJ, Gies RA, Thrall KD. Development of a physiologically based pharmacokinetic model for ethylene glycol and its metabolite, glycolic acid, in rats and humans. Toxicol Sci. 2005 May; 85(1):476-90)." The commenter further state that "…the greatest danger is that such a model structure as EPA used could inadvertently lead to an optimized rate of 'urinary' clearance that exceeds blood flow to the kidney." This commenter notes that "[t]he implications are of lesser concern for methanol, since parent compound concentrations are used in the risk assessment, but could have implications if total metabolism, or levels of a metabolite were a key consideration" and concludes that they "…would not recommend that such a structure be used for other chemicals without better justification than EPA has provided in this document."

Response: As indicated above in response to the Charge A1 Follow-up Peer Review Comments, the commenters are correct in that the lack of an explicit kidney compartment would be a significant factor if total urinary clearance was a significant fraction of renal blood flow, which likely occurs for some other chemicals. However for the methanol model the clearance rate for this pathway in the rat is only 0.24% of renal blood flow [using a renal flow fraction of 0.141 from Brown et al. (1997)] and in the human is only 0.07% of renal blood flow [using a renal flow fraction of 0.175 from Brown et al. (1997)]. Therefore including an explicit kidney compartment with its own flow rate would have a negligible impact on the methanol model results reported here. These calculations and a statement that the approach should only be used

when renal clearance is a small fraction (< 10%) of renal blood flow have been added to appendix B.

Comment 19 – The drinking water scenario used by EPA for derivation of the RfD lacks explanation/justification: One commenter stated the following concerns and suggestions regarding the drinking water scenario that EPA applied to derive the RfD:

- "No references/precedents are cited to justify this scenario."
- "Probabilistic descriptions should be considered."
- "…EPA has not provided any analysis that indicates which of the assumptions embedded in this scenario had an impact on the resulting RfD, and to what degree."
- "My concern is primarily the precedent that may be set (or continued?…) with this assessment;"
- "EPA should better justify this departure from established practice."

Response: The commenter is correct that the drinking water pattern used has not been extensively evaluated, but it was previously used in the posted dichloromethane toxicological review (which underwent extensive peer review) and has been described in the recent peer-reviewed paper by Sasso et al. (2013) for chloroform. While a probabilistic analysis as suggested by the commenter would be ideal, the EPA is not aware of an available published statistical model for water/fluid ingestion in a given day. Variability in total water imbibed from one day to another is likely available, but a description of the detailed drinking pattern within a day (e.g., probabilities of ingestion in a given time increment) would need to be generated, the analysis conducted, and the results subject to peer-review.

As stated in the draft review, the pattern is meant to be representative, rather than exact. Except for individuals receiving medical treatment, an assumption of continuous ingestion over the entire 24 hours of each day, prior standard practice, is clearly unrealistic. At the other extreme, to assume that all water was ingested in a single daily bolus would be equally unrealistic. The proposed scenario is most certainly between those two unrealistic extremes.

A detailed analysis of the uncertainty associated with the assumed pattern could be insightful, but to determine the extent to which the pattern should be varied in such an analysis would require development of the probabilistic model mentioned just above. Future research may provide information to be considered beyond the current assessment. The EPA believes that the assumed pattern is sufficiently more realistic than assuming continuous exposure (the

previous standard approach), that this realism is effectively self-evident, and therefore that it can be used and considered an improvement over prior practice.

Comment 20. One commenter stated that "I am not so content with moving the model source code files to the HERO database since that database is not freely accessible to the public (needing password access and an EPA account)."

Response: The model source code files are available in a publically accessible record of the HERO database via the following citation link, (U.S. EPA, 2012b). This citation here, and elsewhere in the assessment, links directly to the HERO record.

Comment 21: One commenter stated that they "…disagree with the comments made in the Appendix (D26, line12) that 10% is a typically justifiable BMR for non-developmental dichotomous data, and that nested developmental studies are necessarily 'more sensitive' than straightforward non-developmental studies." This commenter noted that "[t]he reason for the nested design in developmental studies is to accommodate the additional complicating litter effects in these data: it allows the analysis to deal with additional variability and bias in the data (which do not exist in the non-developmental data) rather than providing more accuracy or sensitivity." This commenter also noted that "[i]n order to retain comparability with existing assessments, and to retain compatibility with the guidance on UFs, it is necessary to have a BMDL which is at least in general properties similar to a NOAEL, and practical experience has shown that in fact the $BMDL_{05}$ best meets this criterion for dichotomous general toxicity data as well as developmental studies."

Response: The statements in Appendix D referred to by the commenter are taken from the recent EPA BMD technical guidance (U.S. EPA, 2012a), which states that "[t]he 10% response level has customarily been used for comparisons because it is at or near the limit of sensitivity in most cancer bioassays and in noncancer bioassays of comparable size" and further states that "[f]rom a statistical standpoint, most reproductive and developmental studies with nested study designs easily support a BMR of 5%." While EPA agrees that the nested study design of developmental studies does not necessarily makes them more sensitive, in many cases, developmental studies have the advantage of a larger sample size, which can increase statistical power and allow for the use of the lower BMR. Also, as the commenter suggests, a series of papers have shown that when data are expressed as the proportion of affected fetuses per litter (nested dichotomous data), the NOAEL was on average 0.7 times the BMDL for a 10% excess probability of response and was approximately equal, on average, to the BMDL for a 5% excess probability of response (U.S. EPA, 2012a). The text in Appendix D has been modified to remove the suggestion that the nested study design of developmental studies justifies a BMR of 5%.

APPENDIX B. DEVELOPMENT, CALIBRATION, AND APPLICATION OF A METHANOL PBPK MODEL

B.1. Summary

This appendix describes the development, calibration, and approach for application of PBPK models for adult (non-pregnant) Sprague-Dawley (S-D) rats and humans to extrapolate rat methanol inhalation-route internal dose metrics to human equivalent inhalation exposure concentrations (HECs) or oral exposure doses (HEDs) that result in the same internal doses. This model is a revision of the model reported by Ward et al. (1997), reflecting significant simplifications (removal of compartments for placenta, embryo/fetus, and extraembryonic fluid) and several elaborations (details follow), which allow the model to describe methanol blood kinetics. The reasoning for removal of the pregnancy description is given in Section 3.4.1.2, so is not reiterated here.

The model includes compartments for lung/blood methanol exchange, liver, fat, and the rest of the body. A single set of parameters was identified for each species modeled, whereas Ward et al. (1997) employed a number of data-set specific parameters. Fitting parameters to each data set make it difficult at best to apply the model to bioassay conditions (i.e., to extrapolate the model to exposure scenarios not used for model calibration). Other biokinetic methanol models that were considered as starting points for the current model also used varied parameters by data set to achieve model fits to the data. For example, the model of Bouchard et al. (2001) used different respiratory rates and fractional inhalation absorbed for different human exposures. Thus, model re-calibration using a single set of parameters was considered necessary for use in a health assessment.

The model structure common to rats and humans is described in further detail in Section B.2.1. Three model features are species-specific:

- (1) A term to account for observed decreases in respiration rate (and assumed corresponding decrease in cardiac output) was used to match rat data for rats reported by Pollack and Brouwer (1996). Human exposures used for model calibration, and for which model application is expected, are assumed to be low enough that the term is inactive for the human model.
- (2) A urinary bladder compartment is used to simulate urine excretion time-course data in humans. Human urinary data are sufficient to identify a bladder residence-time constant, but no such data are available for rats. Urinary elimination is included in the rat model, but the kinetics of methanol appearance in rat urine were not analyzed.

- (3) For rats, the body:blood PC had to be adjusted to match the short-time i.v. data (i.e., the data indicated that the volume-of-distribution predicted by assuming that body:blood partitioning was identical to muscle:blood was incorrect). But once this was done, the oral data were well predicted with 100% bioavailability. However for humans, since there was a single limited i.v. data set which could not be used to calibrate the body:blood PC, the value for muscle was used, but it was then found that less than 100% oral bioavailability must be used to match the oral PK data.

Further details of and justification for these features are given in corresponding Sections below.

Algebraic functions which approximate the full human PBPK model to within ~1% are also presented. These functions allow one to calculate human oral methanol doses (HEDs) and inhalation concentrations (HECs) yielding internal dose(s) equal to specified maximum concentrations (C_{max} values) or area-under-the-curve (AUC) values, specifically to match internal doses (internal PODs) determined from rat dose-response data.

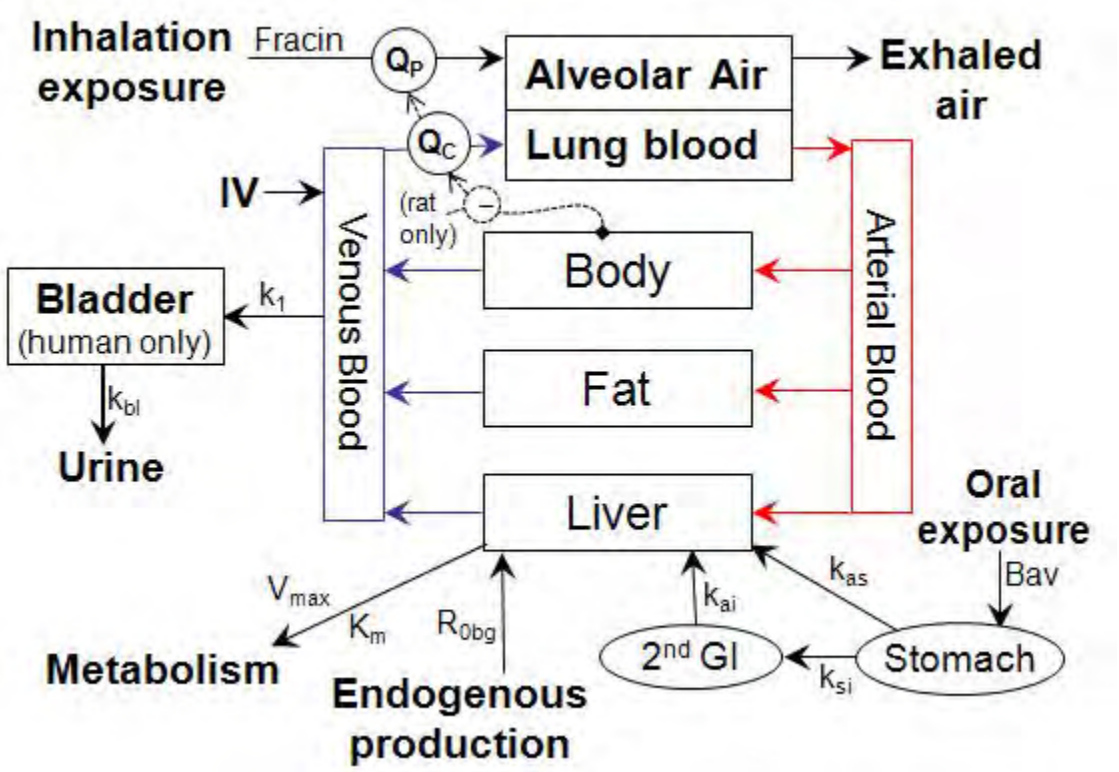
B.2. Model Development

B.2.1. Model Structure

The model structure is shown in Figure B-1. A gas-exchange model for inhalation exposure was added, with an adjustment factor (FRACIN) for methanol absorption/desorption in the conducting airways, as was done by Fisher et al. (2000), to describe delivery of methanol to blood as a function of ventilation, partitioning, and blood flow rather than the less standard approach used by Ward et al. (1997). A second (non-physiological) GI compartment was added to better describe oral uptake in rats. For humans the limited oral PK data were not sufficient to identify the two additional parameters associated with the second GI compartment, but the data were consistent with less than 100% bioavailability. (Rat data were consistent with 100% bioavailability, with the second GI compartment included.) The kidney was lumped with the body compartment because the blood:tissue partition coefficients for these tissues were similar and recent practice in PBPK modeling is to treat urinary clearance as occurring from the blood compartment rather than the kidney tissue [for example, (Loccisano et al., 2011)]. In particular this reflects the biological reality that renal excretion is initiated by filtration of blood flowing through the glomeruli, rather than a partitioning form kidney tissue into the nephrons. Where the current model is not realistic is that it uses venous blood rather than arterial blood concentrations and the rate of clearance is not limited by the fraction of blood flow to the kidney. The use of venous vs. arterial blood could be significant where there is very high clearance or uptake in

various tissues, but examining these two for the experimental studies used for model calibration showed the difference was below 1% for rats and below 4% for humans, with the maximum difference occurring during inhalation exposures. Where the kidney blood-flow limitation would be significant is if the clearance in the kidney was a significant fraction (i.e., greater than 10%) of the kidney blood flow. However for the methanol model the clearance rate for this pathway in the rat is only 0.24% of renal blood flow [using a renal flow fraction of 0.141 from Brown et al. (1997)] and in the human is only 0.07% of renal blood flow [using a renal flow fraction of 0.175 from Brown et al. (1997)]. Therefore including an explicit kidney compartment with its own flow rate would have a negligible impact on the methanol model results reported here.

A fat compartment was included because it is the only tissue with a tissue:blood partitioning coefficient appreciably different than unity, and the liver is included because it is the primary site of metabolism. Background levels of methanol are included through use of a zero-order rate of infusion, $R_{Øbg}$. Equations in the model code allow $R_{Øbg}$ to be calculated as a function of other model parameters to match a user-specified background blood or urine concentration.



Note: Parameters: Fracin (FRACIN), fraction of exposure concentration reaching gas exchange region in lungs; Bav, oral bioavailability; k_{as}, first-order oral absorption rate from stomach; k_{ai}, first-order uptake from 2nd GI compartment; k_{si}, first-order transfer between stomach and 2nd GI; V_{max} and apparent K_m Michaelis-Menten rate constants for metabolism in liver; k_1, first-order rate constant for urinary elimination; k_{bl}, rate constant for urinary excretion from bladder. For the rat only, high levels of methanol in the body compartment lead to respiratory and cardiac depression, indicated by the dashed line. Rat data were consistent with Bav = 100% but humans with Bav = 83%.

Figure B-1 Schematic of the PBPK model used to describe the inhalation, oral, and i.v. route pharmacokinetics of methanol.

Methanol is well absorbed by the inhalation and oral routes, and is readily metabolized to formaldehyde, which is rapidly converted to formate in both rodents and humans. Although the primary enzymes responsible for metabolizing formaldehyde are different in rodents (CAT) and adult humans (ADH); the metabolite, formate, is the same, and the metabolic rates are similar (Clary, 2003). The published rodent kinetic models for methanol differ in how they describe the metabolism of methanol (Bouchard et al., 2001; Fisher et al., 2000; Ward et al., 1997; Horton et al., 1992). Ward et al. (1997) used one saturable and one first-order pathway for mice, and Horton et al. (1992) applied two saturable pathways of metabolism to describe methanol elimination in rats. Bouchard et al. (2001) employed one metabolic pathway and a second pathway described as urinary elimination in rats and humans, both being first-order.

Since metabolic reactions are known to be saturable – the rate is ultimately limited by the amount of enzyme present – and metabolism is known to be the primary route of elimination in rats and humans, the starting point for both rats and humans was to assume the simplest model

form consistent with this biochemistry: a single saturable pathway, described by Michaelis-Menten kinetics. This model structure provided a reasonable fit to a range of data, with the first-order urinary pathway included. If the human PK data, in particular, were completely linear, then attempts to fit this structure would result in a lack of parameter convergence, with the saturation constant (K_m) approaching infinity, which did not occur. Parameter estimation converged to a reasonable value for K_m when the model was fit to the human data, and the resulting fit to the data was slightly but clearly improved versus a forced first-order function (evaluated by both a quantitative measure of fit and visual inspection). Further, when human model optimization was begun with larger values of K_m, which would make the model predictions more linear, the optimization still converged to the value reported here, clearly indicating that the human data are more consistent with a saturable metabolic description than a first-order description. The impact of uncertainty in this model choice is discussed in the corresponding section of the review (Section B.2.6.1). However, that uncertainty would not be reduced by assuming strictly first-order metabolism, given the data available and results described here.

Inclusion of a second metabolic pathway was tested for the rat model, but was found to create problems with parameter convergence and not found to significantly improve model fits.

For the rat, suppression of respiration rate at higher exposure levels was reported by (Perkins et al., 1996a). Therefore, an empirical function was fit to the respiration rate vs. blood data from Perkins et al. (1996a) and, assuming this indicates a parallel depression in both cardiac output and ventilation, the function was applied to the rat cardiac output with ventilation-perfusion-ratio fixed. Further details are given in Section B.2.3 on rat model calibration below.

While the PBPK model explicitly describes the concentration of methanol, it only describes the rate of metabolism or conversion of methanol to its metabolites. Distribution and metabolism of formaldehyde is not considered by the model, and this model does not track formate or formaldehyde. The data needed to parameterize or validate a specific description of either of these metabolites is not available. Since the metabolic conversion of formaldehyde to formate is rapid (< 1 minute) in all species (Kavet and Nauss, 1990), the methanol metabolism rate should approximate a formate production rate, though this has not been verified. Thus the rate of methanol metabolism predicted by the model can be used as a dose metric for either or both of these metabolites, but scaling of that metabolic rate metric to humans requires that the rate be normalized to $BW^{0.75}$, (i.e., scaled rate = $mg/kg^{0.75}$ − time), to account for the general expectation metabolic elimination of the metabolites scales as $BW^{0.75}$, hence is slower in humans. First-order rate constants were scaled as $1/BW^{0.25}$, since the resulting rate is also multiplied by tissue volume which scales as BW^1.

The model was initially coded in acslXtreme v1.4 and was subsequently updated in acslX v 3.0.2.1 (The AEgis Technologies Group, Inc., Huntsville, AL). Most procedures used to generate this report, except those for the optimization, may be run by executing the

corresponding .m files. The model code (acslX .csl file) and supporting .m files are available electronically on the EPA HERO database (U.S. EPA, 2012b). A key identifying .m files associated with figures and tables in this report is also provided in the supporting materials.

B.2.2. Model Parameters

Physiological parameters such as tissue volumes, blood flows, and ventilation rates were obtained from the open literature (Table B-1). Parameters for blood flow, ventilation, and metabolic capacity were scaled as $BW^{0.75}$, according to the methods of Ramsey and Andersen (1984). Pulmonary air-flow (Q_P) was coded as the product of cardiac output (Q_C) and a ventilation-perfusion ratio (VPR) in order to facilitate coding of changes in these quantities due to exercise or respiratory depression in rats. In particular it was generally assumed that VPR remained constant, so Q_P and Q_C varied in proportion to one another during such changes, unless data specifically indicated otherwise.

As briefly described in the summary, when published partition coefficients (PCs) were used for all body compartments for the rat; the predicted blood levels immediately following i.v. doses were not well estimated. Since those blood levels only depend on the tissue partitioning and the rest-of-body compartment is compromised of multiple tissues which have differing partition coefficients, it was therefore decided to initially fit the body:blood PC to the i.v. data and then to the total PK data set in global parameter estimation. This approach is validated by the observation that the resulting fitted PC was in the range of those measured for other tissues and the rat model was then consistent with 100% oral bioavailability. Rat PCs were taken as measured for that species by Horton et al. (1992) for liver:blood and blood:air. The "slow-to-blood" PC (1.1) in rats reported by Horton et al. (1992), is inconsistent with the value for fat:blood (0.083) in mice from Ward et al. (1997), and that determined for rat fat:blood (0.11) partitioning of ethanol by Pastino and Conolly (2000); these other results indicate much lower partitioning of alcohols into fat. Therefore the Ward et al. (1997) PC for mouse fat:blood, was used.

Table B-1 Parameters used in the rat and human PBPK models.

	S-D Rat	Human	Data Source
Body weight (kg)			
	0.275[a]	70	Measured/estimated
Tissue volume (% body weight)			
Liver	3.7	2.6	Brown et al. (1997)
Arterial blood	1.85	1.98	
Venous blood	4.43	5.93	
Fat	7.0	21.4	
Lung	0.50	0.8	
Rest of body	73.9	58.3	Calculated[b]
Flows: Total			
Cardiac output (Q_CC; L/hr/kg$^{0.75}$)[c]	16.4	16.5	Brown et al. (1997); Perkins et al. (1995); U.S. EPA (2000a)
Ventilation-perfusion ratio (VPR)[c]	1	1.45	
Blood Flows: (% Cardiac Output)			
Liver	25.0	22.7	Brown et al. (1997)
Fat	7.0	5.2	
Rest of body	68	72.1	Calculated
Biochemical constants[d]			
V_{maxC} (mg/hr/kg$^{0.75}$)	21.4	41	Fitted, except rat k_1C which is calculated from Pollack and Brouwer (1996).
K_m (mg/L)	29	36	
k_1C (kg$^{0.25}$/hr)	0.153	0.034	
Oral absorption			
k_{as} (hr^{-1})	12.8	0.21	Rat: fitted, except Bav assumed = 1 Human: k_{as}, k_{si}, and k_{ai} are for ethanol [from (Sultatos et al., 2004)]; Bav fitted.
k_{si} (hr^{-1})	3.1	3.17	
k_{ai} (hr^{-1})	0.38	3.28	
Bav (fraction)	1	0.79	

Table B-1 (Continued): Parameters used in the rat and human PBPK models.

	S-D Rat	Human	Data Source
Partition coefficients			
Liver:Blood	1.6	0.583[e]	Human: Fiserova-Bergerova & Diaz (1986) (human "body" assumed = muscle); Rat: Horton et al. (1992);except rat fat:blood assumed equal to mouse (Ward et al., 1997) body:blood was fit to data (estimated), and lung:blood assumed (approximately equal to human)
Fat:Blood	0.083	0.142	
Blood:Air	1,350	1,626	
Body:Blood	0.89	0.805	
Lung:Blood	1	1.07	
Bladder time-constant (k_{bl}, hr^{-1})[f]	NA	0.76	Fitted (human)
Inhalation fractional availability (FRACIN, %)	0.81	0.75	Fitted

[a]The midpoints of rat weights reported for each study was used and ranged from 0.22 to 0.33 kg
[b]The volume of the other tissues was subtracted from 91% (whole body minus a bone volume of approximately 9%) to derive the volume of the remaining tissues.
[c]In the model cardiac output (QC; L/hr) was set as the primary constant, via the scaling constant Q_CC ($QC/BW^{0.75}$), and pulmonary ventilation (Q_P) was defined as the product of QC and the ventilation-perfusion ratio, VPR. Q_CC and VPR for humans were obtained (VPR calculated) from U.S. EPA (2000a).
[d]V_{max} and K_m represent a saturable metabolic process assumed to occur solely in the liver. V_{max} used in the model = V_{maxC} ($mg/kg^{0.75}\cdot hr$)×$BW^{0.75}$. k_1C is the first-order urinary elimination constant (from the blood compartment). k_1 used in the model = $k_1C/BW^{0.25}$.
[e]Human liver:blood partition coefficient estimated by Fiserova-Bergerova and Diaz (1986) from correlation to measured fat:blood partition coefficient, based on data from 27 other solvents.
[f]k_{bl} – a first-order rate constant for elimination from the bladder compartment, used to account for the difference between blood kinetics and urinary excretion data as observed in humans.

NA - Not applicable for that species.

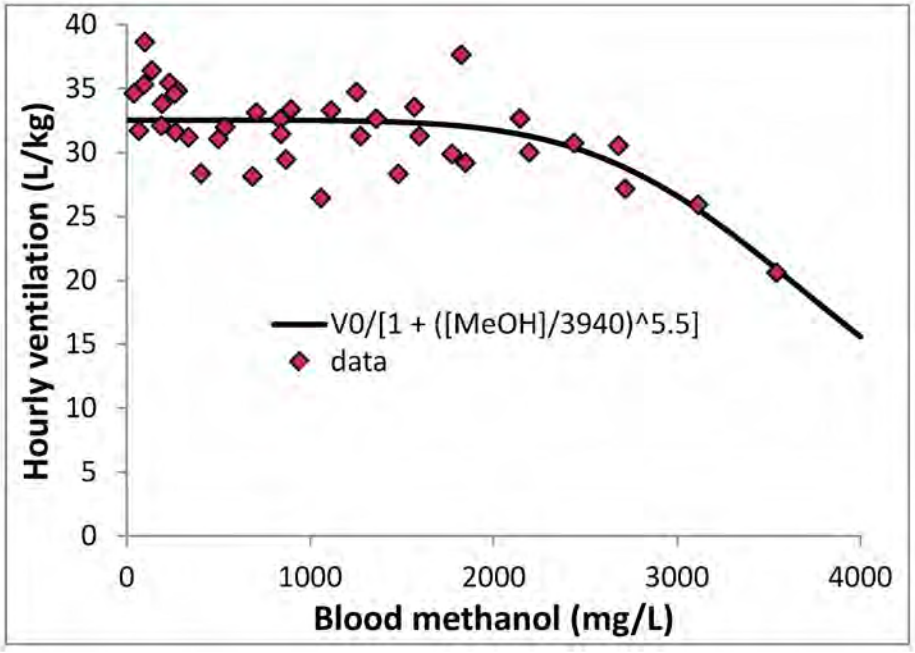
B.2.3. Rat Model Calibration

The S-D rat model was calibrated to fit blood concentration data from intravenous, inhalation, and oral exposures. However, the urinary clearance constant and the respiratory depression function were specified outside of the PBPK model, using separate data. Pollack and Brouwer (1996) used linear regression of urine excretion rates versus blood concentration in non-pregnant rats to obtain a clearance constant, k_1 = 0.00916 L/hr/kg. This was converted to the equivalent k_1C in the PBPK model by normalizing to the venous blood fraction of BW, 0.0443 L/kg, and multiplying by an average SD BW of 0.3 kg (raised to 0.25) to obtain the allometric constant:

$$k_1C = (0.00916\ \text{L/hr/kg}) \times (0.3\ \text{kg})^{0.25} / (0.0443\ \text{L/kg}) = 0.153\ \text{kg}^{0.25}/\text{hr}.$$

As mentioned above, suppression of respiration rate in the rat at higher exposure levels was reported by (Perkins et al., 1996a). An empirical function was therefore fit to the respiration rate versus blood data from that source, shown in Figure B-2. It was assumed that cardiac output decreased proportionately with ventilation, so the inhibition term $\{1+ ([MeOH])/3{,}940)^{5.5}\}^{-1}$, was applied to the rat cardiac output with ventilation-perfusion-ratio fixed. However, when the response was assumed to occur instantaneously due to changes in mixed venous blood

concentration (i.e., the mixed venous blood concentration was used for [MeOH]), the model predicted an unreasonable level of suppression immediately after i.v. dosing because of the short-term spike in blood levels predicted to occur. If instead the concentration in venous blood exiting the "body" compartment was used for [MeOH], reasonable model simulations resulted. Since some (short) time is likely needed for methanol to interact with the neurons involved in respiratory and cardiac control, and for neural processing of the resulting signal to the heart and lungs, the use of this body-tissue-blood concentration, for which the methanol concentration changes are slightly delayed and "smoothed" relative to the mixed venous blood, seems a reasonable option.



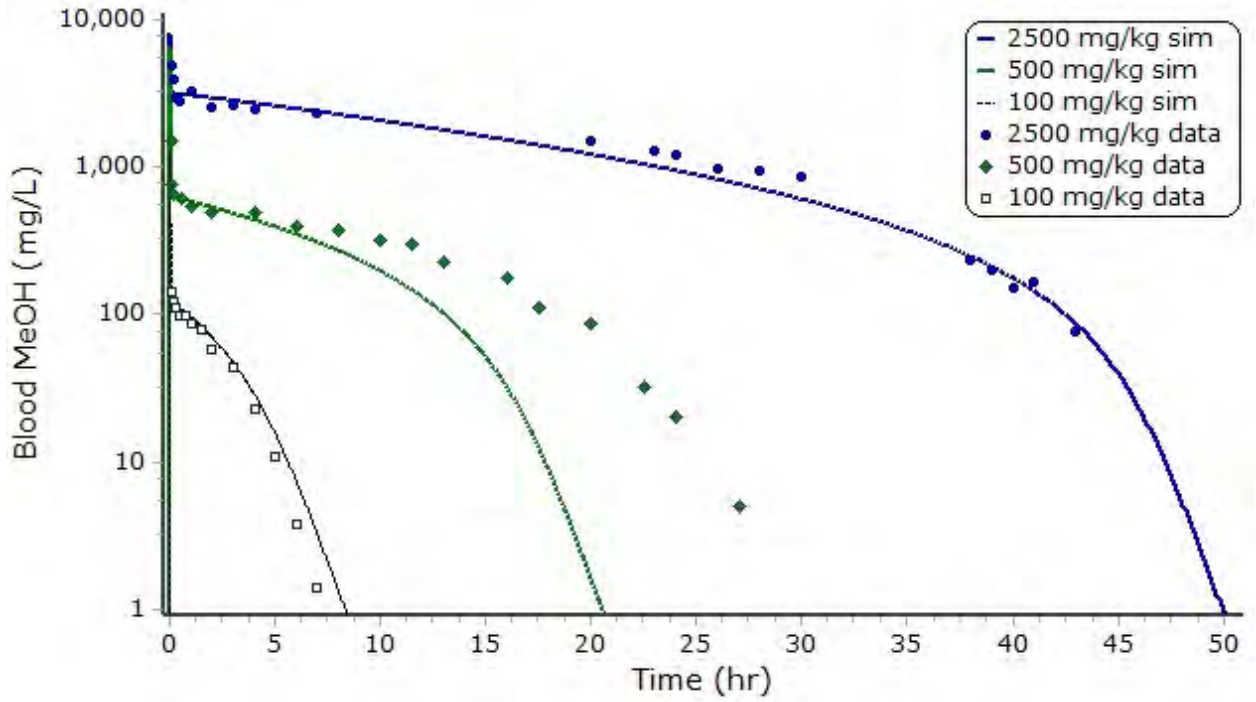
Source: Adapted with permission of Informa Healthcare; Perkins et al. (1996b).

Figure B-2 Respiratory depression in Sprague-Dawley rats as a function of blood methanol concentration. The empirical curve fit (solid line) was selected to describe the data with a minimal number of parameters.

All of the data available for S-D rats are reported with background levels subtracted. These data are from the laboratory of Gary M. Pollack (then at the University of North Carolina, Chapel Hill) and most are also presented in the thesis of Keith W. Ward (1995). The original source reported only values with background subtracted, and neither Dr. Ward nor Dr. Pollack have retained any other records of these experiments (personal communications). Therefore, the methanol blood levels reported by NEDO (NEDO, 1987) in control animals, 3 mg/L, was assumed for all PK experiments analyzed. Rather than adding this number to the reported data, however, this background was subtracted from model simulation result obtained with this background level set to match the reported data. Specifically, model simulations were run with a zero-order endogenous production rate, R_{0bg}, set to produce a concentration in venous blood (C_{VB}) of 3 mg/L in the absence of exposure. This background level is denoted C_{VBbg} and is a constant in the model code. A secondary variable was defined in the code: $C_{VBmb} = C_{VB} - C_{VBbg}$, i.e., the concentration predicted including background, C_{VB}, minus that constant background. Since the rates of metabolism, including saturation, calculated in the model all used the total concentration, which includes that produced from the zero-order term, this approach accounts for background methanol in the animals to the extent possible, given the data, without adjusting the data using an otherwise assumed background level. All of the plots which follow, demonstrating model fits to various data, then show model predictions of C_{VBmb} versus the data as reported in the various publications. Total blood concentrations, C_{VB}, are listed in tables of internal metrics and show in plots depicting internal dosimetry under bioassay conditions.

Initial values V_{maxC}, K_m, and the body:blood partition coefficient (PR) were then obtained by fitting the model to the 100 and 2,500 mg/kg i.v. data provided in the command file of Ward et al. (1997) (holding other parameters constant). As mentioned previously, if PR was not also adjusted, the predicted concentration immediately following the distribution phase, which are only dependent on the partition coefficients, were discrepant from the data. Without adjusting PR, this then created a bias in the metabolic parameters to correct for the error in the distribution phase. Model predictions were also compared to 500 mg/kg i.v. data in the command file of Ward et al. (1997), with additional early time-points reported by Pollack and Brouwer (1996). With PR adjusted this way to fit the 100 and 2,500 mg/kg data, the model matched the initial time points of the 500 mg/kg data quite well (see Figure B-3). However, the subsequent clearance rate fit to these other two dose levels was inconsistent with the 500 mg/kg data. All three data sets with globally-fit model parameters are shown in Figure B-3. If one compares the clearance rate of the 500 mg/kg data at 20 hours and beyond, when the concentration range is the same as the 100 mg/kg data, it is clear that the two data sets are discrepant. Thus no model with a single set of parameters could simultaneously match both data sets. That the model does fit the 2,500 mg/kg data quite well indicates that the discrepancy is not due to a simple dose-dependency. Since it is most important that the model describe the low-dose data well, in the

range of the point-of-departure for toxicity extrapolation, while capturing as much of the high-dose dependency as possible, the 500 mg/kg i.v. data were not used in subsequent model calibration.

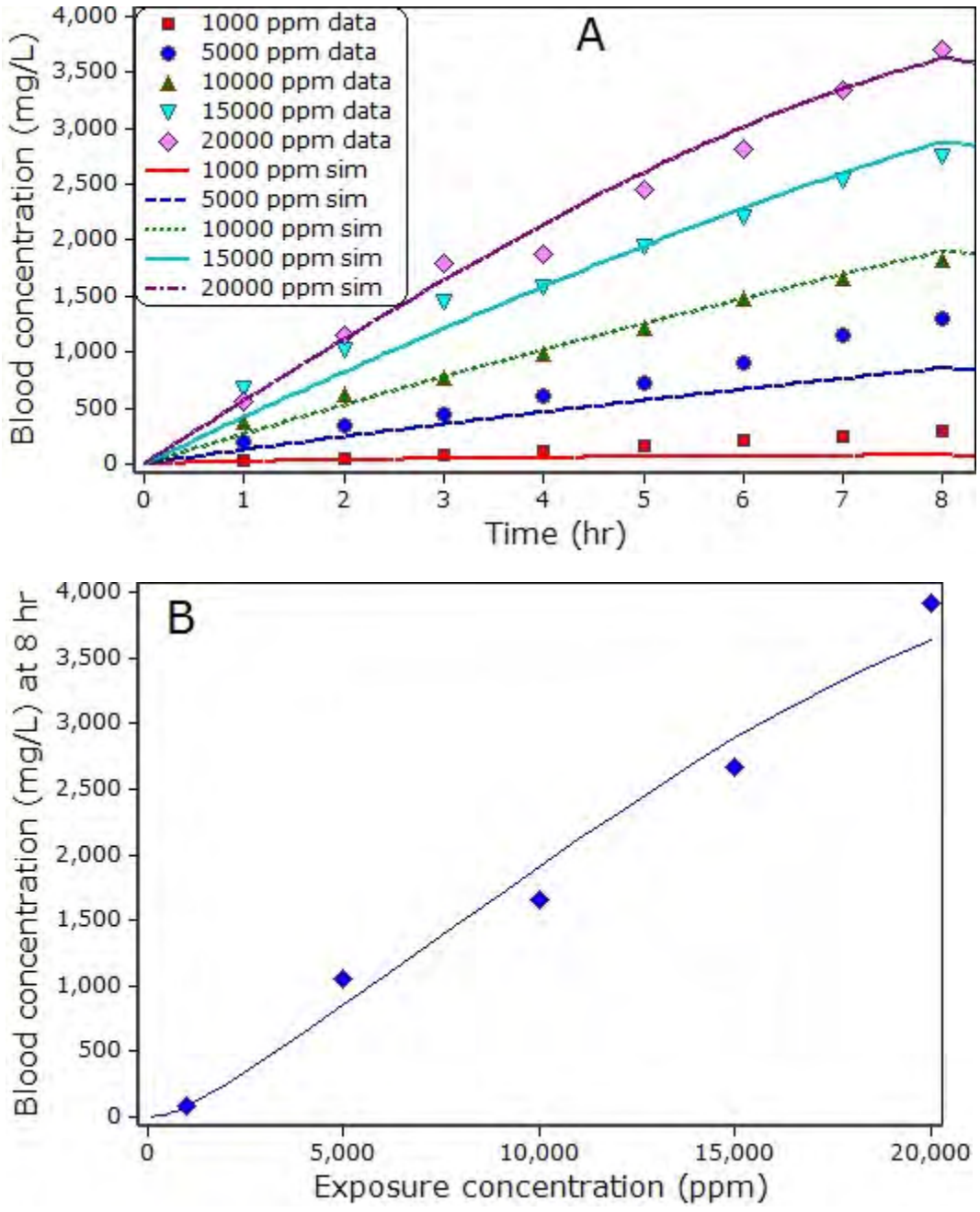


Source: Ward et al. (1997: squares); with additional 500 mg/kg points from Pollack and Brouwer (1996).

Note: MeOH (methanol) was infused into female Sprague-Dawley (S-D) rats at target doses of 100, 500, or 2,500 mg/kg. Data points are measured blood concentrations and lines are PBPK model simulations with metabolic parameters fit to a large set of S-D rat data (see text for further details).

Figure B-3 Rat i.v.-route methanol blood kinetics.

The initial value for the inhalation fractional absorption constant, FRACIN, was then set by fitting it to inhalation PK time-course data from Perkins et al. (1996a), holding other parameters constant. Model fits to these data with the final set of parameters are shown in panel A of Figure B-4. Model predictions are also shown versus end-of-exposure concentrations reported by Pollack and Brouwer (1996) in panel B of Figure B-4. This second data set was not used in model fitting. It is worth noting that while the model significantly under-predicts the 1,000 and 5,000 ppm data shown in panel A of Figure B-4, the model almost exactly matches the end-of-exposure concentration at 1,000 ppm in panel B, and only slightly under-predicts then 5,000 ppm measurement from that data set. Also the downward curvature which is noticeable in the 20,000 ppm simulation and to a lesser extent at 15,000 ppm (panel A), and above ~12,000 ppm in panel B, is due to the respiratory depression term.



Source: (Panel A): Adapted with permission of Springer; Perkins et al. (1996a); (Panel B): Reprinted with the permission of the Health Effects Institute, Boston, MA; from Pollack and Brouwer (1996).

Note**:** (A) Model fits to time-course data for 1,000-20,000 ppm exposures reported by Perkins et al. (1996a). (B) Model predictions versus end-of-exposure data, for 8-hr exposures; data from Pollack and Brouwer (1996), not used for parameter estimation. Model results are with globally fit parameters. The noticeable downward curvatures seen in the 20,000 ppm model prediction (panel A) and above ~12,000 ppm in panel B are the due to the inclusion of the respiratory depression term in the PBPK model.

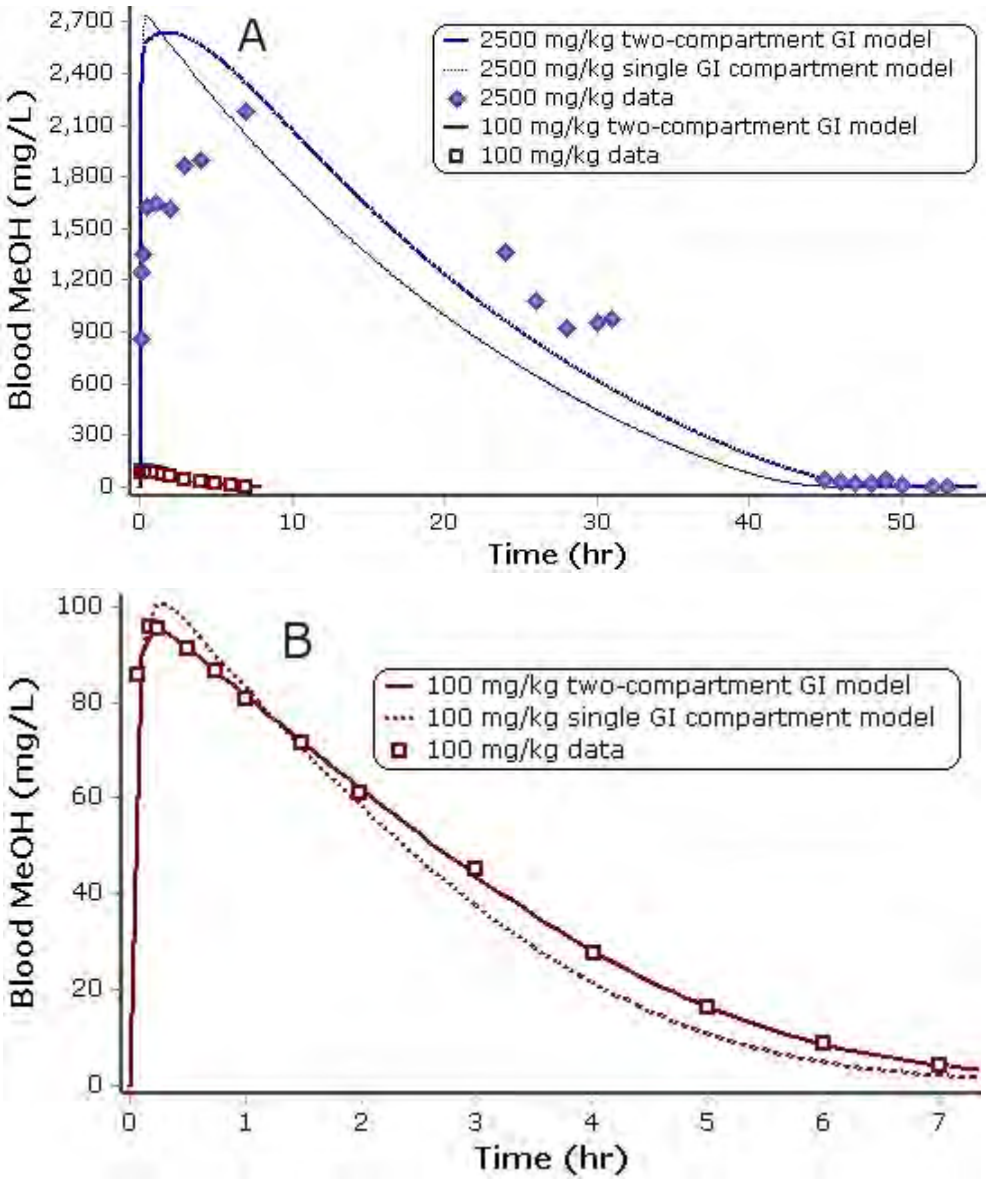
Figure B-4 Model fits to data sets from inhalation exposures in female Sprague-Dawley rats.

Oral absorption parameters were first fit to the lower dose (100 mg/kg) oral absorption data reported by Ward et al. (1997) (with other parameters held constant). The initial fit, with a single GI compartment was not very good, even with the oral bioavailability adjusted at the same time (dashed line in panel B of Figure B-4). Therefore, an empirical (non-physiological) second GI compartment was considered, like that used by Sultatos et al. (2004) for ethanol. With bioavailability fixed at 100%, use of this second compartment gave an excellent fit to the data (solid line in panel B of Figure B-4). Therefore the two-compartment GI structure was used.

While the fit to the 100 mg/kg oral data was quite good, the fit to the 2,500 mg/kg data exhibited a much faster and higher peak than shown by the data and under-predicted the data between 24 and 31 hr, during the clearance phase (Figure B-5, panel A). Notably, the two-compartment model reproduces these high-dose data much better than the single-compartment model. Even when the model was fit to both the high- and low-concentration data simultaneously, the fit to the high-concentration data could not be significantly improved without completely degrading the low-concentration fit (not shown). Several variations of the GI compartment rate equations were tested, in part reflecting data available from the ethanol literature, but none could significantly improve the fit to the 2,500 mg/kg data without introducing otherwise untested parameters and hypotheses. Since the primary concern is with fitting low-dose data, which produce blood concentrations near the point of departure, it was therefore decided to not use the 2,500 mg/kg data for parameter estimation, though comparisons of model predictions to those data are still presented, and to use first-order kinetics with the empirical, two-compartment GI model shown in Figure B-1.

A final set of fitted model parameters for the rat was obtained by allowing all of the adjusted parameters (V_{maxC}, K_m, PR, k_{as}, k_{si}, k_{ai}, and FRACIN) to the data sets as described above: 100 and 2,500 mg/kg i.v. doses [Figure B-3; (Ward et al., 1997), squares]; 1,000 to 20,000 ppm inhalation time-course data [Figure B-4, panel A, (Perkins et al., 1996a)], and 100 mg/kg oral dose data [Figure B-5; (Ward et al., 1997)]. The resulting parameter values are listed in Table B-1 and the simulations with solid lines in Figures B-3 to B-5 all use this global set. Although the model does not fit all of the data as well as one might like, particularly the 1,000 and 5,000 ppm data in Figure B-4, panel A, the overall quality of the fits is considered good. The number of parameters adjusted is considered modest, since could reduce the number of parameters by keeping PR at a value measured for muscle or using a one-compartment GI model. But either of these choices significantly degrades the model fits (shown for GI model), which indicates that the number and variety of data available are sufficient to inform these seven fitted parameters. One can consider the urinary excretion constant, k_1C, and the two parameters used to define the level of respiratory depression, as additional adjusted parameters. These two parameters were fit using additional data on urinary excretion and respiration rate, respectively. So the total number of fitted parameters is considered to be well supported by the corresponding

data used to determine their values, and hence a fairly good level of confidence should be held for model predictions of bioassay dosimetry. To further elucidate the level of confidence one can place in model predictions, evaluation of model sensitivity to these parameters was conducted as described in Section B.2.4.



Source: Ward et al. (1997).

Note: Thick solid lines are PBPK model results using a two-compartment GI model (oral bioavailability = 100%); thin dotted lines use a single GI compartment (bioavailability allowed to vary below 100%).

Figure B-5 Model simulations compared to 100 (squares) or 2,500 (diamonds) mg/kg oral methanol data in female Sprague-Dawley rat (expanded scale in panel B).

B.2.4. Rat Model Sensitivity Analysis

An evaluation of the importance of selected parameters on rat model estimates of blood methanol concentration was performed. Since the rat model was only used to evaluate internal doses during inhalation exposures, the sensitivity to the oral uptake parameters was not evaluated. The parameters which can affect inhalation dosimetry that were identified by matching to PK (and respiratory response) data were V_{maxC}, K_m, k_1C [estimated by Pollack and Brouwer (1996)], PR (body:blood partition coefficient), FRACIN, and k_{iv} (respiratory/cardiac depression constant). For the purpose of comparison, the blood:air partition coefficient (PB) was also included. Sensitivity of the dose metrics, C_{max} and AUC (both above background) was estimated under conditions of the NEDO bioassay (NEDO, 1987), 22 hr/day inhalation exposure, at the bounding levels of 200 and 5,000 ppm. The analysis was conducted by measuring the change in each metric resulting from a ±1% change in a given model parameter when all other parameters were held fixed. The normalized sensitivity coefficient is then:

$$SC = (\Delta \text{ metric}/\text{metric}_0)/\ (\Delta\ p/p_0),$$

where metric_0 and p_0 are the values of the metric and parameter, respectively with the unchanged (as fitted) values and Δ metric and Δ p are the differences between the values obtained with p increased by 1% and decreased by 1%.

A normalized sensitivity coefficient of 1 indicates that there is a one-to-one relationship between the fractional change in the parameter and model output; values close to zero indicate a small effect on model output. A positive value for the normalized sensitivity coefficient indicates that the output and the corresponding model parameter are directly related while a negative value indicates they are inversely related. Results are listed in Table B-2.

Table B-2 Sensitivity of rat model dose metrics to fitted parameters.

	Exposure level, metric			
	200 ppm		5,000 ppm	
Parameter[a]	C_{max}	AUC	C_{max}	AUC
V_{maxC}	-1.1	-1.0	-0.2	-0.2
K_m	0.7	0.7	0.0	0.0
PR	0.0	0.0	0.0	0.0
PB	0.0	0.0	0.3	0.4
k_1C	0.0	0.0	-0.2	-0.2
FRACIN	1.2	1.2	0.8	0.8
k_{iv}	0	0	0.4	0.4

[a]Values are normalized sensitivity coefficients (SCs), as explained in text, for a 22 hr/day inhalation exposure to the concentrations indicated. Parameters with SC absolute values greater than 0.2 are generally considered to be sensitive.

The sensitivity analysis results are mostly not surprising. At the lower concentration of 200 ppm, metabolic elimination has a significant influence, with both V_{maxC} and K_m having high SCs. The SC for V_{maxC} is negative since an increase in its value decrease blood concentration, while K_m is positive for the opposite reason. At 5,000 ppm V_{maxC} is only marginally significant and K_m not at all, but urinary elimination (k_1C) becomes significant, though only slightly. The one somewhat surprising result is that the body:blood partition coefficient, PR, has very little influence on the inhalation dose predictions. However, the analysis was conducted on conditions near steady-state with 22 hr/day exposure. As shown by Chiu and White (2006), the steady-state level predicted in blood by a PBPK model depends on only a small number of parameters: those affecting absorption, elimination (metabolic), and the blood:air partition coefficient (PB). For this model, at 200 ppm the rate of absorption by inhalation is likely limited by respiration rate, hence PB has little influence at that concentration, but it does significantly impact uptake at 5,000 ppm. More importantly, since PR has so little effect on these predictions means that any uncertainty in its value is inconsequential to the outcome of this assessment. (PR is expected to more strongly influence non-steady-state conditions, such as when oral ingestion occurs in boluses.)

The fraction inhaled (FRACIN) is highly sensitive at both dose levels. The respiration inhibition constant, k_{iv}, has no influence at 200 ppm but is sensitive at 5,000 ppm. Since increasing k_{iv} decreases the level of inhibition – increases respiration – its coefficient is positive. Differences in the sensitivities of the two metrics existed in the second decimal place, but otherwise the two are closely correlated for this exposure scenario, hence the SCs are effectively identical.

Thus, all of the adjusted parameters except PR have a significant influence on model predictions over part of the relevant range of concentrations. Of these fitted parameters, k_1C and

k_{iv} were fit to independent data sets, not used to fit any other parameters. Hence a good degree of confidence can be given to their values. Because of the wide range of doses, particularly by the i.v. route, used for the PK data, V_{maxC} and K_m can also be considered fairly well identified. However the model's inability to fit the 500 mg/kg i.v. data (Figure B-3) and 1,000 and 5,000 ppm inhalation data (Figure B-4, panel A) create some level of uncertainty in their values and that of FRACIN. That the model fits rather well both the 100 and 2,500 mg/kg i.v. data, makes it difficult to come up with a simple explanation for the lack-of-fit to the intermediate dose. Since the clearance observations at 500 ppm go beyond 24 hr, it is possible that there is a time-dependent process that reduces clearance in that time range. The 2,500 mg/kg i.v. dose clearance was only measured to 43 hours, when it had just dropped to ~100 mg/L, so one cannot say if the clearance from then on would have been more like the 100 mg/kg data or the 500 mg/kg data.

For FRACIN, the poor fit to the lower two inhalation exposures (Figure B-4, panel A) suggests a concentration-dependence; (i.e., FRACIN is higher at low concentrations. However, even if FRACIN is set to 100%, the later time points for the 1,000 and 5,000 ppm concentration curves are *still* under-predicted (results not shown). One hypothesis is that at low concentrations, deposition in the conducting airways leads to a significant amount of absorption, not accounted for in the standard gas-exchange model used here. Including such a mechanism would increase model complexity significantly, and such a hypothesis should be tested by also comparing model predictions to methanol gas uptake experiments, which would clearly show if methanol is being taken up more efficiently at low concentrations versus an error in the model's description of metabolic elimination or some other systemic process.

This consideration of possible model errors and potential future improvements (with necessary data) should be balanced against the observation that the model-predicted blood level at 8 hours from a 1,000 ppm exposure, 81 mg/L, almost exactly matches the measured concentration reported in Pollack and Brouwer's (1996) (Table 16): 83 ± 15 mg/L. The discrepancy between that result and the value obtained from a plot (digitized) in the same report which also appears in Perkins et al. (1996a), ~290 mg/L, can only be attributed to experimental variability, which no model can fully describe. Since the model does fit the lower-concentration 8-hr data (Figure B-4, panel B) fairly well, it is considered adequate for use in the assessment as is, without further complication and additional parameters, and FRACIN is assumed to provide a reasonable adjustment to the internal doses with the value obtained here.

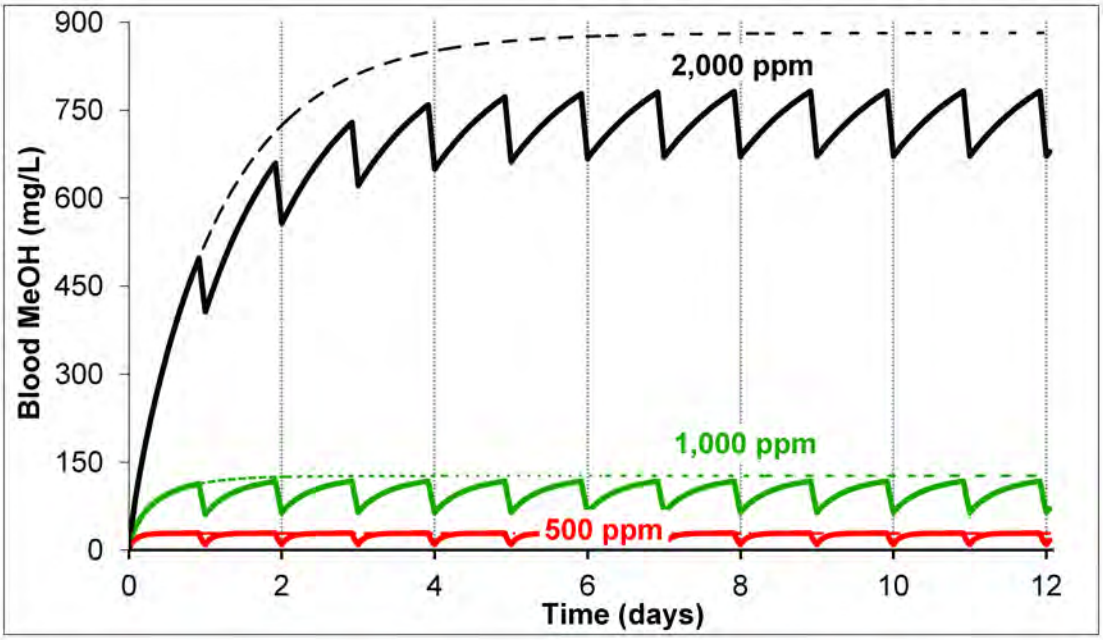
B.2.5. Rat Model Simulations

A range of adverse developmental effects was noted in rat pups exposed to methanol throughout embryogenesis (NEDO, 1987). In particular, model simulations were conducted for S-D rats in utero over different periods of pregnancy and as neonates via inhalation. Inhalation exposures to methanol were carried out for 18–22 hours, depending on the exposure group. Simulations of predicted C_{max} and 22-hour exposures to 500, 1,000, and 2,000 ppm methanol are shown in Figure B-6. Although the exposures in these studies are to rats over long periods and in some cases exposures of the newborn pups, the model simulations are for NP adult rats only, and do not take into account changes is body weight or composition. These simulated values are presumed to be a better surrogate for and predictor of target-tissue concentrations in developing rats, and the corresponding estimated human concentrations a better predictor of developmental risk in humans than would be obtained using the applied concentration or dose and default extrapolations. The logic here is simply that the ratio of actual target tissue concentration (in the developing rat pup or human) to the simulated concentration in the NP adult is expected to be the same in both species and hence, that proportionality drops out in calculating a HEC.

Figure B-6 depicts rat model simulations to determine internal doses for 22 hours/day inhalation exposures at 500, 1,000, or 2,000 ppm. A typical BW of 0.3 kg was used, since predicted inhalation dosimetry is usually insensitive to the exact BW. Simulation results for continuous inhalation exposures are shown for contrast. The simulations show that for all but the highest dose (2,000 ppm) steady-state is reached within 22 hours, and that "periodicity," where the concentration time course is the same for each subsequent day, is reached by the 3rd day of exposure. At 2,000 ppm, however, steady state is not reached until after 8 days for the continuous exposure. Therefore, the C_{max} and 24-hour AUC were calculated by simulating 22 hours/day exposures for 12 days, with the AUC calculated over the last day (24 hours) of that period. The AUC values shown in Figure B-6 are calculated from the concentration increase above the background or endogenous level; (i.e.,

$$AUC = \int_0^{24} (C - C_{bg})dt$$

where the integration is over 24 hours, C is the instantaneous blood concentration, and C_{bg} is the endogenous/background level, set to 3 mg/L for the rat).



Exposure concentration (ppm)	C_{max}; (mg/L)	C_{max} - C_{bg}; (mg/L)	AUC (C - C_{bg}); (mg-hr/L)
500	28.7	25.7	547
1,000	118	115	2,310
2,000	783	780	17,500

Note: Rat BW was set to 0.3 kg. Simulations are shown for both continuous (thin, dashed/dotted lines in plot) and 22 hours/day exposures (thick, solid lines in plot). Simulations shown are total blood concentration (including endogenous/background methanol, C_{bg}). C_{max} and AUC are determined from the 22 hour/day simulations, run for a total of 12 days (288 hours), with the AUC calculated from the total concentration minus background for the last 24 hours of the simulation.

Figure B-6 Simulated Sprague-Dawley rat inhalation exposures to 500, 1,000, or 2,000 ppm methanol.

B.2.6. Human Model Calibration

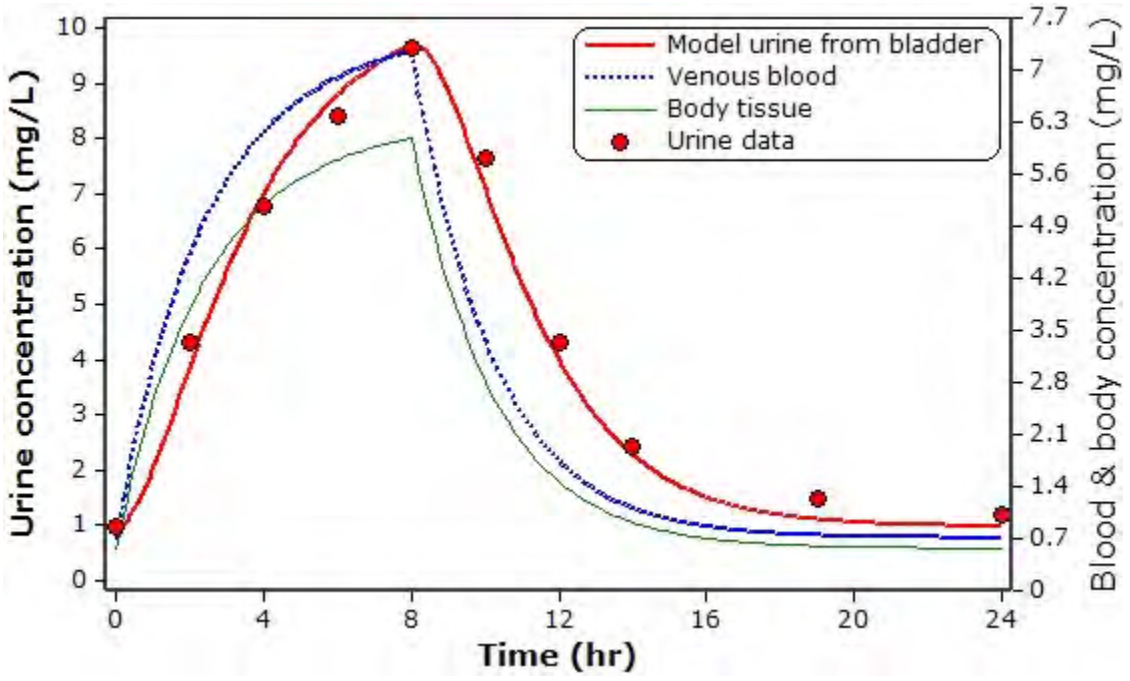
The rat model was scaled to human body weight (70 kg or study-specific average), using human tissue compartment volumes and blood flows, and calibrated to fit the human inhalation-exposure data available from the open literature, which comprised data from four publications (Ernstgård et al., 2005; Batterman et al., 1998; Osterloh et al., 1996; Sedivec et al., 1981), and a single data set for oral exposure (Schmutte et al., 1988). Model predictions are also compared to an i.v. data set that was not used for parameter estimation (Haffner et al., 1992). Since the bulk of the human data were from inhalation exposures, the approach to identifying parameters was to first fit the metabolic (and endogenous level) parameters to those data sets. Initial estimates for the oral uptake parameters were then obtained by fitting the oral PK data with other parameters held constant. Lastly, a global fit over the inhalation and oral data sets combined, with all fitted

parameters varied simultaneously, was performed to obtain final parameter values. The two key differences in model structure and parameters adjusted are discussed below, followed by a more detailed description of the calibration against specific data.

More specifically, the human model calibration differed from the rat calibration in two ways. First, a bladder compartment was included (calibrated) to better describe the kinetics of human urinary data, where both the rise and the drop in excretion rate is slower than the predicted decline in blood and tissue methanol and hence rate of metabolite production. This difference is shown in Figure B-7 for the 231 ppm exposure data of Sedivec et al. (1981). The model-predicted venous blood and body tissue concentration curves show the pattern typical for PBPK models which use the common venous-equilibration equations for tissue distribution (used in this model) for fixed-duration inhalation exposures: an asymptotic rise in concentration during the exposure period and then a sharp decline starting the moment that exposure ends. If urinary excretion was assumed to be proportional to the body tissue concentration (which includes the kidney tissue) or a separate kidney compartment was used wih the same venous-equilibration equations, then the shape of the predicted time-course would simply mirror that of the tissue level shown in Figure B-7, which is clearly a poor representation of the data. However, fitting the one additional parameter introduced for the bladder compartment, the bladder clearance constant, k_{bl}, allows the model to reproduce the distinct kinetics of urinary excretion quite well. Thus this addition is considered both biologically realistic and well justified.

The second difference from the rat calibration is that the body:blood partition coefficient (PR) was not adjusted but the oral bioavailability (Bav) was adjusted. In particular, PR was not adjusted because only limited i.v. dosing data were available (a single dose level with actual data only available for one subject). Instead the value measured for muscle by Fiserova-Bergerova and Diaz (1986) was used for PR without adjustment. However, when attempting to match the model to the oral PK data, model predictions then significantly over-predicted those data (with parameters otherwise consistent with the inhalation data). Therefore the oral bioavailability was allowed to vary to less than 100% to fit the oral PK data.

In summary, the set of key parameters fit for the human model were the metabolic (V_{maxC} and K_m) and urinary elimination (k_1C and k_{bl}) constants, the inhalation fraction (FRACIN), and the oral bioavailability (Bav). In addition, the endogenous background concentration and an increment in background over time were fit to control data from Osterloh et al. (1996). A detailed description of each data set and the parameter(s) that it primarily informs follow. However, as with the rat, the final set of parameters was obtained by global optimization: varying all parameters while fitting all of data sets simultaneously. Other human parameters were set as reported in Table B-1.



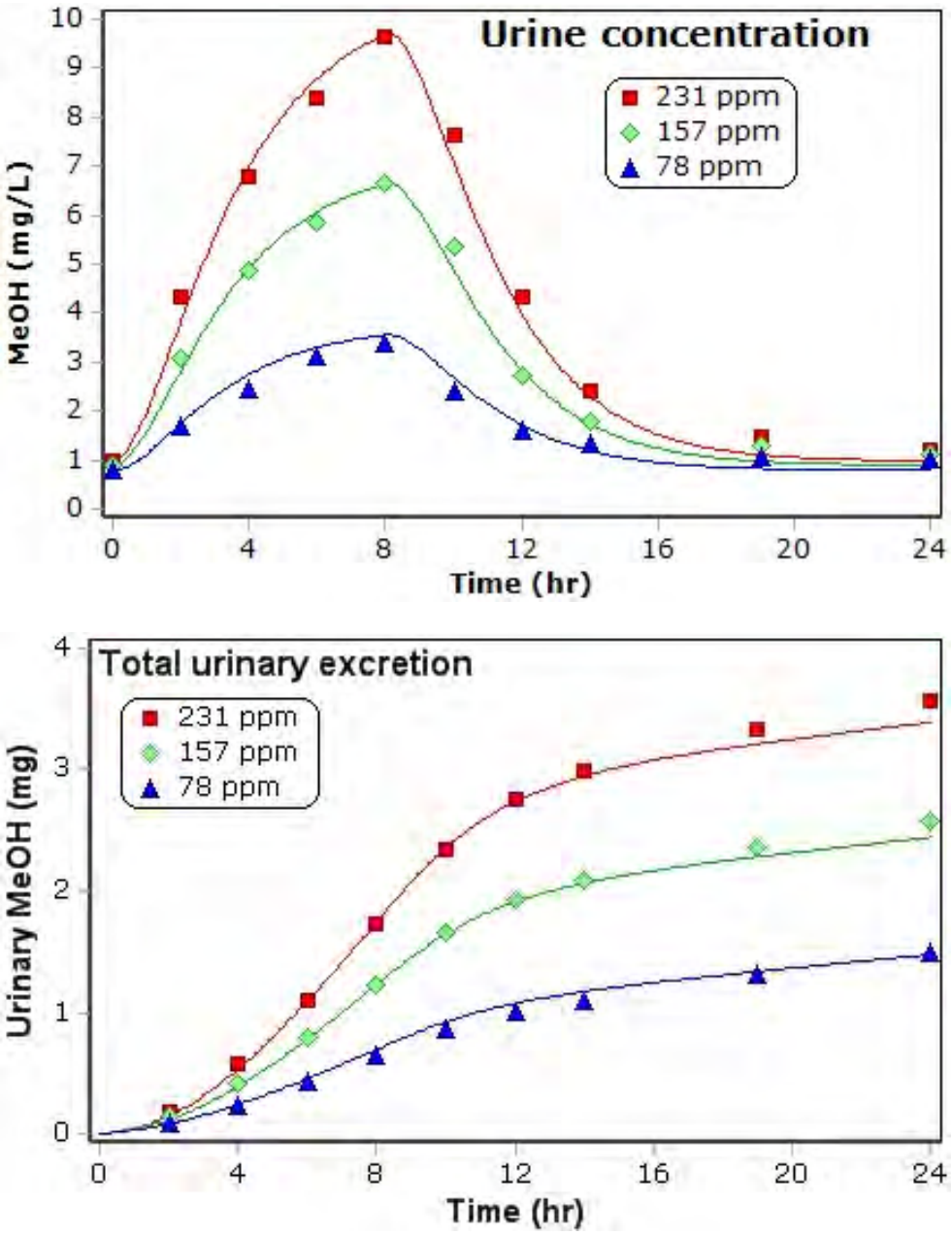
Source: Sedivec et al. (1981).

Figure B-7 Comparison of model predictions of urine concentration (from bladder compartment), venous blood, body tissue, and urine concentration data for a 231 ppm, 8-hour exposure. Right axis provides scale for venous blood and body tissue results.

The first-order rate of clearance of methanol from the blood to urine, k_1C, and first-order bladder compartment time constant, k_{bl}, were used to describe urinary methanol elimination. (See Section B.2.1 on the reasoning for treating urinary elimination as occurring from the blood compartment versus a kidney tissue compartment.) The inhalation-route urinary methanol kinetic data described by Sedivec et al. (1981) (Figure B-8) were used to inform these parameters. The urine methanol concentration data reported by the authors were converted to amount in urine by assuming 0.5 mL/hr/kg total urinary output (Horton et al., 1992). Since the resulting values of k_1C and k_{bl} (Table B-1) are only calibrated using a small data set, they should be considered an estimate. Urine is a minor route of methanol clearance in humans, with little impact on total blood methanol concentration, but changes in urine levels are expected to closely reflect corresponding changes in blood levels, hence the slight nonlinearity in the urine data also inform the apparent metabolic saturation constant, K_m. The potential for this information is lost, however, if the kinetics of urinary elimination are not well matched; i.e., if the bladder compartment is not used.

To estimate both the Michaelis-Menten (hepatic) and first-order (urinary) clearance rates, all human inhalation data under nonworking conditions were used (Batterman et al., 1998; Osterloh et al., 1996; Sedivec et al., 1981).

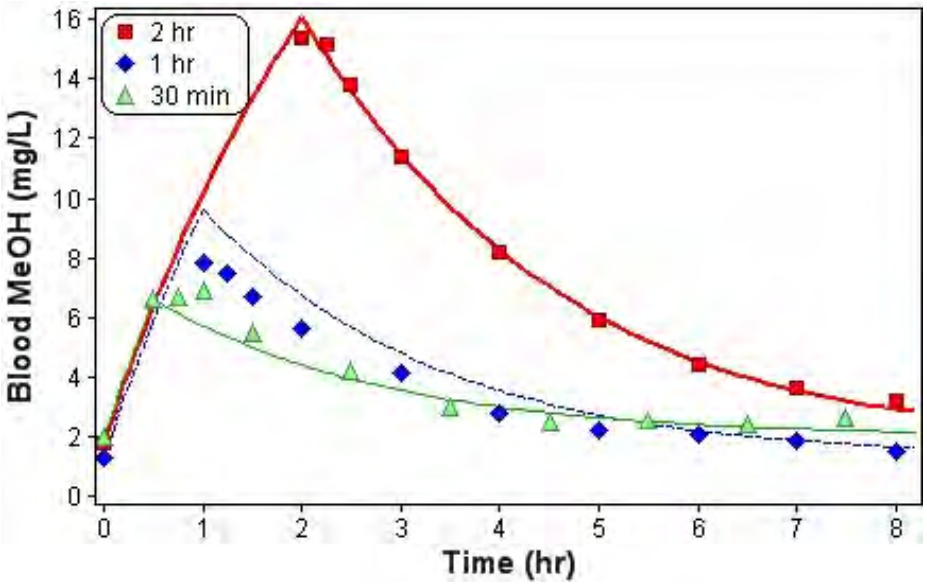
- The initial urine concentrations from Sedivec et al. (1981) (reported at time = 0; see Figure B-8) were assumed to represent endogenous background levels, and therefore were used to set a (constant) endogenous level for each exposure level to match that urinary level (i.e., the endogenous blood level that must exist to match the observed urine concentration, given the urinary clearance constant, k_1C). The endogenous blood concentrations so estimated were 0.6-0.74 mg/L.
- Batterman et al. (1998) subtracted background levels before reporting their results, but also included the exposure-specific background (pre-exposure) concentrations in a separate table. Therefore those background levels were added back to the reported exposure-group values and treated as actual blood concentrations. Results of model fits to the Batterman et al. (1998) data are shown in Figure B-9.
- Osterloh et al. (1996) measured and reported (plotted) blood methanol in nonexposed controls (data shown in Figure B-10). The data for Osterloh et al. (1996) clearly show a time-dependent trend which is close to linear. Therefore, the endogenous methanol production rate was assumed to increase at a constant rate over time when simulating the Osterloh et al. (1996) data (both controls and methanol-exposed), with the rate of increase fit to the control data set. The results shown in Figure B-10 (solid lines) include this increase. For comparison, the thin dashed line shows results for the 200 ppm exposure if the endogenous production is assumed to be constant.



Source: Sedivec et al. (1981).

Note: Data points in lower panel represent estimated total urinary methanol elimination from humans exposed to 78 (diamonds), 157 (triangles), and 231 (circles) ppm methanol for 8 hours, and lines represent PBPK model simulations. Solid lines are model results with the saturable equation for hepatic metabolism.

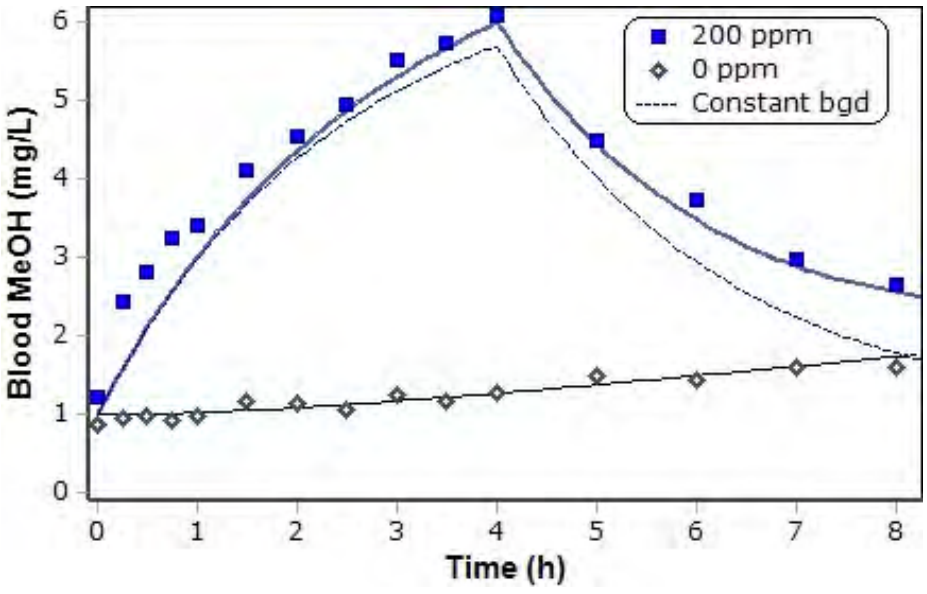
Figure B-8 Urinary methanol elimination concentration (upper panel) and cumulative amount (lower panel), following inhalation exposures to methanol in human volunteers.



Source: Batterman et al. (1998).

Note: Pre-exposure blood background levels as measured for each exposure group were used: 2.0 mg/L for 30 min group; 1.3 mg/L for 1 hr group; and 1.8 mg/L for 2 hr group.

Figure B-9 Blood methanol concentrations in subjects exposed for 30 min, 1 hr, or 2 hr at 800 ppm.

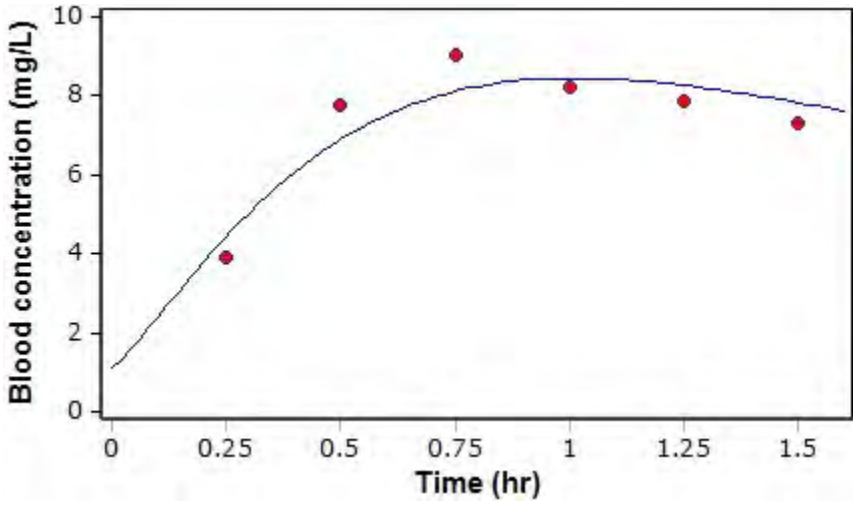


Source: Osterloh et al. (1996).

Note: Symbols are data and lines are model simulations. An initial endogenous background level was set using a constant rate of appearance of methanol in the liver, but this rate was increased linearly over time to match the non-constant level in controls (diamonds); assumed to also apply to exposed subjects (squares). Thin dashed line is a model simulation with this time-dependence turned off.

Figure B-10 Blood methanol concentrations in control (0 ppm) and methanol exposed (200 ppm) subjects.

Oral PK data from Schmutte et al. (1988) from a 10 mg/kg dose was used to set an oral bioavailability for humans and to test the assumption that human oral absorption of methanol could otherwise be described using the simple two-compartment GI model of Sultatos et al. (2004), with parameters fit by Sultatos et al. (2004) to ethanol PK data. Sultatos et al. (2004) included a rate of metabolism for ethanol in the stomach, which would reduce the systemic bioavailability of that compound from 100%. Lacking the data to fit a specific rate constant for methanol metabolism in the GI, the simulated dose was simply reduced using a bioavailability constant (Bav), but the mechanism for less than 100% availability could also be metabolism in the GI. A value of Bav = 0.79 was obtained and the simulation curve matches the data of Schmutte et al. (1988) fairly well (Figure B-11). The initial condition was set to the reported pre-exposure background by Schmutte et al. (1988) (1.1 mg/L). The model reproduces the data well, considering that only one parameter is adjusted for the oral dose route. Data were only collected for 1.5 hours: a longer sampling time would have provided a better evaluation of the model's ability to predict longer-term kinetics from oral exposures.



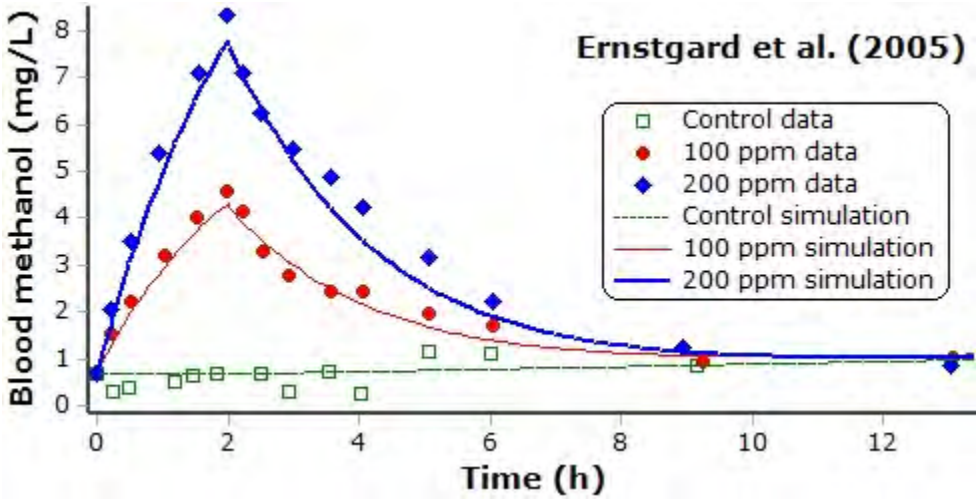
Source: Modified with permission of ; Schmutte et al. (1988)

Note: The endogenous background was set to match the reported pre-exposure blood concentration of 1.1 mg/L and the bioavailability (Bav) was calibrated to fit the data (Bav = 0.79). Otherwise the ethanol absorption parameters for ethanol from Sultatos et al. [(2004),see Table B-1] were used.

Figure B-11 Oral exposure (10 mg/kg) to methanol in human volunteers (points).

The data from Ernstgård et al. (2005) was used to assess the use of the model parameters with a data set collected under conditions of light work. Historical measures of VPR (2.023) and $Q_C C$ (26 L/hr/kg$^{0.75}$) for individuals exposed under conditions of 50 W of work from that laboratory (Ernstgård, 2005; Corley et al., 1994; Johanson et al., 1986) were used for the 2-hour exposure period (Figure B-12). Also, a linear rate of increase in the endogenous production rate

was fit to the control data set, as this set showed an increasing trend over time, like Osterloh et al. (1996), and the initial background level was set to match the observed value at time = 0 for each data set. Otherwise, there were no changes in the model parameters (no fitting to these data). The results are remarkably good, given the lack of parameter adjustment to data collected in a different laboratory, using different human subjects than those to which the model was calibrated.



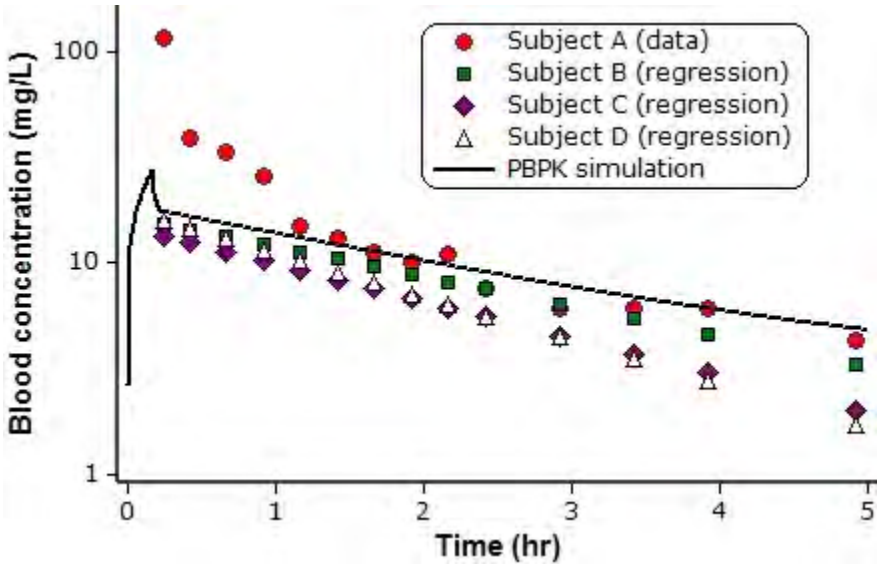
Source: Ernstgàrd et al. (2005)

Note: Data are average measured blood methanol concentrations from 4 men and 4 women exposed to 100 (98.4) ppm or 200 (192.4) ppm target (actual) methanol for 2 hours during light physical activity. Smooth lines are PBPK model simulations using actual concentrations and an estimated BW of 75 kg (see text). The initial concentration for each exposure group was matched to the measured level. A small constant rate of increase in endogenous production was calibrated to fit the control data, but otherwise model parameters were not fitted. For the first 2 hours, a VPR of 2.023 (unitless) and a Q_CC of 26 L/hr/kg$^{0.75}$ were used to match the subjects' light exercise, after which Q_CC is reduced to 15 L/hr/kg$^{0.75}$ and VPR to 1.0 (Corley et al., 1994; Johanson et al., 1986).

Figure B-12 Inhalation exposures to methanol in human volunteers.

A final set of data used for model validation is provided by Haffner et al. (1992) who observed blood kinetics in 4 volunteers after i.v. injections of 10 mg/kg methanol in a 10-minute infusion. Model simulations based on this dosing regimen, with an assumed average BW of 70 kg, are shown in Figure B-13 versus reported data for Subject A and simulated data using reported regression results for Subject B-D. Haffner et al. (1992) only showed data for the first subject, but gave exponential regression equations that they fit to the data for the other subjects. The "regression" results in Figure B-13 are calculated from regression equations provided in the paper for each subject, at the same time points as Subject A data. The model simulation during the first hour of exposure poorly matches the data, with the maximum blood level predicted to only be 27.6 mg/L versus 116 mg/L observed. After 1 hour the simulation matches the data for Subject A well, but over-predicts the regression curves for the other subjects. It is possible that the perfusion-limited PBPK model over-predicts the rate of distribution from the blood to

various body tissues and hence under-predicts blood concentrations in this time period; i.e., that distribution to body tissues is diffusion-limited, with the effect being significant at shorter times. The slope of the simulation line closely matches that of Subject B, indicating similar clearance kinetics. Subjects C and D exhibited faster elimination kinetics than predicted by the model. The authors report elimination rate constants of 0.259, 0.325, 0.406, and 0.475 hr^{-1} for Subjects A-D, respectively, so Subject D has 60% higher clearance than A.



Source: Haffner et al. (1992)

Note: Points for Subject A are actual data. Only regression parameters were reported for Subjects C-D, so simulated data were estimated from the regression results (points shown) at the same times as Subject A's data. See text for further details.

Figure B-13 Intravenous exposure (10 mg/kg) to methanol in human volunteers (points).

B.2.7. Discussion and Sensitivity Analysis of Human Model.

Horton et al. (1992) employed two sets of metabolic rate constants to describe human methanol disposition, but in vitro studies using monkey tissues with non-methanol substrates were used as justification for this approach. Although Bouchard et al. (2001) described their metabolism using Michaelis-Menten metabolism, Starr and Festa (2003) reduced that to an effective first-order equation and showed adequate fits. Perkins et al. (1995) estimated a K_m of 320 ± 1,273 mg/L (mean ± S.E.) by fitting a one-compartment model to data from a single oral poisoning to an estimated dose. In addition to the extremely high standard error, the large standard error for the associated V_{max} (93 ± 87 mg/kg/hr) indicates that the set of Michaelis-Menten constants was not uniquely identifiable using this data. Other Michaelis-Menten constants that have been used to describe methanol metabolism in various models for primates are given in Table B-3. Because the K_m calculated by Perkins et al. (1995) from the high-dose

oral exposure is 320 mg/L, while the highest observed concentration in the data sets considered here is 14 mg/L (Batterman et al., 1998), forcing the model to use this higher K_m would simply result in fits that are effectively indistinguishable from the linear model. The value obtained in this analysis, 36 mg/L, allows the model to describe the slight nonlinearity that exists in the data. For example, the peak urine concentration observed by Sedivec et al. (1981) after the 231 ppm exposure was increased 8.66 mg/L above the time zero value, while that observed after the 78 ppm exposure was 2.63 mg/L above the time zero value, so a 3-fold increase in exposure lead to a 3.3-fold increase (8.66/2.63) increase in the urinary excretion above background. It is possible that a much higher K_m pathway is also operant in humans, but is only significant at much higher concentrations than evaluated here.

Table B-3 Primate K_m values reported in the literature.

K_m (mg/L)	Reference	Note
320 ± 1,273	Perkins et al. (1995)	Human: oral poisoning, estimated dose
716 ± 489	Perkins et al. (1995)	Cynomolgus monkey: 2 g/kg dose
278	Perkins et al. (1995)	Rhesus monkey: 0.05-1 mg/kg dose
252 ± 116	Perkins et al. (1995)	Cynomolgus monkey: 1 g/kg dose
33.9	Horton et al. (1992)	PBPK model: adapted from rat K_m
0.66	Fisher et al. (2000)	PBPK model, Cynomolgus monkey:10-900 ppm
36[a]	(This analysis.)	PBPK model, human: 100-800 ppm

Note: The values from Perkins et al. (1995) are ± S.E.

[a]This K_m was optimized while also varying V_{max}, k_1C, k_{bl}, Bav, FRACIN, and parameters to fit the time-varying control data (endogenous) of Osterloh et al. (1996) (used only for simulating that study), to the full data set.

Sedivec et al. (1981) estimated a fractional uptake of 57.7%, based on total amount inhaled. Since the PBPK model uses alveolar rather than total ventilation and this is typically assumed to be 2/3 of total ventilation, one might correct this value by dividing by 2/3 to obtain a value for FRACIN of 0.8655. Ernstgård et al. (2005) also estimated a fractional uptake, 51% at 100 ppm and 49.3% at 200 ppm under light exercise. It is reasonable to expect uptake efficiency to decrease with more rapid breathing due to exercise, since an inhaled volume element of air spends less time in the respiratory tract, allowing less time for uptake, as respiration increases. Also, while Ernstgård et al. (2005) based their calculation on estimated pulmonary ventilation, they used the difference between inhaled air concentration and exhaled air concentration. Exhaled air will be a mixture of air that was taken into the pulmonary airways and air that only entered the conducting airways. Very little methanol would be absorbed from the later air and hence the mixed exhaled concentration will be higher than that which exits the pulmonary region and the resulting calculation will then under-estimate the fraction of methanol absorbed from

pulmonary air. Thus the "fraction inhaled" estimated from a given data set will depend on which flow rates and concentrations are being used in the calculation, or to which it might be applied; i.e., the value depends on the model "context" in which it is used. Therefore, EPA decided to fit FRACIN with the other parameters estimated in the context of the PBPK model used here, as was done for the rat, and obtained a value of 0.75. This indicates that the concentration entering the pulmonary space is reduced by 25% due to deposition in the conducting airways (with that material assumed to desorb on exhalation), and is not the fraction removed in the pulmonary space. At 200 ppm, for example, the model predicts that 99.9976% of the methanol entering the pulmonary region is absorbed. The value is slightly less than estimated for the rat (rat FRACIN = 81%) which seems reasonable since the larger human airways would reduce uptake efficiency somewhat. Assuming that 2/3 of inhaled air goes to the pulmonary region, the total rate of inhalation would be $1.5*Q_P*CONC$ (rate of inhalation through nose and mouth at air concentration CONC), and the amount removed in the pulmonary region roughly $0.75*Q_P*CONC$ (using FRACIN = 0.75), so the fraction of each breath absorbed is predicted by the model to be:

$$(0.75*Q_P*CONC)/\ (1.5*Q_P*CONC) = 50\%,$$

which closely matches the estimates of Ernstgård et al. (2005). Considering that EPA did not fit FRACIN to the Ernstgård et al. data, this appears to be a good validation of the value obtained for this parameter.

Considering the model simulations versus the data of Haffner et al. (1992) (Figure B-13), it is first evident that the model is not capturing the short-term kinetics shown for Subject A. Since Haffner et al. (1992) did not indicate that the data for this subject were discrepant from the other subjects in the first hour it is assumed that these data represent human distribution, hence that the model does not describe well what happens immediately after such an exposure. Given that i.v. exposures are not a route for which risk is estimated, model failure is not considered critical here; however, it does suggest an area for future research and model improvement. The model does track the longer-term clearance data for Subject A quite well. Since the model is intended to represent an average adult human, it is also not alarming that it does not match so well the individually-fitted clearance curves for Subjects B-D, which indicates a range of human variability. In particular the results for those other subjects indicate that some people will clear methanol more quickly than predicted by the model, which means that the model will somewhat over-estimate internal doses and health effects for those individuals. Since other data to which the model is fit are averages among individuals, and the model does not show a strong bias with regard to those data (Figures B-8 to B-11), neither does it appear that the model is systematically under-predicting clearance for most of the population. Therefore, the model predictions are expected to provide reasonably good estimates of average adult human methanol PK under long-

term exposure scenarios. Caution is suggested, though, in potential use of the model to estimate internal doses shortly after accidental exposures.

A sensitivity analysis for human model predictions to the primary fitted parameters was conducted for continuous inhalation exposures, and results are shown in Table B-4. Normalized sensitivity coefficients are calculated using the method described for the rat (see B.2.4). To bracket the range of likely concern for human exposures, inhalation sensitivities were evaluated at 10 and 200 ppm concentration. The bladder time constant, k_{bl}, was not included in the analysis since it has no influence on blood concentrations. The resulting coefficients (Table B-4) are not surprising. V_{maxC} and K_m both strongly influence model predictions. At these exposure levels the urinary pathway (k_1C) has little effect on blood level. There is essentially a 1:1 correspondence with FRACIN, which follows from the fact that close to 100% of what enters the gas-exchange compartment is absorbed. That all of the sensitivities are slightly higher at 200 ppm than at 10 ppm is due to the slight metabolic saturation.

Table B-4 Human PBPK model sensitivity analysis for steady-state inhalation exposure.

	Exposure level[a]	
Parameter	**10 ppm**	**200 ppm**
V_{maxC}	-0.81	-0.93
K_m	0.74	0.75
k_1C	-0.0024	-0.0029
FRACIN	1.00	1.11

[a]Normalized sensitivity coefficients for steady-state blood levels (increase above background) at the indicated concentrations.

For oral exposures ingestion is assumed to occur in a series of six boluses over the course of the day, with the fraction of the total daily dose and respective times ingested being: 25% at 7 a.m., 10% at 10 a.m., 25% at 12 p.m., 10% at 3 p.m., 25% at 6 p.m., and 5% at 9 p.m. The pattern is meant to be representative of human ingestion patterns, recognizing that this will vary among the population. The impact of changing the pattern on estimated AUC values is fairly small, since the total ingestion remains the same. However the pattern will clearly influence the peak concentration, since an assumption of ingestion in a single bolus would lead to the highest predicted daily peak, while assumption of continuous ingestion would lead to the minimal peak possible, for a given total daily exposure. With the pattern used here, the blood concentration profile predicted at 10 mg/kg-day is shown in Figure B-14 (time is in hours from first bolus). In particular, the model predicts a following peak to occur ~45-55 minutes after each bolus (depending on dose size), with the overall daily peak occurring just before 1 p.m. (~6 and 30 hr time-points in Figure B-14). While the boluses assumed to be ingested at 7 a.m. and 12 p.m. are both 25%, because methanol is predicted to accumulate somewhat over the morning, the later

bolus leads to a peak that is roughly 30% higher than the first of the day. At this exposure level there is a very small residual blood level at 24 hr, about 1% of the mid-day peak. At higher exposure levels more significant day-to-day accumulation would be predicted until a state of "periodicity" is reached, when the day-to-day pattern no longer changes. For example, at 200 mg/kg-day the blood level just prior to the next day's ingestion is predicted to approach 3% of the daily peak (~80 mg/L). At 500 mg/kg-day, the model predicts that it will take about 2 weeks to reach periodicity, where the peak during the first day is ~350 mg/L, but this increases to 740 mg/L after two weeks, and the end-of-day minimum is 460 mg/L.

The model sensitivities to the key fitted parameters at 0.2 and 10 mg/kg-day under this oral exposure scenario are listed in Table B-5. As with the inhalation sensitivity analysis, these exposure levels are selected to bracket the range of primary concern for this assessment. The results are qualitatively the same as for inhalation exposure (Table B-4), with oral bioavailability (Bav) having an effect essentially identical to that of FRACIN for inhalation. The metabolic parameters have slightly less impact for these exposure levels; probably due to blood-flow and oral-absorption limitation, and the increase in sensitivity from 0.2 to 10 mg/L is not as large as in going from 10 to 200 ppm inhalation concentrations.

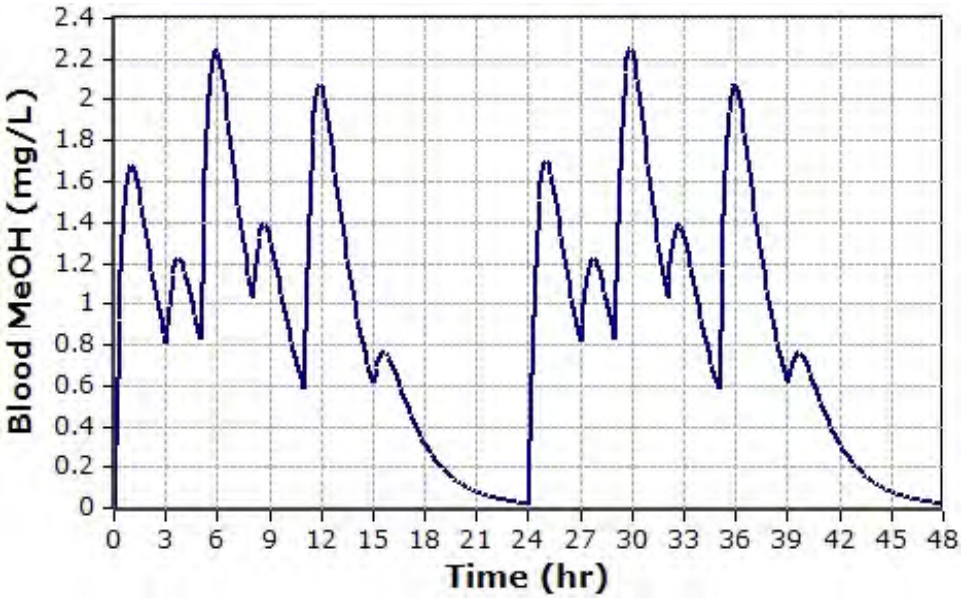


Figure B-14 Predicted human blood concentrations (increase above background) from total daily exposures to 10 mg/kg-day methanol, consumed in a series of 6 boluses. Time is from the first bolus of the day. See text for further details.

Table B-5 PBPK model sensitivity analysis for oral exposure.

	Exposure level, metric[a]			
	0.2 mg/kg-day		10 mg/kg-day	
Parameter	C_{max}	AUC	C_{max}	AUC
V_{maxC}	-0.67	-0.98	-0.68	-1.01
K_m	0.62	0.91	0.59	0.89
k_1C	-0.0014	-0.0024	-0.0015	-0.0025
Bav	1.00	1.00	1.03	1.04

[a]Normalized sensitivity coefficients for methanol blood levels at the indicated oral exposure rates. Human oral exposures are assumed to occur in a series of boluses, with a blood concentration profile as shown in Figure B-14. See text for further details.

Considering the multiple data sets used for human model calibration and validation, there is fairly high confidence in the fitted metabolic/clearance parameters, V_{maxC}, K_m, and k_1C. Since the pharmacokinetics are mostly linear in the range of interest, it is really V_{maxC}/K_m that is the critical determinant of predicted internal doses, but as that is equally true of the model fits to the data, this does not decrease confidence in model predictions. Where more uncertainty and concern exists is with the oral bioavailability, since it is only estimated from a small data set [4 individuals; (Schmutte et al., 1988)], with measurements only extending to 90 minutes after ingestion. However, bioavailability can be no more than 100%, a 25% increase over the fitted value (79%). Hence any under-prediction of human dosimetry after oral exposure should be no greater than that factor, well within the general variability and uncertainty expected for human dosimetry (for which the UF_H of 10 is used).

B.2.8. Inhalation Route HECs and Oral Route HEDs

The atmospheric methanol concentration resulting in a human daily blood methanol AUC (hr × mg/L) or C_{max} (mg/L) equal to that occurring in experimental animals following exposure at the POD concentration is termed the HEC. Similarly, the oral dose (rate) resulting in human daily blood methanol AUC (hr × mg/L) equivalent to that occurring in an experimental animal at the POD concentration is termed the HED. For humans these estimates are made using long-term exposure patterns, after a steady-state is reached from continuous inhalation exposures, or otherwise there is no longer a variation from day-to-day in the blood concentration profile, given an assumed consistent exposure pattern, as indicated in Figure B-14. Internal concentration PODs in mice were estimated by BMD analysis applied to measured (peak) blood concentrations (C_{max} values), as described in Section 5. For the rat, internal C_{max} and AUC values were estimated using the rat PBPK model as described in B.2.5 for bioassay exposures prior to BMD analysis.

To estimate the HEC for specific blood methanol C_{max} and 24-hour AUC values, continuous 1,000-hour exposures were simulated, to assure steady state was achieved, for which the human C_{max} was the steady state blood methanol concentration (C_{ss}) so predicted and the AUC calculated from the last 24 hours of that period. (AUC = 24*C_{ss}.). For oral exposure, the daily ingestion pattern described in B.2.6.1 was used, simulations were again run for 1,000 hours, the C_{max} selected as the maximum achieved over the resulting time-course, and the AUC calculated over the last 24 hours. Results for selected exposure levels are given in Table B-6.

While the PBPK computational code was used to derive the HECs and HEDs used in this assessment, using a computational script that will be described below, an alternative approach was developed that provides an initial approximation, which also allows non-PBPK model users to estimate methanol HECs and HEDs from BMDs in the form of C_{max} (or C_{ss}) and AUC values. This approach uses algebraic equations describing the relationship between predicted methanol C_{max} or 24-hour AUC and the inhalation exposure level (i.e., an HEC in ppm) (Equations 1 or 2 below) or oral exposure rate (i.e., an HED in mg/kg-day) (Equations 3 or 4 below). The equations were derived by generating tables of exposure-dose values like Table B-6, but with more entries to define the relationship, then selecting and fitting equations to interpolate among the simulated points from that table. The resulting approximations match the exact PBPK model results to within a few percent. To use the equations to derive an HEC or HED, the target human C_{max} or AUC is simply plugged into to the appropriate equation.

Table B-6 PBPK model predicted C_{max} (C_{ss}) and 24-hour AUC for humans exposed to Methanol.

Inhalation exposure[a]			Oral exposure[a]		
Concentration (ppm)	AUC (mg-hr/L)	$C_{max} = C_{ss}$ (mg/L)	Dose (mg/kg-day)	AUC (mg-hr/L)	C_{max} (mg/L)
1	0.65	0.03	0.1	0.21	0.02
5	3.27	0.14	1	2.14	0.22
10	6.56	0.27	10	22.2	2.27
50	33.5	1.39	50	130	12.9
100	68.7	2.86	100	320	29.9
200	145	6.04	200	984	81.1
500	437	18.2	500	15,000	751
1,000	1,380	57.3	1,000	80,600	3,610
2,000	15,400	639	2,000	216,000	9,520
5,000	115,000	4,810	5,000	625,000	27,300

[a]Values are increases above background, with an assumed endogenous background of 1.5 mg/L. For example, at 10 ppm inhalation, the total blood steady-state concentration is predicted to be 1.5 + 0.27 = 1.77 mg/L. Human simulation results are considered uncertain above 500 ppm (inhalation) or 50 mg/kg-day (oral), since the blood levels predicted rise above those for which there are calibration data at higher exposures.

$$HEC(ppm) = 0.554 \times Css + \frac{1734 \times Css}{45.73 + Css} \qquad \text{Equation 1}$$

$$HEC(ppm) = 0.02308 \times AUC + \frac{1734 \times AUC}{1098 + AUC} \qquad \text{Equation 2}$$

$$HED(\text{mg/kg-day}) = 0.1904 \times C\max + \frac{440.4 \times C\max}{109.9 + C\max} \qquad \text{Equation 3}$$

$$HED(\text{mg/kg-day}) = 0.007257 \times AUC + \frac{419.0 \times AUC}{1098 + AUC} \qquad \text{Equation 4}$$

In Equations 1-4 above, AUC, C_{ss}, and C_{max} are above endogenous background. The endogenous background blood concentration (C_{max} or C_{ss}) was set to 1.5 mg/L, so the endogenous background AUC = 1.5 (mg/L)×24 (hr) = 36 mg-hr/L. So to identify an HEC or HED that lead to a total daily AUC of 50 mg-hr/L, for example, one would then plug 50 – 36 = 14 mg-hr/L into Equation 2 or 4.

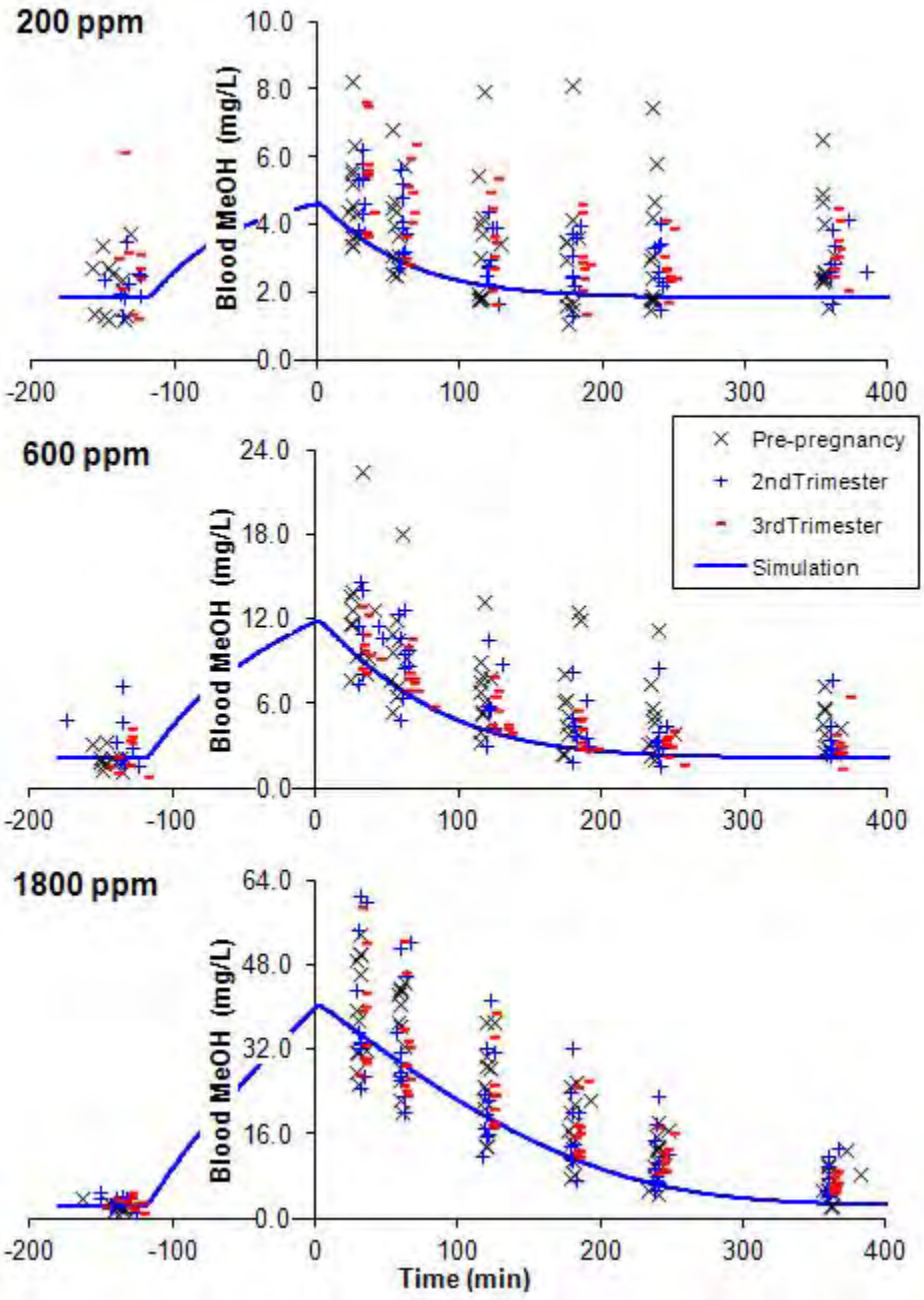
While the preceding equations approximate the PBPK model fairly well, an exact solution is preferred if the full PBPK model can be run. For example, for C_{max} = 20 mg/L (above

background), Equation (1) estimates HEC = 538.6 ppm, but running the PBPK model at this exposure level predicts a (peak) blood level of 20.2 mg/L. An exact HEC (to 4 significant figures) is 535.6 ppm. Two .m file scripts were created as part of the acslX PBPK model workspace for methanol, which calculate HEC and HED values through a simple search algorithm (U.S. EPA, 2012b). These were used to generate all of the HEC and HED values reported in Section 5 of this assessment.

B.3. Monkey PK Data and Model Analysis

In order to estimate internal doses (blood C_{max} values) for the monkey health-effects study of Burbacher et al. (1999b) and further elucidate the potential differences in methanol pharmacokinetics between NP and pregnant individuals (2nd and 3rd trimester), a focused reanalysis of the data of Burbacher et al. (1999a) was performed. The monkeys in this study were exposed for 2.5 hours/day, with the methanol concentration raised to approximately the target concentration for the first 2 hours of each exposure and the last 30 minutes providing a chamber "wash-out" period, when the exposure chamber concentration was allowed to drop to 0. Blood samples were taken and analyzed for methanol concentration at 30 minutes, 1, 2, 3, 4, and 6 hours after removal from the chamber (or 1, 1.5, 2.5, 3.5, 4.5, and 6.5 hours after the end of active exposure). These data were analyzed to compare the PK in NP versus pregnant animals, and fitted with a simple PK model to estimate blood C_{max} values above background for each exposure level. Dr. Burbacher graciously provided the original data, which were used in this analysis.

Two cohorts of monkeys were examined, but the data (plots) did not indicate a systematic difference between the two, so the data from the two cohorts were combined. The data from the scatter plots of Burbacher et al. (1999a) for the NP (pre-pregnancy), first pregnancy (2nd trimester), and second pregnancy (3rd trimester) studies are compared in Figure B-15, along with model simulations (explained below). Since the pregnancy time points were from animals that had been previously exposed for 87 days plus the duration of pregnancy to that time point, the pre-exposed NP animals were used for comparison, rather than naïve animals, with the expectation that effects due to changes in enzyme expression (i.e., induction) from the subchronic exposure would not be a distinguishing factor. Note that each exposure group included a pre-exposure baseline or background measurement, also shown. To aid in distinguishing the data visually, the NP data are plotted at times 5 minutes prior to the actual blood draws and the 3rd trimester at 5 minutes after each blood draw.



Source: Reprinted with permission of Health Effects Institute, Boston, MA; Burbacher et al. (1999a).

Note: NP and 3rd trimester data are plotted, respectively, at 5 minutes before and after actual collection times to facilitate comparison. Solid line is from simple PK model, fit to 2nd trimester data only.

Figure B-15 Blood methanol concentration data from NP and pregnant monkeys.

To analyze and integrate the PK data of Burbacher et al. (1999a), the one-compartment model used by Burbacher et al. (1999b) and Burbacher et al. (1999a) was extended by the addition of a chamber compartment to capture the kinetics of concentration change in the exposure chamber, as shown in Figure B-16. The data in Figure B-16 [digitized from Figure 5 of

Burbacher et al. (1999a)] show an exponential rise to and fall from the approximate target concentration during the exposure period. The use of a single-compartment model for the chamber allows this dynamic exposure period to be captured, so that the full concentration-time course is used in simulating the monkey internal concentration rather than an approximate step function (i.e., rather than assuming an instantaneous rise and fall). The pair of equations representing the time-course in the chamber and monkey are as follows (bolded parameters are fit to data):

$$\text{Chamber: } dC_{ch}/dt = [(C_{CM} \bullet S - C_{ch}) \bullet F_{ch} - R_{inh}]/V_{ch}$$

$$\text{Monkey: } dC_{mk}/dt = [R_{inh} - V_{max} \bullet C_{mk}/(K_m + C_{mk})]/(V_{mk} \bullet BW)$$

$$\text{with } R_{inh} = C_{ch} \bullet R_C (1{,}000 \cdot BW)^{0.74} \bullet F \text{ and } C_{net} = C_{mk} + C_{bg}.$$

d: delta, change

C_{ch}: instantaneous chamber concentration (mg/L)

t: time (hour)

C_{CM}: chamber in-flow methanol concentration (mg/L), which was set to the concentrations corresponding to those reported in Table 2 of Burbacher et al. (1999a), using the "Breeding" column for the NP (87 days pre-exposed; values in Table B-7)

S: exposure switch, set to 1 when exposure is on (first 2 hours) and 0 when off

F_{ch}: chamber air-flow, 25,200 L/hr, as specified by Burbacher et al. (2004a) and Burbacher et al. (2004b)

R_{inh}: net rate of methanol inhalation by the monkeys (mg/hr)

V_{ch} (1,220 L): chamber volume, initially set to 1,380 L ("accessible volume" stated by Burbacher et al. (2004a) and Burbacher et al. (2004b), but allowed to vary below that value to account for volume taken by equipment, monkey, and to empirically fit the mixing time to the observed data (Figure B-16).

C_{mk}: instantaneous inhalation-induced monkey blood methanol concentration (mg/L); this is added to the measured background/endogenous concentration before comparison to data

V_{max} (32.5 mg/hr): fitted (nonscaled) Michaelis-Menten maximum elimination rate

K_m (14.4 mg/L): fitted (nonscaled) Michaelis-Menten saturation constant

V_{mk} (0.623 L/kg): fitted volume of distribution for monkey

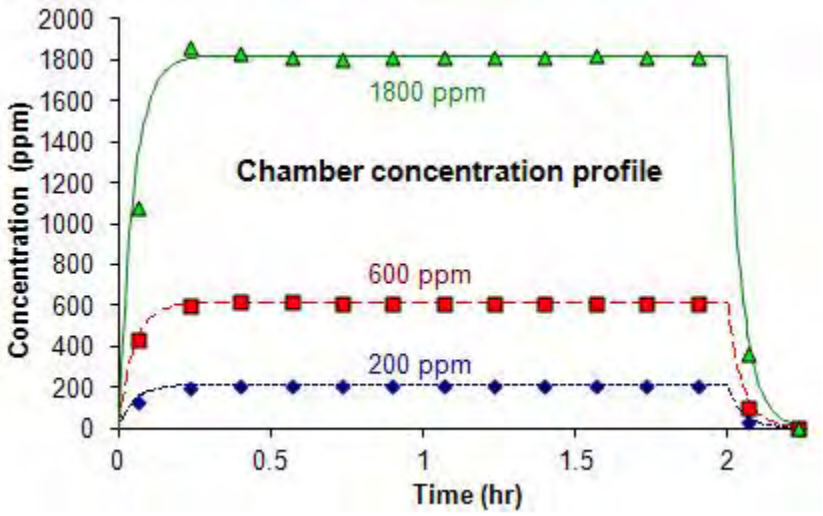
BW: monkey body weight (kg); for NP monkeys set to group average values in data of Burbacher et al. (1999a) and Burbacher et al. (1999b)

R_c: allometric scaling factor for total monkey respiration (0.12 L/hours/$g^{0.74}$ = 2 mL/minute/$g^{0.74}$), as used by Burbacher et al. (1999a; 1999b) (note that scaling is to BW in g, not kg)

F: fractional absorption of inhaled methanol; set to 0.5 (50%), 2/3 the value fitted for humans using the human PBPK model (see Appendix B, Section B.2); for the monkey F and V_{mk} cannot be uniquely identified, given the model structure; since the monkey model uses total ventilation (defined by R_c) as the driver, while the human model uses alveolar ventilation which is assumed to be 2/3 of total ventilation, F was set to 2/3 the human value to obtain a realistic estimate of V_{mk}

C_{net}: net blood concentration, equal to sum of the inhalation-induced concentration (C_{mk}) and the background blood level (C_{bg}) (mg/L)

C_{bg}: background (endogenous) methanol concentration, set to the pre-exposure group-specific mean from the data of Burbacher et al. (1999a) and Burbacher et al. (1999b)



Source: Reprinted with permission of the Health Effects Institute, Boston, MA; Burbacher et al. (1999a).

Note: Lines are model simulations. Indicated concentrations are target concentrations; measured concentrations differed slightly (see Table 3-9).

Figure B-16 Chamber concentration profiles for monkey methanol exposures.

The model was specifically fit to the 2nd trimester monkey data, assuming that the parameters were the same for all the exposure groups and concentrations. While the data show little difference between the NP and two pregnancy groups, the 2nd trimester group was presumed to be most representative of the average internal dosimetry over the entire pregnancy. Further, the results of Mooney and Miller (2001) show that developmental effects on the monkey brain stem following ethanol exposure are essentially identical for monkeys exposed only during early pregnancy versus full-term, indicating that early pregnancy is a primary window of vulnerability.

Model simulation results are the lines shown in Figures B-15 and B-16. The model provides a good fit to the monkey blood and chamber air concentration data. The chamber volume was treated as a fitted parameter, decreasing the "accessible volume" of 1,380 L, provided by Burbacher et al. (1999a) to 1,220 L, which calibrated the mixing time in the chamber to match the chamber concentration data (Figure B-16). An adjustment of the "accessible volume" also accounts for any volume filled by the monkey and other chamber equipment. A detailed description of the chamber set-up is found in Burbacher et al. (1999a). The model does an adequate job of fitting the data for all exposure groups without group-specific parameters. In particular, the data for all exposure levels can be adequately fit using a single value for the volume of distribution (V_{mk}) as well as each of the metabolic parameters. While one may be able to show statistically distinct parameters for different groups or exposure levels (by fitting the model separately to each), as was done by Burbacher et al. (1999a), it is unlikely that such differences are biologically significant, given the fairly large number of data points and the

large variability evident in the blood concentration data. Thus, the single set of parameters listed with the parameter descriptions above will be used to estimate internal blood concentrations (C_{max} above background) for the dose-response analysis described in Appendix D. The chamber concentrations for "pregnancy" exposures recorded by Burbacher et al. (1999a: Table 2) and average body weights for each exposure group at the 2nd trimester time point were used along with the model to calculate C_{max} values above background (Table B-7).

Table B-7 Monkey group exposure characteristics for Burbacher et al. (1999a).

Exposure concentration (ppm)[a]	Group average BW (kg)[b]	C_{max} above background (mg/L)[c]
0	3.93	0
206	3.46	2.87
610	4.08	10.38
1,822	3.83	38.51

[a]Reprinted with the permission of the Health Effects Institute, Boston, MA; from Burbacher et al. (1999a) and Burbacher et al. [(1999b), Table 2, "pregnancy" exposure.]

[b]From Burbacher, original data (personal communication).

[c]The two-compartment PK model described above was used to estimate C_{max} above background [i.e., max (C_{mk})].

Model simulations were also conducted to predict internal doses for the NEDO (1987) monkey studies. Specifically, simulations were conducted for 21 h/d exposures to 10, 100, and 1,000 ppm methanol with an average animal BW of 2.2 kg. Exposures were simulated for 7 days to assure that periodicity had been reached, and internal metrics calculated for the 7th day. Visual inspection of simulated blood levels indicated that periodicity was in fact attained by the 2nd or 3rd day. Results are provided in Table B-8.

Table B-8 Monkey group exposure characteristics ofr NEDO (1987).[a]

Exposure concentration (ppm)	$C_{average}$ above background (mg/L)[b]	C_{max} above background (mg/L)[c]
10	0.09	0.11
100	0.97	1.11
1,000	17.9	21.5

[a]Metrics calculated for 2.2 kg BW animals exposed for 21 h/d, on the 7th day of simulated exposure.
[b]Net (total) blood concentration averaged over 24 hours minus the background level of 2 mg/L.
[c]Peak blood concentration minus the background level of 2 mg/L.

B.4. Conclusions and Discussion

Rat and human methanol PBPK models have been developed and calibrated to data in the open literature. EPA developed its own model because none of the existing models satisfactorily fulfilled all of the criteria specified in Section 3.4.1.2. Further, none of the existing models had been calibrated or tested against the larger collection of data considered for each species here. As a result, while each model may fit the subset of the data to which it had been calibrated better than the final model described here, without adjustment of parameters from those published, each model either had features which made it incompatible with risk extrapolation (e.g., parameters which vary with dose in an unpredictable way) or had an inadequate fit to other data considered critical for establishing overall model soundness. The EPA model simplifies the structure used by Ward et al. (1997) in some aspects while adding specific refinements (e.g., a standard lung compartment and a two-compartment GI tract).

Although the developmental endpoints of concern are effects, which result from in utero and (to a lesser extent) lactational exposure, it is not necessary for a methanol PBPK model to specifically describe pregnancy (i.e., specify a fetal/gestational/conceptus compartment) and lactation in order for it to provide better cross-species extrapolation of risk than default methods. Representation of the unique physiology of pregnancy and the fetus/conceptus would be necessary if methanol pharmacokinetics differed significantly during pregnancy or if the observed partitioning of methanol into the fetus/conceptus versus the mother showed a concentration ratio significantly greater than or less than 1. Further details on the reasoning for not including a pregnancy description are given in Section 3.4.1.2.

While lactational exposure is less direct than fetal exposure and blood or target-tissue levels in the breast-feeding infant or rat pup are likely to differ more from maternal levels, the health-effects data indicate that most of the effects of concern are due to fetal exposure, with only a small influence due to postnatal exposures. Separating out the contribution of postnatal exposure from prenatal exposure to a given endpoint in a way that would allow the risk to be estimated from estimates of both exposure levels would be extremely difficult, even if one had a lactation/child PBPK model that allowed for prediction of blood (or target-tissue) levels in the offspring. Target tissue concentrations in the offspring would still be expected to be closely related to maternal blood levels (which depend on ambient exposure and determine the amount delivered through breast milk), with the relationship between maternal levels and those in the offspring being similar across species.

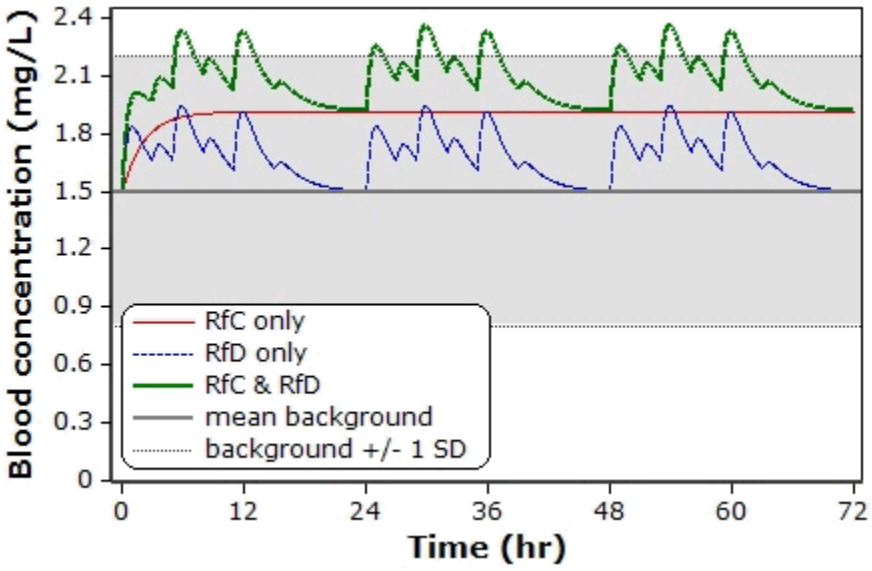
Therefore, the development of a lactation/child PBPK model appears not to be supported, given the minimal change that is likely to result in risk extrapolations and use of (NP) maternal blood levels as a measure of risk in the offspring is still considered preferable over use of default extrapolation methods. In particular, the existing human data allow for accurate predictions of

maternal blood levels, which depend strongly on the rate of maternal methanol clearance. Failing to use the existing data (via PBPK modeling) for human methanol clearance (versus that in other species) would be to ignore this very important determinant of exposure to breast-fed infants. And since bottle-fed infants do *not* receive methanol from their mothers, they are expected to have lower or, at most, similar overall exposures for a given ambient concentration than the breast-fed infant, so that use of maternal blood levels for risk estimation should also be adequately protective for that group.

During model development, several inconsistencies between experimental blood methanol kinetic data embedded in the Ward et al. (1995) model and the published figures first reporting these data were discovered. Therefore, data were digitized from the published literature when a figure was available, and the digitized data was compared to the provided data. When the digitized data and the data embedded in the computational files (i.e., provided to Battelle under contract from EPA) were within 3% of each other, the provided data was used; when the difference was greater than 3%, the digitized data was used. Often, using the published figures as a data source resulted in substantial improvements of the fit to the data in the cases where the published figures were different from the embedded data.

The final methanol PBPK model fits inhalation-route blood kinetic data from separate laboratories in rats and humans fairly well. The low-dose exposures of all routes were considered the most important for model optimization, since these doses are most relevant to a health assessment.

Figure B-17 illustrates the changes in blood methanol concentrations predicted by the human PBPK model for exposures to either the RfC or RfD alone, or a combined exposure to both the RfC and RfD, with oral exposure assumed divided into six daily boluses as described previously (Section B.2.7) and inhalation exposure assumed to be continuous. The predictions are shown for an individual starting with an average background level of 1.5 mg/L blood methanol, relative to one standard deviation of the background (grey area).



Note: The horizontal grey lines and band show the mean background blood concentration (1.5 mg/L) ± one standard deviation (1 SD; 0.7 mg/L). The thin, solid, red curve shows the predicted change in blood concentration given a continuous exposure to the RfC alone, simply rising over ~10 hr to a new steady state at 1.91 mg/L. The thin, dashed, blue curve shows the predicted change given ongoing exposure to the RfD, with ingestion divided among six daily boluses (see Section B.2.7 for details), with a resulting daily pattern which has a peak concentration of 1.94 mg/L (differs slightly from the RfC due to round-off) and average level of 1.68 mg/L. The thick, solid, green curve (upper most) shows the predicted change due to simultaneous exposure to both the RfD (six daily boluses) and RfC (continuous), with a peak predicted concentration of 2.36 mg/L and average concentration of 2.09 mg/L.

Figure B-17 PBPK model predictions of changes in blood methanol levels in humans for exposures at the RfC and RfD.

APPENDIX C. HUMAN CASE STUDIES

An extensive library of case reports has documented the consequences of acute accidental/intentional methanol poisoning. Nearly all have involved ingestion, but a few have involved percutaneous and/or inhalation exposure. As discussed in Section 4.1.1, the CNS damage seen in acute overdose exposures is most likely from acidosis and not from methanol per se. As many of the case reports demonstrate, the association of Parkinson-like symptoms with methanol poisoning is related to the observation that lesions in the putamen are a common feature both in Parkinson's disease and methanol overexposure. A brief discussion of the terms cited in case report literature follows.

Basal ganglia, a group of interconnected subcortical nuclei in each cerebral hemisphere, refers to various structures in the grey matter of the brain that are intimately involved, for example, in coordinating motor function, maintaining ocular and respiratory function, and consciousness. The connectivity within the basal ganglia involves both excitatory and inhibitory neurotransmitters such as dopamine (associated with Parkinson's disease when production is deficient).

The structures comprising the basal ganglia include but are not limited to: the putamen and the globus pallidus (together termed the lentiform nuclei), the pontine tegmentum, and the caudate nuclei. Dystonia or involuntary muscle contraction can result from lesions in the putamina; if there are concomitant lesions in the globus pallidus, Parkinsonism can result (Bhatia and Marsden, 1994). Bhatia and Marsden (1994) have discussed the various behavioral and motor consequences of focal lesions of the basal ganglia from 240 case-study reports. Lesions in the subcortical white matter adjacent to the basal ganglia often occur as well (Airas et al., 2008; Rubinstein et al., 1995; Bhatia and Marsden, 1994). In the case reports of Patankar et al. (1999), it was noted that the severity and extent of necrosis in the lentiform nuclei do not necessarily correlate with clinical outcome.

In one of the earliest reviews of methanol overexposure, Bennett et al. (1953) described a mass accidental poisoning when 323 persons, ranging in age from 10 to 78 years, in Atlanta, Georgia, consumed "whisky" adulterated with as much as 35–40% methanol. In all, 41 people died. Of the 323 individuals, 115 were determined to be acidotic with symptoms (visual impairment, headache [affecting ~62%], dizziness [affecting ~30%], nausea, abdominal pain and others) beginning around 24 hours post exposure. Visual impairment was mostly characterized by blurred or indistinct vision; some who were not acidotic experienced transient visual disturbances. The cardiovascular parameters were unremarkable. The importance of acidosis to outcome is shown in Table C-1. Among the key pathological features were cerebral edema, lung

congestion, gastritis, pancreatic necrosis, fatty liver, epicardial hemorrhages, and congestion of abdominal viscera.

Table C-1 Mortality rate for subjects exposed to methanol-tainted whisky in relation to their level of acidosis.

Subjects[a]	Number	Percent deaths
All patients	323	6.2
Acidotic (CO_2 <20 mEq)	115	19
Severely acidotic (CO_2 <10 mEq)	30	50

[a]These data do not include those who died outside the hospital or who were moribund on arrival.

Source: Reprinted with permission of Lippincott, Williams & Wilkins; Bennett et al. (1953).

Riegel and Wolf (1966), in a case report involving a 60-year-old woman who ingested methanol, noted that nausea and dizziness occurred within 30 minutes of ingestion. She subsequently passed out and remained unconscious for 3 days. Upon awakening she had paralysis of the vocal cords and was clinically blind in one eye after 4 months. Some aspects of Parkinson-like symptoms were evident. There was a pronounced hypokinesia with a mask-like face resembling a severe state of Parkinson's disease. The patient had difficulty walking and could only make right turns with difficulty. There was no memory loss.

Treatment of a 13-year-old girl who ingested an unspecified amount of a windshield-washer solution containing 60% methanol was described by Guggenheim et al. (1971). She displayed profound acidosis; her vital signs, once she was treated for acidosis, were normal by 36 hours after hospital admission. During the ensuing 6 months after discharge from the hospital, visual acuity (20/400, both eyes) worsened, and she experienced muscle tremors, arm pain, and difficulty in walking. A regimen of levodopa treatment greatly improved her ability to function normally.

Ley and Gali (1983) also noted symptoms that are Parkinson like following methanol intoxication. In this case report respiratory support was needed; the woman was in a coma. Once stabilized, she exhibited symptoms similar to those noted in other case study reports, such as blurred vision, movement difficulty, and tremors. Computerized Axial Tomography scan findings highlighted the central nervous system (CNS) as an important site for methanol poisoning.

Rubinstein et al. (1995) presented evidence that a methanol blood level of 360 mg/L is associated with a suite of CNS and ocular deficits that led to a 36-year-old man (who subsequently died) becoming comatose. CT scans at 1-2 days following ingestion were normal. However, MRI scans at day 4 revealed lesions in the putamen and peripheral white matter of the cerebral and cerebellar hemispheres. Bilateral cerebellar cortical lesions had been reported in an earlier case of methanol poisoning by Chen et al. (1991).

Finkelstein and Vardi (2002) reported that long-term inhalation exposure of a woman scientist to methanol without acute intoxication resulted in a suite of delayed neurotoxic symptoms (e.g., hand tremor, dystonia, bradykinesia, and other decrements in body movement). Despite treatment with levodopa, an increase in the frequency and severity of effects occurred. Exposure to bromine fumes was concomitant with exposure to methanol.

Hantson et al. (1997b) found, in four cases, that MRI and brain CT scans were important tools in revealing specific brain lesions (e.g., in the putamina and white matter). The first subject was a 57-year-old woman who complained of blurred vision, diplopia, and weakness 24 hours after ingesting 250 mL of a methanolic antifreeze solution. Upon hospital admission she was comatose and in severe metabolic acidosis. An MRI scan at 9 days indicated abnormal hyperintense foci in the putamina (decreased in size by day 23) and subtle lesions (no change by day 23) in the white matter. Upon her discharge, bilateral deficits in visual acuity and color discrimination persisted.

Similar deficits (metabolic acidosis, visual acuity, and color discrimination) were seen in a man who ingested 300 mL of 75% methanol solution. His blood methanol level was1,630 mg/L. An MRI administered 24 hours after hospital admission revealed abnormal hyperintense foci in the putamina, with less intense lesions in the white matter. Like the first subject, a subsequent MRI indicated the foci decreased in size over time, but visual impairments persisted.

The third individual, a male, ingested an unspecified amount of a methanolic solution. His blood methanol level was 12,900 mg/L, and he was in a coma upon hospital admission. An MRI revealed lesions in the putamina and occipital subcortical white matter. A follow-up CT scan was performed after 1 year and showed regression of the putaminal lesions but no change in the occipital lesions. Upon his discharge, severe visual impairment remained but no extrapyramidal signs were observed.

The last case was a man who became comatose 12 hours after ingesting 100 mL methanol. His blood methanol level at that time was 600 mg/L. An MRI revealed lesions in the putamina; at 3 weeks these lesions were observed to have decreased in size. Upon his discharge, the neurological signs had improved but optic neuropathy (in visual evoked potential) was observed.

In a separate publication, Hantson et al. (1997a) reported a case of a 26-year-old woman who had ingested 250–500 mL methanol during the 38th week of pregnancy. Her initial blood methanol level was 2,300 mg/L (formate was 336 mg/L), yet only a mild metabolic acidosis was indicated. No distress to the fetus was observed upon gynecologic examination. Six days after therapy was initiated (methanol was not present in blood), she gave birth. No further complications with either the mother or newborn were noted.

There have been several case reports involving infant or toddler exposures to methanol (De Brabander et al., 2005; Wu et al., 1995; Brent et al., 1991; Kahn and Blum, 1979). The report by Wu et al. (1995) involved a 5-week-old infant with moderate metabolic acidosis and a serum methanol level of 11,480 mg/L, a level that is ordinarily fatal. However, this infant exhibited no toxic signs and survived without any apparent permanent problems. De Brabander et al. (2005) reported the case of a 3-year-old boy who ingested an unknown amount of pure methanol; at 3 hours after ingestion, the blood methanol level was almost 300 mg/L. Ethanol infusion as a therapeutic measure was not well tolerated; at 8 hours after ingestion, fomepizole (4-methylpyrazole) was administered to inhibit the metabolism of methanol by ADH1, and blood methanol levels stabilized below 200 mg/L, a level above which is considered to be toxic by the American Academy of Clinical Toxicology (Barceloux et al., 2002). Neither metabolic acidosis nor visual impairment was observed in this individual. Hantson et al. (1997b), in their review, touted the efficacy of fomepizole over ethanol in the treatment of methanol poisoning

Bilateral putaminal lesions, suggestive of nonhemorrhagic necrosis in the brain of a man who accidentally ingested methanol, were reported by Arora et al. (2005). Approximately 10 hours after MRI examination, he developed blurred vision and motor dysfunction. After 5 months, visual deficits persisted along with extrapyramidal symptoms. Persistent visual dysfunction was also reported in another methanol poisoning case (Arora et al., 2007); the vision problems developed ~46 hours subsequent to the incident.

Vara-Castrodeza et al. (2007) applied diffusion-weighted MRI on a methanol-induced comatose woman. Diffusion-weighted MRI provides an image contrast distinct from standard imaging in that contrast is dependent on the molecular motion of water (Schaefer et al., 2000). The neuroradiological findings were suggestive of bilateral putaminal hemorrhagic necrosis, cerebral and intraventricular hemorrhage, diffuse cerebral edema, and cerebellar necrosis. Diffusion-weighted MRI allows for differentiation of restricted diffusion which is indicative of nonviable tissue. In this case, treatment for acidosis (blood methanol levels had risen to 1,000 mg/L) was unsuccessful and the patient died.

Emergency treatment was unable to save the life of a 38-year-old man who presented with abdominal pain and convulsions after methanol intoxication (Henderson and Brubacher, 2002). A review of a head CT scan performed before the individual went into respiratory arrest revealed bilateral globus pallidus ischemia.

Discrete lesions of the putamen, cerebral white matter, and corpus callosum were observed upon MRI (8 days post ingestion) in a man exposed to methanol (blood level 370 mg/L) complaining of vision loss (Keles et al., 2007). Standard treatments corrected the acidosis (pH 6.8), and at 1-month follow-up, his cognitive function improved but blindness and bilateral optic atrophy were described as permanent. The follow-up MRI showed persistent putaminal lesions with cortical involvement.

Fontenot and Pelak (2002) described a case of a woman who presented with persistent blurred vision and a worsening mental status 36 hours after ingestion of an unspecified amount of methanol. The initial CT scan revealed mild cerebral edema. The blood methanol level at this time was 860 mg/L. A repeat CT scan 48 hours after presentation showed hypodensities in the putamen and peripheral white matter. One month after discharge, cognitive function improved, and the patient experienced only a mild lower-extremity tremor.

Putaminal necrosis and edema of the deep white matter (the corpus callosum was not affected) was found upon MRI examination of a 50-year-old woman who apparently ingested an unknown amount of what was believed to be pure laboratory methanol (Kuteifan et al., 1998). Her blood methanol level was 1,272 mg/L upon hospital admission and dropped to 1,020 mg/L at 10 hours and to 710 mg/L at 34 hours. The woman, a chronic alcoholic, was in a vegetative state when found and did not improve over the course of a year.

MRI and CT scans performed on a 51-year-old man with generalized seizures who had a blood methanol level of 3,044 mg/L revealed bilateral hemorrhagic necrosis of the putamen and caudate nuclei (Gaul et al., 1995). In addition, there was extensive subcortical necrosis and bilateral necrosis of the pontine tegmentum and optic nerve. The patient died several hours after the scans were performed.

The relation of methanol overexposure to brain hemorrhage was a focus of the report by Phang et al. (1988), which followed the treatment of 7 individuals, 5 of whom died within 72 hours after hospital admission. In two of the deceased individuals, CT scans and autopsy revealed putaminal hemorrhagic necrosis. The investigators postulated that the association of methanol with hemorrhagic necrosis may be complicated by the use of heparin during hemodialysis treatment for acidosis

Treatment of two men who had drunk a solution containing 58% methanol and presented with impaired vision, coma, and seizures was discussed in a case report by Bessell-Browne and Bynevelt (2007). A CT scan, on one individual, revealed bilateral putaminal and cerebral lesions. Blood methanol levels were 21 mg/L. This individual, despite standard treatments, never regained consciousness. The second individual, upon MRI, showed scattered hemorrhage at the grey-white interface of the cerebral hemispheres.

There have been case reports that involved percutaneous and inhalation exposure (Adanir et al., 2005; Downie et al., 1992). Use of a methanol-containing emollient by a woman with chronic pain led to vision loss, hyperventilation and finally, coma (Adanir et al., 2005). Subsequent to standard treatment followed by hospital discharge, some visual impairment and CNS decrements remained. The methanol blood threshold for ocular damage and acidosis appeared to be ~20 mg/L. Dutkiewicz et al. (1980) have determined the skin absorption rate to be 0.192 mg/cm^2/minute. In the case report of Aufderheide et al. (1993), two firefighters were

transiently exposed to methanol by inhalation and the percutaneous route. Both only complained of a mild headache and had blood methanol levels of 230 and 160 mg/L, respectively.

Bebarta et al. (2006) conducted a prospective observational study of seven men who had purposefully inhaled a methanol-containing product. Four had a blood methanol level upon hospital presentation of >240 mg/L; the mean formic acid level was .71 mg/L. One individual had a blood methanol level of 860 mg/L and a blood formic acid level of 250 mg/L upon hospital admission. This latter individual was treated with fomepizole. No patient had an abnormal ophthalmologic examination. All seven stabilized quickly and acidosis was normalized in 4 hours.

Numerous other case reports documenting putaminal necrosis/hemorrhage and/or blindness have been reported (Blanco et al., 2006; Feany et al., 2001; Hsu et al., 1997; Pelletier et al., 1992; Chen et al., 1991).

Hovda et al. (2005) presented a combined prospective and retrospective case series study of 51 individuals in Norway (39 males and 12 females, many of whom were alcoholics) who were hospitalized after consuming tainted spirits containing 20% methanol and 80% ethanol. In general, serum methanol concentrations were highest among those most severely affected. The poor outcome was closely correlated with the degree of metabolic acidosis. It was noted by the investigators that the concomitant consumption of ethanol prevented more serious sequelae in 2/5 individuals who presented with detectable ethanol levels and were not acidotic despite 2 having the highest blood methanol levels. However, others with detectable levels of ethanol along with severe metabolic acidosis (two of whom died) presumably had subtherapeutic levels of ethanol in their system.

In a later report, Hovda et al. (2007) focused on formate kinetics in a 63-year-old male who died 6 days after being admitted to the hospital with headache, vomiting, reduced vision, and dizziness. The investigators speculated that the prolonged metabolic acidosis observed ($T^{1/2}$ for formic acid was 77 hours before dialysis, compared to a typical normal range of 2.5-12 hours) may have been related to retarded formate elimination.

Hovda and colleagues (Hunderi et al., 2006) found a strong correlation between blood methanol concentration and the osmolal gap ($R^2 = 0.92$) among 17 patients undergoing dialysis after consuming methanol-contaminated spirits. They concluded that the osmolal gap could be taken as a priori indication of methanol poisoning and be used to guide initiation and duration of dialysis. As they indicated, many hours of dialysis could be safely dispensed with. The osmolal gap pertains to the effect that methanol (and other alcohols) has on the depression of the freezing point of blood in the presence of normal solutes. Braden et al. (1993) demonstrated in case studies that the disappearance of the osmolal gap correlates with the correction of acidosis; they cautioned that methanol and ethanol should not be assumed to be the main factors in causing

osmolal gap as glycerol and acetone and its metabolites can as well. A more detailed discussion of the anion and osmolal gap has been provided by Henderson and Brubacher (2002).

Hassanian-Moghaddam et al. (2007) compiled data on the prognostic factor relating to outcome in methanol-poisoning cases in Iran. They examined 25 patients, 12 of whom died; 3 of the survivors were rendered blind. There was a significant difference in mean pH of the first arterial blood gas measurements of those who subsequently died compared with survivors. It was concluded that poor prognosis was associated with pH <7, coma upon admission, and >24-hours delay from intake to admission.

The use of blood methanol levels as predictors of outcome is generally not recommended (Barceloux et al., 2002). These investigators cited differences in sampling time, ingestion of ethanol, and levels of toxic (e.g., formic acid) metabolites among the complicating factors. As an illustration, the case report by Prabhakaran et al. (1993) cites two women who ingested a methanol solution (photocopying diluent) at about the same time, were admitted to the hospital about the same time (25-26 hours after ingestion) and had identical plasma methanol concentrations (830 mg/L) upon admission, but different outcomes. Patient #1 was in metabolic acidosis and had an unstable conscious state even after treatment. Upon discharge at day 6, there were no apparent sequelae. Patient #2 had severe metabolic acidosis, fixed and dilated pupils, and no brain stem reflexes. This patient died at day 3 even though therapeutic measures had been administered.

In a discussion of 3 fatal methanol-overexposure cases, Andresen et al. (2008) found antemortem blood methanol levels of 5,400 and 7,400 mg/L in two individuals. At autopsy brain stem blood levels were 7,380 and 10,080 mg/L, respectively. These brain levels were much higher than blood levels postmortem. Autopsy revealed brain and pulmonary edema in all three individuals; in the two who had the longer survival times, there was hemorrhagic necrosis of the putamen and hemorrhages of the tissue surrounding the optic nerve. In their study of 26 chronic users of methylated spirits, Meyer et al. (2000) found that the best predictor of death or a poor outcome in chronic abusers was a pH <7.0; there was no correlation between blood methanol levels and outcome. Mahieu et al. (1989) considered a latency period before treatment exceeding 10 hours and a blood formate level >500 mg/L as predictive of possible permanent sequelae. Liu et al. (1998) in their examination of medical records of 50 patients treated for methanol poisoning over a 10-year period found that: (1) deceased patients had a higher mean blood methanol level than survivors; and (2) initial arterial pH levels <7.0 (i.e., severe metabolic acidosis). Coma or seizure was also associated with higher mortality upon hospital admission.

Numerous cases of methanol poisoning have been documented in a variety of countries. In Tunisia, 16 cases of methanol poisoning were discussed by Brahmi et al. (2007). Irreversible blindness occurred in two individuals, with others reporting CNS symptoms, GI effects, visual disturbances, and acidosis. Putaminal necrosis was also described in case reports from Iran

(Sefidbakht et al., 2007). Of 634 forensic autopsies carried out in Turkey during 1992-2003, 18 deaths appeared to be related to methanol poisoning (Azmak, 2006). Brain edema and focal necrosis of the optic nerve were among various sequelae noted. Dethlefs and colleagues (Naraqi et al., 1979; Dethlefs and Naraqi, 1978) described permanent ocular damage in 8/24 males who ingested methanol in Papua New Guinea.

In summary, most cases of accidental/intentional methanol poisoning reveal a common set of symptoms, many of which are likely to be presented upon hospital admission. See Section 4.1.1 for a list of common symptoms.

APPENDIX D. RFC DERIVATION OPTIONS

D.1. Benchmark Dose Modeling Summary

This appendix provides technical detail on dose-response evaluation and determination of points of departure (POD) for relevant toxicological endpoints. The endpoints were modeled using the U.S. EPA's Benchmark Dose Software (BMDS, version 2.2). Sections D.1.1 and D.1.2 describe the common practices used in evaluating the model fit and selecting the appropriate model for determining the POD, as outlined in the Benchmark Dose Technical Guidance Document (U.S. EPA, 2012a).

D.1.1. Evaluation of Model Fit

For the nested dichotomous endpoint (cervical rib), BMDS nested dichotomous models were fitted to the data using the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test (χ^2 p-value < 0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

For each continuous endpoint (brain weight and VDR), BMDS continuous models[1] were fitted to the data using the maximum likelihood method. Model fit was assessed by a series of tests as follows. For each model, first the homogeneity of the variances was tested using a likelihood ratio test (BMDS Test 2). If Test 2 was not rejected (χ^2 p-value ≥ 0.10), the model was fitted to the data assuming constant variance. If Test 2 was rejected (χ^2 p-value < 0.10), the variance was modeled as a power function of the mean, and the variance model was tested for adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using either constant variance or modeled variance, models for the mean response were tested for adequacy of fit using a likelihood ratio test (BMDS Test 4, with χ^2 p-value < 0.10 indicating inadequate fit). Other factors were also used to assess the model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

[1] Unless otherwise specified, all available BMDS continuous models were fitted. The following parameter restrictions were applied: for the polynomial models, restrict the coefficients b1 and higher to be nonnegative or nonpositive if the direction of the adverse effect is upward or downward, respectively; for the Hill, power, and exponential models, restrict power ≥ 1.

D.1.2. Model Selection

For each endpoint, the BMDL estimate (95% lower confidence limit on the BMD, as estimated by the profile likelihood method) and AIC value were used to select a best-fit model from among the models exhibiting adequate fit. If the BMDL estimates were "sufficiently close," that is, differed by at most 3-fold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

D.2. RfC Derivations Using the NEDO Methanol Report (NEDO, 1987)

In the application of the BMD approach, continuous models in EPA's BMDS, version 2.2 (U.S. EPA, 2011b), were fit to data sets for decreased brain weight in male rats exposed throughout gestation and the postnatal period to 6 weeks and male rats exposed during gestation on days 7–17 only (NEDO, 1987). Although there remains uncertainty surrounding the identification of the proximate teratogen of importance (methanol, formaldehyde, or formate), the dose metrics chosen for the derivation of RfCs were based on blood methanol levels. This decision was primarily based on evidence that the toxic moiety is not likely to be the formate metabolite of methanol (NTP-CERHR, 2004) and evidence that levels of the formaldehyde metabolite following methanol maternal and/or neonate exposure would be much lower in the fetus and neonate than in adults. While recent in vitro evidence indicates that formaldehyde is more embryotoxic than methanol and formate, the high reactivity of formaldehyde would significantly limit its transport from maternal to fetal blood, and the capacity for the metabolism of methanol to formaldehyde is lower in the fetus and neonate versus adults.

D.2.1. Decreased Brain Weight in Male Rats Exposed throughout Gestation and into the Postnatal Period

As discussed in Section 5.1.2.1, brain weight is susceptible to both the level and duration of exposure suggesting that a dose metric that incorporates a time component would be the most appropriate metric to use. For these reasons and because it is more typically used in internal-dose-based assessments and better reflects total exposure within a given day, daily AUC (measured for 22-hour exposure/day) was chosen as the most appropriate dose metric for modeling the effects of methanol exposure on brain weights in rats exposed throughout gestation and continuing into the F_1 generation.

As is discussed in Section 5.1.3.2.2, the additional routes of exposure to the pups in this study (lactation and inhalation) present uncertainties in that the average blood levels in pups is

likely to be greater than those of their dams. The assumption made in this assessment is that, if such differences exist between human mothers and their offspring, they are not significantly greater than that which has been postulated for rats. Assuming this is true, the PBPK model-estimated adult blood methanol level is considered to be an appropriate dose metric for the purpose of this analysis and the estimation of a human equivalent concentration (HEC).

The first step in the current analysis is to convert the inhalation doses, given as ppm values from the studies, to an internal dose surrogate or dose metric using the EPA PBPK model (see Appendix B). Predicted AUC values for methanol in the blood of rats are summarized in Table D-1. The AUC values above background (AUC – control) are then used as the dose metric for the BMD analysis of response data shown in Table D-1 for decreased brain weight at 6 weeks in male rats following gestational and postnatal exposure.[2] Decreases in brain weight at 6 weeks (gestational and postnatal exposure), rather than those seen at 3 and 8 weeks, were chosen as the basis for the RfC derivation because they resulted in lower estimated BMDs and BMDLs. The details of this analysis are reported below. More details concerning the PBPK modeling were presented in Appendix B.

Table D-1 EPA PBPK model estimates of methanol blood levels (AUC)[a] in rat dams following inhalation exposures and reported brain weights of 6 week old male pups

Exposure level (ppm)	Blood methanol AUC (mg-hr/L)[a] in rats	Blood methanol AUC – control (mg-hr/L)[a] in rats	Mean male rat (F_1 generation) brain weight at 6 weeks[b]	N
0	72	0	1.78 ± 0.07	12
500	619	547	1.74 ± 0.09	12
1,000	2,380	2,308	1.69 ± 0.06 [c]	11
2,000	17,600	17,528	1.52 ± 0.07[d]	14

[a]AUC values were obtained by simulating 22 hr/day exposures for 5 days and calculated for the last 24 hours of that period, with a simulated background blood level of 3 mg/L. (See Appendix B for further details.)
[b]Exposed throughout gestation and F_1 generation. Values are means ± SDSD
[c]$p < 0.01$, [d]$p < 0.001$, as calculated by the authors.
Data from NEDO (1987)

The EPA BMD technical guidance (U.S. EPA, 2012a) suggests that in the absence of knowledge as to what level of response to consider adverse, a change in the mean equal to 1 control SDSD from the control mean can be used as a BMR for continuous endpoints. However, it has been suggested that other BMRs, such as 5% change relative to estimated control mean, are also appropriate when performing BMD analyses on fetal weight change as a developmental endpoint (Kavlock et al., 1995). Therefore, in this assessment, both a 1 control mean SD change and a 5% change relative to estimated control mean were considered. All

[2]All BMD assessments in this review were performed using BMDS version 2.2 (U.S. EPA, 2011a)

models were fit using restrictions and option settings suggested in the EPA BMD Technical Guidance Document (U.S. EPA, 2012a).

D.2.1.1. BMD Approach with a BMR of 1 Control Mean SD – Decreased Brain Weight in Male Rats Exposed throughout Gestation and into the Postnatal Period (NEDO, 1987)

A summary of the results most relevant to the development of a POD using the BMD approach (BMD, BMDL, and model fit statistics) for decreased brain weight at 6 weeks in male rats exposed to methanol throughout gestation and continuing into the F_1 generation, with a BMR of 1 control mean SD (NEDO, 1987), is provided in Table D-2. Model fit and was determined by statistics (AIC and χ^2 residuals of individual dose groups) and visual inspection, as recommended by EPA (U.S. EPA, 2012a). There is a 5.1-fold range of BMDL estimates from adequately fitting models, indicating considerable model dependence. In addition, the fit of the Hill and more complex Exponential models are better than the other models in the dose region of interest as indicated by a lower scaled residual at the dose group closest to the BMD (0.18 and 0.16 versus -1.4) and by visual inspection. In accordance with EPA BMD Technical Guidance (U.S. EPA, 2012a), the BMDL from the Hill model (bolded), is selected as the most appropriate basis for an RfC derivation because it results in the lowest BMDL from among a broad range of BMDLs and provides a superior fit in the low dose region nearest the BMD. The detailed results of the Hill model run, including text and a plot (Figure D-1) are shown after Table D-2. The $BMDL_{1SD}$ was determined to be 858 mg-hr/L using the 95% lower confidence limit of the dose-response curve expressed in terms of the AUC above background for methanol in blood.

Table D-2 Comparison of BMD_{1SD} results for decreased brain weight in male rats at 6 weeks of age using modeled AUC above background of methanol as a dose metric

Model	BMD_{1SD} (AUC, mg-hr/L) [a]	$BMDL_{1SD}$ (AUC, mg-hr/L)[a]	*p*-value	AIC[b]	Scaled residual[c]
Linear	5,469.53	4,410.68	0.1385	-201.13	-1.39
2nd degree Polynomial	5,469.53	4,410.68	0.1385	-201.13	-1.39
3rd degree Polynomial	5,469.53	4,410.68	0.1385	-201.13	-1.39
Power	5,469.53	4,410.68	0.1385	-201.13	-1.39
Hill[b]	**1,730.35**	**858.04**	**0.5920**	**-202.79**	**0.179**
Exponential 2	5,159.24	4,118.16	0.1573	-201.38	-1.336
Exponential 3	5,159.24	4,118.16	0.1573	-201.38	-1.336
Exponential 4	1,802.01	997.71	0.5513	-202.72	0.163
Exponential 5	1,802.01	997.71	0.5513	-202.72	0.163

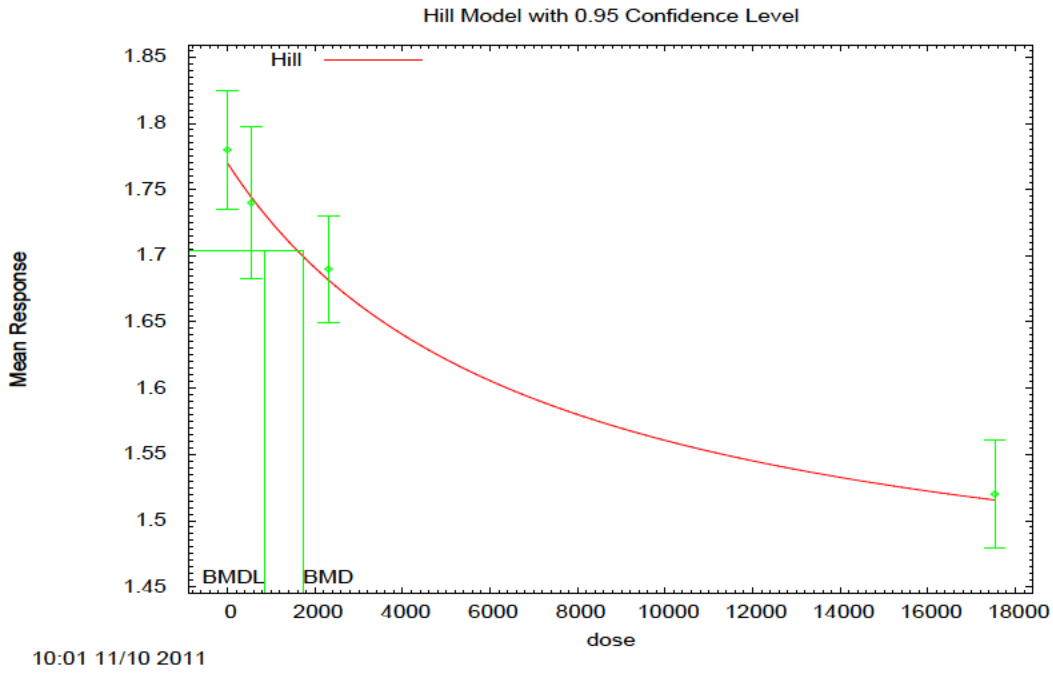
[a]The BMDL is the 95% lower confidence limit on the AUC estimated to decrease brain weight by 1 control mean SD using BMDS 2.2 (U.S. EPA, 2011b) and model options and restrictions suggested by EPA BMD technical guidance (U.S. EPA, 2012a).
[b]AIC = Akaike Information Criterion = -2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters, and P is the number of modeled degrees of freedom (usually the number of parameters estimated).
[c]chi-squared (X^2) residual (measure of how model-predicted responses deviate from the actual data) for the dose group closest to the BMD scaled by an estimate of its SD Provides a comparative measure of model fit near the BMD. Residuals that exceed 2.0 in absolute value should cause one to question model fit in this region.

Data from NEDO (1987).

```
 ================================================================
    Hill Model. (Version: 2.16; Date: 04/06/2011)
    Input Data File: C:/USEPA/BMDS220/Data/Methanol/hil_NEDOrat-6wk-male_Hil-
ConstantVariance-BMR1Std-Restrict.(d)
    Gnuplot Plotting File: C:/USEPA/BMDS220/Data/Methanol/hil_NEDOrat-6wk-male_Hil-
ConstantVariance-BMR1Std-Restrict.plt
                  Tue Mar 27 08:42:04 2012
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

 The form of the response function is:

 Y[dose] = intercept + v*dose^n/(k^n + dose^n)


 Dependent variable = Mean
 Independent variable = Dose
 rho is set to 0
 Power parameter restricted to be greater than 1
 A constant variance model is fit

 Total number of dose groups = 4
 Total number of records with missing values = 0
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



 Default Initial Parameter Values
 alpha = 0.00539333
 rho = 0 Specified
 intercept = 1.78
 v = -0.26
 n = 0.698151
 k = 5889.18


 Asymptotic Correlation Matrix of Parameter Estimates

          (*** The model parameter(s) -rho -n have been estimated at a boundary
          point, or have been specified by the user, and do not appear in the
          correlation matrix )


 alpha intercept v k

 alpha 1 1.7e-008 2.5e-008 -4e-008

 intercept 1.7e-008 1 0.24 -0.62

 v 2.5e-008 0.24 1 -0.85

 k -4e-008 -0.62 -0.85 1



  Parameter Estimates

  95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
alpha 0.00498218 0.00100655 0.00300938 0.00695499
intercept 1.77449 0.0177456 1.73971 1.80927
```

```
 v -0.3555 0.0666435 -0.486119 -0.224881
 n 1 NA
 k 6984.58 4505.13 -1845.31 15814.5

NA - Indicates that this parameter has hit a bound
 implied by some inequality constraint and thus
 has no standard error.



 Table of Data and Estimated Values of Interest

 Dose N Obs Mean Est Mean Obs Std Dev Est Std Dev Scaled Res.
------ --- -------- -------- ----------- ----------- ----------

 0   12 1.78 1.77 0.07 0.0706 0.27
 547 12 1.74 1.75 0.09 0.0706 -0.425
 2308   11 1.69 1.69 0.06 0.0706 0.179
1.753e+004 14 1.52 1.52 0.07 0.0706 -0.0151



 Model Descriptions for likelihoods calculated


 Model A1: Yij = Mu(i) + e(ij)
 Var{e(ij)} = Sigma^2

 Model A2: Yij = Mu(i) + e(ij)
 Var{e(ij)} = Sigma(i)^2

 Model A3: Yij = Mu(i) + e(ij)
 Var{e(ij)} = Sigma^2
 Model A3 uses any fixed variance parameters that
 were specified by the user

 Model R: Yi = Mu + e(i)
 Var{e(i)} = Sigma^2


 Likelihoods of Interest

 Model Log(likelihood) # Param's AIC
 A1 105.539862 5 -201.079724
 A2 106.570724 8 -197.141449
 A3 105.539862 5 -201.079724
 fitted 105.396232 4 -202.792465
 R 77.428662 2 -150.857324


 Explanation of Tests

 Test 1: Do responses and/or variances differ among Dose levels?
 (A2 vs. R)
 Test 2: Are Variances Homogeneous? (A1 vs A2)
 Test 3: Are variances adequately modeled? (A2 vs. A3)
 Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

 Tests of Interest

 Test -2*log(Likelihood Ratio) Test df p-value

 Test 1 58.2841 6 <.0001
 Test 2 2.06173 3 0.5597
```

```
 Test 3 2.06173 3 0.5597
 Test 4 0.287259 1 0.592

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1. The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data


 Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

 BMD = 1730.35

 BMDL = 858.038
```



Data points obtained from NEDO (1987).

Figure D-1 Hill model, BMR of 1 Control Mean SD - Decreased Brain weight in male rats at 6 weeks age versus AUC above background, F1 Generation inhalational study.

D.2.1.2. BMD approach with a BMR of 0.05, change relative to estimated control mean – Decreased brain weight in male rats exposed throughout gestation and into the postnatal period (NEDO, 1987).

A summary of the results most relevant to the development of a POD using the BMD approach (BMD, BMDL, and model fit statistics) for decreased brain weight at 6 weeks in male rats exposed to methanol throughout gestation and continuing into the F_1 generation, with a BMR of 0.05 change relative to estimated control mean, is provided in Table D-3. Model fit was determined by statistics (AIC and χ^2 residuals of individual dose groups) and visual inspection, as recommended by the EPA BMD Technical Guidance (U.S. EPA, 2012a). There is a 4.7-fold range of BMDL estimates from adequately fitting models, indicating considerable model dependence. In addition, the fit of the Hill and more complex Exponential models are better than the other models in the dose region of interest as indicated by a lower scaled residual at the dose group closest to the BMD (0.18 and 0.16 versus -1.4) and visual inspection. In accordance with EPA BMD Technical Guidance (U.S. EPA, 2012a), the BMDL from the Hill model (bolded), is selected as the most appropriate basis for an RfC derivation because it results in the lowest BMDL from among a broad range of BMDLs and provides a superior fit in the low dose region nearest the BMD. Output from the Hill model, including text and plot (Figure D-2), is shown after Table D-3. The $BMDL_{05}$ was determined to be 1,183 mg-hr/L, using the 95% lower confidence limit of the dose-response curve expressed in terms of the AUC above background for methanol in blood.

Table D-3 Comparison of BMD_{05} results for decreased brain weight in male rats at 6 weeks of age using modeled AUC above background of methanol as a dose metric

Model	BMD_{05} (AUC, mg-hr/L)[a]	$BMDL_{05}$ (AUC, mg-hr/L)[a]	*p*-value	AIC[b]	Scaled Residual[c]
Linear[b]	6,537.04	5,614.56	0.1385	-201.13	-1.39
2nd degree Polynomial	6,537.04	5,614.56	0.1385	-201.13	-1.39
3rd degree Polynomial	6,537.04	5,614.56	0.1385	-201.13	-1.39
Power	6,537.04	5,614.56	0.1385	-201.13	-1.39
Hill	**2,322.94**	**1,182.99**	**0.5920**	**-202.79**	**0.179**
Exponential 2	6,212.5	5,270.18	0.1573	-201.38	-1.34
Exponential 3	6,212.5	5,270.18	0.1573	-201.38	-1.34
Exponential 4	2,367.26	1,334.02	0.5513	-202.72	0.163
Exponential 5	2, 367.26	1,334.02	0.5513	-202.72	0.163

[a]The BMDL is the 95% lower confidence limit on the AUC estimated to decrease brain weight by 5% using BMDS 2.2 (U.S. EPA, 2011b) and model options and restrictions suggested by EPA BMD Technical Guidance (U.S. EPA, 2012a).
[b]AIC = Akaike Information Criterion = -2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters, and P is the number of modeled degrees of freedom (usually the number of parameters estimated).
[c]χ^2d residual (measure of how model-predicted responses deviate from the actual data) for the dose group closest to the BMD scaled by an estimate of its SD Provides a comparative measure of model fit near the BMD. Residuals that exceed 2.0 in absolute value should cause one to question model fit in this region.

Data from NEDO (1987)

```
================================================================================
    Hill Model. (Version: 2.16; Date: 04/06/2011)
    Input Data File: C:/USEPA/BMDS220/Data/Methanol/hil_NEDOrat-6wk-male_Hil-
ConstantVariance-BMR05-Restrict.(d)
    Gnuplot Plotting File: C:/USEPA/BMDS220/Data/Methanol/hil_NEDOrat-6wk-male_Hil-
ConstantVariance-BMR05-Restrict.plt
                  Tue Mar 27 10:57:37 2012
 ================================================================================

 BMDS Model Run
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 The form of the response function is:

 Y[dose] = intercept + v*dose^n/(k^n + dose^n)


 Dependent variable = Mean
 Independent variable = Dose
 rho is set to 0
 Power parameter restricted to be greater than 1
 A constant variance model is fit

 Total number of dose groups = 4
 Total number of records with missing values = 0
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
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 Default Initial Parameter Values
 alpha = 0.00539333
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 Asymptotic Correlation Matrix of Parameter Estimates

          (*** The model parameter(s) -rho -n have been estimated at a boundary
          point, or have been specified by the user, and do not appear in the
          correlation matrix )


 alpha intercept v k

 alpha 1 1.7e-008 2.5e-008 -4e-008

 intercept 1.7e-008 1 0.24 -0.62

 v 2.5e-008 0.24 1 -0.85

 k -4e-008 -0.62 -0.85 1



  Parameter Estimates

  95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
alpha 0.00498218 0.00100655 0.00300938 0.00695499
intercept 1.77449 0.0177456 1.73971 1.80927
```

```
v -0.3555 0.0666435 -0.486119 -0.224881
n 1 NA
k 6984.58 4505.13 -1845.31 15814.5

NA - Indicates that this parameter has hit a bound
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 2308 11 1.69 1.69 0.06 0.0706 0.179
1.753e+004 14 1.52 1.52 0.07 0.0706 -0.0151



 Model Descriptions for likelihoods calculated


 Model A1: Yij = Mu(i) + e(ij)
 Var{e(ij)} = Sigma^2

 Model A2: Yij = Mu(i) + e(ij)
 Var{e(ij)} = Sigma(i)^2

 Model A3: Yij = Mu(i) + e(ij)
 Var{e(ij)} = Sigma^2
 Model A3 uses any fixed variance parameters that
 were specified by the user

 Model R: Yi = Mu + e(i)
 Var{e(i)} = Sigma^2


 Likelihoods of Interest

 Model Log(likelihood) # Param's AIC
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 A2 106.570724 8 -197.141449
 A3 105.539862 5 -201.079724
 fitted 105.396232 4 -202.792465
 R 77.428662 2 -150.857324


 Explanation of Tests

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 Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

 Tests of Interest

 Test -2*log(Likelihood Ratio) Test df p-value

 Test 1 58.2841 6 <.0001
 Test 2 2.06173 3 0.5597
```

```
 Test 3 2.06173 3 0.5597
 Test 4 0.287259 1 0.592

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The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1. The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data


 Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Relative risk

Confidence level = 0.95

 BMD = 2322.94

 BMDL = 1182.99
```
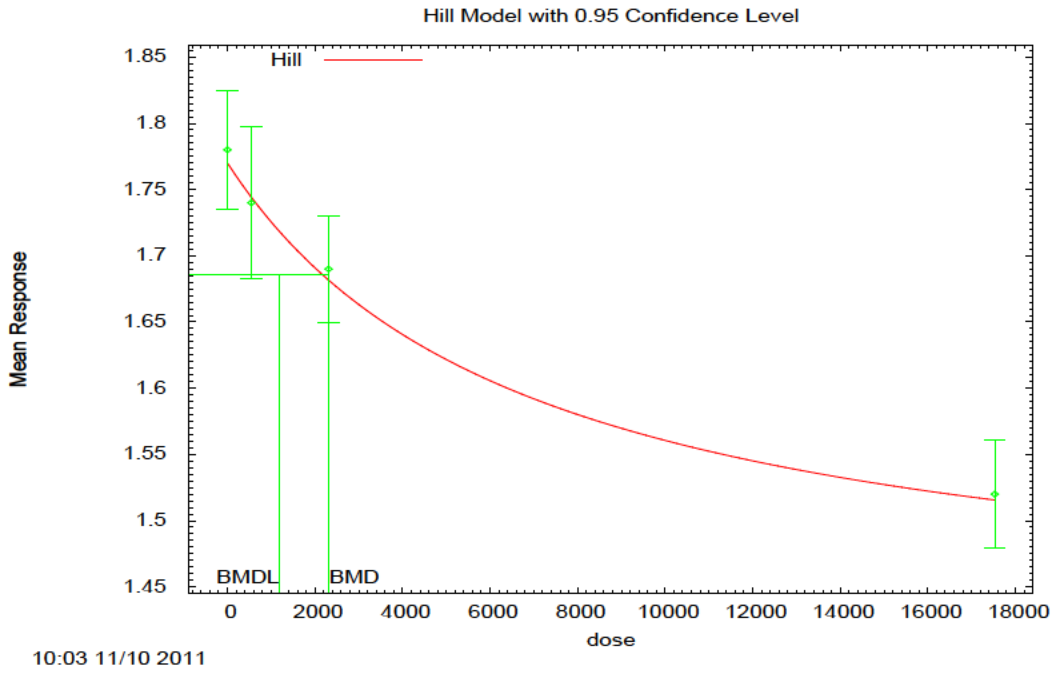



Data points obtained from NEDO (1987).

Figure D-2 Hill model, BMR of 0.05 relative risk - decreased brain weight in male rats at 6 weeks age versus AUC above background of methanol, F_1 generation inhalational study.

D.2.2. Decreased Brain Weight in Male Rats Exposed During Gestation Only (GD7-GD17)

As discussed in Section 5.1.2.1, C_{max}, as calculated by EPA's PBPK model, was selected as the dose metric for this exposure scenario. Exposures occurred only during the major period of organogenesis, during which the level of exposure is believed to be more important than the duration of exposure.

The first step in the current analysis is to convert the inhalation doses, given as ppm values from the studies, to an internal dose surrogate or dose metric using the EPA PBPK model (see Appendix B). Predicted C_{max} values for methanol in the blood of rats, with and without background methanol levels, are summarized in Table D-4.

Table D-4 EPA PBPK model estimates of methanol blood levels (C_{max}) in rat pups at 8 weeks following inhalation exposures during gestation

Exposure level (ppm)	Blood methanol C_{max} (mg /L)[a] in rats	Blood methanol C_{max} – control (mg/L)[a] in rats	Mean male rat brain weight at 8 weeks[b]	N
0	3	0	2.00 ± 0.047	11
200	10.41	7.41	2.01 ± 0.075	11
1,000	117.6	114.6	1.99 ± 0.072	12
5,000	2,989	2,986	1.81 ± 0.161[c]	10

[a]C_{max} values were obtained by simulating 22 hr/day exposures with a simulated background blood level of 3 mg/L. (See Appendix B for further details).
[b]Exposed throughout gestation. Values are means ± SD
[c]$p < 0.01$, as calculated by the authors.
Data from NEDO (1987).

The BMD technical guidance (U.S. EPA, 2012a) suggests that in the absence of knowledge as to what level of response to consider adverse, a change in the mean equal to 1 control SD from the control mean can be used as a BMR for continuous endpoints. However, it has been suggested that other BMRs, such as 5% change relative to estimated control mean, are also appropriate when performing BMD analyses on fetal weight change as a developmental endpoint (Kavlock et al., 1995). Therefore, in this assessment, both a 1 control mean SD change and a 5% change relative to estimated control mean were considered. All models were fit using restrictions and option settings suggested in the EPA's BMD Technical Guidance Document (U.S. EPA, 2012a).

D.2.2.1. BMD Approach with a BMR of 1 Control Mean SD (GD7-GD17)

A summary of the results most relevant to the development of a POD using the BMD approach (BMD, BMDL, and model fit statistics) (NEDO, 1987) for decreased brain weight at 8

weeks in male rats exposed to methanol during gestation from days 7–17, with a BMR of 1 control mean S.D, is provided in Table D-5. Male brain weight responses were chosen because they resulted in lower BMD and BMDL estimates than female responses (data not shown). Model fit was determined by statistics (AIC and χ^2 residuals of individual dose groups) and visual inspection, as recommended by EPA (U.S. EPA, 2012a). The Polynomial and Power models reduced to Linear model and returned identical modeling results. There is a greater than 5-fold range of BMDL estimates from adequately fitting models, indicating considerable model dependence. In addition, the fit of the Hill and Exponential 4 and 5 models are better than the other models in the dose region of interest as indicated by a lower scaled residual at the dose group closest to the BMD (~0.09 versus ~-0.3) and visual inspection. In accordance with EPA BMD Technical Guidance (U.S. EPA, 2012a), the BMDL from the Exponential 4 and 5 models (bolded), is selected as the most appropriate basis for an RfC derivation because it results in the lowest BMDL from among a broad range of BMDLs and provides a superior fit in the low dose region nearest the BMD. Output from the Exponential 4 model, including text and plot (Figure D-3), is shown after Table D-5. The $BMDL_{1SD}$ was determined to be 115 mg/L, using the 95% lower confidence limit of the dose-response curve expressed in terms of the C_{max} above background for methanol in blood.

Table D-5 Comparison of BMD_{1SD} results for decreased brain weight in male rats at 8 weeks of age using modeled C_{max} above background of methanol as a dose metric

Model	BMD_{1SD} (C_{max}, mg/L)[a]	$BMDL_{1SD}$ (C_{max}, mg/L)[a]	*p*-value	AIC[b]	Scaled residual[c]
Linear	960.78	626.64	0.8837	-173.347015	-0.28
2nd degree Polynomial	960.78	626.64	0.8837	-173.347015	-0.28
3rd degree Polynomial	960.78	626.64	0.8837	-173.347015	-0.28
Power	960.78	626.64	0.8837	-173.347015	-0.28
Hill[b]	449.28	115.97	0.9272	-171.586011	0.0944
Exponential 2	925.82	589.97	0.8910	-173.3635	-0.2674
Exponential 3	925.92	589.97	0.8910	-173.3635	-0.2674
Exponential 4	**433.46**	**114.86**	**0.9266**	**-171.5859**	**0.09421**
Exponential 5	**433.46**	**114.86**	**0.9266**	**-171.5859**	**0.09421**

[a]The BMDL is the 95% lower confidence limit on the C_{max} estimated to decrease brain weight by 1 control mean SD using BMDS 2.1.1 (U.S. EPA, 2009) and model options and restrictions suggested by EPA BMD technical guidance (U.S. EPA, 2012a).
[b]AIC = Akaike Information Criterion = -2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters, and P is the number of modeled degrees of freedom (usually the number of parameters estimated).
[c]chi-squared (X^2) residual (measure of how model-predicted responses deviate from the actual data) for the dose group closest to the BMD scaled by an estimate of its SD Provides a comparative measure of model fit near the BMD. Residuals that exceed 2.0 in absolute value should cause one to question model fit in this region.

Data from NEDO (1987)

```
================================================================
    Exponential Model. (Version: 1.7; Date: 12/10/2009)
    Input Data File: C:/USEPA/BMDS220/Data/Methanol/exp_NEDOrat-Gest-Cmax-Std_Exp-
ModelVariance-BMR1Std-Down.(d)
    Gnuplot Plotting File:
                  Tue Mar 27 12:45:12 2012
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

 The form of the response function by Model:
 Model 2: Y[dose] = a * exp{sign * b * dose}
 Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
 Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
 Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

 Note: Y[dose] is the median response for exposure = dose;
 sign = +1 for increasing trend in data;
 sign = -1 for decreasing trend.

 Model 2 is nested within Models 3 and 4.
 Model 3 is nested within Model 5.
 Model 4 is nested within Model 5.


 Dependent variable = Mean
 Independent variable = Dose
 Data are assumed to be distributed: normally
 Variance Model: exp(lnalpha +rho *ln(Y[dose]))
 The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

 Total number of dose groups = 4
 Total number of records with missing values = 0
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

 MLE solution provided: Exact


 Initial Parameter Values

 Variable Model 4
 -------- --------
 lnalpha 7.32457
 rho -18.5236
 a 2.1105
 b 0.000507001
 c 0.816778
 d 1



 Parameter Estimates

 Variable Model 4
 -------- -------
 lnalpha 6.99305
 rho -18.0776
 a 2.00632
 b 0.000758964
 c 0.891583
 d 1
```

Table of Stats From Input Data

Dose	N Obs	Mean Obs	Std Dev
0	11	2	0.047
7.41	11	2.01	0.075
114.6	12	1.99	0.072
2986	10	1.81	0.161

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	2.006	0.06098	-0.3437
7.41	2.005	0.06132	0.2651
114.6	1.988	0.06619	0.09421
2986	1.811	0.1536	-0.02792

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	83.20596	5	-156.4119
A2	92.06049	8	-168.121
A3	90.61606	6	-169.2321
R	70.76186	2	-137.5237
4	90.79294	5	-171.5859

Additive constant for all log-likelihoods = -40.43. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

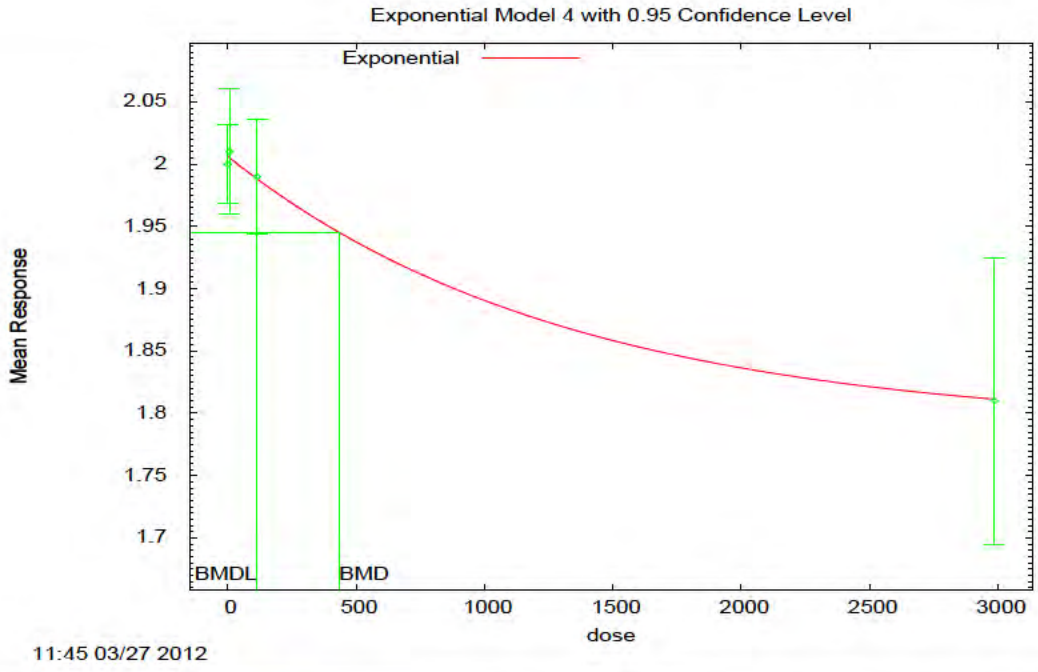
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	42.6	6	< 0.0001
Test 2	17.71	3	0.000505
Test 3	2.889	2	0.2359
Test 6a	-0.3538	1	N/A

```
The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose
levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous
variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled
variance appears to be appropriate here.

The p-value for Test 6a is less than .1. Model 4 may not adequately
describe the data; you may want to consider another model.


Benchmark Dose Computations:

Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD = 433.456

BMDL = 114.856
```



Data points obtained from NEDO (1987).

Figure D-3 Exponential model 4, BMR of 1 control mean SD - Decreased brain weight in male rats at 8 weeks of age versus C_{max} above background, gestation only inhalational study.

D.2.3. C.1.2.2. BMD Approach with a BMR of 0.05 Change Relative to Control Mean (GD7-GD17)

A summary of the results most relevant to the development of a POD using the BMD approach (BMD, BMDL, and model fit statistics) for decreased brain weight at 8 weeks in male rats exposed to methanol during gestation from days 7 to 17, with a BMR of 0.05 change relative to estimated control mean, is provided in Table D-6. Model fit was determined by statistics (AIC and χ^2 residuals of individual dose groups) and visual inspection, as recommended by EPA (2012a). Modeling considerations and uncertainties for this data set were discussed in C.1.2.1 and, as was done for the BMR of 1 SD, the lowest BMDL was chosen for use in the RfC derivation (NEDO, 1987), which in this case was the $BMDL_{05}$ of 119.51 mg methanol/L in blood estimated by the Exponential 5 model. Results from the Exponential 5 model, including text and plot (see Figure D-4), are shown after Table D-6.

Table D-6 Comparison of BMD_{05} modeling results for decreased brain weight in male rats at 8 weeks of age using modeled C_{max} above background of methanol as a common dose metric

Model	BMD_{05} (C_{max}, mg/L)[a]	$BMDL_{05}$ (C_{max}, mg/L)[a]	*p*-value	AIC[c]	Scaled residual[d]
Linear[b]	1,542.49	1,061.91	0.8837	-173.347015	-0.28
2nd degreePolynomial	1,542.49	1,061.91	0.8837	-173.347015	-0.28
3rd degree Polynomial	1,542.49	1,061.91	0.8837	-173.347015	-0.28
Power	1,542.49	1,061.91	0.8837	-173.347015	-0.28
Hill[b]	871.996	Not Reported	0.9272	-171.586011	0.0944
Exponential 2	1,502.61	1,009.52	0.8910	-173.3635	-0.2674
Exponential 3	1,502.61	1,009.52	0.8910	-173.3635	-0.2674
Exponential 4	814.76	233.33	0.9266	-171.5859	0.09421
Exponential 5	**814.76**	**119.51**	**0.9266**	**-171.5859**	**0.09421**

[a]The BMDL is the 95% lower confidence limit on the C_{max} estimated to decrease brain weight by 5% using BMDS 2.2 (U.S. EPA, 2011b) and model options and restrictions suggested by EPA BMD Technical Guidance (2012a).

[c]AIC = Akaike Information Criterion = -2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters, and P is the number of modeled degrees of freedom (usually the number of parameters estimated).

[d]chi-squared (X^2) residual (measure of how model-predicted responses deviate from the actual data) for the dose group closest to the BMD scaled by an estimate of its SD Provides a comparative measure of model fit near the BMD. Residuals that exceed 2.0 in absolute value should cause one to question model fit in this region.

Data from NEDO (1987).

```
================================================================
    Exponential Model. (Version: 1.7; Date: 12/10/2009)
    Input Data File: C:/USEPA/BMDS220/Data/Methanol/exp_NEDOrat-Gest-Cmax-Std_Exp-
ModelVariance-BMR05-Down.(d)
    Gnuplot Plotting File:
                  Tue Mar 27 15:30:45 2012
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

 The form of the response function by Model:
 Model 2: Y[dose] = a * exp{sign * b * dose}
 Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
 Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
 Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

 Note: Y[dose] is the median response for exposure = dose;
 sign = +1 for increasing trend in data;
 sign = -1 for decreasing trend.

 Model 2 is nested within Models 3 and 4.
 Model 3 is nested within Model 5.
 Model 4 is nested within Model 5.


 Dependent variable = Mean
 Independent variable = Dose
 Data are assumed to be distributed: normally
 Variance Model: exp(lnalpha +rho *ln(Y[dose]))
 The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

 Total number of dose groups = 4
 Total number of records with missing values = 0
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

 MLE solution provided: Exact


 Initial Parameter Values

 Variable Model 5
 -------- --------
 lnalpha 7.32457
 rho -18.5236
 a 2.1105
 b 0.000507001
 c 0.816778
 d 1



 Parameter Estimates

 Variable Model 5
 -------- -------
 lnalpha 6.99305
 rho -18.0776
 a 2.00632
 b 0.000758964
 c 0.891583
 d 1
```

Table of Stats From Input Data

Dose	N Obs	Mean Obs	Std Dev
0	11	2	0.047
7.41	11	2.01	0.075
114.6	12	1.99	0.072
2986	10	1.81	0.161

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	2.006	0.06098	-0.3437
7.41	2.005	0.06132	0.2651
114.6	1.988	0.06619	0.09421
2986	1.811	0.1536	-0.02792

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	83.20596	5	-156.4119
A2	92.06049	8	-168.121
A3	90.61606	6	-169.2321
R	70.76186	2	-137.5237
5	90.79294	5	-171.5859

Additive constant for all log-likelihoods = -40.43. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

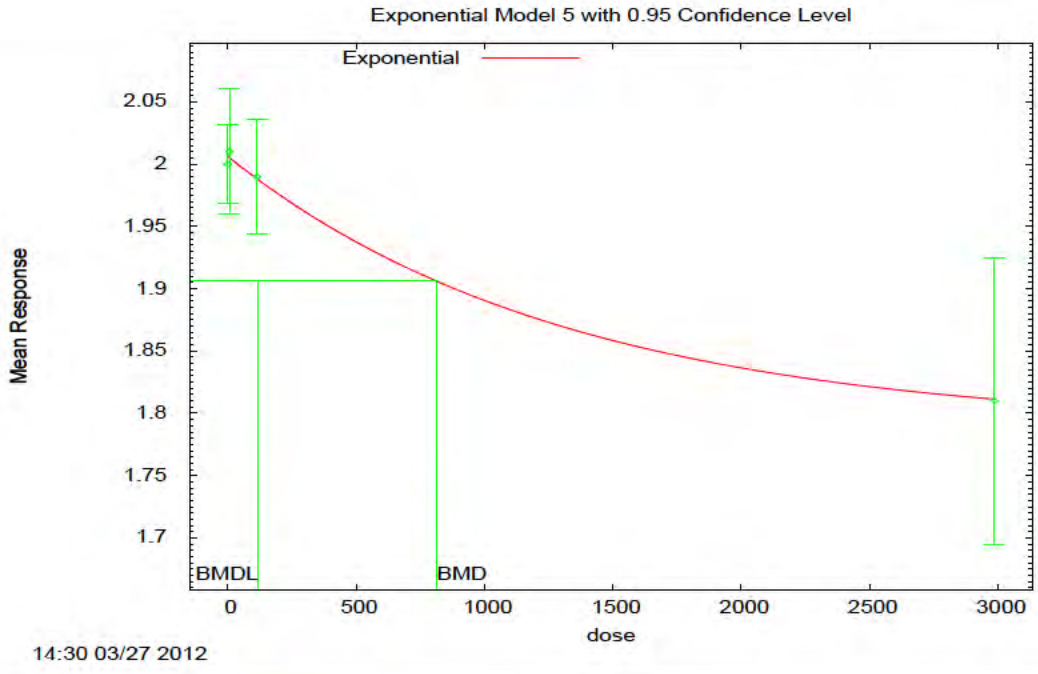
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 7a: Does Model 5 fit the data? (A3 vs 5)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	42.6	6	< 0.0001
Test 2	17.71	3	0.000505
Test 3	2.889	2	0.2359
Test 7a	-0.3538	1	N/A

```
The p-value for Test 1 is less than .05. There appears to be a
 difference between response and/or variances among the dose
 levels, it seems appropriate to model the data.

 The p-value for Test 2 is less than .1. A non-homogeneous
 variance model appears to be appropriate.

 The p-value for Test 3 is greater than .1. The modeled
 variance appears to be appropriate here.

 The p-value for Test 7a is less than .1. Model 5 may not adequately
 describe the data; you may want to consider another model.


 Benchmark Dose Computations:

 Specified Effect = 0.050000

 Risk Type = Relative deviation

 Confidence Level = 0.950000

 BMD = 814.763

 BMDL = 119.505
```



Data points obtained from NEDO (1987).

Figure D-4 Exponential model 4, BMR of 0.05 relative risk - Decreased brain weight in male rats at 8 weeks age versus C_{max} above background, gestation only inhalational study.

D.3. RfC Derivations Using Rogers et al. (1993b)

For the purposes of deriving an RfC for methanol from developmental endpoints using the BMD method and mouse data, cervical rib incidence data were evaluated from Rogers et al. (1993b). In this paper, Rogers et al. (1993b) also utilized a BMD methodology, examining the dosimetric threshold for cervical ribs and other developmental impacts by applying a log-logistic maximum likelihood model to the dose-response data. Using air exposure concentrations (ppm) as their dose metric, a value for the lower 95% confidence limit on the benchmark dose for 5% additional risk in mice was 305 ppm (400 mg/m^3), using the log-logistic model. Although the teratology portion of the NEDO study (1987) also reported increases in cervical rib incidence in Sprague-Dawley rats, the Rogers et al. (1993b) study was chosen for dose-response modeling because effects were seen at lower doses, it was peer-reviewed and published in the open literature, and data on individual animals were available for a more statistically robust analysis utilizing nested models available in BMDS 2.2 (U.S. EPA, 2011b).

As described in Section 5.1.2.1, because exposure was during gestation only and due to the small critical gestational window for cervical rib abnormalities, C_{max} of methanol in blood (mg/L) is chosen as the appropriate internal dose metric. Because the critical window for methanol induction of cervical rib malformations in CD-1 mice is between GD6 and GD7 (Rogers and Mole, 1997; Rogers et al., 1993a), the measured C_{max} plasma methanol levels for gestation day 6 from the Rogers study are used with background levels (1.6 g/L) subtracted. C_{max} values for methanol in the blood of mice are summarized in Table D-7. These C_{max} values are then used as the dose metric for the BMD analysis of the litter-specific cervical rib response. The overall cervical rib/litter (%) reported by Rogers et al. (1993b) is shown in Table D-7, but litter-specific response data from this study (170 litters) obtained from John Rogers (personal communication) was used for the nested BMD analysis. Due to high mortality, the high (15,000 ppm) dose group (5 litters) was excluded from this analysis. The individual animal response data for the four dose groups are displayed below in the text output files for the NLogistic model.

Table D-7 Methanol blood levels (C_{max} above background) in mice following inhalation exposures

Exposure (ppm)	Methanol in blood C_{max} (mg/L)[a] in mice	Cervical Rib/Litter (%)
0	0	28
1,000	61.4	33.6
2,000	485.4	49.6
5,000	2,124.4	74.4

[a]Reported C_{max} background levels of 1.6 mg/L were subtracted from reported C_{max} values.
Source: Rogers et al. (1993b)

A 10% BMR level is the value typically calculated for comparisons across chemicals and endpoints for dichotomous responses because this level is near the low end of the observable range for many types of toxicity studies. However, from a statistical standpoint most reproductive and developmental studies involve a large enough sample size to support a 5% BMR for determination of a POD (U.S. EPA, 2012a; Allen et al., 1994a). Rogers et al. (1993b) utilized a 5% added risk for the BMR in the original study. This assessment utilizes both a 10% and 5% extra risk level as a BMR for the determination of a POD.[3] The nested suite of models available in BMDS 2.2 (U.S. EPA, 2011b) was used to model the cervical rib data. In general, data from developmental toxicity studies are best modeled using nested models, as these models account for any intralitter correlation (i.e., the tendency of littermates to respond similarly to one another, relative to other litters in a dose group). All models were fit using restrictions and option settings suggested in the EPA's BMD Technical Guidance Document (U.S. EPA, 2012a).

D.3.1. BMD Approach with a BMR of 0.10 Extra Risk

A summary of the results most relevant to the development of a POD using the BMD approach (BMD, BMDL, and model fit statistics) for increased incidence of cervical rib in mice exposed to methanol during gestation from days 6 to 15, with a BMR of 0.10 extra risk, is provided in Table D-8. Model fit was determined by statistics (AIC and χ^2 residuals of individual dose groups) and visual inspection, as recommended by U.S. EPA (U.S. EPA, 2012a). The best model fit to these data (from visual inspection and comparison of AIC values) was obtained using the Nested Logistic (NLogistic) model. The textual and graphic (see Figure D-5) output from this model follows Table D-8. The $BMDL_{10}$ was determined to be 90.9972 mg/L using the

[3] Starr and Festa (2003) have argued that the Rogers, et al. (1993b) study's experimental design lacked the statistical power to detect a 5% risk and that a 5% level lay below the observable response data. However, EPA's BMD guidance (U.S. EPA, 2012a) does not preclude the use of a BMR that is below observable response data and EPA has deemed that Rogers et al. (1993b) is adequate for the consideration of a 5% BMR.

95% lower confidence limit of the dose-response curve expressed in terms of the C_{max} for methanol in blood (Rogers et al., 1993b).

Table D-8 Comparison of BMD modeling results for 10% cervical rib incidence in mice using modeled C_{max} above background of methanol as a common dose metric

Model	BMD_{10} (C_{max}, mg/L)[a]	$BMDL_{10}$ (C_{max}, mg/L)[a]	*p*-value	AIC[c]	Scaled residual[d]
NLogistic[b]	140.75	91.00	0.3359	1,047.37	0.5395
NCTR	223.55	111.78	0.2705	1,050.32	0.5640
Rai and Van Ryzin	233.61	116.81	0.2625	1,052.14	0.6043

[a]C_{max} values are the blood levels of the dams on GD6 with background subtracted; the BMDL is the 95% lower confidence limit on the C_{max} for 10% extra risk (dichotomous endpoints) estimated by the model using the likelihood profile method (U.S. EPA, 2012a).

[b]Model choice based on adequate *p* value (> 0.1), visual inspection, low AIC, and low (absolute) scaled residual.

[c]AIC = Akaike Information Criterion = -2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters, and P is the number of modeled degrees of freedom (usually the number of parameters estimated).

[d]X^2d residual (measure of how model-predicted responses deviate from the actual data) for the dose group closest to the BMD scaled by an estimate of its SD Provides a comparative measure of model fit near the BMD. Residuals exceeding 2.0 in absolute value should cause one to question model fit in this region.

Data from Rogers et al. (1993b).

```
 ========================================================================
    NLogistic Model. (Version: 2.15; Date: 10/28/2009)
    Input Data File: C:/Documents and
Settings/llowe/Desktop/ROGERS_CMAX_BMD/ROGERS_CMAX_BMD10/NLog_CR_10. (d)
                  Fri Dec 16 10:48:13 2011
 ========================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

 The probability function is:


 Prob. = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/

 [1+exp (-beta-theta2*Rij-rho*log (Dose))],

 where Rij is the litter specific covariate.

 Restrict Power rho >= 1.



 Total number of observations = 166
 Total number of records with missing values = 0
 Total number of parameters in model = 9
 Total number of specified parameters = 0


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



 Default Initial Parameter Values
 alpha = 0.302379
 beta = -7.2579
 theta1 = 0
 theta2 = 0
 rho = 1
 phi1 = 0.214334
 phi2 = 0.304943
 phi3 = 0.220179
 phi4 = 0.370088



 Parameter Estimates

 Variable Estimate Std. Err.
 alpha 0.127131 *
 beta -4.62736 *
 theta1 0.0297845 *
 theta2 -0.467856 *
 rho 1 *
 phi1 0.203691 *
 phi2 0.305429 *
 phi3 0.212663 *
 phi4 0.363199 *

* - Indicates that this value is not calculated.

 Log-likelihood: -515.686 AIC: 1047.37
```

Litter Data

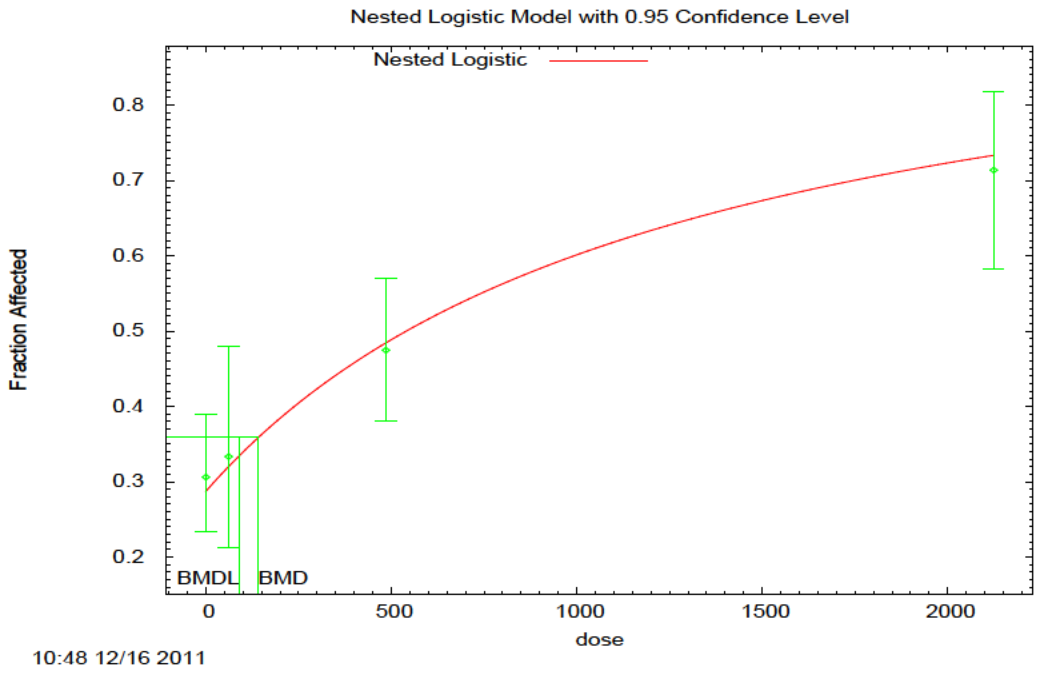
Dose	Lit.-Spec. Cov.	Litter Est._Prob.	Scaled Size	Expected	Observed	Residual
0.0000	1.0000	0.157	1	0.157	0	-0.4314
0.0000	1.0000	0.157	1	0.157	0	-0.4314
0.0000	2.0000	0.187	2	0.373	0	-0.6176
0.0000	2.0000	0.187	2	0.373	2	2.6904
0.0000	2.0000	0.187	2	0.373	0	-0.6176
0.0000	2.0000	0.187	2	0.373	0	-0.6176
0.0000	2.0000	0.187	2	0.373	1	1.0364
0.0000	3.0000	0.216	3	0.649	1	0.4142
0.0000	3.0000	0.216	3	0.649	0	-0.7674
0.0000	3.0000	0.216	3	0.649	1	0.4142
0.0000	3.0000	0.216	3	0.649	0	-0.7674
0.0000	4.0000	0.246	4	0.985	0	-0.9007
0.0000	4.0000	0.246	4	0.985	1	0.0136
0.0000	4.0000	0.246	4	0.985	0	-0.9007
0.0000	4.0000	0.246	4	0.985	1	0.0136
0.0000	5.0000	0.276	5	1.380	0	-1.0250
0.0000	5.0000	0.276	5	1.380	1	-0.2824
0.0000	5.0000	0.276	5	1.380	1	-0.2824
0.0000	5.0000	0.276	5	1.380	3	1.2028
0.0000	5.0000	0.276	5	1.380	0	-1.0250
0.0000	5.0000	0.276	5	1.380	0	-1.0250
0.0000	5.0000	0.276	5	1.380	0	-1.0250
0.0000	5.0000	0.276	5	1.380	1	-0.2824
0.0000	5.0000	0.276	5	1.380	1	-0.2824
0.0000	5.0000	0.276	5	1.380	1	-0.2824
0.0000	6.0000	0.306	6	1.835	0	-1.1444
0.0000	6.0000	0.306	6	1.835	5	1.9738
0.0000	6.0000	0.306	6	1.835	3	0.7265
0.0000	6.0000	0.306	6	1.835	3	0.7265
0.0000	6.0000	0.306	6	1.835	0	-1.1444
0.0000	6.0000	0.306	6	1.835	3	0.7265
0.0000	6.0000	0.306	6	1.835	5	1.9738
0.0000	6.0000	0.306	6	1.835	3	0.7265
0.0000	6.0000	0.306	6	1.835	1	-0.5208
0.0000	6.0000	0.306	6	1.835	3	0.7265
0.0000	6.0000	0.306	6	1.835	2	0.1029
0.0000	6.0000	0.306	6	1.835	2	0.1029
0.0000	6.0000	0.306	6	1.835	1	-0.5208
0.0000	6.0000	0.306	6	1.835	6	2.5975
0.0000	6.0000	0.306	6	1.835	0	-1.1444
0.0000	6.0000	0.306	6	1.835	0	-1.1444
0.0000	7.0000	0.336	7	2.349	1	-0.7245
0.0000	7.0000	0.336	7	2.349	5	1.4233
0.0000	7.0000	0.336	7	2.349	1	-0.7245
0.0000	7.0000	0.336	7	2.349	2	-0.1876
0.0000	7.0000	0.336	7	2.349	1	-0.7245
0.0000	7.0000	0.336	7	2.349	2	-0.1876
0.0000	7.0000	0.336	7	2.349	5	1.4233
0.0000	7.0000	0.336	7	2.349	2	-0.1876
0.0000	7.0000	0.336	7	2.349	3	0.3494
0.0000	7.0000	0.336	7	2.349	3	0.3494
0.0000	7.0000	0.336	7	2.349	0	-1.2615
0.0000	7.0000	0.336	7	2.349	0	-1.2615
0.0000	7.0000	0.336	7	2.349	2	-0.1876
0.0000	8.0000	0.365	8	2.923	4	0.5076
0.0000	8.0000	0.365	8	2.923	2	-0.4352
0.0000	8.0000	0.365	8	2.923	8	2.3932
0.0000	8.0000	0.365	8	2.923	3	0.0362

```
0.0000 8.0000 0.365 8 2.923 1 -0.9066

61.4000 1.0000 0.387 1 0.387 0 -0.7951
61.4000 1.0000 0.387 1 0.387 0 -0.7951
61.4000 2.0000 0.342 2 0.684 2 1.7177
61.4000 2.0000 0.342 2 0.684 0 -0.8919
61.4000 3.0000 0.317 3 0.952 1 0.0472
61.4000 3.0000 0.317 3 0.952 3 2.0021
61.4000 3.0000 0.317 3 0.952 1 0.0472
61.4000 3.0000 0.317 3 0.952 2 1.0246
61.4000 4.0000 0.310 4 1.240 3 1.3743
61.4000 4.0000 0.310 4 1.240 0 -0.9685
61.4000 5.0000 0.316 5 1.578 0 -1.0189
61.4000 5.0000 0.316 5 1.578 0 -1.0189
61.4000 5.0000 0.316 5 1.578 1 -0.3733
61.4000 5.0000 0.316 5 1.578 4 1.5633
61.4000 5.0000 0.316 5 1.578 0 -1.0189
61.4000 6.0000 0.330 6 1.981 3 0.5566
61.4000 6.0000 0.330 6 1.981 2 0.0105
61.4000 7.0000 0.350 7 2.453 2 -0.2131
61.4000 7.0000 0.350 7 2.453 2 -0.2131
61.4000 7.0000 0.350 7 2.453 3 0.2577
61.4000 7.0000 0.350 7 2.453 0 -1.1545
61.4000 7.0000 0.350 7 2.453 2 -0.2131
61.4000 7.0000 0.350 7 2.453 2 -0.2131
61.4000 8.0000 0.374 8 2.994 2 -0.4101
61.4000 8.0000 0.374 8 2.994 8 2.0644
61.4000 8.0000 0.374 8 2.994 0 -1.2350

485.4000 2.0000 0.716 2 1.432 2 0.8091
485.4000 3.0000 0.638 3 1.915 3 1.0920
485.4000 4.0000 0.564 4 2.258 1 -0.9912
485.4000 4.0000 0.564 4 2.258 2 -0.2032
485.4000 5.0000 0.503 5 2.517 5 1.6327
485.4000 5.0000 0.503 5 2.517 1 -0.9972
485.4000 5.0000 0.503 5 2.517 3 0.3178
485.4000 5.0000 0.503 5 2.517 3 0.3178
485.4000 6.0000 0.460 6 2.763 2 -0.4350
485.4000 6.0000 0.460 6 2.763 5 1.2756
485.4000 6.0000 0.460 6 2.763 2 -0.4350
485.4000 6.0000 0.460 6 2.763 3 0.1352
485.4000 6.0000 0.460 6 2.763 2 -0.4350
485.4000 6.0000 0.460 6 2.763 0 -1.5754
485.4000 6.0000 0.460 6 2.763 4 0.7054
485.4000 6.0000 0.460 6 2.763 0 -1.5754
485.4000 6.0000 0.460 6 2.763 5 1.2756
485.4000 6.0000 0.460 6 2.763 2 -0.4350
485.4000 6.0000 0.460 6 2.763 4 0.7054
485.4000 6.0000 0.460 6 2.763 3 0.1352
485.4000 6.0000 0.460 6 2.763 6 1.8458
485.4000 6.0000 0.460 6 2.763 3 0.1352
485.4000 6.0000 0.460 6 2.763 5 1.2756
485.4000 6.0000 0.460 6 2.763 3 0.1352
485.4000 7.0000 0.437 7 3.057 4 0.4762
485.4000 7.0000 0.437 7 3.057 5 0.9813
485.4000 7.0000 0.437 7 3.057 0 -1.5443
485.4000 7.0000 0.437 7 3.057 5 0.9813
485.4000 7.0000 0.437 7 3.057 1 -1.0392
485.4000 7.0000 0.437 7 3.057 4 0.4762
485.4000 7.0000 0.437 7 3.057 3 -0.0289
485.4000 7.0000 0.437 7 3.057 4 0.4762
485.4000 7.0000 0.437 7 3.057 1 -1.0392
485.4000 7.0000 0.437 7 3.057 3 -0.0289
485.4000 7.0000 0.437 7 3.057 3 -0.0289
485.4000 7.0000 0.437 7 3.057 1 -1.0392
```

```
 485.4000 8.0000 0.430 8 3.436 7 1.6134
 485.4000 8.0000 0.430 8 3.436 5 0.7079
 485.4000 8.0000 0.430 8 3.436 0 -1.5558
 485.4000 9.0000 0.435 9 3.915 0 -1.6016
 485.4000 9.0000 0.435 9 3.915 6 0.8530

2124.4000 1.0000 0.940 1 0.940 1 0.2530
2124.4000 1.0000 0.940 1 0.940 1 0.2530
2124.4000 1.0000 0.940 1 0.940 1 0.2530
2124.4000 2.0000 0.911 2 1.822 2 0.3783
2124.4000 2.0000 0.911 2 1.822 1 -1.7500
2124.4000 3.0000 0.872 3 2.615 3 0.5058
2124.4000 3.0000 0.872 3 2.615 3 0.5058
2124.4000 3.0000 0.872 3 2.615 1 -2.1218
2124.4000 3.0000 0.872 3 2.615 1 -2.1218
2124.4000 4.0000 0.820 4 3.282 4 0.6473
2124.4000 4.0000 0.820 4 3.282 4 0.6473
2124.4000 4.0000 0.820 4 3.282 2 -1.1551
2124.4000 4.0000 0.820 4 3.282 4 0.6473
2124.4000 4.0000 0.820 4 3.282 4 0.6473
2124.4000 4.0000 0.820 4 3.282 3 -0.2539
2124.4000 4.0000 0.820 4 3.282 4 0.6473
2124.4000 5.0000 0.759 5 3.795 1 -1.8656
2124.4000 5.0000 0.759 5 3.795 5 0.8047
2124.4000 5.0000 0.759 5 3.795 5 0.8047
2124.4000 5.0000 0.759 5 3.795 4 0.1371
2124.4000 5.0000 0.759 5 3.795 4 0.1371
2124.4000 5.0000 0.759 5 3.795 3 -0.5305
2124.4000 6.0000 0.692 6 4.153 5 0.4466
2124.4000 6.0000 0.692 6 4.153 6 0.9736
2124.4000 6.0000 0.692 6 4.153 6 0.9736
2124.4000 6.0000 0.692 6 4.153 3 -0.6074
2124.4000 6.0000 0.692 6 4.153 6 0.9736
2124.4000 6.0000 0.692 6 4.153 2 -1.1344
2124.4000 6.0000 0.692 6 4.153 4 -0.0804
2124.4000 6.0000 0.692 6 4.153 0 -2.1885
2124.4000 6.0000 0.692 6 4.153 5 0.4466
2124.4000 6.0000 0.692 6 4.153 0 -2.1885
2124.4000 6.0000 0.692 6 4.153 5 0.4466
2124.4000 6.0000 0.692 6 4.153 4 -0.0804
2124.4000 7.0000 0.628 7 4.396 5 0.2650
2124.4000 7.0000 0.628 7 4.396 5 0.2650
2124.4000 7.0000 0.628 7 4.396 7 1.1421
2124.4000 7.0000 0.628 7 4.396 6 0.7036
2124.4000 7.0000 0.628 7 4.396 7 1.1421
2124.4000 8.0000 0.575 8 4.598 0 -1.7470


Combine litters with adjacent levels of the litter-specific covariate
within dose groups until the expected count exceeds 3.0, to help improve
the fit of the X^2 statistic to chi-square.


 Grouped Data


 Mean Scaled
 Dose Lit.-Spec. Cov. Expected Observed Residual
 ---------------------------------------------------------------
 0.0000 1.0000 0.314 0 -0.6101
 0.0000 2.0000 1.867 3 0.8381
 0.0000 3.0000 2.598 2 -0.3532
 0.0000 4.0000 3.940 2 -0.8870
 0.0000 5.0000 4.141 2 -0.9178
 0.0000 5.0000 4.141 3 -0.4891
```

```
0.0000 5.0000 4.141 2 -0.9178
0.0000 5.0000 1.380 1 -0.2824
0.0000 6.0000 3.670 5 0.5865
0.0000 6.0000 3.670 6 1.0275
0.0000 6.0000 3.670 3 -0.2955
0.0000 6.0000 3.670 8 1.9094
0.0000 6.0000 3.670 4 0.1455
0.0000 6.0000 3.670 4 0.1455
0.0000 6.0000 3.670 7 1.4685
0.0000 6.0000 3.670 0 -1.6184
0.0000 7.0000 4.699 6 0.4941
0.0000 7.0000 4.699 3 -0.6450
0.0000 7.0000 4.699 3 -0.6450
0.0000 7.0000 4.699 7 0.8738
0.0000 7.0000 4.699 6 0.4941
0.0000 7.0000 4.699 0 -1.7840
0.0000 7.0000 2.349 2 -0.1876
0.0000 8.0000 5.847 6 0.0512
0.0000 8.0000 5.847 11 1.7178
0.0000 8.0000 2.923 1 -0.9066

61.4000 1.0000 0.775 0 -1.1245
61.4000 2.0000 1.367 2 0.5840
61.4000 3.0000 3.807 7 1.5606
61.4000 4.0000 2.480 3 0.2870
61.4000 5.0000 3.157 0 -1.4409
61.4000 5.0000 3.157 5 0.8414
61.4000 5.0000 1.578 0 -1.0189
61.4000 6.0000 3.962 5 0.4010
61.4000 7.0000 4.905 4 -0.3013
61.4000 7.0000 4.905 3 -0.6342
61.4000 7.0000 4.905 4 -0.3013
61.4000 8.0000 5.989 10 1.1697
61.4000 8.0000 2.994 0 -1.2350

485.4000 2.0000 1.432 2 0.8091
485.4000 3.0000 1.915 3 1.0920
485.4000 4.0000 4.516 3 -0.8446
485.4000 5.0000 5.033 6 0.4494
485.4000 5.0000 5.033 6 0.4494
485.4000 6.0000 5.526 7 0.5944
485.4000 6.0000 5.526 5 -0.2120
485.4000 6.0000 5.526 2 -1.4216
485.4000 6.0000 5.526 4 -0.6152
485.4000 6.0000 5.526 7 0.5944
485.4000 6.0000 5.526 7 0.5944
485.4000 6.0000 5.526 9 1.4008
485.4000 6.0000 5.526 8 0.9976
485.4000 7.0000 3.057 4 0.4762
485.4000 7.0000 3.057 5 0.9813
485.4000 7.0000 3.057 0 -1.5443
485.4000 7.0000 3.057 5 0.9813
485.4000 7.0000 3.057 1 -1.0392
485.4000 7.0000 3.057 4 0.4762
485.4000 7.0000 3.057 3 -0.0289
485.4000 7.0000 3.057 4 0.4762
485.4000 7.0000 3.057 1 -1.0392
485.4000 7.0000 3.057 3 -0.0289
485.4000 7.0000 3.057 3 -0.0289
485.4000 7.0000 3.057 1 -1.0392
485.4000 8.0000 3.436 7 1.6134
485.4000 8.0000 3.436 5 0.7079
485.4000 8.0000 3.436 0 -1.5558
485.4000 9.0000 3.915 0 -1.6016
485.4000 9.0000 3.915 6 0.8530
```

```
2124.4000 1.0000 2.820 3 0.4382
2124.4000 2.0000 3.645 3 -0.9699
2124.4000 3.0000 5.230 6 0.7153
2124.4000 3.0000 5.230 2 -3.0007
2124.4000 4.0000 3.282 4 0.6473
2124.4000 4.0000 3.282 4 0.6473
2124.4000 4.0000 3.282 2 -1.1551
2124.4000 4.0000 3.282 4 0.6473
2124.4000 4.0000 3.282 4 0.6473
2124.4000 4.0000 3.282 3 -0.2539
2124.4000 4.0000 3.282 4 0.6473
2124.4000 5.0000 3.795 1 -1.8656
2124.4000 5.0000 3.795 5 0.8047
2124.4000 5.0000 3.795 5 0.8047
2124.4000 5.0000 3.795 4 0.1371
2124.4000 5.0000 3.795 4 0.1371
2124.4000 5.0000 3.795 3 -0.5305
2124.4000 6.0000 4.153 5 0.4466
2124.4000 6.0000 4.153 6 0.9736
2124.4000 6.0000 4.153 6 0.9736
2124.4000 6.0000 4.153 3 -0.6074
2124.4000 6.0000 4.153 6 0.9736
2124.4000 6.0000 4.153 2 -1.1344
2124.4000 6.0000 4.153 4 -0.0804
2124.4000 6.0000 4.153 0 -2.1885
2124.4000 6.0000 4.153 5 0.4466
2124.4000 6.0000 4.153 0 -2.1885
2124.4000 6.0000 4.153 5 0.4466
2124.4000 6.0000 4.153 4 -0.0804
2124.4000 7.0000 4.396 5 0.2650
2124.4000 7.0000 4.396 5 0.2650
2124.4000 7.0000 4.396 7 1.1421
2124.4000 7.0000 4.396 6 0.7036
2124.4000 7.0000 4.396 7 1.1421
2124.4000 8.0000 4.598 0 -1.7470

 Chi-square = 101.30 DF = 96 P-value = 0.3359


To calculate the BMD and BMDL, the litter specific covariate is fixed
 at the mean litter specific covariate of all the data: 5.379518

 Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

 BMD = 140.749

 BMDL = 90.9972
```



Data points obtained from Rogers et al. (1993b).

Figure D-5 Nested logistic model, 0.1 extra risk - Incidence of cervical rib in mice versus C_{max} above background of methanol, GD6-GD15 inhalational study.

D.3.2. BMD Approach with a BMR of 0.05 Extra Risk

A summary of the results most relevant to the development of a POD using the BMD approach (BMD, BMDL, and model fit statistics) for increased incidence of cervical rib in mice exposed to methanol during gestation from days 6 to 15, with a BMR of 0.05 extra risk, is provided in Table D-9. Model fit was determined by statistics (AIC and χ^2 residuals of individual dose groups) and visual inspection, as recommended by U.S. EPA (2012a). The best model fit to these data (from visual inspection and comparison of AIC values) was obtained using the NLogistic model. The text and graphic (see Figure D-6) output from this model follow Table D-6. The $BMDL_{05}$ was determined to be 43.1039 mg/L using the 95% lower confidence limit of the dose-response curve expressed in terms of the C_{max} for methanol in blood (Rogers et al., 1993b).

Table D-9 Comparison of BMD modeling results for 5% cervical rib incidence in mice using modeled C_{max} above background of methanol as a common dose metric

Model	BMD_{05} (C_{max}, mg/L)[a]	$BMDL_{05}$ (C_{max}, mg/L)[a]	*p*-value	AIC[c]	Scaled residual[d]
NLogistic[b]	66.67	43.10	0.3359	1047.37	0.5395
NCTR	108.83	54.42	0.2705	1050.32	0.5640
Rai and Van Ryzin	113.73	56.87	0.2625	1052.14	0.6043

[a]C_{max} are the blood levels of the dams on GD6 with background subtracted; the BMDL is the 95% lower confidence limit on the C_{max} for a 5% extra risk (dichotomous endpoints) estimated by the model using the likelihood profile method (U.S. EPA, 2012a).

[b]Model choice based on adequate *p* value (> 0.1), visual inspection, low AIC, and low (absolute) scaled residual.

[c]AIC = Akaike Information Criterion = -2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters, and P is the number of modeled degrees of freedom (usually the number of parameters estimated).

[d]chi-squared (X^2) residual (measure of how model-predicted responses deviate from the actual data) for the dose group closest to the BMD scaled by an estimate of its SD Provides a comparative measure of model fit near the BMD. Residuals exceeding 2.0 in absolute value should cause one to question model fit in this region.

Data from Rogers et al. (1993b).

```
================================================================
    NLogistic Model. (Version: 2.15; Date: 10/28/2009)
    Input Data File: C:/Documents and
Settings/llowe/Desktop/ROGERS_CMAX_BMD/ROGERS_CMAX_BMD05/NLog_CR_5. (d)
                  Fri Dec 16 10:56:05 2011
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

 The probability function is:


 Prob. = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/

 [1+exp (-beta-theta2*Rij-rho*log (Dose))],

 where Rij is the litter specific covariate.

 Restrict Power rho >= 1.



 Total number of observations = 166
 Total number of records with missing values = 0
 Total number of parameters in model = 9
 Total number of specified parameters = 0


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



 Default Initial Parameter Values
 alpha = 0.302379
 beta = -7.2579
 theta1 = 0
 theta2 = 0
 rho = 1
 phi1 = 0.214334
 phi2 = 0.304943
 phi3 = 0.220179
 phi4 = 0.370088



 Parameter Estimates

 Variable Estimate Std. Err.
 alpha 0.127131 *
 beta -4.62736 *
 theta1 0.0297845 *
 theta2 -0.467856 *
 rho 1 *
 phi1 0.203691 *
 phi2 0.305429 *
 phi3 0.212663 *
 phi4 0.363199 *

* - Indicates that this value is not calculated.

 Log-likelihood: -515.686 AIC: 1047.37
```

Litter Data

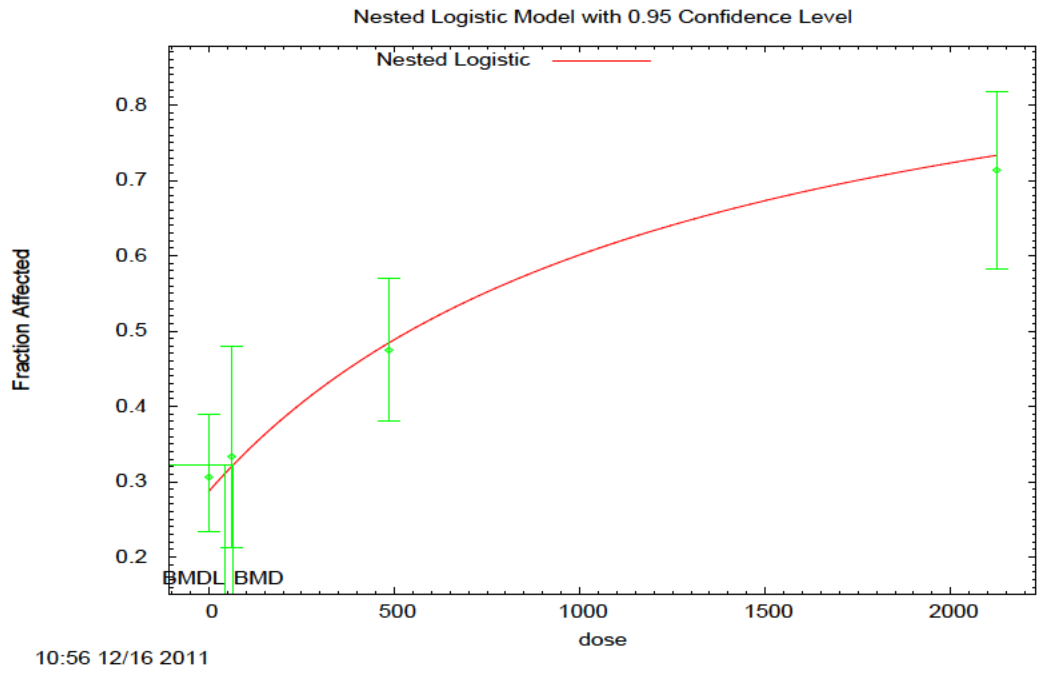
Dose	Lit.-Spec. Cov.	Litter Est._Prob.	Scaled Size	Expected	Observed	Residual
0.0000	1.0000	0.157	1	0.157	0	-0.4314
0.0000	1.0000	0.157	1	0.157	0	-0.4314
0.0000	2.0000	0.187	2	0.373	0	-0.6176
0.0000	2.0000	0.187	2	0.373	2	2.6904
0.0000	2.0000	0.187	2	0.373	0	-0.6176
0.0000	2.0000	0.187	2	0.373	0	-0.6176
0.0000	2.0000	0.187	2	0.373	1	1.0364
0.0000	3.0000	0.216	3	0.649	1	0.4142
0.0000	3.0000	0.216	3	0.649	0	-0.7674
0.0000	3.0000	0.216	3	0.649	1	0.4142
0.0000	3.0000	0.216	3	0.649	0	-0.7674
0.0000	4.0000	0.246	4	0.985	0	-0.9007
0.0000	4.0000	0.246	4	0.985	1	0.0136
0.0000	4.0000	0.246	4	0.985	0	-0.9007
0.0000	4.0000	0.246	4	0.985	1	0.0136
0.0000	5.0000	0.276	5	1.380	0	-1.0250
0.0000	5.0000	0.276	5	1.380	1	-0.2824
0.0000	5.0000	0.276	5	1.380	1	-0.2824
0.0000	5.0000	0.276	5	1.380	3	1.2028
0.0000	5.0000	0.276	5	1.380	0	-1.0250
0.0000	5.0000	0.276	5	1.380	0	-1.0250
0.0000	5.0000	0.276	5	1.380	0	-1.0250
0.0000	5.0000	0.276	5	1.380	1	-0.2824
0.0000	5.0000	0.276	5	1.380	1	-0.2824
0.0000	5.0000	0.276	5	1.380	1	-0.2824
0.0000	6.0000	0.306	6	1.835	0	-1.1444
0.0000	6.0000	0.306	6	1.835	5	1.9738
0.0000	6.0000	0.306	6	1.835	3	0.7265
0.0000	6.0000	0.306	6	1.835	3	0.7265
0.0000	6.0000	0.306	6	1.835	0	-1.1444
0.0000	6.0000	0.306	6	1.835	3	0.7265
0.0000	6.0000	0.306	6	1.835	5	1.9738
0.0000	6.0000	0.306	6	1.835	3	0.7265
0.0000	6.0000	0.306	6	1.835	1	-0.5208
0.0000	6.0000	0.306	6	1.835	3	0.7265
0.0000	6.0000	0.306	6	1.835	2	0.1029
0.0000	6.0000	0.306	6	1.835	2	0.1029
0.0000	6.0000	0.306	6	1.835	1	-0.5208
0.0000	6.0000	0.306	6	1.835	6	2.5975
0.0000	6.0000	0.306	6	1.835	0	-1.1444
0.0000	6.0000	0.306	6	1.835	0	-1.1444
0.0000	7.0000	0.336	7	2.349	1	-0.7245
0.0000	7.0000	0.336	7	2.349	5	1.4233
0.0000	7.0000	0.336	7	2.349	1	-0.7245
0.0000	7.0000	0.336	7	2.349	2	-0.1876
0.0000	7.0000	0.336	7	2.349	1	-0.7245
0.0000	7.0000	0.336	7	2.349	2	-0.1876
0.0000	7.0000	0.336	7	2.349	5	1.4233
0.0000	7.0000	0.336	7	2.349	2	-0.1876
0.0000	7.0000	0.336	7	2.349	3	0.3494
0.0000	7.0000	0.336	7	2.349	3	0.3494
0.0000	7.0000	0.336	7	2.349	0	-1.2615
0.0000	7.0000	0.336	7	2.349	0	-1.2615
0.0000	7.0000	0.336	7	2.349	2	-0.1876
0.0000	8.0000	0.365	8	2.923	4	0.5076
0.0000	8.0000	0.365	8	2.923	2	-0.4352
0.0000	8.0000	0.365	8	2.923	8	2.3932
0.0000	8.0000	0.365	8	2.923	3	0.0362

```
0.0000 8.0000 0.365 8 2.923 1 -0.9066

61.4000 1.0000 0.387 1 0.387 0 -0.7951
61.4000 1.0000 0.387 1 0.387 0 -0.7951
61.4000 2.0000 0.342 2 0.684 2 1.7177
61.4000 2.0000 0.342 2 0.684 0 -0.8919
61.4000 3.0000 0.317 3 0.952 1 0.0472
61.4000 3.0000 0.317 3 0.952 3 2.0021
61.4000 3.0000 0.317 3 0.952 1 0.0472
61.4000 3.0000 0.317 3 0.952 2 1.0246
61.4000 4.0000 0.310 4 1.240 3 1.3743
61.4000 4.0000 0.310 4 1.240 0 -0.9685
61.4000 5.0000 0.316 5 1.578 0 -1.0189
61.4000 5.0000 0.316 5 1.578 0 -1.0189
61.4000 5.0000 0.316 5 1.578 1 -0.3733
61.4000 5.0000 0.316 5 1.578 4 1.5633
61.4000 5.0000 0.316 5 1.578 0 -1.0189
61.4000 6.0000 0.330 6 1.981 3 0.5566
61.4000 6.0000 0.330 6 1.981 2 0.0105
61.4000 7.0000 0.350 7 2.453 2 -0.2131
61.4000 7.0000 0.350 7 2.453 2 -0.2131
61.4000 7.0000 0.350 7 2.453 3 0.2577
61.4000 7.0000 0.350 7 2.453 0 -1.1545
61.4000 7.0000 0.350 7 2.453 2 -0.2131
61.4000 7.0000 0.350 7 2.453 2 -0.2131
61.4000 8.0000 0.374 8 2.994 2 -0.4101
61.4000 8.0000 0.374 8 2.994 8 2.0644
61.4000 8.0000 0.374 8 2.994 0 -1.2350

485.4000 2.0000 0.716 2 1.432 2 0.8091
485.4000 3.0000 0.638 3 1.915 3 1.0920
485.4000 4.0000 0.564 4 2.258 1 -0.9912
485.4000 4.0000 0.564 4 2.258 2 -0.2032
485.4000 5.0000 0.503 5 2.517 5 1.6327
485.4000 5.0000 0.503 5 2.517 1 -0.9972
485.4000 5.0000 0.503 5 2.517 3 0.3178
485.4000 5.0000 0.503 5 2.517 3 0.3178
485.4000 6.0000 0.460 6 2.763 2 -0.4350
485.4000 6.0000 0.460 6 2.763 5 1.2756
485.4000 6.0000 0.460 6 2.763 2 -0.4350
485.4000 6.0000 0.460 6 2.763 3 0.1352
485.4000 6.0000 0.460 6 2.763 2 -0.4350
485.4000 6.0000 0.460 6 2.763 0 -1.5754
485.4000 6.0000 0.460 6 2.763 4 0.7054
485.4000 6.0000 0.460 6 2.763 0 -1.5754
485.4000 6.0000 0.460 6 2.763 5 1.2756
485.4000 6.0000 0.460 6 2.763 2 -0.4350
485.4000 6.0000 0.460 6 2.763 4 0.7054
485.4000 6.0000 0.460 6 2.763 3 0.1352
485.4000 6.0000 0.460 6 2.763 6 1.8458
485.4000 6.0000 0.460 6 2.763 3 0.1352
485.4000 6.0000 0.460 6 2.763 5 1.2756
485.4000 6.0000 0.460 6 2.763 3 0.1352
485.4000 7.0000 0.437 7 3.057 4 0.4762
485.4000 7.0000 0.437 7 3.057 5 0.9813
485.4000 7.0000 0.437 7 3.057 0 -1.5443
485.4000 7.0000 0.437 7 3.057 5 0.9813
485.4000 7.0000 0.437 7 3.057 1 -1.0392
485.4000 7.0000 0.437 7 3.057 4 0.4762
485.4000 7.0000 0.437 7 3.057 3 -0.0289
485.4000 7.0000 0.437 7 3.057 4 0.4762
485.4000 7.0000 0.437 7 3.057 1 -1.0392
485.4000 7.0000 0.437 7 3.057 3 -0.0289
485.4000 7.0000 0.437 7 3.057 3 -0.0289
485.4000 7.0000 0.437 7 3.057 1 -1.0392
```

```
 485.4000 8.0000 0.430 8 3.436 7 1.6134
 485.4000 8.0000 0.430 8 3.436 5 0.7079
 485.4000 8.0000 0.430 8 3.436 0 -1.5558
 485.4000 9.0000 0.435 9 3.915 0 -1.6016
 485.4000 9.0000 0.435 9 3.915 6 0.8530

2124.4000 1.0000 0.940 1 0.940 1 0.2530
2124.4000 1.0000 0.940 1 0.940 1 0.2530
2124.4000 1.0000 0.940 1 0.940 1 0.2530
2124.4000 2.0000 0.911 2 1.822 2 0.3783
2124.4000 2.0000 0.911 2 1.822 1 -1.7500
2124.4000 3.0000 0.872 3 2.615 3 0.5058
2124.4000 3.0000 0.872 3 2.615 3 0.5058
2124.4000 3.0000 0.872 3 2.615 1 -2.1218
2124.4000 3.0000 0.872 3 2.615 1 -2.1218
2124.4000 4.0000 0.820 4 3.282 4 0.6473
2124.4000 4.0000 0.820 4 3.282 4 0.6473
2124.4000 4.0000 0.820 4 3.282 2 -1.1551
2124.4000 4.0000 0.820 4 3.282 4 0.6473
2124.4000 4.0000 0.820 4 3.282 4 0.6473
2124.4000 4.0000 0.820 4 3.282 3 -0.2539
2124.4000 4.0000 0.820 4 3.282 4 0.6473
2124.4000 5.0000 0.759 5 3.795 1 -1.8656
2124.4000 5.0000 0.759 5 3.795 5 0.8047
2124.4000 5.0000 0.759 5 3.795 5 0.8047
2124.4000 5.0000 0.759 5 3.795 4 0.1371
2124.4000 5.0000 0.759 5 3.795 4 0.1371
2124.4000 5.0000 0.759 5 3.795 3 -0.5305
2124.4000 6.0000 0.692 6 4.153 5 0.4466
2124.4000 6.0000 0.692 6 4.153 6 0.9736
2124.4000 6.0000 0.692 6 4.153 6 0.9736
2124.4000 6.0000 0.692 6 4.153 3 -0.6074
2124.4000 6.0000 0.692 6 4.153 6 0.9736
2124.4000 6.0000 0.692 6 4.153 2 -1.1344
2124.4000 6.0000 0.692 6 4.153 4 -0.0804
2124.4000 6.0000 0.692 6 4.153 0 -2.1885
2124.4000 6.0000 0.692 6 4.153 5 0.4466
2124.4000 6.0000 0.692 6 4.153 0 -2.1885
2124.4000 6.0000 0.692 6 4.153 5 0.4466
2124.4000 6.0000 0.692 6 4.153 4 -0.0804
2124.4000 7.0000 0.628 7 4.396 5 0.2650
2124.4000 7.0000 0.628 7 4.396 5 0.2650
2124.4000 7.0000 0.628 7 4.396 7 1.1421
2124.4000 7.0000 0.628 7 4.396 6 0.7036
2124.4000 7.0000 0.628 7 4.396 7 1.1421
2124.4000 8.0000 0.575 8 4.598 0 -1.7470


Combine litters with adjacent levels of the litter-specific covariate
within dose groups until the expected count exceeds 3.0, to help improve
the fit of the X^2 statistic to chi-square.


 Grouped Data


 Mean Scaled
 Dose Lit.-Spec. Cov. Expected Observed Residual
 ---------------------------------------------------------------
 0.0000 1.0000 0.314 0 -0.6101
 0.0000 2.0000 1.867 3 0.8381
 0.0000 3.0000 2.598 2 -0.3532
 0.0000 4.0000 3.940 2 -0.8870
 0.0000 5.0000 4.141 2 -0.9178
 0.0000 5.0000 4.141 3 -0.4891
```

```
0.0000 5.0000 4.141 2 -0.9178
0.0000 5.0000 1.380 1 -0.2824
0.0000 6.0000 3.670 5 0.5865
0.0000 6.0000 3.670 6 1.0275
0.0000 6.0000 3.670 3 -0.2955
0.0000 6.0000 3.670 8 1.9094
0.0000 6.0000 3.670 4 0.1455
0.0000 6.0000 3.670 4 0.1455
0.0000 6.0000 3.670 7 1.4685
0.0000 6.0000 3.670 0 -1.6184
0.0000 7.0000 4.699 6 0.4941
0.0000 7.0000 4.699 3 -0.6450
0.0000 7.0000 4.699 3 -0.6450
0.0000 7.0000 4.699 7 0.8738
0.0000 7.0000 4.699 6 0.4941
0.0000 7.0000 4.699 0 -1.7840
0.0000 7.0000 2.349 2 -0.1876
0.0000 8.0000 5.847 6 0.0512
0.0000 8.0000 5.847 11 1.7178
0.0000 8.0000 2.923 1 -0.9066

61.4000 1.0000 0.775 0 -1.1245
61.4000 2.0000 1.367 2 0.5840
61.4000 3.0000 3.807 7 1.5606
61.4000 4.0000 2.480 3 0.2870
61.4000 5.0000 3.157 0 -1.4409
61.4000 5.0000 3.157 5 0.8414
61.4000 5.0000 1.578 0 -1.0189
61.4000 6.0000 3.962 5 0.4010
61.4000 7.0000 4.905 4 -0.3013
61.4000 7.0000 4.905 3 -0.6342
61.4000 7.0000 4.905 4 -0.3013
61.4000 8.0000 5.989 10 1.1697
61.4000 8.0000 2.994 0 -1.2350

485.4000 2.0000 1.432 2 0.8091
485.4000 3.0000 1.915 3 1.0920
485.4000 4.0000 4.516 3 -0.8446
485.4000 5.0000 5.033 6 0.4494
485.4000 5.0000 5.033 6 0.4494
485.4000 6.0000 5.526 7 0.5944
485.4000 6.0000 5.526 5 -0.2120
485.4000 6.0000 5.526 2 -1.4216
485.4000 6.0000 5.526 4 -0.6152
485.4000 6.0000 5.526 7 0.5944
485.4000 6.0000 5.526 7 0.5944
485.4000 6.0000 5.526 9 1.4008
485.4000 6.0000 5.526 8 0.9976
485.4000 7.0000 3.057 4 0.4762
485.4000 7.0000 3.057 5 0.9813
485.4000 7.0000 3.057 0 -1.5443
485.4000 7.0000 3.057 5 0.9813
485.4000 7.0000 3.057 1 -1.0392
485.4000 7.0000 3.057 4 0.4762
485.4000 7.0000 3.057 3 -0.0289
485.4000 7.0000 3.057 4 0.4762
485.4000 7.0000 3.057 1 -1.0392
485.4000 7.0000 3.057 3 -0.0289
485.4000 7.0000 3.057 3 -0.0289
485.4000 7.0000 3.057 1 -1.0392
485.4000 8.0000 3.436 7 1.6134
485.4000 8.0000 3.436 5 0.7079
485.4000 8.0000 3.436 0 -1.5558
485.4000 9.0000 3.915 0 -1.6016
485.4000 9.0000 3.915 6 0.8530
```

```
2124.4000 1.0000 2.820 3 0.4382
2124.4000 2.0000 3.645 3 -0.9699
2124.4000 3.0000 5.230 6 0.7153
2124.4000 3.0000 5.230 2 -3.0007
2124.4000 4.0000 3.282 4 0.6473
2124.4000 4.0000 3.282 4 0.6473
2124.4000 4.0000 3.282 2 -1.1551
2124.4000 4.0000 3.282 4 0.6473
2124.4000 4.0000 3.282 4 0.6473
2124.4000 4.0000 3.282 3 -0.2539
2124.4000 4.0000 3.282 4 0.6473
2124.4000 5.0000 3.795 1 -1.8656
2124.4000 5.0000 3.795 5 0.8047
2124.4000 5.0000 3.795 5 0.8047
2124.4000 5.0000 3.795 4 0.1371
2124.4000 5.0000 3.795 4 0.1371
2124.4000 5.0000 3.795 3 -0.5305
2124.4000 6.0000 4.153 5 0.4466
2124.4000 6.0000 4.153 6 0.9736
2124.4000 6.0000 4.153 6 0.9736
2124.4000 6.0000 4.153 3 -0.6074
2124.4000 6.0000 4.153 6 0.9736
2124.4000 6.0000 4.153 2 -1.1344
2124.4000 6.0000 4.153 4 -0.0804
2124.4000 6.0000 4.153 0 -2.1885
2124.4000 6.0000 4.153 5 0.4466
2124.4000 6.0000 4.153 0 -2.1885
2124.4000 6.0000 4.153 5 0.4466
2124.4000 6.0000 4.153 4 -0.0804
2124.4000 7.0000 4.396 5 0.2650
2124.4000 7.0000 4.396 5 0.2650
2124.4000 7.0000 4.396 7 1.1421
2124.4000 7.0000 4.396 6 0.7036
2124.4000 7.0000 4.396 7 1.1421
2124.4000 8.0000 4.598 0 -1.7470

 Chi-square = 101.30 DF = 96 P-value = 0.3359


To calculate the BMD and BMDL, the litter specific covariate is fixed
 at the mean litter specific covariate of all the data: 5.379518

 Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

 BMD = 66.6706

 BMDL = 43.1039
```



Data points obtained from Rogers et al.(1993b).

Figure D-6 Nested logistic model, 0.05 extra risk - Incidence of cervical rib in mice versus C_{max} above background of methanol, GD6-GD15 inhalational study.

D.4. RfC-Derivations Using Burbacher et al. (1999a; 1999b)

The BMD approach was utilized in the derivation of potential chronic inhalation reference values from effects seen in monkeys due to prenatal methanol exposure. Deficits in VDR were evaluated from Burbacher et al. (1999a; 1999b). In the application of the BMD approach, continuous models in EPA's BMDS 2.2 were fit to the data set for increased latency in VDR in neonatal monkeys. The maximum blood methanol values (C_{max}) above background estimated using the PK model described in Appendix B were used as the dose metric.

The VDR test, which assesses time (from birth) it takes for an infant to grasp for a brightly colored object containing an applesauce-covered nipple, is a measure of sensorimotor development. Beginning at 2 weeks after birth, infants were tested 5 times/day, 4 days/week. Performance on that test, measured as age from birth at achievement of test criterion (successful object retrieval on 8/10 consecutive trials over 2 testing sessions), was reduced in all treated male infants. The times (days after birth) to achieve the criteria for the VDR test in means ± SD were 23.7 ± 8.3 (n = 3), 32.4 ± 9.2 (n = 5), 42.7 ± 13.9 (n = 3), and 40.5 ± 17.7 (n = 2) days for males and 34.2 ± 4.0 (n = 5), 33.0 ± 5.8 (n = 4), 27.6 ± 6.0 (n = 5), and 40.0 ± 10.6 (n = 7) days for females in the control to 1,800 ppm groups, respectively. As discussed in Section 4.4.2, this type

of response data is sometimes adjusted to account for premature births by subtracting time (days) premature from the time (days from birth) needed to meet the test criteria (Wilson and Cradock, 2004). When this type of adjustment is applied, the times (days after birth or, if shorter, days after control mean gestation length) to achieve the criteria for VDR test in means ± SD were 22.0 ± 16.5 (n = 3), 26.2 ± 19.3 (n = 5), 33.3 ± 17.3 (n = 3), and 39.5 ± 23.1 (n = 2) days for males and 32.0 ± 9.6 (n = 5), 21.8 ± 11.2 (n = 4), 24.0 ± 12.7 (n = 5), and 32.0 ± 39.2 (n = 7) days for females in the control to 1,800 ppm groups, respectively. When these data were modeled within BMDS 2.1.1 (U.S. EPA, 2009), there was no significant difference between unadjusted responses and/or variances among the dose levels (indicating lack of a dose-response) for males and females combined ($p = 0.244$), for males only ($p = 0.321$) and for males only with the high-dose group excluded ($p = 0.182$), or for adjusted responses of males and females combined ($p = 0.12$), males only ($p = 0.448$) and males only with the high-dose group excluded ($p = 0.586$).[4] The only data that offered a significant dose-response trend was that for unadjusted ($p = 0.0265$) and adjusted ($p = 0.009$) female responses, largely because of the much larger overall sample size across dose groups for females versus males (21 females versus 13 males). However, the model fits for the adjusted female response data were unacceptable. Only the unadjusted female VDR response data (Table D-10) offered both a dose-response trend and acceptable model fits.

Table D-10 EPA PK model estimates of methanol blood levels (C_{max}) above background in monkeys following inhalation exposures and VDR test results for their offspring

Exposure concentration (ppm)[a]	Blood methanol C_{max} above background (mg/L)[b]	Days After Birth to Achieve VDR Test Criteria[d]	N
0	0	34.2 ± 4.0	5
206	2.87	33.0 ± 5.8	4
610	10.4	27.6 ± 6.0	5
1,822	38.4	40.0 ± 10.6	7

[a]Reprinted with permission of the Health Effects Institute, Boston, MA; from Burbacher et al. (1999a) and Burbacher et al. [(1999b), Table 2].

[b]Estimated from the two-compartment PK monkey model described in Appendix B.

[c]Data reported in means ± standard deviation.

The BMD technical guidance (U.S. EPA, 2012a) suggests that in the absence of knowledge as to what level of response to consider adverse, a change in the mean equal to 1 control SD from the control mean can be used as a BMR for continuous endpoints. A summary

[4] BMDS (U.S. EPA, 2011a) continuous models contain a test for dose-response trend, test 1, which compares a model that fits a distinct mean and variance for each dose group to a model that contains a single mean and variance. The dose response is considered to be significant if this comparison returns a p value < 0.05.

of the results most relevant to the development of a POD using the BMD approach (BMD, BMDL, and model fit statistics) for increased latency of VDR in female neonatal monkeys exposed to methanol with a BMR of 1 control mean SD is provided in Table D-11. Model fit was determined by statistics (AIC and χ^2 residuals of individual dose groups) and visual inspection, as recommended by EPA (2012a). The Power model returned a lower AIC than the other models.[5] The text and graphic (see Figure D-7) output from this model follows Table D-10. The $BMDL_{1SD}$ was determined to be 19.59 mg/L, using the 95% lower confidence limit of the dose-response curve expressed in terms of the ppm of external methanol concentration.

Table D-11 Comparison of BMD modeling results for VDR in female monkeys using C_{max} above background of blood methanol as the dose metric

Model	BMD_{1SD} (C_{max}, mg/L)[a]	$BMDL_{1SD}$ (C_{max}, mg/L)[a]	p-value	AIC[c]	Scaled residual[d]
Linear	38.92	15.19	0.13	110.51	0.746
2nd degree Polynomial	32.27	17.59	0.2166	109.49	0.177
3rd degree Polynomial	33.53	18.94	0.2646	109.09	0.0461
Power[b]	**37.50**	**19.59**	**0.2862**	**108.93**	**7.35E-08**
Hill	36.90	Not Reported	0.1137	110.93	7.65E-07
Exponential 2	36.54	16.22	0.133	110.46	0.6748
Exponential 3	37.32	20.00	0.1137	110.93	-2.28E-07
Exponential 4	38.92	15.18	0.0433	112.51	0.7457
Exponential 5	37.19	10.81	Not Reported	112.93	1.71E-07

[a]C_{max} was estimated using the monkey PK model described in Appendix B of the methanol toxicological review; the BMDL is the 95% lower confidence limit on the C_{max} of a decrease of 1 control mean SD estimated by the model using the likelihood profile method (U.S. EPA, 2012a).

[b]Model choice based on adequate *p* value (> 0.1), visual inspection, low AIC, and low (absolute) scaled residual.
[c]AIC = Akaike Information Criterion = -2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters, and P is the number of modeled degrees of freedom (usually the number of parameters estimated).
[d]χ^2d residual (measure of how model-predicted responses deviate from the actual data) for the dose group closest to the BMD scaled by an estimate of its SD Provides a comparative measure of model fit near the BMD. Residuals that exceed 2.0 in absolute value should cause one to question model fit in this region.

Data from Burbacher et al. (1999a).

[5] A detailed analysis of this dose response revealed that modeling results, particularly the BMDL estimation, are very sensitive to the high-dose response. There is no data to inform the shape of the curve between the mid- and high-exposure levels, making the derivation of a BMDL very uncertain. The data were analyzed without the high dose to determine if the downward trend in the low- and mid-exposure groups is significant. It was not, so nonnegative restriction on the β coefficients of the polynomial models was retained.

```
================================================================
    Power Model. (Version: 2.16; Date: 10/28/2009)
    Input Data File: C:/USEPA/BMDS220/Data/pow_monkey_Pow-ModelVariance-BMR1Std-
Restrict. (d)
    Gnuplot Plotting File: C:/USEPA/BMDS220/Data/pow_monkey_Pow-ModelVariance-BMR1Std-
Restrict.plt
                  Wed Nov 16 11:02:04 2011
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

 The form of the response function is:

 Y[dose] = control + slope * dose^power


 Dependent variable = Mean
 Independent variable = Dose
 The power is restricted to be greater than or equal to 1
 The variance is to be modeled as Var (i) = exp (lalpha + log (mean (i)) * rho)

 Total number of dose groups = 4
 Total number of records with missing values = 0
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



 Default Initial Parameter Values
 lalpha = 4.05748
 rho = 0
 control = 27.6
 slope = 3.85161
 power = 0.320501


 Asymptotic Correlation Matrix of Parameter Estimates

 ( *** The model parameter (s) -power
 have been estimated at a boundary point, or have been specified by the user,
 and do not appear in the correlation matrix )

 lalpha rho control slope

 lalpha 1 -1 -0.29 0.6

 rho -1 1 0.27 -0.6

 control -0.29 0.27 1 -0.37

 slope 0.6 -0.6 -0.37 1



 Parameter Estimates

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
 lalpha -13.0645 12.1112 -36.8021 10.673
 rho 4.77979 3.44065 -1.96376 11.5233
 control 31.5 1.4819 28.5955 34.4045
 slope 2.57903e-028 1.21193e-028 2.03691e-029 4.95437e-028
 power 18 NA
```

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N Obs	Mean Est	Mean Obs	Std Dev Est	Std Dev	Scaled Res.
0	5	34.2	31.5	4	5.54	1.09
2.87	4	33	31.5	5.8	5.54	0.541
10.4	5	27.6	31.5	6	5.54	-1.57
38.4	7	40	40	10.6	9.81	7.35e-008

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu (i) + e (ij)
Var {e (ij)} = Sigma^2

Model A2: Yij = Mu (i) + e (ij)
Var {e (ij)} = Sigma (i)^2

Model A3: Yij = Mu (i) + e (ij)
Var {e (ij)} = exp (lalpha + rho*ln (Mu (i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e (i)
Var {e (i)} = Sigma^2

Likelihoods of Interest

Model	Log (likelihood)	# Param's	AIC
A1	-50.884765	5	111.769529
A2	-47.717070	8	111.434139
A3	-49.215263	6	110.430526
fitted	-50.466380	4	108.932759
R	-54.905426	2	113.810852

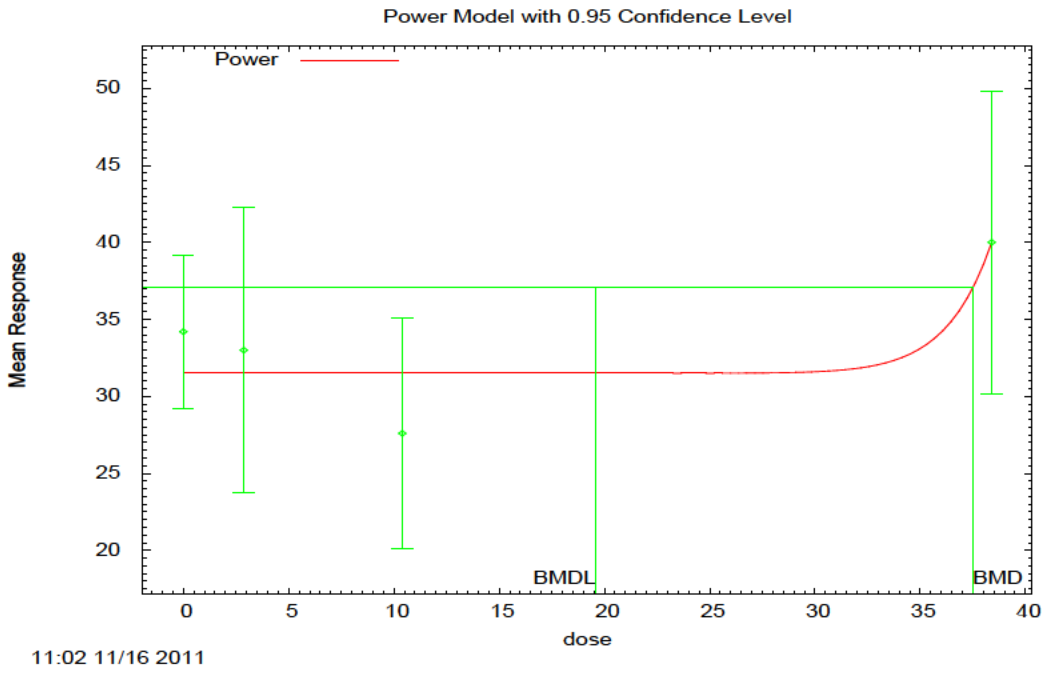
Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log (Likelihood Ratio)	Test df	p-value
Test 1	14.3767	6	0.0257
Test 2	6.33539	3	0.09639
Test 3	2.99639	2	0.2235
Test 4	2.50223	2	0.2862

```
The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data


 Benchmark Dose Computation

Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

 BMD = 37.4993


 BMDL = 19.5918
```



Data points obtained from Burbacher et al. (1999a; 1999b)

Figure D-7 Third (3rd) degree Polynomial model, BMR of 1 control mean SD – VDR in female monkeys using C_{max} above background of blood methanol as the dose metric.

APPENDIX E. DOCUMENTATION OF IMPLEMENTATION OF THE 2011 NATIONAL RESEARCH COUNCIL RECOMMENDATIONS

Background: On December 23, 2011, The Consolidated Appropriations Act, 2012, was signed into law (U.S. Congress, 2011). The report language included direction to EPA for the IRIS Program related to recommendations provided by the National Research Council (NRC) in their review of EPA's draft IRIS assessment of formaldehyde (NRC, 2011). The report language included the following:

"The Agency shall incorporate, as appropriate, based on chemical-specific datasets and biological effects, the recommendations of Chapter 7 of the National Research Council's Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde into the IRIS process…For draft assessments released in fiscal year 2012, the Agency shall include documentation describing how the Chapter 7 recommendations of the National Academy of Sciences (NAS) have been implemented or addressed, including an explanation for why certain recommendations were not incorporated."

The NRC's recommendations, provided in Chapter 7 of their review report, offered suggestions to EPA for improving the development of IRIS assessments. Consistent with the direction provided by Congress, documentation of how the recommendations from Chapter 7 of the NRC report have been implemented in this assessment is provided in the table below. Where necessary, the documentation includes an explanation for why certain recommendations were not incorporated.

The IRIS Program's implementation of the NRC recommendations is following a phased approach that is consistent with the NRC's "Roadmap for Revision" as described in Chapter 7 of the formaldehyde review report. The NRC stated that "the committee recognizes that the changes suggested would involve a multi-year process and extensive effort by the staff at the National Center for Environmental Assessment and input and review by the EPA Science Advisory Board and others."

The IRIS methanol (noncancer) assessment is in Phase 1 of implementation, which focuses on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data in assessments. Phase 1 also focuses on assessments near the end of the development process and close to final posting. Chemical assessments in Phase 2 of implementation will address all of the short-term recommendations from Table E-1. The IRIS Program is implementing all of these recommendations but recognizes that achieving full and robust implementation of certain recommendations will be an evolving process with input and

feedback from the public, stakeholders, and external peer review committees. Chemical assessments in Phase 3 of implementation will incorporate the longer-term recommendations made by the NRC as outlined below in Table E-2, including the development of a standardized approach to describe the strength of evidence for noncancer effects . On May 16, 2012, EPA announced (U.S. EPA, 2012c)[6] that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. This effort is included in Phase 3 of EPA's implementation plan.

Table E-1. National Research Council recommendation that EPA is implementing in the short-term

NRC RECOMMENDATIONS THAT EPA IS IMPLEMENTING IN THE SHORT-TERM	IMPLEMENTATION IN THE METHANOL (NONCANCER) ASSESSMENT
General recommendations for completing the IRIS formaldehyde assessment that EPA will adopt for all IRIS assessments (see p. 152 of the NRC Report)	
1. To enhance the clarity of the document, the draft IRIS assessment needs rigorous editing to reduce the volume of text substantially and address redundancies and inconsistencies. Long descriptions of particular studies should be replaced with informative evidence tables. When study details are appropriate, they could be provided in appendices.	**Partially Implemented.** Methanol is a post-peer review, Phase 1 chemical; as such, implementation has focused on a subset of the short-term recommendations, such as editing and streamlining, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data. For example: • details of EPA PBPK models were moved from Chapter 3 to Appendix B, • descriptions of human case studies were moved to Appendix C, • tables were added to Chapter 4, replacing textual descriptions, and • details of benchmark dose analyses were moved to Appendix D.

[6]EPA Announces NAS' Review of IRIS Assessment Development Process (www.epa.gov/iris)

NRC RECOMMENDATIONS THAT EPA IS IMPLEMENTING IN THE SHORT-TERM	IMPLEMENTATION IN THE METHANOL (NONCANCER) ASSESSMENT
2. Chapter 1 needs to be expanded to describe more fully the methods of the assessment, including a description of search strategies used to identify studies with the exclusion and inclusion criteria articulated and a better description of the outcomes of the searches and clear descriptions of the weight-of-evidence approaches used for the various noncancer outcomes. The committee emphasizes that it is not recommending the addition of long descriptions of EPA guidelines to the introduction, but rather clear concise statements of criteria used to exclude, include, and advance studies for derivation of the RfCs and unit risk estimates.	**Partially Implemented.** Text in Chapter 1 has been added that describes the literature search and study evaluation process in greater detail. This section also provides a link to EPA's Health and Environmental Research Online (HERO) database (www.epa.gov/hero) that contains the references that were cited in the document, along with those that were considered but not cited. As indicated in the comment for recommendation #1, methanol is a post-peer review, Phase 1 chemical. Consequently, literature search and study evaluation processes were not substantially revised.
3. Standardized evidence tables for all health outcomes need to be developed. If there were appropriates tables, long text descriptions of studies could be moved to an appendix of deleted.	**Partially Implemented.** The methanol (noncancer) assessment contains evidence tables for relevant study types, including oral, inhalation, i.p., in vitro study designs. Additional tables with study specific health outcomes have been added to Chapter 4 in response to this recommendation. Standardized evidence tables are being developed as a part of Phase 2 of the implementation process.
4. All critical studies need to be thoroughly evaluated with standardized approaches that are clearly formulated and based on the type of research, for example, observational epidemiologic or animal bioassays. The findings of the reviews might be presented in tables to ensure transparency.	**Partially Implemented.** All critical studies are thoroughly evaluated. Study design, results, and limitations are described in Chapter 4, and the basis for their selection, along with uncertainties, are discussed in Chapter 5. Standardized approaches for evaluating studies are under development as a part of Phase 2 and 3.

NRC RECOMMENDATIONS THAT EPA IS IMPLEMENTING IN THE SHORT-TERM	IMPLEMENTATION IN THE METHANOL (NONCANCER) ASSESSMENT
5. The rationales for the selection of the studies that are advanced for consideration in calculating the RfCs and unit risks need to be expanded. All candidate RfCs should be evaluated together with the aid of graphic displays that incorporate selected information on attributes relevant to the database.	**Implemented.** The Dose-Response Analysis section of the methanol (noncancer) assessment provides a clear explanation of the rationale used and uncertainties considered in selecting and advancing studies that were considered for calculating toxicity values. Rationales for the selection of studies advanced for reference value derivation are informed by the weight-of-evidence for hazard identification. In support of the RfC and RfD derivations, exposure-response arrays were included that compare effect levels for several toxicological effects following oral (Figure 4-1) and inhalation (Figure 4-2) exposure. The exposure-response arrays provide a visual representation of points of departure for various effects resulting from exposure to methanol. The arrays inform the identification of doses associated with specific effects, and the choice of principal studies and critical effects. In the case of methanol, the database supported development of multiple candidate RfCs and RfDs. The candidate RfCs and RfDs are presented in Tables 5-1, 5-3 and 5-4. Uncertainties with the RfD and RfC derivations are summarized in Table 5-7.
6. Strengthened, more integrative and more transparent discussions of weight-of-evidence are needed. The discussions would benefit from more rigorous and systematic coverage of the various determinants of weight-of-evidence, such as consistency.	**Partially implemented.** Weight-of-evidence considerations were added or revised based on peer review comments (see Appendix A). Table 5-7 summarizes considerations and uncertainties in the assessment and their potential impact on the RfC/RfD. Additional discussion of approaches to ensure systematic coverage of the various determinants of weight-of-evidence will be added to Phase 2 chemicals.
General Guidance for the Overall Process (p. 164 of the NRC Report)	
7. Elaborate an overall, documented, and quality-controlled process for IRIS assessments.	**Partially Implemented.** A team approach has been utilized for the development of the methanol (noncancer) assessment to help ensure that the necessary disciplinary expertise is available for assessment development and review, to provide a forum for identifying and addressing key issues. Due to timing, and because methanol is a post-peer review, Phase 1 chemical, the methanol team was not able to make use of the "overall, documented, and quality-controlled process" that is now being developed in response to the NRC recommendations.
8. Ensure standardization of review and evaluation approaches among contributors and teams of contributors; for example, include standard approaches for reviews of various types of studies to ensure uniformity.	
9. Assess disciplinary structure of teams needed to conduct the assessments.	

NRC RECOMMENDATIONS THAT EPA IS IMPLEMENTING IN THE SHORT-TERM	IMPLEMENTATION IN THE METHANOL (NONCANCER) ASSESSMENT
Evidence Identification: Literature Collection and Collation Phase (p. 164 of the NRC Report)	
10. Select outcomes on the basis of available evidence and understanding of mode of action.	**Partially Implemented.** More detailed information on the literature search strategy used for the methanol (noncancer) assessment has been added to Chapter 1. Information on how studies were selected to be included in the document is presented, along with a link to an external database (www.epa.gov/hero) that contains the references that were cited in the document, along with those that were considered but not cited. Each citation in the Toxicological Review is linked to HERO such that the public can access the references and abstracts to the scientific studies used in the assessment. Outcomes have been selected on the basis of available evidence and understanding mode of action in accordance EPA guidelines (U.S. EPA, 2002, 1994). Uncertainties associated with the available evidence are described in Section 5.3. Available evidence played an important role in the selection of candidate studies and endpoints. For example, questions concerning the Burbacher et al. (2004b; 1999b) monkey study endpoint and dose-response are considered serious enough to preclude its use for RfC/D derivation, despite the possibility that a lower BMDL POD would have been derived (Section 5.3.1 and Appendix D). Standard protocols for evidence identification and templates for describing the search approach are being implemented as a part of Phase 2.
11. Establish standard protocols for evidence identification.	
12. Develop a template for description of the search approach.	
13. Use a database, such as the Health and Environmental Research Online (HERO) database, to capture study information and relevant quantitative data.	
Evidence Evaluation: Hazard Identification and Dose-Response Modeling (p. 165 of the NRC Report)	
14. Standardize the presentation of reviewed studies in tabular or graphic form to capture the key dimensions of study characteristics, weight-of- evidence, and utility as a basis for deriving reference values and unit risks.	**Partially Implemented.** Tables have been developed that provide summaries of key study design information and results by health effect. In addition, exposure-response arrays are utilized in the assessment to provide a graphical representation of points of departure for various effects resulting from exposure to methanol. The exposure-response arrays inform the identification of doses associated with specific effects and the weight-of-evidence for those effects. The use of standardized tables and graphics will be included in assessments that are part of Phase 2 of the implementation process.
15. Develop templates for evidence tables, forest plots, or other displays.	**Not Implemented.** Evidence table templates and templates for other graphics are being implemented as a part of Phase 2.

NRC RECOMMENDATIONS THAT EPA IS IMPLEMENTING IN THE SHORT-TERM	IMPLEMENTATION IN THE METHANOL (NONCANCER) ASSESSMENT
16. Establish protocols for review of major types of studies, such as epidemiologic and bioassay.	**Partially Implemented.** Formalized protocols for review of studies will be developed as a part of Phase 2 and 3. The study evaluation processes was not revised because methanol is a Phase 1 chemical. However, the methanol (noncancer) assessment was developed using standard protocols for evidence identification that are provided in existing EPA guidance.
Selection of Studies for Derivation of Reference Values and Unit Risks (p. 165 of the NRC Report)	
17. Establish clear guidelines for study selection. a. Balance strengths and weaknesses. b. Weigh human vs. experimental evidence c. Determine whether combining estimates among studies is warranted.	**Partially Implemented.** The basis for study selection is described in Sections 5.1.1 (RfC) and 5.2.1 (RfD). Existing EPA guidelines for study selection were applied to inform the evaluation of the weight-of-evidence across health effects and the strengths and weaknesses of individual studies. Sections 5.1.2, 5.1.3, and 5.2.2 discuss uncertainties that are addressed quantitatively via uncertainty factors. Section 5.3 provides an additional discussion of the uncertainties associated with the RfC and RfD derivation. A summary of these uncertainties is presented in Table 5-7. Section 5.3.1 specifically addresses the uncertainties associated with the choice of study and endpoint. Other aspects besides the choice of study and endpoint that can impact RfC/D derivation that are discussed include dose-response modeling (5.3.2), route-to-route extrapolation (5.3.3), statistical uncertainty at the POD (5.3.4), choice of species and gender (5.3.5) and the relationship of the RfC and RfD with endogenous methanol blood Levels (5.3.6). In the case of methanol, the database did not support the combination of estimates across studies. In future assessments, combining estimates across studies will be routinely considered.
Calculation of Reference Values and Unit Risks (pp. 165-166 of the NRC Report)	
18. Describe and justify assumptions and models used. This step includes review of dosimetry models and the implications of the models for uncertainty factors; determination of appropriate points of departure (such as benchmark dose, no-observed-adverse-effect level, and lowest observed-adverse-effect level), and assessment of the analyses that underlie the points of departure.	**Implemented.** Appendix B documents EPA's PBPK model. Appendix D documents the benchmark dose modeling analyses used to derive candidate points of departure. The implications of the models for uncertainty factors are described in Sections 5.1.3 and 5.2.2, and the impact of model choices are further described in Section 5.3.
19. Provide explanation of the risk-estimation modeling processes (for example, a statistical or biologic model fit to the data) that are used to develop a unit risk estimate.	**Not applicable.** A cancer unit risk estimate was not derived in this assessment.

NRC RECOMMENDATIONS THAT EPA IS IMPLEMENTING IN THE SHORT-TERM	IMPLEMENTATION IN THE METHANOL (NONCANCER) ASSESSMENT
20. Provide adequate documentation for conclusions and estimation of reference values and unit risks. As noted by the committee throughout the present report, sufficient support for conclusions in the formaldehyde draft IRIS assessment is often lacking. Given that the development of specific IRIS assessments and their conclusions are of interest to many stakeholders, it is important that they provide sufficient references and supporting documentation for their conclusions. Detailed appendixes, which might be made available only electronically, should be provided when appropriate.	**Implemented.** Chapter 5 documents the approach taken for the estimation of reference values and provides support for the conclusions drawn. As recommended, supplementary information is provided in the accompanying appendices. Appendix D documents the benchmark dose modeling analyses used to derive candidate points of departure.

Table E-2. National Research Council recommendations that the EPA is generally implementing in the long-term

NRC RECOMMENDATIONS THAT THE EPA IS GENERALLY IMPLEMENTING IN THE LONG-TERM	IMPLEMENTATION IN THE METHANOL (NONCANCER) ASSESSMENT
Weight-of-Evidence Evaluation: Synthesis of Evidence for Hazard Identification (p. 165 of the NRC Report) 1. Review use of existing weight-of-evidence guidelines. 2. Standardize approach to using weight-of-evidence guidelines. 3. Conduct agency workshops on approaches to implementing weight-of-evidence guidelines. 4. Develop uniform language to describe strength of evidence on noncancer effects. 5. Expand and harmonize the approach for characterizing uncertainty and variability. 6. To the extent possible, unify consideration of outcomes around common modes of action rather than considering multiple outcomes separately.	As indicated above, Phase 3 of EPA's implementation plan will incorporate the longer-term recommendations made by the NRC, including the development of a standardized approach to describe the strength of evidence for noncancer effects. On May 16, 2012, EPA announced (U.S. EPA, 2012c) that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. In addition, EPA held a workshop on August 26, 2013, on issues related to weight-of-evidence to inform future assessments.
Calculation of Reference Values and Unit Risks (pp. 165-166 of the NRC Report) 7. Assess the sensitivity of derived estimates to model assumptions and end points selected. This step should include appropriate tabular and graphic displays to illustrate the range of the estimates and the effect of uncertainty factors on the estimates.	**Partially Implemented.** Chapter 5 describes the derivation of **c**andidate RfCs and RfDs from data for multiple endpoints in multiple species. In addition, a sensitivity analysis on model parameters used in the rat and human PBPK models has been conducted and results are tabulated in Appendix B, Sections B.2.4 and B.2.7. However, such analyses can only partly inform the question of model adequacy, which is addressed in more detail in the response to Charge A1 Comment 1 of Appendix A.

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