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TOXICOLOGICAL REVIEW OF METHANOL (NONCANCER) APPENDICES

(CAS No. 67-56-1)

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

July 2013

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U.S. Environmental Protection Agency
Washington, DC

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LIST OF ABBREVIATIONS AND ACRONYMS

ACGIH	American Conference of Governmental and Industrial Hygienists	CH ₃ OH	methanol
ADH	alcohol dehydrogenase	CHL	Chinese hamster lung (cells)
ADH1	alcohol dehydrogenase-1	CI	confidence interval
ADH3	formaldehyde dehydrogenase-3	Cl _s	clearance rate
AIC	Akaike Information Criterion	C _{max}	peak concentration
ALD	aldehyde dehydrogenase	CNS	central nervous system
ALDH2	mitochondrial aldehyde dehydrogenase-2	CO ₂	carbon dioxide
ALT	alanine aminotransferase	con-A	concanavalin-A
ANOVA	analysis of variance	CR	crown-rump length
AP	alkaline phosphatase	CSF	Cancer slope factor
AST	aspartate aminotransferase	C _{ss}	steady-state concentration
ATP	adenosine triphosphate	CT	computed tomography
ATSDR	Agency for Toxic Substances and Disease Registry	C _{VB}	concentration in venous blood
AUC	area under the curve, representing the cumulative product of time and concentration for a substance in the blood	C _{VBbg}	background concentration in venous blood
β-NAG	N-acetyl-beta-D-glucosaminidase	C _{VBmb}	concentration in venous blood minus constant background
Bav	oral bioavailability	CYP450	cytochrome P450
BMD	benchmark dose(s)	d, δ, Δ	delta, difference, change
BMD _{1SD}	BMD for response one standard deviation from control mean	D ₂	dopamine receptor
BMDL	95% lower bound confidence limit on BMD (benchmark dose)	DA	dopamine
BMDL _{1SD}	BMDL for response one standard deviation from control mean	DIPE	diisopropyl ether
BMDS	benchmark dose software	DMDC	dimethyl dicarbonate
BMR	benchmark response	DNA	deoxyribonucleic acid
BSO	butathione sulfoximine	DNT	developmental neurotoxicity test(ing)
BUN	blood urea nitrogen	DOPAC	dihydroxyphenyl acetic acid
BW, bw	body weight	DPC	days past conception
C ₁ pool	one carbon pool	DTH	delayed-type hypersensitivity
C _{max}	peak concentration of a substance in the blood during the exposure period	EFSA	European Food Safety Authority
C-section	Cesarean section	EKG	electrocardiogram
CA	chromosomal aberrations	EO	Executive Order
CAR	conditioned avoidance response	EPA	U.S. Environmental Protection Agency
CASRN	Chemical Abstracts Service Registry Number	ERF	European Ramazzini Foundation
CAT	catalase	EtOH	ethanol
CERHR	Center for the Evaluation of Risks to Human Reproduction at the NTP	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	k_1C	first-order urinary clearance scaling constant; first order clearance of methanol from the blood to the bladder for urinary elimination
FR	folate reduced	k_{ai}	first order uptake from the intestine
FRACIN	fraction inhaled	k_{as}	first order methanol oral absorption rate from stomach
FS	folate sufficient	k_{bl}	rate constant for urinary excretion from bladder
FSH	follicular stimulating hormone	k_{iv}	respiratory/cardiac depression constant
γ -GT	gamma glutamyl transferase	KLH	keyhole limpet hemocyanin
g	gravity	KLL	alternate first order rate constant
g, kg, mg, μ g	gram, kilogram, milligram, microgram	K_m	apparent Michaelis-Menten constant; substrate concentration at half the enzyme maximum velocity (V_{max})
G6PD	glucose-6-phosphate dehydrogenase	k_{si}	first order transfer between stomach and intestine
GAP43	growth-associated protein (neuronal growth cone)	L, dL, mL	liter, deciliter, milliliter
GD	gestation day	LD ₅₀	median lethal dose
GFR	glomerular filtration rate	LDH	lactate dehydrogenase
GI	gastrointestinal track	LH	luteinizing hormone
GLM	generalized linear model	LLF	(maximum) log likelihood function
GLP	good laboratory practice	LMI	leukocyte migration inhibition (assay)
GSH	glutathione	LOAEL	lowest-observed-adverse-effect level
HAP	hazardous air pollutant	M, mM, μ M	molar, millimolar, micromolar
HCHO	formaldehyde	MeOH	methanol
HCOO	formate	MLE	maximum likelihood estimate
Hct	hematocrit	M-M	Michaelis-Menten
HEC	human equivalent concentration	MN	micronuclei
HED	human equivalent dose	MOA	mode of action
HEI	Health Effects Institute	4-MP	4-methylpyrazole (fomepizole)
HERO	Health and Environmental Research Online (database system)	MRI	magnetic resonance imaging
HH	hereditary hemochromatosis	mRNA	messenger RNA
5-HIAA	5-hydroxyindolacetic acid	MTBE	methyl tertiary butyl ether
HMGS	S-hydroxymethylglutathione	MTX	methotrexate
Hp	haptoglobin	N_2O/O_2	nitrous oxide
HPA	hypothalamus-pituitary-adrenal (axis)	NAD^+	nicotinamide adenine dinucleotide
HPLC	high-performance liquid chromatography	NADH	reduced form of nicotinamide adenine dinucleotide
HSDB	Hazardous Substances Databank	NBT	nitroblue tetrazolium (test)
HSP70	biomarker of cellular stress	NCEA	National Center for Environmental Assessment
5-HT	serotonin	ND	not determined
IL	interleukins	NEDO	New Energy Development Organization (of Japan)
i.p.	intraperitoneal	NIEHS	National Institute of Environmental Health Sciences
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		

NIOSH	National Institute of Occupational Safety and Health	S9	microsomal fraction from liver
nmol	nanomole	SAP	serum alkaline phosphatase
NOAEL	no-observed-adverse-effect level	s.c.	subcutaneous
NOEL	no-observed-effect level	SCE	sister chromatid exchange
NP	nonpregnant	S-D	Sprague-Dawley rat strain
NR	not reported	SD	standard deviation
NRC	National Research Council	S.E.	standard error
NS	not specified	SEM	standard error of mean
NTP	National Toxicology Program at NIEHS	SGPT	serum glutamate pyruvate transaminase
NZW	New Zealand White (rabbit strain)	SHE	Syrian hamster embryo
OR	osmotic resistance	SOD	superoxide dismutase
ORD	Office of Research and Development	SOP	standard operating procedure(s)
OSF	oral slope factor	t; T _{1/2} , t _{1/2}	time; half-life
OU	oculus uterque (each eye)	T wave	the next deflection in the electrocardiogram after the QRS complex; represents ventricular repolarization
OXA	oxazolone	TAME	tertiary amyl methyl ether
P, p	probability	TAS	total antioxidant status
PB	blood:air partition coefficient	Tau	taurine
PBPK	physiologically based pharmacokinetic model	THF	tetrahydrofolate
PC	partition coefficient	TLV	threshold limit value
PEG	polyethylene glycol	TNF α	tumor necrosis factor-alpha
PFC	plaque-forming cell	TNP-LPS	trinitrophenyl-lipopolysaccharide
PK	pharmacokinetic	TRI	Toxic Release Inventory
PMN	polymorphonuclear leukocytes	U83836E	vitamin E derivative
PND	postnatal day	UF(s)	uncertainty factor(s)
POD	point of departure	UF _A	UF associated with interspecies (animal to human) extrapolation
ppb, ppm	parts per billion, parts per million	UF _D	UF associated with deficiencies in the toxicity database
PR	body:blood partition coefficient	UF _H	UF associated with variation in sensitivity within the human population
PWG	Pathology Working Group of the NTP of NIEHS	UF _S	UF associated with subchronic to chronic exposure
Q wave	the initial deflection of the QRS complex	V _d	volume of distribution
Q _C C	cardiac output scaling constant	V _{max}	pseudo-maximal velocity of metabolism
Q _P	pulmonary (alveolar) ventilation	V _{max} C	multiplier for allometric scaling of V _{max}
QRS	portion of electrocardiogram corresponding to the depolarization of ventricular cardiac cells.	VDR	visually directed reaching test
R ²	square of the correlation coefficient, a measure of the reliability of a linear relationship.	VitC	vitamin C
RBC	red blood cell	VPR	ventilation perfusion ratio
RfC	reference concentration	v/v	volume of solute/volume of solution
RfD	reference dose	VYS	visceral yolk sac
RNA	ribonucleic acid	WBC	white blood cell
R _{0bg}	zero-order endogenous production rate	WOE	weight of evidence
ROS	reactive oxygen species	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

1 The [draft toxicological review](#) of methanol ([U.S. EPA, 2011b](#)) has undergone a formal
2 external peer review performed by scientists in accordance with EPA guidance on peer review
3 ([U.S. EPA, 2006b](#)), ([U.S. EPA, 2000b](#)). An external peer-review meeting was held July 22, 2011.
4 There were seven external peer reviewers. The external peer reviewers were tasked with
5 providing written answers to general questions on the overall assessment and on chemical-
6 specific questions in areas of scientific controversy or uncertainty. At the workshop, they
7 discussed their responses to each of the charge questions and consensus was not sought. A
8 summary of significant comments made by the external reviewers and EPA's responses to these
9 comments arranged by charge question follow.

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

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

23 EPA received comments from the public on the 2011 and 2013 draft toxicological
24 reviews prior to the 2011 peer review and the 2013 follow-up peer review, which were
25 distributed to both peer review panels for their consideration. These public comments and EPA's
26 responses are included in Section A.2 of this appendix.

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

1 **Comment 1:** One reviewer stated that “The revised ([May 2013](#)) version of
2 ‘Toxicological Review of Methanol (noncancer)’ has been improved significantly in comparison
3 to its external peer-review draft ([2011](#)) version. It addressed the key recommendations,
4 comments, and suggestions provided in my Post-Meeting Comments of 7/31/2011.”

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

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

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

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

22 **Response:** A short statement has been added to the beginning of Appendix C, as
23 requested, to indicate that CNS damage seen in the acute overdose exposures most likely results
24 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.

25 **Summary of Comments:** *In general, four reviewers stated that the PBPK model structure*
26 *was sound for the purposes of this assessment, a fifth reviewer stated that they noticed no*
27 *obvious flaws but could not comment on a technical level due to a lack of expertise, and two*

1 *reviewers did not explicitly state whether the PBPK models were sound but provided comments.*
2 *Several reviewers commented that the models were comprehensively documented and that stated*
3 *assumptions are justified for the purposes of this assessment. One reviewer commended the*
4 *Agency for “developing a consistent model framework and sets of species-specific parameters*
5 *which have been validated across several somewhat diverse data sets.” Another reviewer*
6 *commented that “the Sprague-Dawley (S-D) rat PBPK model is inappropriately parameterized*
7 *(or insufficiently validated) for the inhalation route” and provided specific suggestions to*
8 *improve or validate the rat, mouse and human PBPK models developed by EPA for the purposes*
9 *of this assessment. Specific comments or suggestions made by the reviewers with respect to this*
10 *charge are described below, along with EPA responses.*

11 **Comment 1:** One reviewer asked for “clarification of the process for evaluating the
12 usefulness of each model for the assessment and why the nonhuman primate model was not
13 included.”

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

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

29 Regarding the nonhuman primate model, EPA had incorrectly stated [in Appendix C,
30 Section C.3 of the draft assessment ([U.S. EPA, 2011b](#))] that external concentrations were used
31 for dose-response modeling for the monkey. However, a nonhuman primate classical PK model
32 (not a PBPK model) was adapted for use in the draft assessment and is used in the final
33 assessment to estimate internal doses (blood methanol C_{max} values) for derivation of internal
34 dose BMDLs associated with the Burbacher et al. ([2004b](#); [1999b](#)) monkey study. See Appendix
35 D, Section D.3, and Table D-10 in the final assessment.

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

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

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

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

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

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

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

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

5 **Response:** The monkey PK model description and analysis is now found in Appendix B,
6 Section B.3.1 of this final assessment. Information regarding the chamber volume adjustment for
7 the PK model was added to this section. Briefly, the chamber volume was fit to the chamber
8 concentration data to allow for a better fit to the “mixing time” in the chamber (mixing time is
9 the time it takes for the chamber concentration to rise or fall after the inlet concentration is
10 turned on or off) and to account for the volume filled by the monkey and other chamber
11 equipment. The “accessible [chamber] volume” reported by Burbacher et al. (1999b) was
12 1,380 L, and the model fitted volume was 1,220 L. A detailed description of the chamber set-up
13 is included in the original Burbacher et al. (1999b) report and was not included in Appendix B of
14 this assessment.

15 Since methanol chamber time-course data were available for this study, and model
16 predictions did not match the data when the assumption of perfect mixing was used (chamber
17 volume of 1,380 L), EPA considered it reasonable to use the available data to calibrate the
18 residence- or mixing-time for the model of the chamber concentration. The text in Appendix B,
19 Section B.3.1 has been modified to indicate why V_{ch} was varied.

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

24 **Response:** In the external peer review draft document the derivation of the value of
25 FRACIN used for humans was described in detail in Appendix B, p. B-29. The exact mean
26 absorption measured by Sedivec et al. (1981) was 57.7%, but this was based on total ventilation.
27 However the human PBPK model uses alveolar ventilation, assumed to be 2/3 of total
28 ventilation, with the remaining 1/3 of each breath assumed to not enter the gas exchange area.
29 Therefore, to yield the same net uptake as Sedivec et al., 57.7% was divided by 2/3 to yield
30 86.55% for the human model parameter, FRACIN.

31 In contrast the monkey model used total respiration rather than alveolar ventilation. This
32 detail was included at the bottom of p. 3-49, where the monkey model parameter R_c was defined
33 as “allometric scaling factor for **total** monkey respiration” (emphasis added). However the
34 parameter symbol “F” was used for the monkey “fraction of inhaled” to further distinguish it

1 from the human FRACIN, given that they are applied to different portions of total ventilation.
2 Further, in the description of the monkey parameter “F” on p. 3-50 (immediately after the value
3 of 60% is given), the document noted that 60% was the “(rounded)” value from Sedivec et al.
4 (1981) and went on to state, “F and V_{mk} cannot be uniquely identified, given the model structure,
5 so F was set to the (approximate) human value to obtain a realistic estimate of V_{mk} . For example,
6 if both F and V_{mk} are increased by 50%, then also increasing the fitted V_{max} by 50% would yield
7 identical model fits. Any positive value could be assigned to F and it would not affect the
8 resulting model fits. Given that the airways in a 2-4 kg monkey are much smaller than those in a
9 70 kg human, it is unlikely that the transport characteristics for which F and FRACIN account
10 are identical in the two species. But a realistic value was considered desirable for the monkey, so
11 the approximate *total ventilation* human value was assumed to be sufficient.

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

19 **Comment 7:** Two reviewers noted that the blood methanol levels predicted by the EPA
20 rat PBPK model are much lower than the levels reported for S-D rats on recently located
21 (supplied by industry at the peer review meeting) pages from the NEDO (1987) report and
22 Perkins et al. (1995). One of the reviewers further noted that if the fraction inhaled (FRACIN)
23 model parameter is changed from 20% to a value more consistent with the mouse (66.5%) and
24 human (86.6%) estimates, the 1,000 ppm blood prediction is in agreement with Perkins et al.
25 (1995).

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

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

1 **Response:** When the data and fits shown in Figure 3-14 were evaluated, EPA noted that
2 overall there appears to be no significant or systematic difference among the NP and pregnant
3 groups. The solid lines, in the figure, are model simulations calibrated to only the 2nd trimester
4 data (details below), but they just as adequately represent average concentrations for the NP and
5 3rd trimester data. Likewise, a PK model calibrated to the NP PK data adequately predicted the
6 maternal methanol concentrations in the pregnant monkeys (results not shown). Since any
7 maternal:fetal methanol differences are expected to be similar in experimental animals and
8 humans (with the maternal:fetal ratio being close to one due to methanol's high aqueous
9 solubility and relatively limited metabolism by the fetus), the predicted levels for the 2nd
10 trimester maternal blood are used in place of measured or predicted fetal concentrations.

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

22 **Comment 9:** One reviewer suggested that the K_m values estimated by the rat and human
23 models “don’t seem to reflect the true Michaelis values of the metabolic enzymes themselves.”

24 **Response:** Since methanol is metabolized by multiple enzymes with differing K_m values,
25 at best one would expect the empirical K_m values identified here to represent an average of the
26 enzyme-specific values (weighted by the contribution of each enzyme to total metabolism).
27 Further, it is quite typical to find that in vivo PK data are not well-predicted when K_m values
28 measured in vitro are used in a model, hence K_m values estimated from in vivo data are not
29 expected to be identical to values measured in vitro. The K_m values identified for the revised
30 PBPK models described here are 28 mg/L for rats and 36 mg/L for humans. Pollack and Brouwer
31 ([1996](#)) analyzed the kinetics of formaldehyde formation in vitro and estimated $K_m = 39.3$ mg/L
32 using nonpregnant adult rat liver homogenates and $K_m = 35.5$ mg/L with GD 20 homogenates:
33 quite similar to the revised value for the rat used in the final assessment. Mani et al. ([1970](#))
34 measured methanol kinetics with human liver ADH and obtained a K_m of 48 mg/L. This is
35 likewise quite similar to the K_m estimated here with the human PBPK model.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1 **Response:** The reviewer is correct that by not including an explicit kidney compartment,
2 where urinary clearance would be limited by the arterial blood concentration and flow to the
3 kidney, the model does not include the limitation to clearance that occurs because renal flow is
4 only a fraction of total cardiac output. This limitation would be a significant factor if total urinary
5 clearance was a significant fraction of renal blood flow, which likely occurs for some other
6 chemicals. However for the methanol model the clearance rate for this pathway in the rat is only
7 0.24% of renal blood flow (using a renal flow fraction of 0.141 from Brown et al. 1997) and in
8 the human is only 0.07% of renal blood flow (using a renal flow fraction of 0.175 from Brown et
9 al. 1997). Therefore including an explicit kidney compartment with its own flow rate would have
10 a negligible impact on the methanol model results reported here. These calculations and a
11 statement that the approach should only be used when renal clearance is a small fraction (<10%)
12 of renal blood flow have been added to Appendix B.

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

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

27 **Response:** The EPA appreciates the reviewer’s support for the proposed model structure
28 and has chosen to retain this description of metabolism as a saturable process in the PBPK model
29 for both rats and humans. The EPA agrees that there is not an exact correspondence between the
30 parameters, V_{max} and K_M , as used and calibrated in the model versus values obtained from initial
31 rate experiments *in vitro*. Therefore the descriptions of these parameters in the text and glossary
32 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 non-cancer risks.

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

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

23 **Response:** The language in the draft assessment may have confused this issue and has
24 been clarified in Section 3.4.3.2. EPA acknowledges that endogenous/background methanol
25 concentrations can be a contributing factor in health effects that are associated with exogenous
26 methanol exposure. As indicated in response to Comment 2 below, for the sake of obtaining
27 more accurate and reliable toxicokinetic estimates, the PBPK models used in the final assessment
28 incorporate background/endogenous concentrations of methanol. Background estimates on
29 which the models were calibrated are described in Appendix B, Sections B.2.3 (rats), B.2.8
30 (humans) and B.3.1 (monkeys). However, for BMD modeling of laboratory animal dose-
31 response data, the species-specific background estimates were subtracted from the dose metric
32 predicted under the relevant bioassay conditions. This approach takes into account the impact of

1 endogenous/background levels on the toxicokinetics of methanol, and allows for the derivation
2 of an RfC (or RfD) that is, by definition, a population level estimate (including sensitive
3 populations) of the amount of a substance that a person can inhale (or ingest) every day over the
4 course of a lifetime [above endogenous/background levels] without an appreciable risk of harm.

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

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

21 **Response:** As described in the response to Comment 1 above, EPA re-calibrated the
22 PBPK models to account for species-specific estimates of background/endogenous production of
23 methanol. For humans the model was tuned to have an average background level of 1.5 mg/L
24 determined from the corresponding human data in Table 3-1; for rats the model was tuned to a
25 background level of 3 mg/L from the corresponding (control) rat data in Table 3-5. These revised
26 PBPK models were used in estimation of internal dose metrics for the derivation of the RfD and
27 RfC. This addition did increase the model complexity by including an additional term (R_{0bg} , a
28 zero-order endogenous/background production rate, see Appendix B, Section B.2.1) for
29 endogenous/background production of methanol; however, this term was estimated using human
30 data for background blood methanol concentrations (Table 3-1). Since the background term was
31 tuned to match average observed background levels in rats and humans for the corresponding
32 models, there should be minimal systematic error or bias due to the incorporation of the
33 background term; i.e., the average background level is neither under- nor over-predicted by the
34 model. Moreover, adding the term resulted in only minor changes, less than 20%, in model-
35 predicted blood levels at higher exposure levels (i.e., in the range of the bioassays for rats or the

1 HEC and HED values estimated for humans). Hence model predictions are not sensitive to the
2 inclusion of these average background levels vs. no background at all, and so the effect of and
3 uncertainty due to possible small changes (or errors) in the background term will be minimal.

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

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

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

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

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

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

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

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

10 **Response:** EPA appreciates the affirmation of sufficient revisions to the Toxicological
11 Review in response to previous peer-review comments and has retained the species-specific
12 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.

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

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

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

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

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

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

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

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

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

8 Several research studies recommended by the reviewers in response to Charge D3
9 (below), including "dual labeled material" studies to "confirm fetal exposure" and "resolve
10 whether formaldehyde is involved in the developmental effects following perinatal methanol
11 exposure" and studies "to confirm the low activity of methanol metabolism in fetal tissues,"
12 could help to resolve the "principal sources of uncertainty" referred to by the reviewer. However,
13 as indicated in response to the Charge D3 comments, and consistent with the follow-up
14 comments to EPA's Charge D3 responses, EPA is not planning to delay the completion of this
15 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.

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

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

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

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

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

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

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

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

18 **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.

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

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

3 **Response:** EPA agrees with the majority of the reviewers that the extrapolation approach
4 employed is justified given the available data. The reviewer concerns regarding the model
5 evaluation process and the perceived lack of a nonhuman primate model are addressed in
6 response to Charge A1 Comment 1. The lack of data on lactational and early postnatal inhalation
7 exposure to methanol is a recognized data gap that led to the current approach. As discussed in
8 response to comments under Charge A3 above, gestational and lactational compartment may be
9 considered in a future assessment, but they are not necessary at this time for the purposes of this
10 toxicological review. Concerns over use of rodent studies stem from the Sweeting et al. (2011)
11 study. The relevance of the Sweeting et al. (2011) to these concerns is discussed in Section 5.3.5
12 and elsewhere in the toxicological review and in response to Comment 1 of Charge A4 and
13 Comment 1 of Charge D2. As discussed in response to Charge A3 Comment 2 and Section
14 5.1.3.2.2 of the toxicological review, the uncertainty surrounding the assumption of similar
15 maternal/offspring methanol concentration ratios between rats and humans is recognized, but the
16 ratio of blood concentrations between a human infant and its mother is not expected to be
17 significantly greater than the approximate 2-fold difference that has been observed between rat
18 pups and dams. Clarifications have been added in this regard to Sections 5.1.3.2.2 and 5.3.5.

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

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

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

1 developing infant would depend on when and to what extent metabolic capacity develops in rat
2 pups versus human infants. So while it would be possible to extrapolate the existing adult models
3 to those life-stages, such extrapolations, for the infant in particular, would be quite speculative
4 and uncertain. In response to this comment, clarification has been added to Section 5.1.3.2.2 to
5 indicate why EPA does not believe that adding additional analyses would substantially reduce the
6 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.

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

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

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

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

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

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

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

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

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

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

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

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

9 **Comment 2:** Regarding the Burbacher et al. monkey study, four reviewers had no
10 comment on its potential for use as the principal study and two reviewers stated the following
11 reasons why it should not be used as the principal study:

- 12 ▪ “The lack of a dose-response function for the major effects.”
- 13 ▪ No “convincing evidence of an effect, given the inconsistencies in dose-response, multiple
14 comparisons, and the potential for unreliable identification of ‘effects’ in small studies.”

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

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

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

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

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

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

20 **Comment 3:** Regarding the Rogers et al. mouse study, two reviewers supported the use
21 of this study over the NEDO rat study and noted the following advantages:

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

26 **Response:** EPA agrees with reviewer comments regarding the advantages of the Rogers
27 et al. mouse developmental study. EPA also agrees with reviewer comments (see Charge B1
28 Comment 1 above) regarding the advantages of the NEDO rat study, including the use of a
29 continuous, nearly full day exposure regimen and the adequacy of the reported response data for
30 dose-response analysis. As a result, EPA decided to treat both the Rogers and NEDO studies as
31 candidate principal studies and derived candidate RfCs and RfDs for the most sensitive endpoint
32 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 than 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/bmnds>) 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

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

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.

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

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

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

26 **Comment 2:** One reviewer commented that there is a “general lack of transparency”
27 regarding the basis for the selection of the critical effect and stated that EPA chose “the one that

1 led to the lowest RfC,” without regard to the limitations of the NEDO study, including
2 inappropriate use of statistical methods as described in a 2009 EPA-sponsored external peer
3 reviewer of the NEDO study. Another reviewer also commented that EPA had not acknowledged
4 errors in the NEDO statistical analysis and recommended that EPA conduct its own analysis of
5 variance (ANOVA) to determine “if there were an overall effect on brain weight” and “which
6 time frame and which methanol level are used in the BMD analysis.”

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

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

26 **Response:** A benchmark dose analysis of brain weight reductions in male and female rats
27 was performed for all postnatal time frames (3, 6 and 8 weeks). In accordance with EPA
28 guidelines ([U.S. EPA, 2002, 1998a](#)), the most sensitive developmental time point in the most
29 sensitive gender was used as the basis for the RfC/D. In order to achieve an increase in statistical
30 power by combining data together from separate ages, the animals must be exchangeable
31 (required for Bayesian statistics) or represent the same population (i.e., the brain weights from 3-
32 8 week old S-D rats would have to represent the same population; required of frequentist
33 statistics). The data for the more sensitive gender (males) suggests that each age represents a
34 separate subpopulation (3 wks (mean \pm standard deviation): 1.45g \pm 0.06; 6 wks: 1.78g \pm 0.07; 8
35 wks: 1.99g \pm 0.06). Randomly permuting the individuals across the groups would not yield the

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

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

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

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

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

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

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

20 **Response:** EPA appreciates the reviewer’s affirmation of the revised NEDO study
21 characterization. As indicate in response to the Charge B1 Follow-up Peer Review Comments,
22 EPA agrees that the EPA analysis of the NEDO rat brain weight data would benefit from, and has
23 therefore applied and described in the assessment, a more reliable trend test to confirm that the
24 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.

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

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

16 **Response:** When performing BMD analyses, it is important to choose a reliably
17 measured or estimated dose metric that has a close relationship to the health effects under
18 consideration. For the BMD analyses of the mouse cervical rib endpoint, which has been shown
19 to result from just one day of gestational exposure, it is assumed that the level of exposure is
20 more important than duration. Internal methanol blood concentrations reported by Rogers et al.
21 ([1993b](#)) for the dams of each dose group at day 6 of gestation were assumed to be approximately
22 equivalent to C_{max} levels and were used as the modeled dose metric. For the BMD analyses of
23 the rat brain weight endpoint following gestational and lactational exposure, PBPK model
24 estimates of AUC methanol in blood for the dams of each dose group were used as the modeled
25 dose metric. As described in Section 5.1.2.1, the decision to use AUC as the dose metric for the
26 gestationally and postnatally exposed rats was made because under this exposure regimen, brain
27 weight is susceptible to both the level and duration of exposure. It is true that the results of

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

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

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

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

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

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

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

8 **Response:** The reviewer’s comments with respect to the selection of a BMR are correct
9 and consistent with EPA BMD Technical Guidance ([U.S. EPA, 2012a](#)). In the case of the
10 methanol toxicological review, all BMR levels considered for RfC or RfD derivation lie well
11 within the range of the dose-response observations. As indicated in the EPA BMD guidance ([U.S.](#)
12 [EPA, 2012a](#)), a series of papers ([Allen et al., 1994a, b](#); [Faustman et al., 1994](#)) suggest that a 5%
13 BMR is appropriate for dichotomous response data from well designed nested developmental
14 studies such as the Rogers et al. ([1993b](#)). For continuous response data, EPA guidance ([U.S.](#)
15 [EPA, 2012a](#)) suggests that “if there is an accepted level of change in the endpoint that is
16 considered to be biologically significant then that amount of change is the BMR.” For
17 continuous response data from developmental studies, comparisons with the NOAEL showed
18 that several cutoff values, including a 5% change in mean fetal weight, could be used to give
19 values similar to the NOAEL ([Kavlock et al., 1995](#)). If a 5% change in fetal weight is considered
20 biologically significant, it is reasonable to assume that a 5% brain weight change should also be
21 considered biologically significant. However, in a recent report on the statistical power in the
22 analyses of brain weight measures in pesticide neurotoxicity testing, Weichenthal et al. ([2010](#))
23 state that “if toxicological experts ultimately decide that brain weight changes in the range of 5%
24 are physiologically meaningful, a larger [than 10 per dose group] sample size will be needed to
25 consistently achieve reasonable power to detect this magnitude of effect.” EPA BMD guidance
26 ([U.S. EPA, 2012a](#)) states that “in the absence of any other idea of what level of response to
27 consider adverse, a change in the mean equal to one control SD from the control mean can be
28 used.” Because there is no clear biological basis for choosing one over the other, both are
29 considered and deference is given to the BMR that results in the lower RfC or RfD (see Tables 5-
30 4 and 5-6).

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

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

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

3 **Response:** EPA has responded to the comment regarding use of methanol concentration
4 in maternal blood as surrogate dose metric for evaluating postnatal changes under Charge A3
5 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.

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

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

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

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

8 Toxicodynamic variability can only be discussed qualitatively. As discussed in Section
9 4.9, there are a number of issues that may lead to sensitive human subpopulations. Potentially
10 sensitive subpopulations would include individuals with polymorphisms in the enzymes involved
11 in the metabolism of methanol and individuals with significant folate deficiencies. The effects
12 used to derive the candidate RfCs are observed in a potentially susceptible and sensitive
13 fetal/neonatal subpopulation. However, there is also variability across fetuses and neonates that
14 need to be taken into account. Children vary in their ability to metabolize and eliminate methanol
15 and in their sensitivity to methanol's toxic developmental effects. Consequently, there exists
16 considerable uncertainty pertaining to human population variability in methanol metabolism,
17 which provides justification for the 10-fold intraspecies UF used to derive the RfC and RfD.

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

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

25 **Comment 3:** Regarding the UF_D , three reviewers provided the following reasons why a
26 3-fold UF_D was not needed:

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

- 1 ○ “BMD analysis itself favors the conservative numbers” and
- 2 ○ “when given the choice of alternative BMD numbers such as those obtained from
- 3 the 3 versus 6 versus 8 week data, the lowest (i.e., most conservative) number is
- 4 chosen.”
- 5 ▪ “The key endpoint is developmental toxicity, which has been evaluated in multiple species,
- 6 including primate, and special endpoints such as neurotoxicity and immunotoxicity have
- 7 been evaluated.”
- 8 ▪ “There is no need to have a UF because ‘there is uncertainty regarding which test species is
- 9 most relevant to humans’—the lowest, high-quality point of departure was used.”
- 10 ▪ “There is also no need to have a UF_D for “dose spacing” because the BMD analysis counters
- 11 this potential design deficiency.

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

29 **Comment 4:** Regarding the application of all UFs, one reviewer stated that EPA should
30 "apply the uncertainty factors to the internal dose point of departure, prior to interspecies
31 extrapolation with the pharmacokinetic model to account for non-linearities in external versus
32 internal dose relationships." This reviewer suggested that EPA should discuss their choice of
33 applying UFs to the HEC/D rather than the BMDL. The reviewer estimated that if UFs are
34 applied first to the mouse cervical rib BMDL₀₅, then converted to the candidate RfC using the

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

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

12 The concern expressed by the second reviewer regarding departure from EPA practice is
13 recognized, given the uncertainty associated with dividing internal dose BMDLs by UFs that are
14 at least partially based on empirical analyses of ratios of NOAELs obtained from external oral
15 exposures ([U.S. EPA, 1994](#); [Dourson and Stara, 1983](#)). In the methanol (noncancer) assessment,
16 the general EPA practice of applying the human PBPK model to derive HEC/D values prior to
17 applying UFs ([U.S. EPA, 2002, 1994](#)) would result in RfC/Ds lower than if the PBPK model was
18 used to derive HEC/D estimates after dividing the BMDL internal doses by UFs. However, this
19 general practice if applied to methanol would result in greater model uncertainty because the
20 HECs (1,042 to 1,604 mg/m³) and HEDs (133 to 220 mg/kg-day) estimated from the BMDLs by
21 the revised PBPK model are well above the inhalation concentrations (655 mg/m³) and oral
22 exposures (50 mg/kg-day) for which there are human data to calibrate the PBPK model (see
23 Appendix B, Section B.2.7, Table B-6).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

12 Finally, for comparison purposes, EPA has performed an analysis of the alternative RfD
13 and RfC that would have been derived if a UF_D of 1 had been applied instead of a UF_D of 3.
14 Tables A-1 and A-2 correspond to Tables 5-4 and 5-6 of the assessment and demonstrate that a
15 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
16 to single digit significance). The EPA has decided against this approach because it believes there
17 is ample evidence that additional data from appropriate studies could result in the derivation of a
18 reference values considerably lower than 60 mg/m^3 and a 6 mg/kg-day . With respect to DNT
19 effects, in addition to the 12-fold higher methanol blood LOAELs in rodents versus primates
20 noted above, a BMD analysis of the VDR DNT effect reported by Burbacher et al. ([2004b](#);
21 [1999b](#)) resulted in a methanol blood C_{max} BMDL of 19.59 mg/L , less than half the methanol
22 blood level PODs shown in Table A-1 and A-2 for rodents. Potential chronic neurotoxicity
23 (fibrosis of "responsive stellate cells") were reported by NEDO ([1987](#)) at a 100 ppm exposure
24 level that EPA's monkey PK model estimates corresponds to a methanol blood level of 3 mg/L .
25 While Figure 5-4 of the assessment illustrates that a RfD or RfC exposure would not increase the
26 methanol blood levels of anyone with an background methanol blood level below 2.5 mg/L to
27 above 3 mg/L (see discussion in Section 5.3.6), Figure A-1 shows that a 6 mg/kg-day alternative
28 RfD or a 60 mg/m^3 alternative RfC exposure would result in blood levels above 3 m/L for a
29 substantial percentage (~25%) of those who started out with background blood levels below 2.5
30 mg/L . These analyses suggest that an alternative RfD of 6 mg/kg-day and an alternative RfC of
31 60 mg/m^3 associated with a UF_D of 1 are likely to be under protective with respect to the DNT
32 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/m³)^b	134.5	65.7	74.6	54.6

^aUF_A = 3; UF_D = 1; UF_H = 10; UF_S = 1; UF_L = 1; product of all UFs = 30.

^bEach 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 1600 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

^aUF_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.

^bEach 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 1600 mg/day (COT, 2011) in a 70-kg person (see Section 5.3.6); the final RfC is rounded to one significant figure.

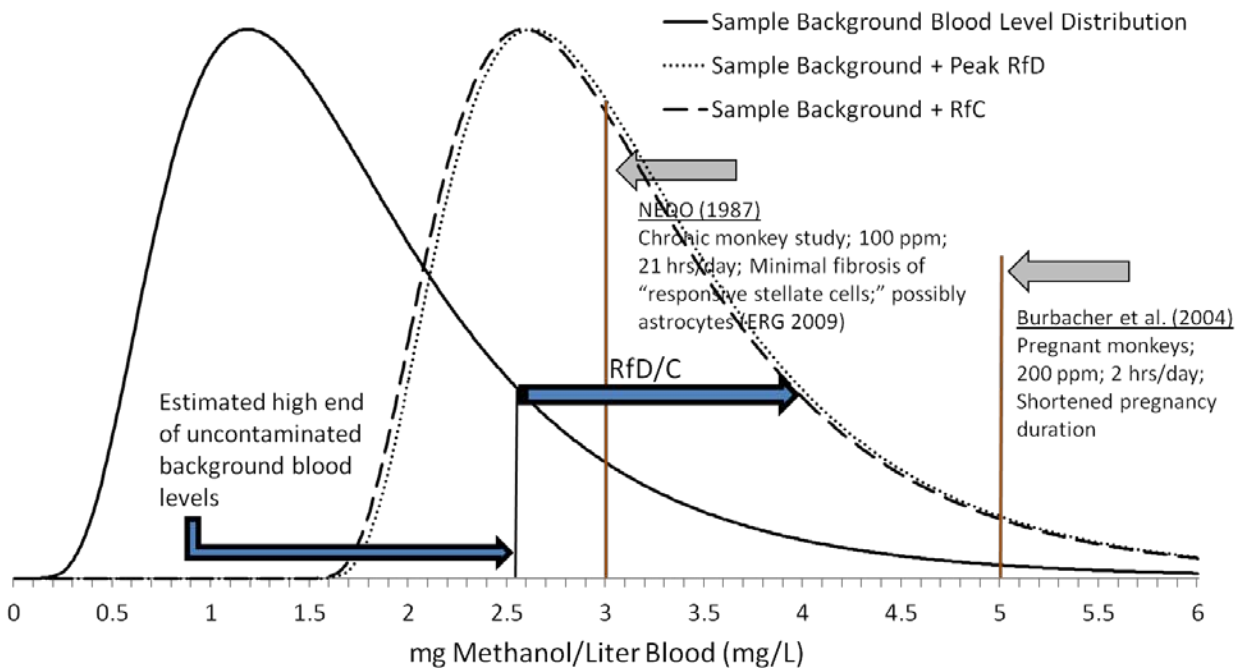


Figure A-1 Relationship of monkey blood levels associated with effects of uncertain adversity with projected impact of daily peak alternative RfC and RfD exposures [derived using aUF_D of 1] on sample background methanol blood levels (mg MeOH/Liter [mg/L] blood) in humans.

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.

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

1 **Comment 1:** One reviewer recommended that EPA “provide alternative RfC estimates
2 that would be derived using traditional approaches.”

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

12 **Comment 2:** One reviewer stated that “Human model validation using the oral data of
13 Schmutte et al. ([1988](#)) (see Charge D2) could further strengthen confidence in the route-to-route
14 extrapolation.”

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

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

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

20 **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.

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

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

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

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

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

7 **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.**

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

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

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

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

21 **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?

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

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

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

18 **Comment 2:** Two reviewers suggested that EPA make edits consistent with recent NRC
19 ([2011](#)) recommendations for the draft EPA formaldehyde assessment to:

- 20 a. Reduce text volume, narrative approach, redundancies and inconsistencies,
- 21 b. rely more heavily on tables and not repeat individual study descriptions,
- 22 c. include “inclusion and exclusion criteria” for cited references, and
- 23 d. reduce “extraneous information” contained in Appendices.

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

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

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

- 11 b. In response to the suggestion to “rely more heavily on tables and not repeat
12 individual study descriptions,” EPA has created new tables for whole embryo
13 studies described in Section “4.3.3 Other Reproductive and Developmental
14 Studies” and the i.p. studies described in Section “4.4.3 Neurotoxicity Studies
15 Employing In Vitro and I.P. Methanol Exposures” and has edited all Sections to
16 reduce unnecessary repetition of individual study descriptions. EPA has also
17 added an exposure-response array (Figure 4-1) to the assessment.
- 18 c. In response to the suggestion to include ‘inclusion and exclusion criteria’ for cited
19 references,” EPA has added text to the Preface of the methanol toxicological
20 review that describes how EPA evaluates the quality of studies.
- 21 d. In response to the suggestion to “reduce ‘extraneous information’ contained in
22 Appendices,” EPA has removed Appendix D, having determined it to be
23 extraneous due to the stronger biological basis for the choice of a Cmax dose
24 metric described in Section 5.1.2.1, and removed the source code text from
25 Appendix B. The source code text is posted on the EPA HERO database ([U.S.
26 EPA, 2012b](#)), as part of a Windows zip file containing a complete package of
27 acsIX code necessary to run all of the developed models.

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

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

1 **Comment 4:** One reviewer asked whether EPA considers the Fagan test results from the
2 Burbacher et al. monkey study to be “biologically significant despite the lack of a statistically
3 significant response?”

4 **Response:** There is uncertainty regarding both the biological and statistical significance
5 of the Fagan test results from the Burbacher et al. monkey study. As explained in Section 4.4.2,

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

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

18 “Although not statistically significant and not quantifiable, the results of this test
19 need to be considered, in conjunction with VDR test results and brain weight
20 changes noted in the NEDO ([1987](#)) rat study, as a possible indication of CNS
21 effects.”

22 **Comment 5:** One reviewer found Section 3.4.2.4 confusing, and suggested that other
23 models that have been developed with inhaled manganese ([Schroeter et al., 2011](#); [Yoon et al.,](#)
24 [2011](#); [Yoon et al., 2009a, b](#)) “could form the basis for a gestational and lactational model.”

25 **Response:** To reduce text volume in response to Charge D1 Comment 2a, Section 3.2.4
26 has been removed from the toxicological review. It contained an unnecessary discussion of the
27 rat and human isopropanol models described by Gentry et al. ([2003](#); [2002](#)) and Clewell et al.
28 ([2001](#)). It was originally included because it was thought to be a possible guide had EPA decided
29 to develop a more complex gestational and lactational model. The reviewer is right in that, had
30 EPA decided to take this approach, other gestational and lactational models, such as the one
31 developed for manganese, could have been considered. However, EPA has determined, and the
32 peer reviewers generally agreed (see “Summary of Comments” under Charges A3 and A5), that
33 such a model was unnecessary for the purposes of the methanol toxicological review.

1 **Comment 6:** One reviewer stated that the “the discussion of a two compartment stomach
2 (page 3-28 and elsewhere) for rodents need additional justification (squamous and epithelial
3 portions?)” and questioned whether this structure is “appropriate for people (as indicated on page
4 3-51).”

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

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

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

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

16 **Response:** In general, both units are given, with mg/m³ values provided parenthetically
17 after the ppm values, except for RfC/D and point of departure (e.g., BMDL) values discussed in
18 Section 5.

19 **Comment 9:** One reviewer requested a discussion of the use of alcohol dehydrogenase
20 inhibitors as a clinical ‘antidote’ on “page 4-7 (and possibly elsewhere).”

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

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

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

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

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

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

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

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

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

20 **Comment 14:** One reviewer noted that the “clarity of the document is hampered by the
21 lack of a clear synthesis of evidence regarding plausible modes of action for developmental
22 toxicity.”

23 **Response:** The mode of action discussions previously divided between Section 4.6 and
24 4.8 have been revised for clarity and consolidated into Section 4.7.

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

26 **Comment 1:** One reviewer stated that “[t]he overall document is much more concise and
27 direct in presenting the key features of the risk assessment that has been conducted.” Another
28 reviewer stated that “The organization and presentation of the information is greatly improved,
29 creating a much more readable document” and that “[t]he information seems to flow better,
30 redundancies are minimized, and tables are used to more effectively summarize information.”
31 Another reviewer stated that “[t]he revised (May, 2013) version of ‘Toxicological Review of Methanol

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

3 **Response:** EPA appreciates the affirmation of the Agency’s revisions to make the
4 methanol (noncancer) toxicological review more clear and concise. The revised format, including
5 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 non-cancer health effects of methanol.

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

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

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

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

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

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

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

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

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

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

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

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

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

3 **Response:** Edits have been made to the subject paragraph to emphasize that formate is
4 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.

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

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

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

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

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

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

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

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.

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

22 *Two reviewers suggested that estimates of the increased blood levels associated with the*
23 *RfD/C values should be compared with either an upper bound or a standard deviation for*

1 existing or normal physiological background levels of methanol. However, another reviewer
2 warned that “in view of the uncertainties as to fetal metabolism, mode of action and contribution
3 of diet and individual metabolic or toxicodynamic differences which are identified in the report it
4 seems very unwise to conclude that high-end [of the distribution of background] exposures which
5 are apparently safe for some individuals are necessarily safe for all.”

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

15 **Summary Response:** The RfC and RfD have increased by several-fold due to PBPK
16 model revisions made in response to the comments received during external peer review. The
17 final RfD of 2 mg/kg-day and RfC of 20 mg/m³ are not overly conservative because they (1) are
18 well above the levels associated with common exposures to methanol such as from a glass of
19 orange juice and (2) need to account for uncertainty regarding the sensitivity of primates to the
20 reproductive and developmental neurotoxic effects of methanol.

21 EPA addressed the recommendation of a reviewer that estimates of the increased blood
22 levels associated with the RfD and RfC be compared with a standard deviation for existing or
23 normal physiological (endogenous blood) background levels of methanol. As described in
24 Section 5.3.6, the methanol blood levels of individuals receiving both an RfC and RfD exposure
25 would increase by a daily maximum of 0.86 mg/L and a daily average of 0.59 mg/L. As shown in
26 Figures 5-3, 5-4 and B-17, these increases are comparable to the 0.7 mg/L standard deviation
27 estimated for the average methanol blood levels (1.5 ± 0.7 mg/L) in humans. Thus, the estimated
28 increase in blood levels of methanol from exogenous exposures at the level of the RfD or the
29 RfC (or from the RfC + RfD) are distinguishable from natural background variation. These RfC
30 and RfD methanol blood level increases are also more than 100-fold higher than the increase that
31 would be associated with a “common exposure” such as from a glass of pasteurized orange juice
32 and about 10-fold higher than the increase that would be associated with exposure to a glass of
33 unpasteurized orange juice (note that this is a relatively rare exposure and FDA requires warning
34 labels on unpasteurized juice that state “This product has not been pasteurized and therefore may
35 contain harmful bacteria that can cause serious illness in children, the elderly, and persons with

1 weakened immune systems”). Hence there is consistency with NTP CERHR ([2003](#)) in this
2 regard.

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

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

32 As discussed in response to Charge A2 Comment 1, the discussion of the RfC and RfD
33 and their relation to endogenous/background blood levels has been clarified in the revised draft
34 assessment (see Section 5.3.6). In summary, EPA does not feel that the RfD of 2 mg/kg-day and
35 RfC of 20 mg/m³ are overly conservative. They are well above the levels associated with

1 common exposures to methanol and they appropriately account for uncertainty regarding the
2 sensitivity of primates to the reproductive and developmental neurotoxic effects of methanol.

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

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

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

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

21 **Response:** In response to these reviewer and public comments on this topic, EPA has
22 revised the methanol (noncancer) assessment, particularly Section 5.3.6, to more clearly reflect
23 the limitations of the data shown in Table 3-1 for estimating population blood levels of methanol
24 (restricted diets, small numbers of studies and individuals, differing results by study), and
25 supplemented its analysis with data and conclusions from the United Kingdom (UK) Food
26 Standards Agency “COT Statement on the effects of chronic dietary exposure to methanol”
27 ([COT, 2011](#)). In Section 5.3.6, EPA has derived a sample lognormal distribution of methanol
28 blood levels that is consistent with data from study groups in Table 3-1 that did not involve
29 dietary restrictions other than alcohol. EPA compares this sample distribution with data for
30 background (endogenous and dietary) “exposure” rates estimated by the UK ([COT, 2011](#)). The
31 EPA sample background distribution and UK background methanol estimates are consistent with
32 one another in that the methanol blood level predicted by EPA’s PBPK model for the UK’s 23
33 mg/kg-day maximum exposure rate estimate and the EPA sample background distribution’s mean
34 + 2xSD are similar, 2.5 mg/L and 2.9 mg/L, respectively. EPA recognizes that some individuals

1 may have background methanol blood levels above 2.5 mg/L (~7% according to the sample
2 distribution), but believes that methanol blood level of 2.5 mg/L represents a reasonable
3 approximation of the upper end of the range of background blood levels associated with a diet
4 that includes fruits and vegetables (as discussed in Section 5.3.6).

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

20 **Response:** By definition, RfDs and RfCs are intended to protect "sensitive subgroups"
21 from "appreciable risk." In the case of methanol, sensitive subgroups would include pregnant
22 females and the elderly (toxicodynamic susceptibility) with lifetime methanol blood levels that
23 are at the high end of the range of background methanol blood levels associated with a diet that
24 includes fruits and vegetables (toxicokinetic susceptibility), estimated to be approximately 2.5
25 mg/L. A determination of whether the daily exogenous exposures at a RfD or RfC are
26 "distinguishable" relative to endogenous methanol blood levels is not a primary consideration.
27 However, EPA has shown in Section 5.3.6 that the shift in EPA's sample background methanol
28 blood level distribution that would be associated with daily exposures of the entire population to
29 methanol at the RfC or the RfD would result in approximately 85% and 94% increases,
30 respectively, in the number of individuals with daily peak methanol blood levels equal to or
31 greater than 2.5 mg/L. As discussed in Section 5.3.6, these estimates are not precise and do not
32 account for interindividual variability. However, they illustrate that the increase in individuals
33 with higher than 2.5 mg/L methanol blood levels (i.e., higher than the upper range of background
34 methanol blood levels associated with a diet that includes fruits and vegetables) following a RfD
35 or RfC exposure would not be negligible.

1 EPA also believes that the RfD and RfC are not “excessively conservative.” As stated in
2 response to the Charge B4 Follow-up Peer Review Comment 3, EPA guidance places particular
3 emphasis on database deficiencies in the area of developmental toxicity, the primary focus of the
4 methanol (noncancer) assessment, stating that “[i]f data from the available toxicology studies
5 raise suspicions of developmental toxicity and signal the need for developmental data on specific
6 organ systems (e.g., detailed nervous system, immune system, carcinogenesis, or endocrine
7 system), then the database factor should take into account whether or not these data are available
8 and used in the assessment and their potential to affect the POD for the particular duration RfD
9 or RfC under development.” As discussed in Section 5.1.3.2.3, a database uncertainty factor of 3-
10 fold has been applied to account for deficiencies in the methanol database, particularly with
11 respect to deficiencies that do not allow the Agency to full assess methanol’s potential to cause
12 developmental neurotoxicity. As discussed in Section 5.3.7, uncertain, but potentially adverse
13 effects have been observed in monkeys at blood levels as low as 3 mg/L. As discussed in Section
14 5.3.6, a RfC or RfD exposure is expected to raise the methanol blood level of an individual with
15 a high end background blood level of approximately 2.5 mg/L to just below 3 mg/L. A higher
16 exposure would raise background levels above the lowest methanol blood level that has been
17 associated with uncertain, but potentially adverse effects.

A.2. Public Comments

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

18 The following public comments and EPA responses pertain to comments received on the
19 2011 draft of the methanol (noncancer) toxicological review during the April 18, 2011 to July 6,
20 2011 public comment period.

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

24 **Response:** As discussed above in response to external peer review Comment 1 of Charge
25 A2 and comments associated with the Bonus Charge Question, the decision to base the RfC/D on
26 exposures that increase the body burden of an individual above their naturally occurring
27 endogenous/background blood levels was not arbitrary. The PBPK models used in the final
28 assessment incorporate endogenous/background concentrations of methanol; however, for BMD
29 modeling, the PBPK model estimate of background concentration is subtracted from the

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

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

12 **Response:** The approach taken by EPA in deriving the RfC and the RfD assumes that
13 endogenous/background blood levels of methanol in a human population with normal
14 background variation do not elicit adverse health effects. There is currently little evidence,
15 epidemiological or otherwise, to challenge this assumption. Given this assumption and lack of
16 evidence to the contrary, if the 2 mg/kg-day RfD or 2×10^1 mg/m³ RfC were so low that the
17 resulting (predicted) change in methanol blood levels was only a small fraction of the normal
18 variation in background levels (e.g., 1% of one standard deviation), one could argue that this
19 would be indistinguishable from natural variation and toxicologically irrelevant. Therefore, a
20 comparison of the expected increase in methanol levels in blood resulting from exposure to
21 methanol at the level of the RfC or RfD to the variation in endogenous/background levels of
22 methanol observed in humans is provided in Section 5.3.6 to determine if this might be the case.
23 As shown in Figures 5-3, 5-4 and B 17, the estimated increase in blood levels of methanol
24 resulting from exposure to methanol at the RfC alone, at the RfD alone, or at the RfC + RfD
25 combined is comparable to the variability (represented as one standard deviation) observed in the
26 average estimated methanol blood levels (1.5 ± 0.7 mg/L) in humans (see Table 3-1 and Section
27 5.3.6). This then demonstrates that the estimated increase in levels of methanol from the RfD or
28 the RfC (or from the RfC + RfD) are distinguishable from natural background variation, but the
29 overall derivation of the RfD and RfC ensures that these increases will not significantly increase
30 adverse health outcomes.

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

33 **Response:** As explained above in response to external peer review Comment 2 of Charge
34 A2, EPA has re-calibrated the PBPK models to account for background levels and has used them

1 to derive the revised RfD and RfC. Hence, the justification for not including a background term
2 in the PBPK models has been removed from the toxicological review.

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

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

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

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

26 **Comment 6:** “Failure to confirm the results of EPA’s PBPK modeling against blood
27 methanol concentration data collected in both [Rogers et al. ([1993b](#)) and NEDO ([1987](#))] studies.”

28 **Response:** The mouse PBPK model has now been removed from the assessment and the
29 blood concentration data from Rogers et al. ([1993b](#)) used directly for the benchmark dose
30 analysis. For example, the BMDL₁₀ for the mouse cervical rib effect based on the blood C_{max}
31 metric has thereby changed from 94.3 mg/L to 90.9 mg/L (both representing concentration
32 increases above background). Data were not available for validating the rat PBPK model
33 predictions prior to the methanol external peer review. Subsequent to the methanol external peer

1 review, EPA received blood measurements from the NEDO ([1987](#)) rat study and has validated
2 model predictions against them (Appendix B, Sections B.2.3, B.2.4 and B.2.5).

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

7 **Response:** As explained above in response to comments made under external peer review
8 Charge D2, a detailed discussion of the University of Toronto findings have been added to
9 Section 5.3 “UNCERTAINTIES IN THE INHALATION RfC AND ORAL RfD” of the
10 toxicological review (see Section “5.3.5 Choice of Species/Gender”). The in-vitro study of Miller
11 and Wells ([2011](#)) demonstrated that methanol-induced developmental effects are enhanced in
12 mouse embryos with low catalase activity and reduced in mouse embryos with high catalase
13 activity. The authors propose that this observation is related to methanol’s impact on the ability
14 of catalase to control the damaging effects of reactive oxygen species (ROS) activity, which
15 would be greater in mouse embryos with low catalase activity. However, as discussed in Section
16 5.3.5, there are several problems with this interpretation, including that in vivo results from the
17 same laboratory ([Siu et al., 2013](#)) do not support the Miller and Wells ([2011](#)) in vitro findings.
18 The University of Toronto studies are informative, but do not demonstrate conclusively that
19 rodent developmental studies are irrelevant to humans.

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

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

1 observed, in the supplementary study. While this supplementary study no longer forms the basis
2 of the RfD, it does form the basis for the RfC because its limitations are not considered serious
3 enough to preclude its consideration as a candidate principal study and because it documents a
4 clear dose-response for a relevant endpoint for a critical organ system, brain weight reduction,
5 which is consistent with its parent two-generation study and with other teratogenicity ([NEDO,
6 1987](#)) and subchronic ([TRL, 1986](#)) study findings with respect to the effect of methanol exposure
7 on brain weight.

8 **Comment 9:** “The lack of utility of the NEDO ([1987](#)) reproductive study for the purpose
9 of human health risk assessment, as judged by other authoritative bodies.”

10 **Response:** By “authoritative bodies” the commenter refers specifically to a 2002 report
11 from the National Toxicology Program’s Center for the Evaluation of Risks to Human
12 Reproduction (NTP CERHR) on the reproductive and developmental toxicity of methanol. The
13 methanol toxicological review cites the subsequently peer reviewed and published version of this
14 report ([NTP-CERHR, 2004](#)). In the more recent, published version of the report, the NTP-
15 CERHR panel states that “a summary of a two-generation rat reproductive toxicity study done by
16 the Japanese NEDO was received, but data were not available in sufficient detail for Expert
17 Panel review.” The NEDO summary reviewed by the NTP-CERHR panel did not contain the
18 more detailed supplementary study data, with pup brain weight means and standard deviations
19 that EPA evaluated in its benchmark dose analysis (Appendix D). This information, as well as
20 supplemental methanol blood measurements, was obtained by EPA after the NTP-CERHR panel
21 completed its report. In addition, EPA sponsored an external peer review ([ERG, 2009](#)) of the
22 NEDO ([1987](#)) report that contained this information, along with two other NEDO chronic rat and
23 mouse studies ([NEDO, 1985a, b](#)). This expert peer review panel of five scientists was asked
24 specifically to “Describe the reliability of the subject NEDO studies for consideration in the
25 derivation of EPA IRIS quantitative health benchmarks.” With respect to this charge and the two-
26 generation study, including the supplementary study, the main concerns expressed by the peer
27 reviewers were that they “may be useful for RfD derivation if brain weight changes persist when
28 normalized by body weight” and that the authors “should have used ANOVA plus multiple
29 comparison tests to analyze these data.” With respect to the former concern, NEDO only reported
30 means and standard deviations for absolute brain weight change and did not report body weight
31 data for the supplementary study. However, body weight data reported for the parent, two-
32 generation study did not indicate a body weight effect in the exposed F1 or F2 generation pups.
33 Further, the absolute brain weights reported by NEDO are an appropriate basis for a dose-
34 response assessment. With respect to the concern over the lack of appropriate statistical testing,
35 EPA did not rely on the NEDO statistical determinations, but performed its own more definitive

1 benchmark dose analysis of the data (see response to external peer review Comment 1 of Charge
2 B1). Hence, while the NEDO report of the two-generation supplementary study results has
3 limitations, particularly with respect to reporting of methods (see discussion in Section 5.3.1), it
4 was not evaluated by the NTP-CERHR (2004) expert panel, and it was not deemed inadequate,
5 for the purposes of RfC derivation, by a panel of expert peer reviewers (ERG, 2009).

6 **Comment 10:** “The use of exposure regimens in both the reproductive study and the
7 24-month rat study that confound the estimates of exposure.”

8 **Response:** The stated basis for this comment is the concern over (1) “consumption of
9 [methanol] contaminated feed,” (2) “ingestion of methanol during the act of preening,” (3)
10 dermal absorption in adult rats, and (4) “increased dermal absorption of neonatal animals [over
11 adults]” because “the epidermal layers of neonatal rats are thinner than those of adult animals,
12 they lack fur for the first week after birth.” With respect to the first concern, EPA estimates that
13 data on a rodent breeder diet (<http://www.labdiet.com/pdf/5013.pdf>) indicates 10% moisture
14 content. If one assumes that chamber methanol concentrations equilibrate with this moisture
15 content, using the blood:air PC for methanol, and uses a typical pregnancy food consumption of
16 30 g/day in rats, then the amount of methanol ingested in the chow would be about 3% of that
17 inhaled during a 22 hr/day exposure. This is likely an upper bound since it would take some time
18 for methanol to diffuse into and through a container of chow (equilibration with the chow would
19 take time), and fresh chow is provided each day. Thus the amount ingested by this route is not
20 considered significant and dosimetry calculations have not been adjusted to reflect that
21 possibility.

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

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

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

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

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

34 **Comment 12:** “Use of statistical methods in both the reproductive study and the 24-
35 month rat study that, by today’s standards, are considered inadequate.”

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

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

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

20 **Comment 14:** “Lack of proper consideration of species differences in sensitivity to
21 developmental toxicity due to methanol exposures in the RfC derivation.”

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

30 **Comment 15:** “Failure of EPA to consider more robust developmental toxicity data in
31 derivation of an RfC value.” Specifically, the commenter suggests that the Rogers et al. ([1993b](#))
32 study would be the more appropriate study on which to base an assessment of the developmental
33 toxicity of methanol.

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

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

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

17 **Comment 17:** “The draft assessment states that Sweeting et al. ([2011](#)) suggests that low
18 ADH activity in mouse embryos could lead to a ‘*greater depletion of catalase.*’ The assessment
19 further states (line 7,8) ‘If ROS accumulation due to this *catalase consumption...*’ Sweeting et al.
20 do not postulate a depletion or consumption of catalase.”

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

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

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

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

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

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

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

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

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

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

20 **Comment 1:** Compliments/Affirmations:

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

- 1 • “EPA is also to be commended for eliminating the mouse PBPK model altogether
2 from its updated assessment.”
- 3 • “EPA is to be commended for their responsiveness to concerns raised by the external
4 peer review panel and the public regarding the previous external peer review draft
5 (2011).”
- 6 • “I would also like to note that I am very pleased that EPA is applying the uncertainty
7 factors to the internal dose prior to using the human PBPK model for conversion of
8 the internal dose to an acceptable external dose (Section 5.1.3.2, pages 5-15- and
9 5-16).”
- 10 • “...the revised draft assessment demonstrates a clear intent to address the significant
11 concerns that were raised during reviews of the initial draft, and does significantly
12 raise the inhalation reference concentration (RfC) and oral reference dose (RfD).”
- 13 • “Given the availability (subsequent to the original draft review) of the toxicokinetic
14 data for S-D rats which are the strain used in the critical experiments for the RfC, it
15 makes sense to use these data as the basis for rat PBPK modeling, and apparently this
16 has been successful in that blood levels reported in the NEDO experiments are
17 matched by the model.”
- 18 • “It appears to me that it is perfectly reasonable to apply the dose metric conversion
19 after the UFs as has been done in the revised toxicity review. This ensures that the
20 dose metric conversion is made at the actual concentration of interest, i.e. the
21 RfC/RfD. This would also usually be done for a cancer risk assessment, where the
22 dose metric conversion would be done at an observed or estimated concentration or a
23 risk specific level.”
- 24 • “The change in critical study for the RfD to the Rogers et al. ([1993b](#)) is a natural
25 response to the change in relative sensitivities of this and the NEDO studies based on
26 the revised toxicokinetic model. This study is of adequate quality and is reported in
27 detail. Although the data used are from the inhalation study (the oral experiment
28 being much more limited) it has the advantage of having actual blood level
29 measurements which are useable as the internal dose metric, reducing the
30 uncertainties associated with application of the animal PBPK model (although the
31 human PBPK model is still needed to convert to an external oral dose metric for the
32 RfD.)”

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

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

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

8 **Comment 2:** One commenter noted that “EPA’s revised draft assessment provides a brief
9 discussion of some potential exposure pathways (e.g., foodstuff or commercial products) but it
10 does not provide information on the specific levels of methanol that humans are likely to be
11 exposed to on a daily basis” and that “[t]he United Kingdom (UK) Food Standards Agency has
12 stated that endogenous methanol production ranges from 300 to 600 mg/day and that up to 1000
13 mg/day methanol can be consumed in food, particularly fruit and vegetables.”

14 **Response:** EPA has added the UK estimates of endogenous methanol production and
15 consumption in food, particularly fruits and vegetables, to Section 2 of the assessment. Also,
16 discussion of how these estimates compare to EPA’s estimate of background blood levels has
17 been added to section 5.3.6. The UK report referred to by the commenter ([COT, 2011](#)) is now
18 used to support the EPA’s estimate of the upper end of the range of methanol blood levels
19 associated with a diet that includes fruits and vegetables.

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

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

1 Thus, studies that substantially restricted the consumption of fruits and vegetables ([Ernstgård et](#)
2 [al., 2005](#); [Osterloh et al., 1996](#); [Cook et al., 1991](#); [Davoli et al., 1986](#)) are considered
3 inappropriate under this definition. The remaining studies, Batterman and Franzblau ([1997](#)),
4 Batterman et al. ([1998](#)), Lee et al. ([1992](#)), Sarkola and Eriksson ([2001](#)), Turner et al. ([2006](#)) and
5 Woo et al. ([2005](#)), are considered appropriate for the purpose of this analysis as they did not
6 involve substantial fasting (i.e., only two involved fasting, one overnight and one for 4 hours) or
7 dietary restrictions (i.e., only one involved a minimal dietary restriction, no aspartame-containing
8 cereals and no juice on the morning of the test). The analysis of these six studies (see details in
9 Section 5.3.6), after weighting them in accordance with the extent to which they represent the
10 U.S. population (see footnote 61), yields an estimate for the mean and SD for endogenous
11 background methanol blood of 1.3 mg/L and 0.8 mg/L, respectively. As discussed in Section
12 5.3.6 and in response to Bonus Charge Follow-up Peer Review Comment 1, these estimates are
13 consistent with endogenous methanol production plus dietary exposure ranges reported by the
14 UK Food Standards Agency ([COT, 2011](#)).

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

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

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

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

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

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

1 substantially increased susceptibility from ‘background’ exposures.” This commenter added that
2 “[t]hese considerations are adequately addressed in the revised toxicity review.”

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

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

27 **Response:** As indicated in response to the Bonus Charge Follow-up Peer Review
28 Comments and Comment 4 above, the primary consideration in deriving the methanol RfD and
29 RfC is whether they represent daily exogenous exposures that, when added to background levels
30 of methanol associated with a diet that includes fruits and vegetables (see discussion in Section
31 5.3.6), are not likely to result in an appreciable health risk, even to sensitive subgroups.
32 Consistent with the view of the second commenter above, a determination of whether the daily
33 exogenous exposures at a RfD or RfC are “distinguishable” relative to background methanol
34 blood levels is not a primary consideration for an IRIS risk assessment. However, reviewers of
35 the 2011 draft methanol (noncancer) toxicological review asked EPA to investigate this topic and

1 EPA responded by adding Section 5.3.6 to the assessment. As discussed in Section 5.3.6, the RfC
2 and RfD exposures would result in approximately 85% and 94% increases, respectively, in the
3 number of individuals with peak methanol blood levels equal to or greater than 2.5 mg/L. These
4 estimates are not precise and do not account for interindividual variability. However, they
5 illustrate that the increase in individuals with higher than 2.5 mg/L methanol blood levels (i.e.,
6 higher than the upper range of background methanol blood levels associated with a diet that
7 includes fruits and vegetables) following a RfD or RfC exposure would not be negligible.

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

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

23 **Response:** As was discussed in response to the Bonus Charge Follow-up Peer Review
24 and 2013 Public Comments 4 and 6 above, the primary consideration in deriving the methanol
25 RfD and RfC is whether they represent daily exogenous exposures that, when added to
26 background levels of methanol associated with a diet that includes fruits and vegetables (as
27 defined in Section 5.3.6), are not likely to result in an appreciable health risk, even to sensitive
28 subgroups. The RfD and RfC are not estimates of exposures that are health hazards, and the risk
29 associated with exposures above the RfD will vary from individual to individual, with the
30 greatest risk experienced by sensitive subgroups. Nevertheless, the revised assessment includes
31 an expanded discussion of the relationship of the RfD and RfC to methanol blood levels that
32 have been associated with effects in monkeys and humans. This discussion has been moved to a
33 new Section 5.3.7 titled "The Relationship of the RfC and RfD to Methanol Blood Levels In
34 Monkeys Associated with Unquantifiable Effects of Uncertain Adversity." Section 5.3.7
35 discusses the reasons EPA believes that blood levels of methanol below 10 mg/L, but above 3

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

11 **Comment 8:** One commenter noted that “...there does not appear to be a full discussion
12 in the document regarding what study quality criteria were applied to each study in determining
13 its ‘acceptable quality.’”

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

21 **Comment 9:** Concerning all IRIS chemicals, one commenter stated that “...to date, EPA
22 has not indicated which substances, under review by the IRIS program, will benefit from
23 implementation of the NRC recommendations...[n]or has EPA provided an updated timeline of
24 when it anticipates having each phase completed and fully implemented.” The commenter
25 suggests that “EPA should provide this information as soon as possible and expeditiously move
26 forward with fully implementing all of the NRC’s recommendations, regardless of the phase”
27 that has been assigned by EPA to each assessment for progressive implementation of the NRC
28 recommendations. Concerning the methanol (noncancer) assessment, the commenter noted that
29 “[t]he revised draft assessment only provides a general overview of the process utilized in EPA’s
30 literature search strategy” and suggests that “[i]t would have been more useful for EPA to have
31 included the search terms it used to select appropriate studies for inclusion in the literature search
32 as well as listing the specific inclusion/exclusion criteria.” The commenter further suggested that
33 “EPA should be consistent and clear in identifying which elements it will focus on with regard to
34 ‘partial implementation’ [of the NRC recommendations]” and noted that “...the necessary, major

1 substantive changes that are needed, such as more fully considering an integrated weight of
2 evidence approach, that includes mode of action, as part of IRIS, remain largely unaddressed.”

3 **Response:** In April 2011, the National Research Council (NRC), in their report Review
4 of the Environmental Protection Agency’s Draft IRIS Assessment of Formaldehyde, made
5 several recommendations to EPA for improving IRIS assessments and the IRIS Program. The
6 NRC’s recommendations were focused on Step 1 of the IRIS process, the development of draft
7 assessments. All IRIS assessments currently under review by the IRIS Program will benefit from
8 implementation of the NRC recommendations, and consistent with the advice of the NRC, the
9 IRIS Program is implementing these recommendations using a phased approach and is making
10 the most extensive changes to assessments that are in the earlier stages of the IRIS process. The
11 methanol (noncancer) assessment is in Phase 1 of implementation of the NRC
12 recommendations. Phase 1 focuses on a subset of the shorter-term recommendations for
13 assessments near the end of the document development process or close to final posting.
14 Consistent with the focus of this phase of implementation of NRC recommendations, the
15 Toxicological Review was edited to be more concise, the rationales for decisions have been made
16 more transparent, and the description of the literature search strategy has been augmented for
17 clarity. Additionally, consistent with direction provided by Congress in The Consolidated
18 Appropriations Act of 2012, the Agency will include documentation in the final methanol
19 (noncancer) IRIS assessment describing how the recommendations of the NRC have been
20 implemented or addressed in the methanol (noncancer) assessment, including an explanation for
21 why certain recommendations were not incorporated.

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

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

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

11 **Comment 11:** One commenter noted that "...in Table B-1, only central estimates of these
12 two parameters [VmaxC and Km] are presented for both the rat and human PBPK models."

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

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

32 **Response:** It should first be noted that the values of Km reported previously for monkeys
33 and one human subject by Perkins et al. (1995a) were estimated using a classical (non-
34 physiological) model. For one of the studies analyzed by Perkins et al. (1995a), Makar et al.
35 (1968), blood concentrations in the monkey were not measured but were estimated from the total

1 dose and clearance was estimated based on exhaled CO₂ and urinary excretion. For all monkey
2 studies analyzed the initial doses ranged from 1-6 g/kg and for the human subject the initial
3 blood level was estimated to be 1.4 g/L. None of the blood data used by Perkins et al. (1995a) to
4 estimate Km values fell below ~ 100 mg/L. Thus none of those other data sets were particularly
5 suited to identifying a potential Km below 100 mg/L, even if that exists. By contrast, the analysis
6 of the much lower, more relevant monkey exposure data from Burbacher et al. (2004a,b) shown
7 in B.3.1 yielded an estimated Km of 14.4 mg/L, a value which none of the commenters has
8 called into question. (This result was also obtained with a classical PK model structure.)
9 Therefore, EPA concluded that the prior Km results from a single published analysis show that
10 the value obtained for humans is likely incorrect. The model as parameterized is clearly
11 consistent with (fits well) the existing human data. The difference between the previously
12 reported values for monkeys and EPA's value for humans is explained by the use of a different
13 data set which covers a much lower range of internal concentrations, in humans.

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

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

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

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

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

33 **Response:** The inter-species (animal-human) uncertainty factor, UF_A was not set to 10,
34 but was set to 3, the default value for pharmacodynamic differences between animals and
35 humans (i.e., allowing that humans may be more sensitive to a given internal dose). The UF_A is 3

1 instead of 10 because the PBPK model is used to capture inter-species differences in
2 pharmacokinetics. As discussed in response to Charge B4 Follow-up Peer Review Comments 2
3 and 3, in the revised Section 5.1.3.2.3, EPA has clarified that the UF_D is based on deficiencies in
4 the methanol toxicological database, particularly with respect to the interpretation of the
5 importance and relevance reproductive, developmental neurotoxicity and chronic CNS effects
6 observed in monkeys. Thus, the UF_D does not have the same basis as the UF_A , and was not
7 “double counted.”

8 With respect to the need for an additional developmental neurotoxicity (DNT) study, EPA
9 ([2002](#)) guidance places particular emphasis in this regard on database deficiencies in the area of
10 developmental toxicity, the primary focus of the methanol (noncancer) assessment, stating that
11 “[i]f data from the available toxicology studies raise suspicions of developmental toxicity and
12 signal the need for developmental data on specific organ systems (e.g., detailed nervous system,
13 immune system, carcinogenesis, or endocrine system), then the database factor should take into
14 account whether or not these data are available and used in the assessment and their potential to
15 affect the POD for the particular duration RfD or RfC under development.” EPA believes that,
16 with respect to the methanol database, the available toxicology studies in monkeys “raise
17 suspicions of developmental toxicity and signal the need for developmental data,” particularly
18 with respect to DNT. Table 5-5 of Section 5.1.3.2.3 indicates that methanol blood levels
19 associated with DNT effects are a 12-fold higher in rodents versus primates. Some of this
20 dissimilarity may be due to differences in species sensitivity, for which the UF_A of 3-fold is
21 intended to account, but some of the difference may be due to other factors, including whether
22 appropriate and comparable endpoints were examined and whether appropriate study designs and
23 quality control measures were used. To account for these additional uncertainties, a 3-fold UF_D is
24 applied.

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

32 EPA’s response to Comment 15 below addresses the general concern regarding EPA’s
33 choice of “...the most conservative decision at several points.” It should be noted here, however,
34 that in consideration of the credibility of the available scientific information, EPA did not derive
35 lower RfD and RfC values using the monkey dose-response data. For instance, as can be seen

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

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

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

34 **Response:** Consistent with EPA guidelines for the development of RfDs and RfCs ([U.S.](#)
35 [EPA, 2002, 1994](#)), use of the best available, sound science has been a key focus of the methanol

1 assessment. The credibility of the available science was an important consideration at every step
2 of the RfD/RfC derivation process. EPA’s commitment to use the best scientific and most
3 credible toxicological approach available resulted in several choices that do not represent “the
4 lowest reference value that is scientifically defensible,” including the use of:

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

23 With respect to the suggestion that the Agency has calculated “a reference concentration
24 below background,” as described in numerous places in the methanol (noncancer) toxicological
25 review, including the Executive Summary, the RfD and RfC are exposures above background
26 blood levels of methanol associated with a diet that includes fruits and vegetables, which are
27 estimated to be below approximately 2.5 mg/L (see Section 5.3.6). As discussed in the response
28 to the Bonus Charge Follow-up Peer Review Comments and Comments 4 and 6 above, the
29 primary consideration in deriving the methanol RfD and RfC is whether they represent daily
30 exogenous exposures that, when added to background levels of methanol associated with a diet
31 that includes fruits and vegetables, are not likely to result in an appreciable health risk, even to
32 sensitive subgroups. Consistent with the view of the second commenter above, a determination
33 of whether the daily exogenous exposures at a RfD or RfC are “distinguishable” relative to

1 background methanol blood levels is not a primary consideration for an IRIS risk assessment but
2 has been accounted for in this assessment.

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

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

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

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

31 **Comment 17 – EPA has not made its PBPK model publically available:** One
32 commenter stated that, as of June 12, 2013, they were unable to locate the PBPK model source
33 code files, which EPA states will be “available electronically on the IRIS website

1 [www.epa.gov/iris}” in one place (page A-9) and “will be posted on the EPA IRIS website, along
2 with the final methanol (noncancer) assessment” (Page A-32).

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

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

23 **Response:** As indicated above in response to the Charge A1 Follow-up Peer Review
24 Comments, the commenters are correct in that the lack of an explicit kidney compartment would
25 be a significant factor if total urinary clearance was a significant fraction of renal blood flow,
26 which likely occurs for some other chemicals. However for the methanol model the clearance
27 rate for this pathway in the rat is only 0.24% of renal blood flow (using a renal flow fraction of
28 0.141 from Brown et al. 1997) and in the human is only 0.07% of renal blood flow (using a renal
29 flow fraction of 0.175 from Brown et al. 1997). Therefore including an explicit kidney
30 compartment with its own flow rate would have a negligible impact on the methanol model
31 results reported here. These calculations and a statement that the approach should only be used
32 when renal clearance is a small fraction (< 10%) of renal blood flow have been added to
33 appendix B.

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

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

12 **Response:** The commenter is correct that the drinking water pattern used has not been
13 extensively evaluated, but it was previously used in the posted dichloromethane toxicological
14 review (which underwent extensive peer review) and has been described in the recent peer-
15 reviewed paper by Sasso et al. (2013;
16 <http://toxsci.oxfordjournals.org/content/early/2012/11/09/toxsci.kfs320.short?rss=1>) for
17 chloroform. While a probabilistic analysis as suggested by the commenter would be ideal, the
18 EPA is not aware of an available published statistical model for water/fluid ingestion in a given
19 day. Variability in total water imbibed from one day to another is likely available, but a
20 description of the detailed drinking pattern within a day (e.g., probabilities of ingestion in a given
21 time increment) would need to be generated, the analysis conducted, and the results subject to
22 peer-review.

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

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

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

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

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

9 **Comment 21:** One commenter stated that they “...disagree with the comments made in
10 the Appendix (D26, line12) that 10% is a typically justifiable BMR for non-developmental
11 dichotomous data, and that nested developmental studies are necessarily ‘more sensitive’ than
12 straightforward non-developmental studies.” This commenter noted that “[t]he reason for the
13 nested design in developmental studies is to accommodate the additional complicating litter
14 effects in these data: it allows the analysis to deal with additional variability and bias in the data
15 (which do not exist in the non-developmental data) rather than providing more accuracy or
16 sensitivity.” This commenter also noted that “[i]n order to retain comparability with existing
17 assessments, and to retain compatibility with the guidance on UFs, it is necessary to have a
18 BMDL which is at least in general properties similar to a NOAEL, and practical experience has
19 shown that in fact the BMDL₀₅ best meets this criterion for dichotomous general toxicity data as
20 well as developmental studies.”

21
22 **Response:** The statements in Appendix D referred to by the commenter are taken from
23 the recent EPA BMD technical guidance ([U.S. EPA, 2012a](#)), which states that “[t]he 10%
24 response level has customarily been used for comparisons because it is at or near the limit of
25 sensitivity in most cancer bioassays and in noncancer bioassays of comparable size” and further
26 states that “[f]rom a statistical standpoint, most reproductive and developmental studies with
27 nested study designs easily support a BMR of 5%.” While EPA agrees that the nested study
28 design of developmental studies does not necessarily makes them more sensitive, in many cases,
29 developmental studies have the advantage of a larger sample size, which can increase statistical
30 power and allow for the use of the lower BMR. Also, as the commenter suggests, a series of
31 papers have shown that when data are expressed as the proportion of affected fetuses per litter
32 (nested dichotomous data), the NOAEL was on average 0.7 times the BMDL for a 10% excess
33 probability of response and was approximately equal, on average, to the BMDL for a 5% excess

- 1 probability of response ([U.S. EPA, 2012a](#)). The text in Appendix D has been modified to remove
- 2 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

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

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

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

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

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

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

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

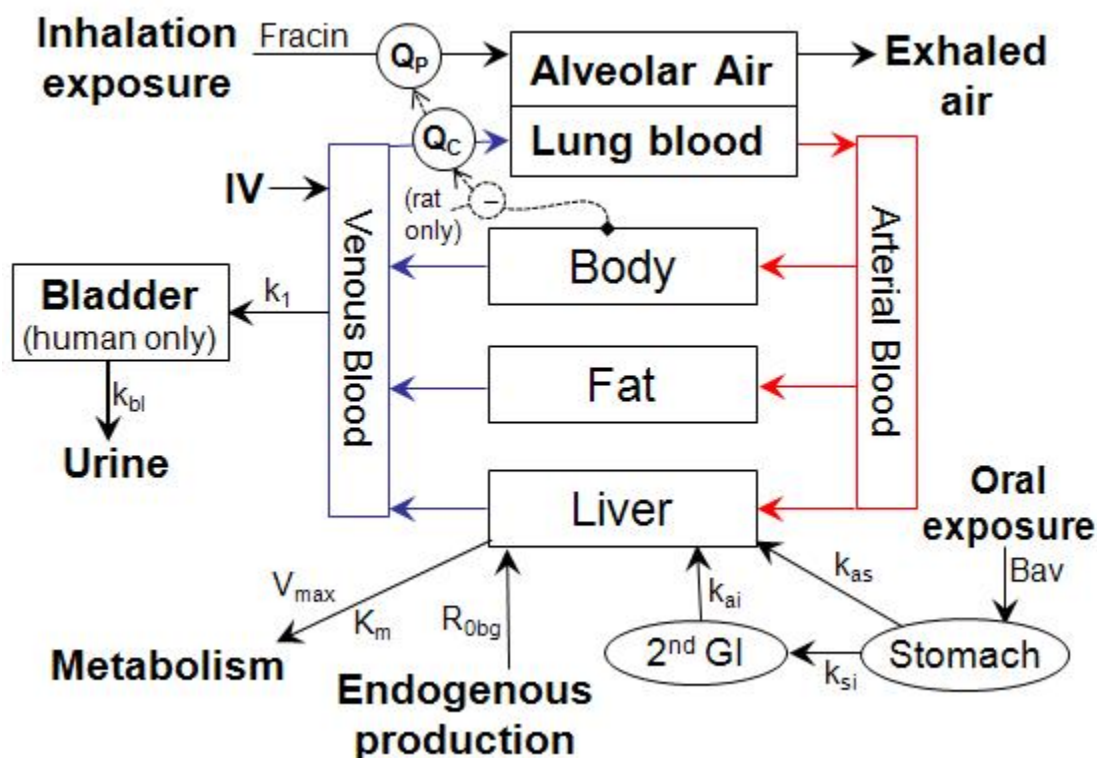
B.2. Model Development

B.2.1. Model Structure

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

1 various tissues, but examining these two for the experimental studies used for model calibration
2 showed the difference was below 1% for rats and below 4% for humans, with the maximum
3 difference occurring during inhalation exposures. Where the kidney blood-flow limitation would
4 be significant is if the clearance in the kidney was a significant fraction (i.e., greater than 10%)
5 of the kidney blood flow. However for the methanol model the clearance rate for this pathway in
6 the rat is only 0.24% of renal blood flow (using a renal flow fraction of 0.141 from Brown et al.
7 1997) and in the human is only 0.07% of renal blood flow (using a renal flow fraction of 0.175
8 from Brown et al. 1997). Therefore including an explicit kidney compartment with its own flow
9 rate would have a negligible impact on the methanol model results reported here.

10 A fat compartment was included because it is the only tissue with a tissue:blood
11 partitioning coefficient appreciably different than unity, and the liver is included because it is the
12 primary site of metabolism. Background levels of methanol are included through use of a zero-
13 order rate of infusion, R_{0bg} . Equations in the model code allow R_{0bg} to be calculated as a
14 function of other model parameters to match a user-specified background blood or urine
15 concentration.



Note: Parameters: Fr_{acin} (FRACIN), fraction of exposure concentration reaching gas exchange region in lungs; B_{av} , 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 $B_{av} = 100\%$ but humans with $B_{av} = 83\%$.

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

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

11 Since metabolic reactions are known to be saturable – the rate is ultimately limited by the
 12 amount of enzyme present – and metabolism is known to be the primary route of elimination in

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

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

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

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

1 The model was initially coded in acslXtreme v1.4 and was subsequently updated in acslX
2 v 3.0.2.1 (The AEGIS Technologies Group, Inc., Huntsville, AL). Most procedures used to
3 generate this report, except those for the optimization, may be run by executing the
4 corresponding .m files. The model code (acslX .csl file) and supporting .m files are available
5 electronically on the EPA HERO database ([U.S. EPA, 2012b](#)). A key identifying .m files
6 associated with figures and tables in this report is also provided in the supporting materials.

B.2.2. Model Parameters

7 Physiological parameters such as tissue volumes, blood flows, and ventilation rates were
8 obtained from the open literature (Table B-1). Parameters for blood flow, ventilation, and
9 metabolic capacity were scaled as $BW^{0.75}$, according to the methods of Ramsey and Andersen
10 ([1984](#)). Pulmonary air-flow (Q_P) was coded as the product of cardiac output (Q_C) and a
11 ventilation-perfusion ratio (VPR) in order to facilitate coding of changes in these quantities due
12 to exercise or respiratory depression in rats. In particular it was generally assumed that VPR
13 remained constant, so Q_P and Q_C varied in proportion to one another during such changes, unless
14 data specifically indicated otherwise.

15 As briefly described in the summary, when published partition coefficients (PCs) were
16 used for all body compartments for the rat; the predicted blood levels immediately following i.v.
17 doses were not well estimated. Since those blood levels only depend on the tissue partitioning
18 and the rest-of-body compartment is comprised of multiple tissues which have differing
19 partition coefficients, it was therefore decided to initially fit the body: blood PC to the i.v. data
20 and then to the total PK data set in global parameter estimation. This approach is validated by the
21 observation that the resulting fitted PC was in the range of those measured for other tissues and
22 the rat model was then consistent with 100% oral bioavailability. Rat PCs were taken as
23 measured for that species by Horton et al. ([1992](#)) for liver: blood and blood: air. The “slow-to-
24 blood” PC (1.1) in rats reported by Horton et al. ([1992](#)), is inconsistent with the value for
25 fat: blood (0.083) in mice from Ward et al. ([1997](#)), and that determined for rat fat: blood (0.11)
26 partitioning of ethanol by Pastino and Conolly ([2000](#)); these other results indicate much lower
27 partitioning of alcohols into fat. Therefore the Ward et al. ([1997](#)) PC for mouse fat: blood, was
28 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 B_{av} assumed = 1
k_{si} (hr ⁻¹)	3.1	3.17	Human: k_{as} , k_{si} , and k_{ai} are for ethanol [from (Sultatos et al., 2004)]; B_{av} fitted.
k_{ai} (hr ⁻¹)	0.38	3.28	
B_{av} (fraction)	1	0.79	

	S-D Rat	Human	Data Source
Partition coefficients			
Liver:Blood	1.6	0.583 ^c	Human: Fiserova-Bergerova & Diaz (1986) (human “body” assumed = muscle);
Fat:Blood	0.083	0.142	
Blood:Air	1350	1626	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)
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

^aThe midpoints of rat weights reported for each study was used and ranged from 0.22 to 0.33 kg

^bThe 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.

^cIn the model cardiac output (Q_C ; L/hr) was set as the primary constant, via the scaling constant $Q_C C$ ($Q_C/BW^{0.75}$), and pulmonary ventilation (Q_P) was defined as the product of Q_C and the ventilation-perfusion ratio, VPR. $Q_C C$ 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 = $V_{max} C$ ($mg/kg^{0.75} \cdot hr$) $\times BW^{0.75}$. $k_1 C$ is the first-order urinary elimination constant (from the blood compartment). k_1 used in the model = $k_1 C/BW^{0.25}$.

^eHuman 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.

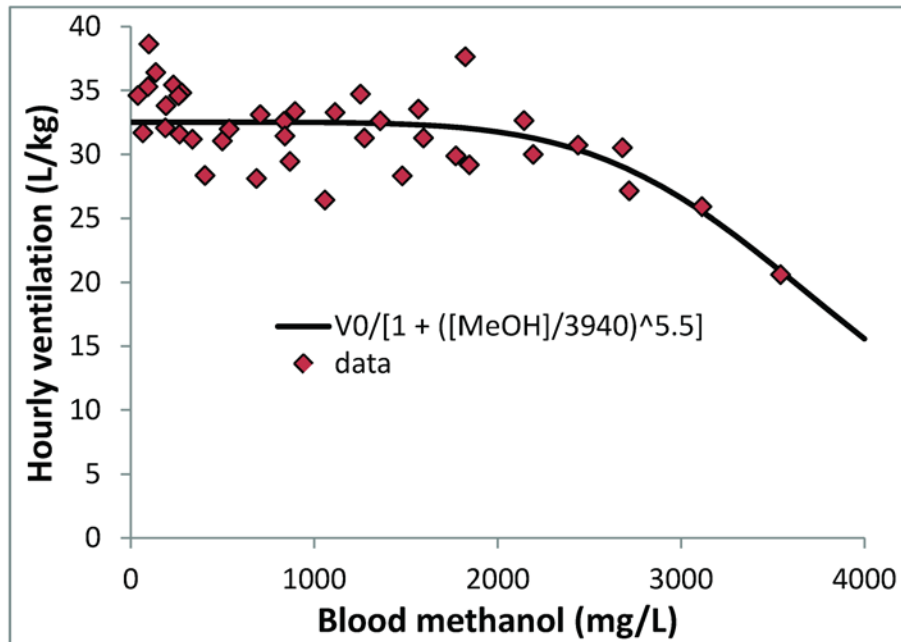
NA - Not applicable for that species.

B.2.3. Rat Model Calibration

1 The S-D rat model was calibrated to fit blood concentration data from intravenous,
2 inhalation, and oral exposures. However, the urinary clearance constant and the respiratory
3 depression function were specified outside of the PBPK model, using separate data. Pollack and
4 Brouwer (1996) used linear regression of urine excretion rates versus blood concentration in
5 non-pregnant rats to obtain a clearance constant, $k_1 = 0.00916$ L/hr/kg. This was converted to the
6 equivalent $k_1 C$ in the PBPK model by normalizing to the venous blood fraction of BW,
7 0.0443 L/kg, and multiplying by an average SD BW of 0.3 kg (raised to 0.25) to obtain the
8 allometric constant:

$$9 \quad k_1 C = (0.00916 \text{ L/hr/kg}) \times (0.3 \text{ kg})^{0.25} / (0.0443 \text{ L/kg}) = 0.153 \text{ kg}^{0.25}/\text{hr}.$$

1 As mentioned above, suppression of respiration rate in the rat at higher exposure levels
2 was reported by (Perkins et al., 1996a). An empirical function was therefore fit to the respiration
3 rate versus blood data from that source, shown in Figure B-2. It was assumed that cardiac output
4 decreased proportionately with ventilation, so the inhibition term $\{1 + ([\text{MeOH}]/3,940)^{5.5}\}^{-1}$, was
5 applied to the rat cardiac output with ventilation-perfusion-ratio fixed. However, when the
6 response was assumed to occur instantaneously due to changes in mixed venous blood
7 concentration (i.e., the mixed venous blood concentration was used for [MeOH]), the model
8 predicted an unreasonable level of suppression immediately after i.v. dosing because of the short-
9 term spike in blood levels predicted to occur. If instead the concentration in venous blood exiting
10 the “body” compartment was used for [MeOH], reasonable model simulations resulted. Since
11 some (short) time is likely needed for methanol to interact with the neurons involved in
12 respiratory and cardiac control, and for neural processing of the resulting signal to the heart and
13 lungs, the use of this body-tissue-blood concentration, for which the methanol concentration
14 changes are slightly delayed and “smoothed” relative to the mixed venous blood, seems a
15 reasonable option.



Source: Perkins et al. (1996b).

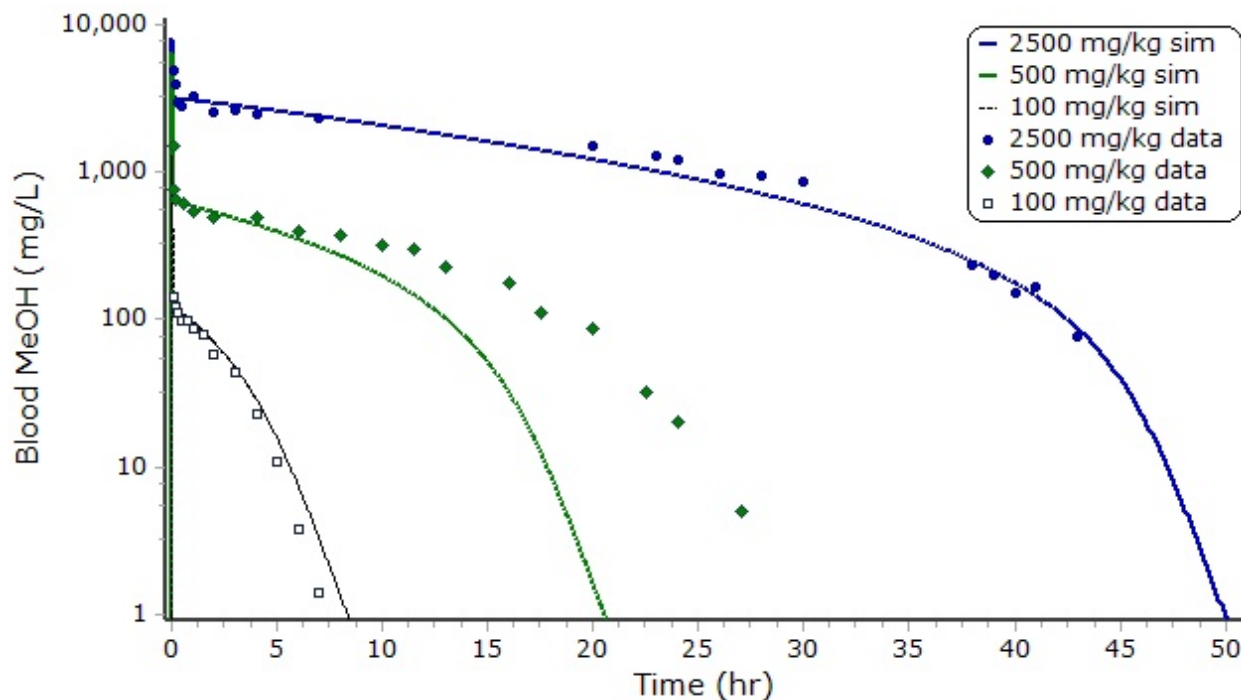
Figure B-2 Respiratory depression in Sprague-Dawley rats as a function of blood methanol concentration. The empirical curve fit (solid line) was selected to describe the data with a minimal number of parameters.

16 All of the data available for S-D rats are reported with background levels subtracted.
17 These data are from the laboratory of Gary M. Pollack (then at the University of North Carolina,

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

19 Initial values $V_{max}C$, K_m , and the body:blood partition coefficient (PR) were then
20 obtained by fitting the model to the 100 and 2,500 mg/kg i.v. data provided in the command file
21 of Ward et al. ([1997](#)) (holding other parameters constant). As mentioned previously, if PR was
22 not also adjusted, the predicted concentration immediately following the distribution phase,
23 which are only dependent on the partition coefficients, were discrepant from the data. Without
24 adjusting PR, this then created a bias in the metabolic parameters to correct for the error in the
25 distribution phase. Model predictions were also compared to 500 mg/kg i.v. data in the command
26 file of Ward et al. ([1997](#)), with additional early time-points reported by Pollack and Brouwer
27 ([1996](#)). With PR adjusted this way to fit the 100 and 2,500 mg/kg data, the model matched the
28 initial time points of the 500 mg/kg data quite well (see Figure B-3). However, the subsequent
29 clearance rate fit to these other two dose levels was inconsistent with the 500 mg/kg data. All
30 three data sets with globally-fit model parameters are shown in Figure B-3. If one compares the
31 clearance rate of the 500 mg/kg data at 20 hours and beyond, when the concentration range is the
32 same as the 100 mg/kg data, it is clear that the two data sets are discrepant. Thus no model with a
33 single set of parameters could simultaneously match both data sets. That the model does fit the
34 2,500 mg/kg data quite well indicates that the discrepancy is not due to a simple dose-
35 dependency. Since it is most important that the model describe the low-dose data well, in the
36 range of the point-of-departure for toxicity extrapolation, while capturing as much of the high-

- 1 dose dependency as possible, the 500 mg/kg i.v. data were not used in subsequent model
- 2 calibration.

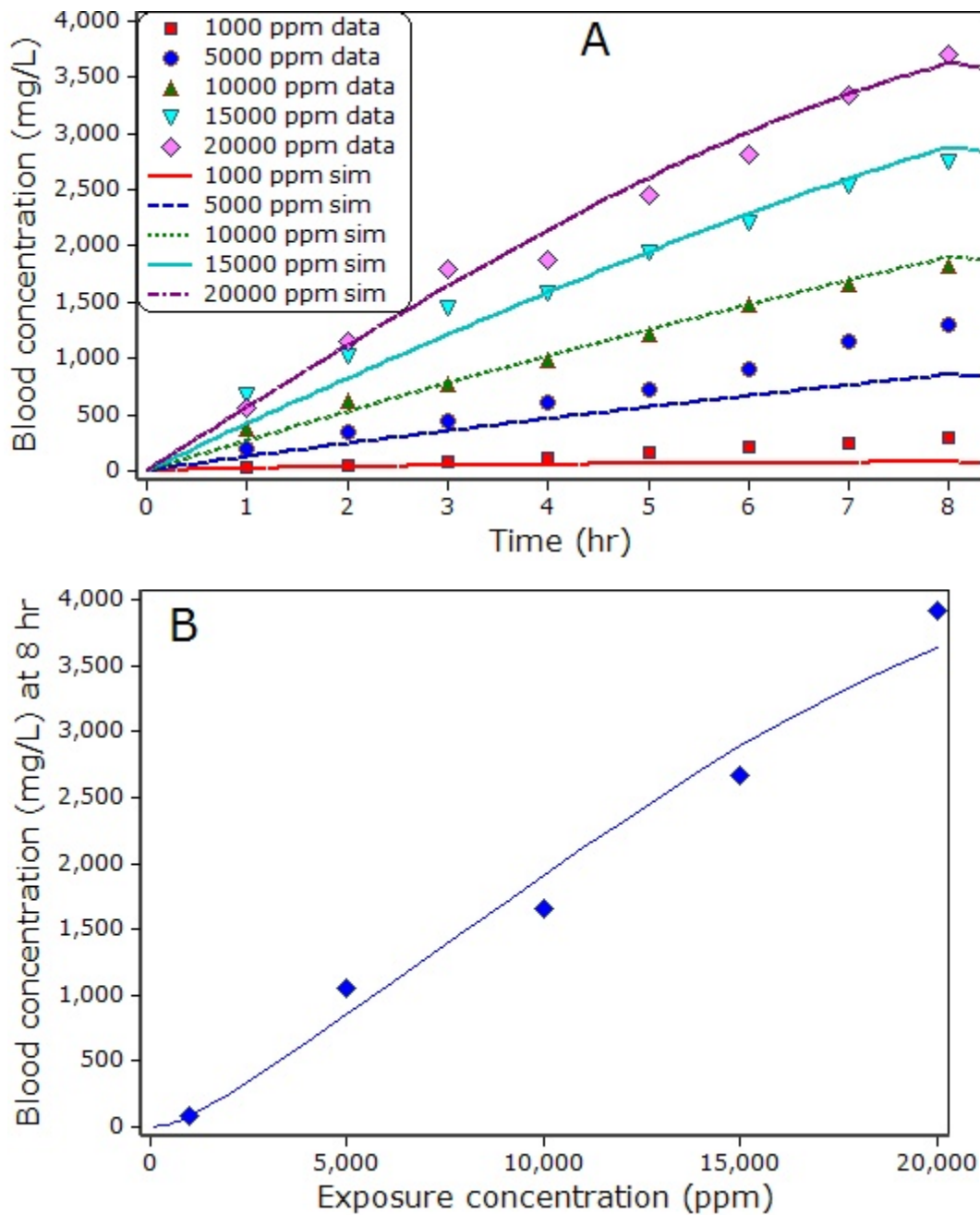


Source: Ward et al. (1997: squares); with additional 500 mg/kg points from Pollack and Brouwer (1996).

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.

- 3 The initial value for the inhalation fractional absorption constant, FRACIN, was then set
- 4 by fitting it to inhalation PK time-course data from Perkins et al. (1996a), holding other
- 5 parameters constant. Model fits to these data with the final set of parameters are shown in panel
- 6 A of Figure B-4. Model predictions are also shown versus end-of-exposure concentrations
- 7 reported by Pollack and Brouwer (1996) in panel B of Figure B-4. This second data set was not
- 8 used in model fitting. It is worth noting that while the model significantly under-predicts the
- 9 1,000 and 5,000 ppm data shown in panel A of Figure B-4, the model almost exactly matches the
- 10 end-of-exposure concentration at 1,000 ppm in panel B, and only slightly under-predicts then
- 11 5,000 ppm measurement from that data set. Also the downward curvature which is noticeable in
- 12 the 20,000 ppm simulation and to a lesser extent at 15,000 ppm (panel A), and above ~
- 13 12,000 ppm in panel B, is due to the respiratory depression term.



Source: (Panel A): Perkins et al. (1996a); (Panel B): 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.

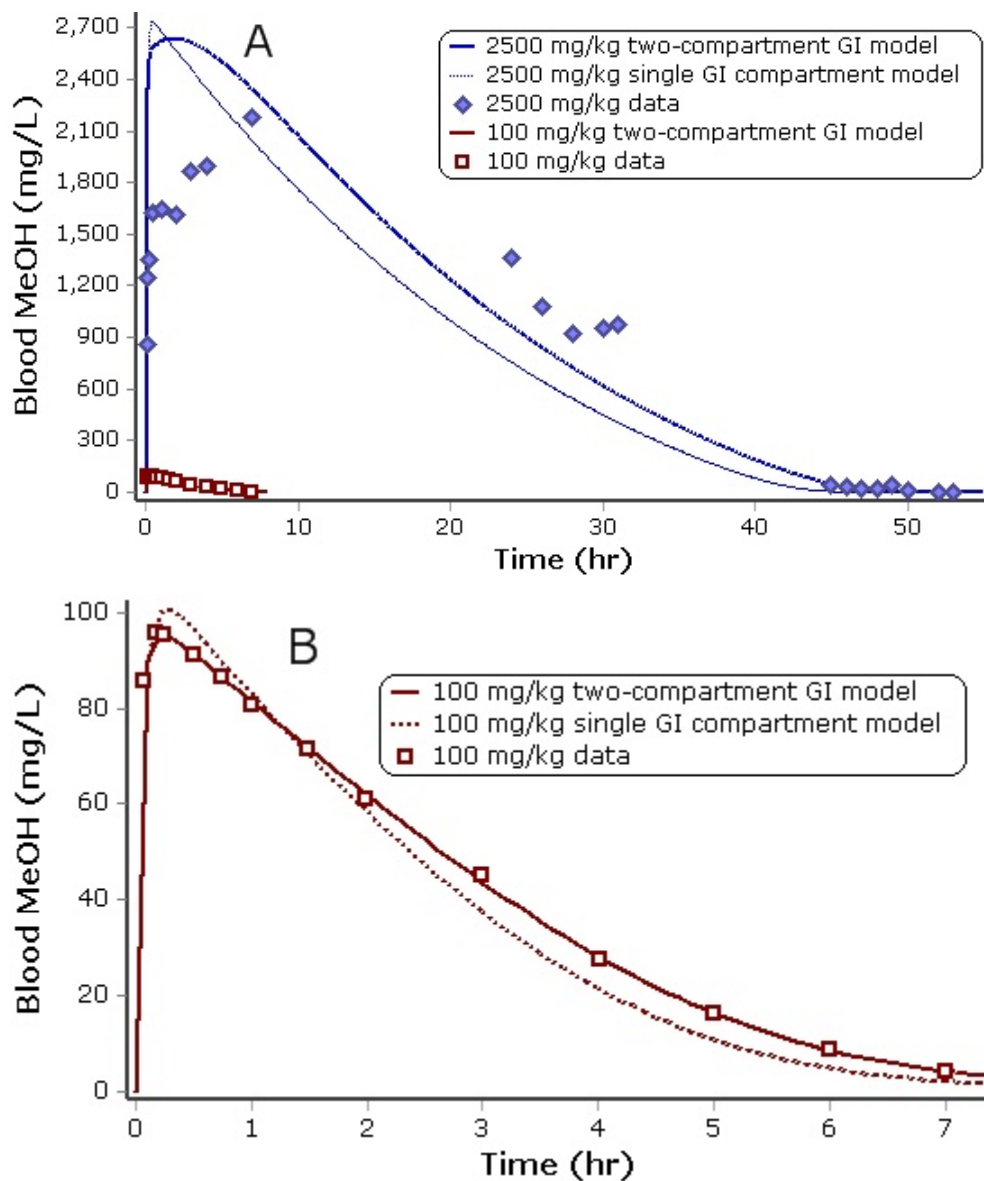
Figure B-4 Model fits to data sets from inhalation exposures in female Sprague-Dawley rats.

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

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

22 A final set of fitted model parameters for the rat was obtained by allowing all of the
23 adjusted parameters ($V_{\max}C$, K_m , PR, k_{as} , k_{si} , k_{ai} , and FRACIN) to the data sets as described
24 above: 100 and 2,500 mg/kg i.v. doses [Figure B-3; (Ward et al., 1997), squares]; 1,000 to
25 20,000 ppm inhalation time-course data [Figure B-4, panel A, (Perkins et al., 1996a)], and
26 100 mg/kg oral dose data [Figure B-5; (Ward et al., 1997)]. The resulting parameter values are
27 listed in Table B-1 and the simulations with solid lines in Figures B-3 to B-5 all use this global
28 set. Although the model does not fit all of the data as well as one might like, particularly the
29 1,000 and 5,000 ppm data in Figure B-4, panel A, the overall quality of the fits is considered
30 good. The number of parameters adjusted is considered modest, since could reduce the number
31 of parameters by keeping PR at a value measured for muscle or using a one-compartment GI
32 model. But either of these choices significantly degrades the model fits (shown for GI model),
33 which indicates that the number and variety of data available are sufficient to inform these seven
34 fitted parameters. One can consider the urinary excretion constant, k_1C , and the two parameters
35 used to define the level of respiratory depression, as additional adjusted parameters. These two
36 parameters were fit using additional data on urinary excretion and respiration rate, respectively.
37 So the total number of fitted parameters is considered to be well supported by the corresponding

1 data used to determine their values, and hence a fairly good level of confidence should be held
2 for model predictions of bioassay dosimetry. To further elucidate the level of confidence one can
3 place in model predictions, evaluation of model sensitivity to these parameters was conducted as
4 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

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

$$13 \quad SC = (\Delta \text{metric}/\text{metric}_0) / (\Delta p/p_0),$$

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

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

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

Parameter ^a	Exposure level, metric			
	200 ppm		5,000 ppm	
	C _{max}	AUC	C _{max}	AUC
V _{max} C	-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 ₁ C	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

^aValues 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.

1 The sensitivity analysis results are mostly not surprising. At the lower concentration of
2 200 ppm, metabolic elimination has a significant influence, with both V_{max}C and K_m having high
3 SCs. The SC for V_{max}C is negative since an increase in its value decrease blood concentration,
4 while K_m is positive for the opposite reason. At 5,000 ppm V_{max}C is only marginally significant
5 and K_m not at all, but urinary elimination (k₁C) becomes significant, though only slightly. The
6 one somewhat surprising result is that the body:blood partition coefficient, PR, has very little
7 influence on the inhalation dose predictions. However, the analysis was conducted on conditions
8 near steady-state with 22 hr/day exposure. As shown by Chiu and White (2006), the steady-state
9 level predicted in blood by a PBPK model depends on only a small number of parameters: those
10 affecting absorption, elimination (metabolic), and the blood:air partition coefficient (PB). For
11 this model, at 200 ppm the rate of absorption by inhalation is likely limited by respiration rate,
12 hence PB has little influence at that concentration, but it does significantly impact uptake at
13 5,000 ppm. More importantly, since PR has so little effect on these predictions means that any
14 uncertainty in its value is inconsequential to the outcome of this assessment. (PR is expected to
15 more strongly influence non-steady-state conditions, such as when oral ingestion occurs in
16 boluses.)

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

1 Thus, all of the adjusted parameters except PR have a significant influence on model
2 predictions over part of the relevant range of concentrations. Of these fitted parameters, k_1C and
3 k_{iv} were fit to independent data sets, not used to fit any other parameters. Hence a good degree of
4 confidence can be given to their values. Because of the wide range of doses, particularly by the
5 i.v. route, used for the PK data, $V_{max}C$ and K_m can also be considered fairly well identified.
6 However the model's inability to fit the 500 mg/kg i.v. data (Figure B-3) and 1,000 and
7 5,000 ppm inhalation data (Figure B-4, panel A) create some level of uncertainty in their values
8 and that of FRACIN. That the model fits rather well both the 100 and 2,500 mg/kg i.v. data,
9 makes it difficult to come up with a simple explanation for the lack-of-fit to the intermediate
10 dose. Since the clearance observations at 500 ppm go beyond 24 hr, it is possible that there is a
11 time-dependent process that reduces clearance in that time range. The 2,500 mg/kg i.v. dose
12 clearance was only measured to 43 hours, when it had just dropped to ~ 100 mg/L, so one cannot
13 say if the clearance from then on would have been more like the 100 mg/kg data or the
14 500 mg/kg data.

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

25 This consideration of possible model errors and potential future improvements (with
26 necessary data) should be balanced against the observation that the model-predicted blood level
27 at 8 hours from a 1,000 ppm exposure, 81 mg/L, almost exactly matches the measured
28 concentration reported in Pollack and Brouwer's (1996) (Table 16): 83 ± 15 mg/L. The
29 discrepancy between that result and the value obtained from a plot (digitized) in the same report
30 which also appears in Perkins et al. (1996a), ~ 290 mg/L, can only be attributed to experimental
31 variability, which no model can fully describe. Since the model does fit the lower-concentration
32 8-hr data (Figure B-4, panel B) fairly well, it is considered adequate for use in the assessment as
33 is, without further complication and additional parameters, and FRACIN is assumed to provide a
34 reasonable adjustment to the internal doses with the value obtained here.

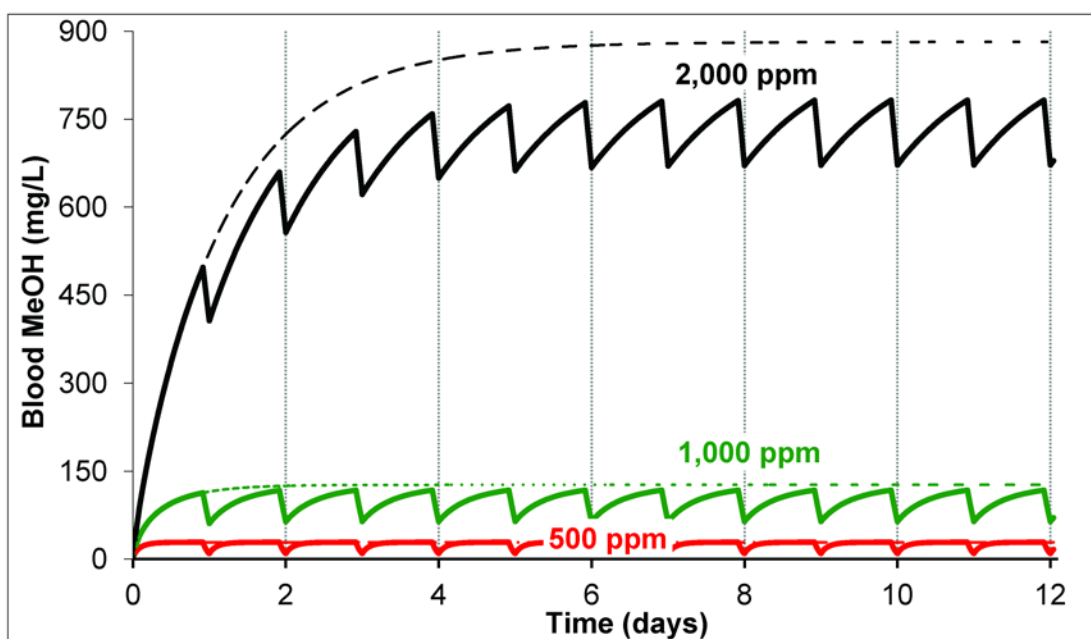
B.2.5. Rat Model Simulations

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

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

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

26 where the integration is over 24 hours, C is the instantaneous blood concentration, and C_{bg} is the
27 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

1 The rat model was scaled to human body weight (70 kg or study-specific average), using
 2 human tissue compartment volumes and blood flows, and calibrated to fit the human inhalation-
 3 exposure data available from the open literature, which comprised data from four publications
 4 ([Ernstgård et al., 2005](#); [Batterman et al., 1998](#); [Osterloh et al., 1996](#); [Sedivec et al., 1981](#)), and a
 5 single data set for oral exposure ([Schmutte et al., 1988](#)). Model predictions are also compared to
 6 an i.v. data set that was not used for parameter estimation ([Haffner et al., 1992](#)). Since the bulk of
 7 the human data were from inhalation exposures, the approach to identifying parameters was to

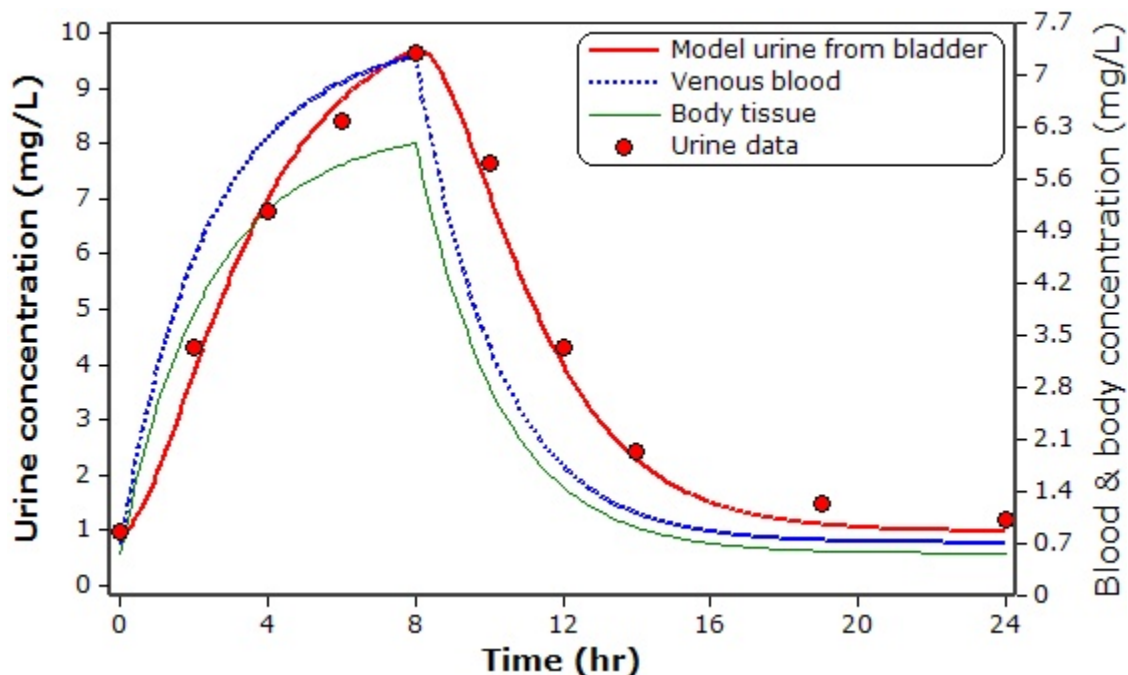
1 first fit the metabolic (and endogenous level) parameters to those data sets. Initial estimates for
2 the oral uptake parameters were then obtained by fitting the oral PK data with other parameters
3 held constant. Lastly, a global fit over the inhalation and oral data sets combined, with all fitted
4 parameters varied simultaneously, was performed to obtain final parameter values. The two key
5 differences in model structure and parameters adjusted are discussed below, followed by a more
6 detailed description of the calibration against specific data.

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

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

31 In summary, the set of key parameters fit for the human model were the metabolic ($V_{max}C$
32 and K_m) and urinary elimination (k_1C and k_{bl}) constants, the inhalation fraction (FRACIN), and
33 the oral bioavailability (B_{av}). In addition, the endogenous background concentration and an
34 increment in background over time were fit to control data from Osterloh et al. ([1996](#)). A detailed
35 description of each data set and the parameter(s) that it primarily informs follow. However, as
36 with the rat, the final set of parameters was obtained by global optimization: varying all

1 parameters while fitting all of data sets simultaneously. Other human parameters were set as
2 reported in Table B-1.



Source: Sedivec et al. (1981).

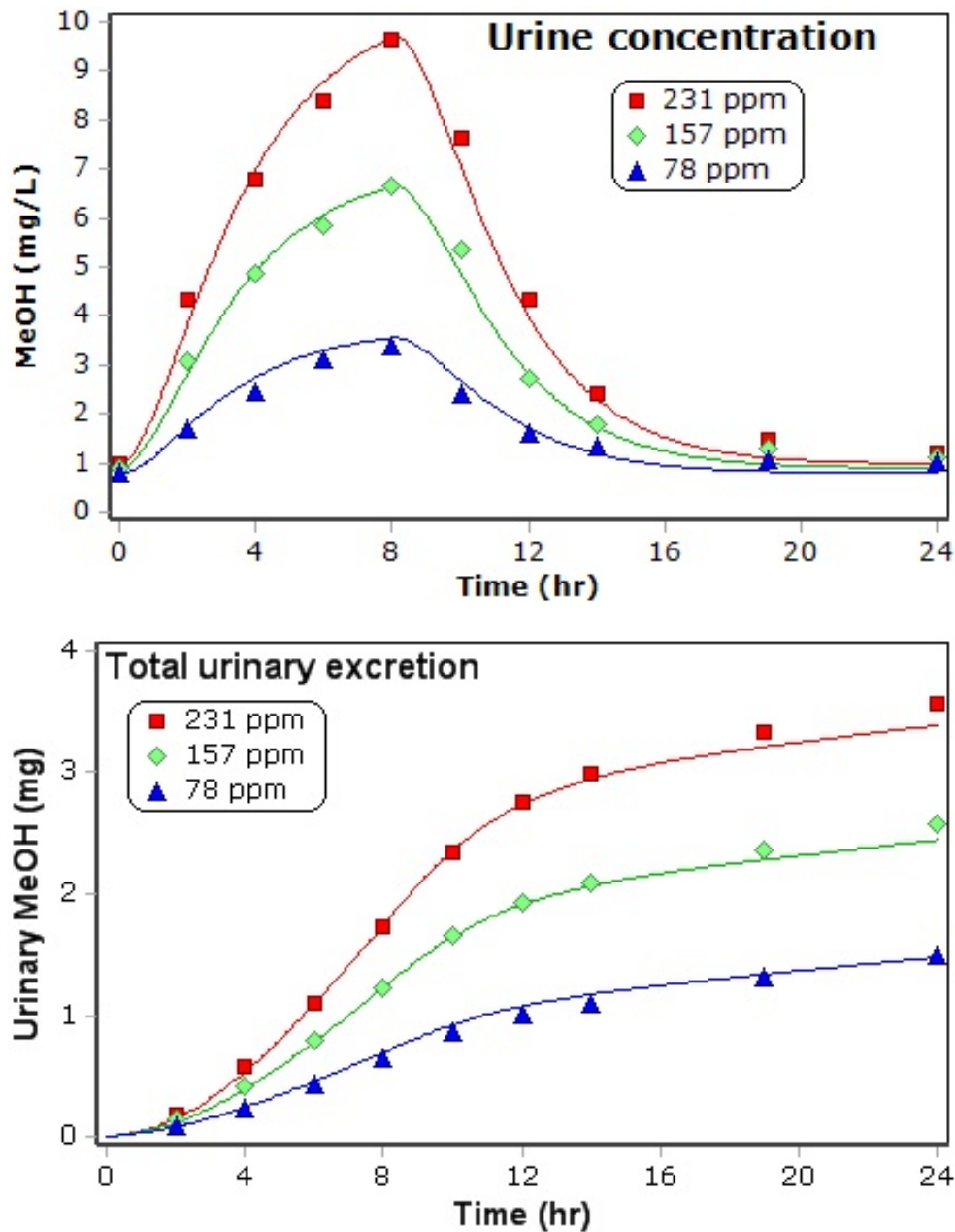
Figure B-7 Comparison of model predictions of urine concentration (from bladder compartment), venous blood, body tissue, and urine concentration data for a 231 ppm, 8-hour exposure. Right axis provides scale for venous blood and body tissue results.

3 The first-order rate of clearance of methanol from the blood to urine, k_1C , and first-order
4 bladder compartment time constant, k_{bl} , were used to describe urinary methanol elimination. (See
5 Section B.2.1 on the reasoning for treating urinary elimination as occurring from the blood
6 compartment versus a kidney tissue compartment.) The inhalation-route urinary methanol kinetic
7 data described by Sedivec et al. (1981) (Figure B-8) were used to inform these parameters. The
8 urine methanol concentration data reported by the authors were converted to amount in urine by
9 assuming 0.5 mL/hr/kg total urinary output (Horton et al., 1992). Since the resulting values of
10 k_1C and k_{bl} (Table B-1) are only calibrated using a small data set, they should be considered an
11 estimate. Urine is a minor route of methanol clearance in humans, with little impact on total
12 blood methanol concentration, but changes in urine levels are expected to closely reflect
13 corresponding changes in blood levels, hence the slight nonlinearity in the urine data also inform
14 the apparent metabolic saturation constant, K_m . The potential for this information is lost,

1 however, if the kinetics of urinary elimination are not well matched; i.e., if the bladder
2 compartment is not used.

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

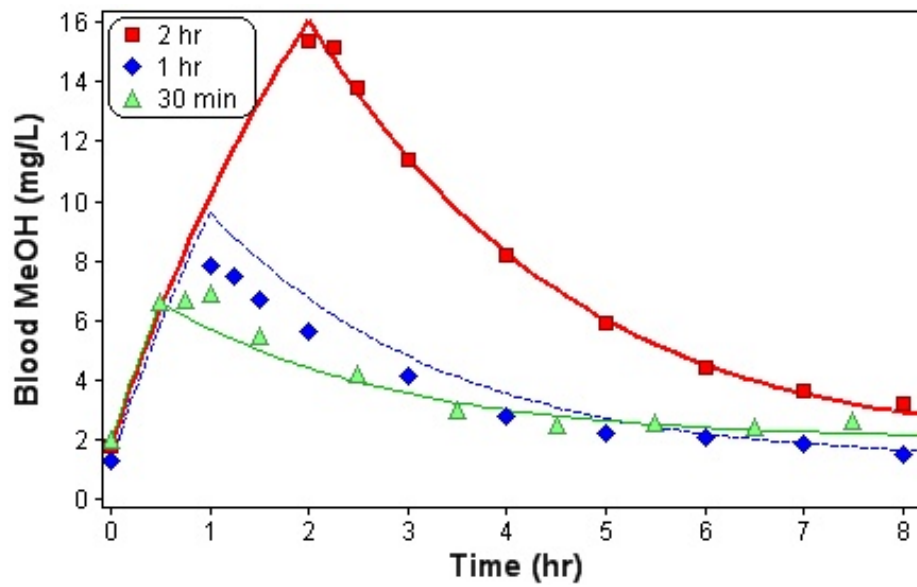
- 6 ■ The initial urine concentrations from Sedivec et al. ([1981](#)) (reported at time = 0; see
7 Figure B-8) were assumed to represent endogenous background levels, and therefore were
8 used to set a (constant) endogenous level for each exposure level to match that urinary level
9 (i.e., the endogenous blood level that must exist to match the observed urine concentration,
10 given the urinary clearance constant, k_1C). The endogenous blood concentrations so
11 estimated were 0.6-0.74 mg/L.
- 12 ■ Batterman et al. ([1998](#)) subtracted background levels before reporting their results, but also
13 included the exposure-specific background (pre-exposure) concentrations in a separate table.
14 Therefore those background levels were added back to the reported exposure-group values
15 and treated as actual blood concentrations. Results of model fits to the Batterman et al.
16 ([1998](#)) data are shown in Figure B-9.
- 17 ■ Osterloh et al. ([1996](#)) measured and reported (plotted) blood methanol in nonexposed
18 controls (data shown in Figure B-10). The data for Osterloh et al. ([1996](#)) clearly show a time-
19 dependent trend which is close to linear. Therefore, the endogenous methanol production rate
20 was assumed to increase at a constant rate over time when simulating the Osterloh et al.
21 ([1996](#)) data (both controls and methanol-exposed), with the rate of increase fit to the control
22 data set. The results shown in Figure B-10 (solid lines) include this increase. For comparison,
23 the thin dashed line shows results for the 200 ppm exposure if the endogenous production is
24 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.

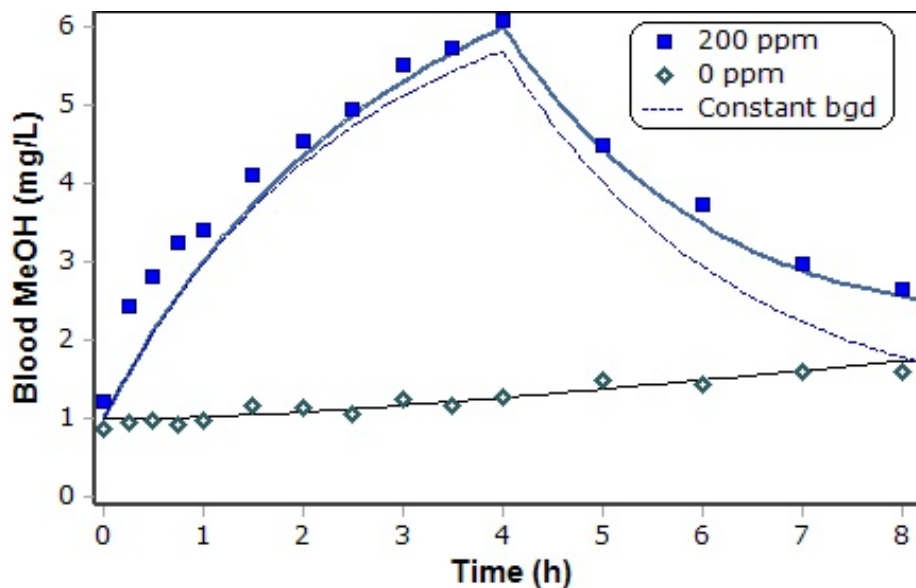
Figure B-8 Urinary methanol elimination concentration (upper panel) and cumulative amount (lower panel), following inhalation exposures to methanol in human volunteers.



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.

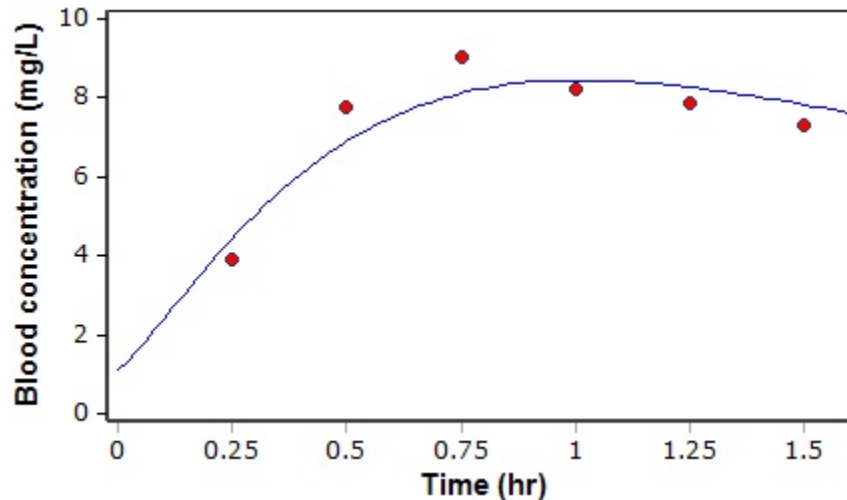


Source: Osterloh et al. (1996).

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.

1 Oral PK data from Schmutte et al. (1988) from a 10 mg/kg dose was used to set an oral
 2 bioavailability for humans and to test the assumption that human oral absorption of methanol
 3 could otherwise be described using the simple two-compartment GI model of Sultatos et al.
 4 (2004), with parameters fit by Sultatos et al. to ethanol PK data. Sultatos et al. (2004) included a
 5 rate of metabolism for ethanol in the stomach, which would reduce the systemic bioavailability
 6 of that compound from 100%. Lacking the data to fit a specific rate constant for methanol
 7 metabolism in the GI, the simulated dose was simply reduced using a bioavailability constant
 8 (B_{av}), but the mechanism for less than 100% availability could also be metabolism in the GI.
 9 A value of $B_{av} = 0.79$ was obtained and the simulation curve matches the data of Schmutte et al.
 10 (1988) fairly well (Figure B-11). The initial condition was set to the reported pre-exposure
 11 background by Schmutte et al. (1988) (1.1 mg/L). The model reproduces the data well,
 12 considering that only one parameter is adjusted for the oral dose route. Data were only collected
 13 for 1.5 hours: a longer sampling time would have provided a better evaluation of the model's
 14 ability to predict longer-term kinetics from oral exposures.

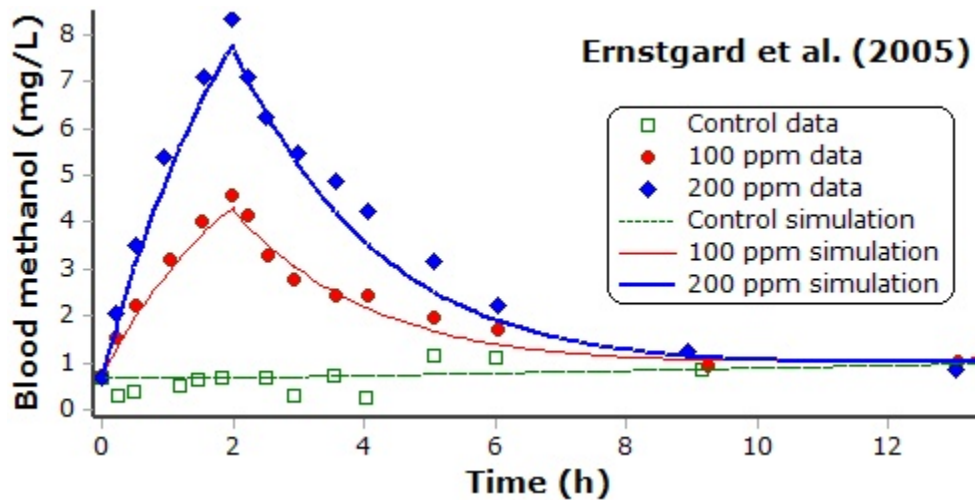


Source: Schmutte et al. (1988)

Note: The endogenous background was set to match the reported pre-exposure blood concentration of 1.1 mg/L and the bioavailability (B_{av}) was calibrated to fit the data ($B_{av} = 0.79$). 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).

1 The data from Ernstgård et al. (2005) was used to assess the use of the model parameters
2 with a data set collected under conditions of light work. Historical measures of VPR (2.023) and
3 Q_{cC} (26 L/hr/kg^{0.75}) for individuals exposed under conditions of 50 W of work from that
4 laboratory (Ernstgård, 2005; Corley et al., 1994; Johanson et al., 1986) were used for the 2-hour
5 exposure period (Figure B-12). Also, a linear rate of increase in the endogenous production rate
6 was fit to the control data set, as this set showed an increasing trend over time, like Osterloh
7 et al. (1996), and the initial background level was set to match the observed value at time = 0 for
8 each data set. Otherwise, there were no changes in the model parameters (no fitting to these
9 data). The results are remarkably good, given the lack of parameter adjustment to data collected
10 in a different laboratory, using different human subjects than those to which the model was
11 calibrated.



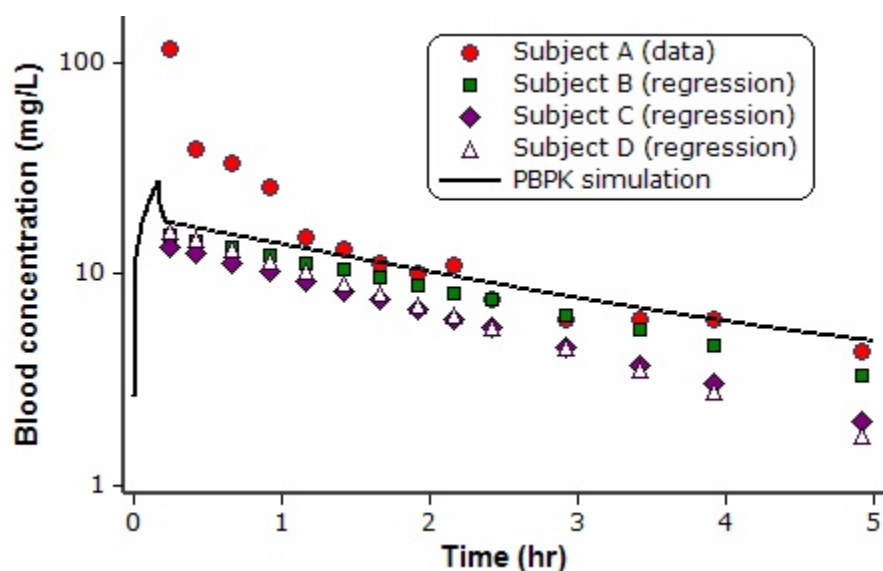
Source: Ernstgård et al. (2005)

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 $26 \text{ L/hr/kg}^{0.75}$ were used to match the subjects' light exercise, after which Q_{cC} is reduced to $15 \text{ L/hr/kg}^{0.75}$ and VPR to 1.0 (Corley et al., 1994; Johanson et al., 1986).

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

1 A final set of data used for model validation is provided by Haffner et al. (1992) who
 2 observed blood kinetics in 4 volunteers after i.v. injections of 10 mg/kg methanol in a 10-minute
 3 infusion. Model simulations based on this dosing regimen, with an assumed average BW of 70
 4 kg, are shown in Figure B-13 versus reported data for Subject A and simulated data using
 5 reported regression results for Subject B-D. Haffner et al. (1992) only showed data for the first
 6 subject, but gave exponential regression equations that they fit to the data for the other subjects.
 7 The “regression” results in Figure B-13 are calculated from regression equations provided in the
 8 paper for each subject, at the same time points as Subject A data. The model simulation during
 9 the first hour of exposure poorly matches the data, with the maximum blood level predicted to
 10 only be 27.6 mg/L versus 116 mg/L observed. After 1 hour the simulation matches the data for
 11 Subject A well, but over-predicts the regression curves for the other subjects. It is possible that
 12 the perfusion-limited PBPK model over-predicts the rate of distribution from the blood to
 13 various body tissues and hence under-predicts blood concentrations in this time period; i.e., that
 14 distribution to body tissues is diffusion-limited, with the effect being significant at shorter times.
 15 The slope of the simulation line closely matches that of Subject B, indicating similar clearance
 16 kinetics. Subjects C and D exhibited faster elimination kinetics than predicted by the model. The
 17 authors report elimination rate constants of 0.259, 0.325, 0.406, and 0.475 hr^{-1} for Subjects A-D,

1 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.

2 Horton et al. (1992) employed two sets of metabolic rate constants to describe human
3 methanol disposition, but in vitro studies using monkey tissues with non-methanol substrates
4 were used as justification for this approach. Although Bouchard et al. (2001) described their
5 metabolism using Michaelis-Menten metabolism, Starr and Festa (2003) reduced that to an
6 effective first-order equation and showed adequate fits. Perkins et al. (1995) estimated a K_m of
7 $320 \pm 1,273$ mg/L (mean \pm S.E.) by fitting a one-compartment model to data from a single oral
8 poisoning to an estimated dose. In addition to the extremely high standard error, the large
9 standard error for the associated V_{max} (93 ± 87 mg/kg/hr) indicates that the set of Michaelis-
10 Menten constants was not uniquely identifiable using this data. Other Michaelis-Menten
11 constants that have been used to describe methanol metabolism in various models for primates
12 are given in Table B-3. Because the K_m calculated by Perkins et al. (1995) from the high-dose
13 oral exposure is 320 mg/L, while the highest observed concentration in the data sets considered
14 here is 14 mg/L (Batterman et al., 1998), forcing the model to use this higher K_m would simply

1 result in fits that are effectively indistinguishable from the linear model. The value obtained in
 2 this analysis, 36 mg/L, allows the model to describe the slight nonlinearity that exists in the data.
 3 For example, the peak urine concentration observed by Sedivec et al. (1981) after the 231 ppm
 4 exposure was increased 8.66 mg/L above the time zero value, while that observed after the
 5 78 ppm exposure was 2.63 mg/L above the time zero value, so a 3-fold increase in exposure lead
 6 to a 3.3-fold increase (8.66/2.63) increase in the urinary excretion above background. It is
 7 possible that a much higher K_m pathway is also operant in humans, but is only significant at
 8 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.

^aThis K_m was optimized while also varying V_{max} , k_1C , k_{bl} , B_{av} , 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.

9 Sedivec et al. (1981) estimated a fractional uptake of 57.7%, based on total amount
 10 inhaled. Since the PBPK model uses alveolar rather than total ventilation and this is typically
 11 assumed to be 2/3 of total ventilation, one might correct this value by dividing by 2/3 to obtain a
 12 value for FRACIN of 0.8655. Ernstgård et al. (2005) also estimated a fractional uptake, 51% at
 13 100 ppm and 49.3% at 200 ppm under light exercise. It is reasonable to expect uptake efficiency
 14 to decrease with more rapid breathing due to exercise, since an inhaled volume element of air
 15 spends less time in the respiratory tract, allowing less time for uptake, as respiration increases.
 16 Also, while Ernstgård et al. (2005) based their calculation on estimated pulmonary ventilation,
 17 they used the difference between inhaled air concentration and exhaled air concentration.
 18 Exhaled air will be a mixture of air that was taken into the pulmonary airways and air that only
 19 entered the conducting airways. Very little methanol would be absorbed from the later air and
 20 hence the mixed exhaled concentration will be higher than that which exits the pulmonary region
 21 and the resulting calculation will then under-estimate the fraction of methanol absorbed from
 22 pulmonary air. Thus the “fraction inhaled” estimated from a given data set will depend on which
 23 flow rates and concentrations are being used in the calculation, or to which it might be applied;

1 i.e., the value depends on the model “context” in which it is used. Therefore, EPA decided to fit
2 FRACIN with the other parameters estimated in the context of the PBPK model used here, as
3 was done for the rat, and obtained a value of 0.75. This indicates that the concentration entering
4 the pulmonary space is reduced by 25% due to deposition in the conducting airways (with that
5 material assumed to desorb on exhalation), and is not the fraction removed in the pulmonary
6 space. At 200 ppm, for example, the model predicts that 99.9976% of the methanol entering the
7 pulmonary region is absorbed. The value is slightly less than estimated for the rat (rat FRACIN =
8 81%) which seems reasonable since the larger human airways would reduce uptake efficiency
9 somewhat. Assuming that 2/3 of inhaled air goes to the pulmonary region, the total rate of
10 inhalation would be $1.5 \cdot Q_p \cdot \text{CONC}$ (rate of inhalation through nose and mouth at air
11 concentration CONC), and the amount removed in the pulmonary region roughly
12 $0.75 \cdot Q_p \cdot \text{CONC}$ (using FRACIN = 0.75), so the fraction of each breath absorbed is predicted by
13 the model to be:

14
$$(0.75 \cdot Q_p \cdot \text{CONC}) / (1.5 \cdot Q_p \cdot \text{CONC}) = 50\%,$$

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

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

1 A sensitivity analysis for human model predictions to the primary fitted parameters was
 2 conducted for continuous inhalation exposures, and results are shown in Table B-4. Normalized
 3 sensitivity coefficients are calculated using the method described for the rat (see B.2.4). To
 4 bracket the range of likely concern for human exposures, inhalation sensitivities were evaluated
 5 at 10 and 200 ppm concentration. The bladder time constant, k_{bl} , was not included in the analysis
 6 since it has no influence on blood concentrations. The resulting coefficients (Table B-4) are not
 7 surprising. $V_{max}C$ and K_m both strongly influence model predictions. At these exposure levels the
 8 urinary pathway (k_1C) has little effect on blood level. There is essentially a 1:1 correspondence
 9 with FRACIN, which follows from the fact that close to 100% of what enters the gas-exchange
 10 compartment is absorbed. That all of the sensitivities are slightly higher at 200 ppm than at
 11 10 ppm is due to the slight metabolic saturation.

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

Parameter	Exposure level ^a	
	10 ppm	200 ppm
$V_{max}C$	-0.81	-0.93
K_m	0.74	0.75
k_1C	-0.0024	-0.0029
FRACIN	1.00	1.11

^aNormalized sensitivity coefficients for steady-state blood levels (increase above background) at the indicated concentrations.

12 For oral exposures ingestion is assumed to occur in a series of six boluses over the course
 13 of the day, with the fraction of the total daily dose and respective times ingested being: 25% at 7
 14 a.m., 10% at 10 a.m., 25% at 12 p.m., 10% at 3p.m., 25% at 6 p.m., and 5% at 9 p.m. The pattern
 15 is meant to be representative of human ingestion patterns, recognizing that this will vary among
 16 the population. The impact of changing the pattern on estimated AUC values is fairly small,
 17 since the total ingestion remains the same. However the pattern will clearly influence the peak
 18 concentration, since an assumption of ingestion in a single bolus would lead to the highest
 19 predicted daily peak, while assumption of continuous ingestion would lead to the minimal peak
 20 possible, for a given total daily exposure. With the pattern used here, the blood concentration
 21 profile predicted at 10 mg/kg-day is shown in Figure B-14 (time is in hours from first bolus). In
 22 particular, the model predicts a following peak to occur ~ 45-55 minutes after each bolus
 23 (depending on dose size), with the overall daily peak occurring just before 1 p.m. (~ 6 and 30 hr
 24 time-points in Figure B-14). While the boluses assumed to be ingested at 7 a.m. and 12 p.m. are
 25 both 25%, because methanol is predicted to accumulate somewhat over the morning, the later
 26 bolus leads to a peak that is roughly 30% higher than the first of the day. At this exposure level
 27 there is a very small residual blood level at 24 hr, about 1% of the mid-day peak. At higher

1 exposure levels more significant day-to-day accumulation would be predicted until a state of
2 “periodicity” is reached, when the day-to-day pattern no longer changes. For example, at
3 200 mg/kg-day the blood level just prior to the next day’s ingestion is predicted to approach 3%
4 of the daily peak (~ 80 mg/L). At 500 mg/kg-day, the model predicts that it will take about 2
5 weeks to reach periodicity, where the peak during the first day is ~ 350 mg/L, but this increases
6 to 740 mg/L after two weeks, and the end-of-day minimum is 460 mg/L.

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

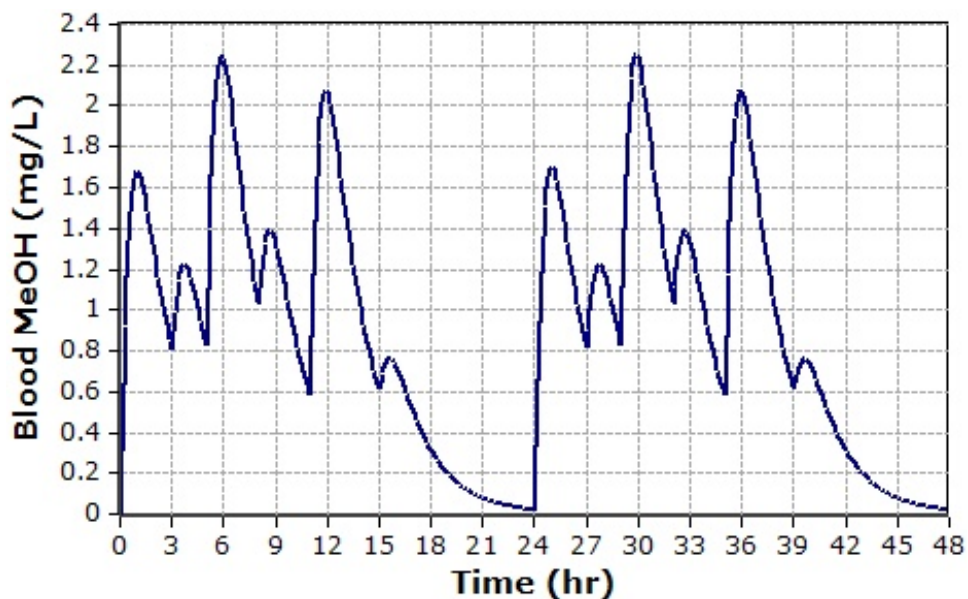


Figure B-14 Predicted human blood concentrations (increase above background) from total daily exposures to 10 mg/kg-day methanol, consumed in a series of 6 boluses. Time is from the first bolus of the day. See text for further details.

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

Parameter	Exposure level, metric ^a			
	0.2 mg/kg-day		10 mg/kg-day	
	C _{max}	AUC	C _{max}	AUC
V _{max} C	-0.67	-0.98	-0.68	-1.01
K _m	0.62	0.91	0.59	0.89
k ₁ C	-0.0014	-0.0024	-0.0015	-0.0025
Bav	1.00	1.00	1.03	1.04

^aNormalized 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.

1 Considering the multiple data sets used for human model calibration and validation, there
2 is fairly high confidence in the fitted metabolic/clearance parameters, V_{max}C, K_m, and k₁C.
3 Since the pharmacokinetics are mostly linear in the range of interest, it is really V_{max}C/K_m that
4 is the critical determinant of predicted internal doses, but as that is equally true of the model fits
5 to the data, this does not decrease confidence in model predictions. Where more uncertainty and
6 concern exists is with the oral bioavailability, since it is only estimated from a small data set
7 [4 individuals; ([Schmutte et al., 1988](#))], with measurements only extending to 90 minutes after
8 ingestion. However, bioavailability can be no more than 100%, a 25% increase over the fitted
9 value (79%). Hence any under-prediction of human dosimetry after oral exposure should be no
10 greater than that factor, well within the general variability and uncertainty expected for human
11 dosimetry (for which the UF_H of 10 is used).

B.2.8. Inhalation Route HECs and Oral Route HEDs

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

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

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

Table B-6PBPK 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

^aValues 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.

$$HEC(ppm) = 0.554 \times C_{ss} + \frac{1734 \times C_{ss}}{45.73 + C_{ss}} \quad \text{Equation 1}$$

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

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

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

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

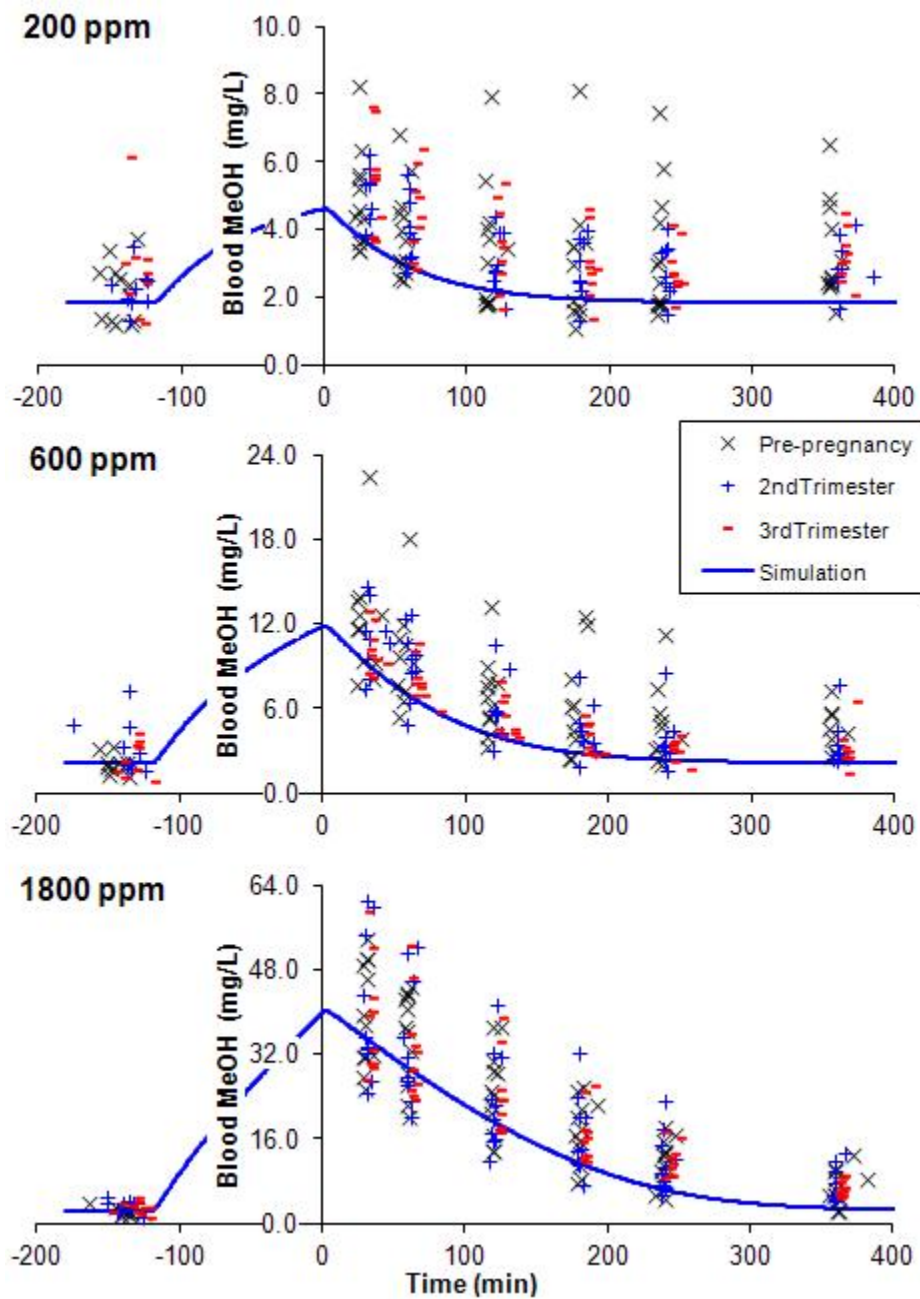
6 While the preceding equations approximate the PBPK model fairly well, an exact

1 solution is preferred if the full PBPK model can be run. For example, for $C_{\max} = 20$ mg/L (above
2 background), Equation (1) estimates HEC = 538.6 ppm, but running the PBPK model at this
3 exposure level predicts a (peak) blood level of 20.2 mg/L. An exact HEC (to 4 significant
4 figures) is 535.6 ppm. Two .m file scripts were created as part of the acslX PBPK model
5 workspace for methanol, which calculate HEC and HED values through a simple search
6 algorithm ([U.S. EPA, 2012b](#)). These were used to generate all of the HEC and HED values
7 reported in Section 5 of this assessment.

B.3. Monkey PK Data and Analysis

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

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



Source: 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.

B.3.1. PK Model Analysis for Monkeys

1 To analyze and integrate the PK data of Burbacher et al. (1999a), the one-compartment
2 model used by Burbacher et al. (1999b) and Burbacher et al. (1999a) was extended by the
3 addition of a chamber compartment to capture the kinetics of concentration change in the
4 exposure chamber, as shown in Figure B-16. The data in Figure B-16 [digitized from Figure 5 of
5 Burbacher et al. (1999a)] show an exponential rise to and fall from the approximate target
6 concentration during the exposure period. The use of a single-compartment model for the
7 chamber allows this dynamic exposure period to be captured, so that the full concentration-time
8 course is used in simulating the monkey internal concentration rather than an approximate step
9 function (i.e., rather than assuming an instantaneous rise and fall). The pair of equations
10 representing the time-course in the chamber and monkey are as follows (bolded parameters are
11 fit to data):

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

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

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

15 **d:** delta, change

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

17 t : time (hour)

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

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

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

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

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

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

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

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

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

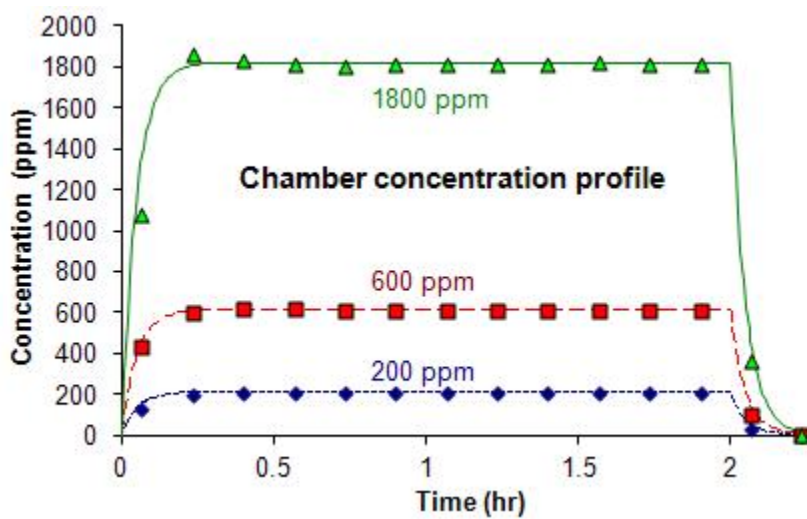
32 BW : monkey body weight (kg); for NP monkeys set to group average values in data of Burbacher et al. (1999a) and
33 Burbacher et al. (1999b)

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

36 F : fractional absorption of inhaled methanol; set to 0.5 (50%), 2/3 the value fitted for humans using the human

37 PBPK model (see Appendix B, Section B.2); for the monkey F and V_{mk} cannot be uniquely identified, given

1 the model structure; since the monkey model uses total ventilation (defined by R_c) as the driver, while the
2 human model uses alveolar ventilation which is assumed to be 2/3 of total ventilation, F was set to 2/3 the
3 human value to obtain a realistic estimate of V_{mk}
4 C_{net} : net blood concentration, equal to sum of the inhalation-induced concentration (C_{mk}) and the background blood
5 level (C_{bg}) (mg/L)
6 C_{bg} : background (endogenous) methanol concentration, set to the pre-exposure group-specific mean from the data of
7 Burbacher et al. (1999a) and Burbacher et al. (1999b)



Source: 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.

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

16 Model simulation results are the lines shown in Figures B-15 and B-16. The model
17 provides a good fit to the monkey blood and chamber air concentration data. The chamber
18 volume was treated as a fitted parameter, decreasing the “accessible volume” of 1,380 L,
19 provided by Burbacher et al. (1999a) to 1,220 L, which calibrated the mixing time in the
20 chamber to match the chamber concentration data (Figure B-16). An adjustment of the

1 “accessible volume” also accounts for any volume filled by the monkey and other chamber
 2 equipment. A detailed description of the chamber set-up is found in Burbacher et al. (1999a). The
 3 model does an adequate job of fitting the data for all exposure groups without group-specific
 4 parameters. In particular, the data for all exposure levels can be adequately fit using a single
 5 value for the volume of distribution (V_{mk}) as well as each of the metabolic parameters. While one
 6 may be able to show statistically distinct parameters for different groups or exposure levels (by
 7 fitting the model separately to each), as was done by Burbacher et al. (1999a), it is unlikely that
 8 such differences are biologically significant, given the fairly large number of data points and the
 9 large variability evident in the blood concentration data. Thus, the single set of parameters listed
 10 with the parameter descriptions above will be used to estimate internal blood concentrations
 11 (C_{max} above background) for the dose-response analysis described in Appendix D. The chamber
 12 concentrations for “pregnancy” exposures recorded by Burbacher et al. (1999a: Table 2) and
 13 average body weights for each exposure group at the 2nd trimester time point were used along
 14 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

^aFrom Burbacher et al. (1999a) and Burbacher et al. [(1999b), Table 2, “pregnancy” exposure.]

^bFrom Burbacher, original data (personal communication).

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

15 Model simulations were also conducted to predict internal doses for the NEDO (1987)
 16 monkey studies. Specifically, simulations were conducted for 21 h/d exposures to 10, 100, and
 17 1000 ppm methanol with an average animal BW of 2.2 kg. Exposures were simulated for 7 days
 18 to assure that periodicity had been reached, and internal metrics calculated for the 7th day. Visual
 19 inspection of simulated blood levels indicated that periodicity was in fact attained by the 2nd or
 20 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

^aMetrics calculated for 2.2 kg BW animals exposed for 21 h/d, on the 7th day of simulated exposure.

^bNet (total) blood concentration averaged over 24 hours minus the background level of 2 mg/L.

^cPeak blood concentration minus the background level of 2 mg/L.

B.3.2. Conclusions and Discussion

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

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

21 While lactational exposure is less direct than fetal exposure and blood or target-tissue
22 levels in the breast-feeding infant or rat pup are likely to differ more from maternal levels, the
23 health-effects data indicate that most of the effects of concern are due to fetal exposure, with
24 only a small influence due to postnatal exposures. Separating out the contribution of postnatal

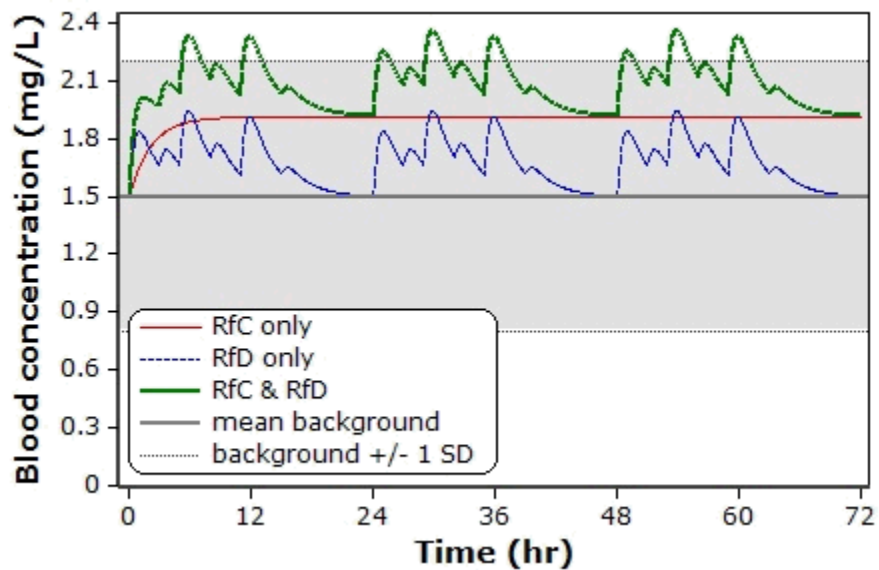
1 exposure from prenatal exposure to a given endpoint in a way that would allow the risk to be
2 estimated from estimates of both exposure levels would be extremely difficult, even if one had a
3 lactation/child PBPK model that allowed for prediction of blood (or target-tissue) levels in the
4 offspring. Target tissue concentrations in the offspring would still be expected to be closely
5 related to maternal blood levels (which depend on ambient exposure and determine the amount
6 delivered through breast milk), with the relationship between maternal levels and those in the
7 offspring being similar across species.

8 Therefore, the development of a lactation/child PBPK model appears not to be supported,
9 given the minimal change that is likely to result in risk extrapolations and use of (NP) maternal
10 blood levels as a measure of risk in the offspring is still considered preferable over use of default
11 extrapolation methods. In particular, the existing human data allow for accurate predictions of
12 maternal blood levels, which depend strongly on the rate of maternal methanol clearance. Failing
13 to use the existing data (via PBPK modeling) for human methanol clearance (versus that in other
14 species) would be to ignore this very important determinant of exposure to breast-fed infants.
15 And since bottle-fed infants do *not* receive methanol from their mothers, they are expected to
16 have lower or, at most, similar overall exposures for a given ambient concentration than the
17 breast-fed infant, so that use of maternal blood levels for risk estimation should also be
18 adequately protective for that group.

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

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

32 Figure B-17 illustrates the changes in blood methanol concentrations predicted by the
33 human PBPK model for exposures to either the RfC or RfD alone, or a combined exposure to
34 both the RfC and RfD, with oral exposure assumed divided into six daily boluses as described
35 previously (Section B.2.7) and inhalation exposure assumed to be continuous. The predictions
36 are shown for an individual starting with an average background level of 1.5 mg/L blood
37 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) \pm 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 \sim 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-17PBPK model predictions of changes in blood methanol levels in humans for exposures at the RfC and RfD.

APPENDIX C. HUMAN CASE STUDIES

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

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

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

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

1 congestion, gastritis, pancreatic necrosis, fatty liver, epicardial hemorrhages, and congestion of
2 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
Acidotic (CO ₂ <10 mEq)	30	50

^aThese data do not include those who died outside the hospital or who were moribund on arrival.

Source: Bennett et al. (1953).

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

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

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

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

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

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

14 Similar deficits (metabolic acidosis, visual acuity, and color discrimination) were seen in
15 a man who ingested 300 mL of 75% methanol solution. His blood methanol level
16 was 1,630 mg/L. An MRI administered 24 hours after hospital admission revealed abnormal
17 hyperintense foci in the putamina, with less intense lesions in the white matter. Like the first
18 subject, a subsequent MRI indicated the foci decreased in size over time, but visual impairments
19 persisted.

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

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

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

1 There have been several case reports involving infant or toddler exposures to methanol
2 ([De Brabander et al., 2005](#); [Wu et al., 1995](#); [Brent et al., 1991](#); [Kahn and Blum, 1979](#)). The
3 report by Wu et al. ([1995](#)) involved a 5-week-old infant with moderate metabolic acidosis and a
4 serum methanol level of 11,480 mg/L, a level that is ordinarily fatal. However, this infant
5 exhibited no toxic signs and survived without any apparent permanent problems. De Brabander
6 et al. ([2005](#)) reported the case of a 3-year-old boy who ingested an unknown amount of pure
7 methanol; at 3 hours after ingestion, the blood methanol level was almost 300 mg/L. Ethanol
8 infusion as a therapeutic measure was not well tolerated; at 8 hours after ingestion, fomepizole
9 (4-methylpyrazole) was administered to inhibit the metabolism of methanol by ADH1, and blood
10 methanol levels stabilized below 200 mg/L, a level above which is considered to be toxic by the
11 American Academy of Clinical Toxicology ([Barceloux et al., 2002](#)). Neither metabolic acidosis
12 nor visual impairment was observed in this individual. Hantson et al. ([1997b](#)), in their review,
13 touted the efficacy of fomepizole over ethanol in the treatment of methanol poisoning

14 Bilateral putaminal lesions, suggestive of nonhemorrhagic necrosis in the brain of a man
15 who accidentally ingested methanol, were reported by Arora et al. ([2005](#)). Approximately
16 10 hours after MRI examination, he developed blurred vision and motor dysfunction. After
17 5 months, visual deficits persisted along with extrapyramidal symptoms. Persistent visual
18 dysfunction was also reported in another methanol poisoning case ([Arora et al., 2007](#)); the vision
19 problems developed ~46 hours subsequent to the incident.

20 Vara-Castrodeza et al. ([2007](#)) applied diffusion-weighted MRI on a methanol-induced
21 comatose woman. Diffusion-weighted MRI provides an image contrast distinct from standard
22 imaging in that contrast is dependent on the molecular motion of water ([Schaefer et al., 2000](#)).
23 The neuroradiological findings were suggestive of bilateral putaminal hemorrhagic necrosis,
24 cerebral and intraventricular hemorrhage, diffuse cerebral edema, and cerebellar necrosis.
25 Diffusion-weighted MRI allows for differentiation of restricted diffusion which is indicative of
26 nonviable tissue. In this case, treatment for acidosis (blood methanol levels had risen to
27 1,000 mg/L) was unsuccessful and the patient died.

28 Emergency treatment was unable to save the life of a 38-year-old man who presented
29 with abdominal pain and convulsions after methanol intoxication ([Henderson and Brubacher,](#)
30 [2002](#)). A review of a head CT scan performed before the individual went into respiratory arrest
31 revealed bilateral globus pallidus ischemia.

32 Discrete lesions of the putamen, cerebral white matter, and corpus callosum were
33 observed upon MRI (8 days post ingestion) in a man exposed to methanol (blood level
34 370 mg/L) complaining of vision loss ([Keles et al., 2007](#)). Standard treatments corrected the
35 acidosis (pH 6.8), and at 1-month follow-up, his cognitive function improved but blindness and
36 bilateral optic atrophy were described as permanent. The follow-up MRI showed persistent
37 putaminal lesions with cortical involvement.

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

7 Putaminal necrosis and edema of the deep white matter (the corpus callosum was not
8 affected) was found upon MRI examination of a 50-year-old woman who apparently ingested an
9 unknown amount of what was believed to be pure laboratory methanol ([Kuteifan et al., 1998](#)).
10 Her blood methanol level was 1,272 mg/L upon hospital admission and dropped to 1,020 mg/L at
11 10 hours and to 710 mg/L at 34 hours. The woman, a chronic alcoholic, was in a vegetative state
12 when found and did not improve over the course of a year.

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

18 The relation of methanol overexposure to brain hemorrhage was a focus of the report by
19 Phang et al. ([1988](#)), which followed the treatment of 7 individuals, 5 of whom died within
20 72 hours after hospital admission. In two of the deceased individuals, CT scans and autopsy
21 revealed putaminal hemorrhagic necrosis. The investigators postulated that the association of
22 methanol with hemorrhagic necrosis may be complicated by the use of heparin during
23 hemodialysis treatment for acidosis

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

30 There have been case reports that involved percutaneous and inhalation exposure ([Adanir
31 et al., 2005](#); [Downie et al., 1992](#)). Use of a methanol-containing emollient by a woman with
32 chronic pain led to vision loss, hyperventilation and finally, coma ([Adanir et al., 2005](#)).
33 Subsequent to standard treatment followed by hospital discharge, some visual impairment and
34 CNS decrements remained. The methanol blood threshold for ocular damage and acidosis
35 appeared to be ~20 mg/L. Dutkiewicz et al. ([1980](#)) have determined the skin absorption rate to be
36 0.192 mg/cm²/minute. In the case report of Aufderheide et al. ([1993](#)), two firefighters were

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

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

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

13 Hovda et al. (2005) presented a combined prospective and retrospective case series study
14 of 51 individuals in Norway (39 males and 12 females, many of whom were alcoholics) who
15 were hospitalized after consuming tainted spirits containing 20% methanol and 80% ethanol. In
16 general, serum methanol concentrations were highest among those most severely affected. The
17 poor outcome was closely correlated with the degree of metabolic acidosis. It was noted by the
18 investigators that the concomitant consumption of ethanol prevented more serious sequelae in
19 2/5 individuals who presented with detectable ethanol levels and were not acidotic despite 2
20 having the highest blood methanol levels. However, others with detectable levels of ethanol
21 along with severe metabolic acidosis (two of whom died) presumably had subtherapeutic levels
22 of ethanol in their system.

23 In a later report, Hovda et al. (2007) focused on formate kinetics in a 63-year-old male
24 who died 6 days after being admitted to the hospital with headache, vomiting, reduced vision,
25 and dizziness. The investigators speculated that the prolonged metabolic acidosis observed ($T^{1/2}$
26 for formic acid was 77 hours before dialysis, compared to a typical normal range of
27 2.5-12 hours) may have been related to retarded formate elimination.

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

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

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

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

20 In a discussion of 3 fatal methanol-overexposure cases, Andresen et al. (2008) found
21 antemortem blood methanol levels of 5,400 and 7,400 mg/L in two individuals. At autopsy brain
22 stem blood levels were 7,380 and 10,080 mg/L, respectively. These brain levels were much
23 higher than blood levels postmortem. Autopsy revealed brain and pulmonary edema in all three
24 individuals; in the two who had the longer survival times, there was hemorrhagic necrosis of the
25 putamen and hemorrhages of the tissue surrounding the optic nerve. In their study of 26 chronic
26 users of methylated spirits, Meyer et al. (2000) found that the best predictor of death or a poor
27 outcome in chronic abusers was a pH <7.0; there was no correlation between blood methanol
28 levels and outcome. Mahieu et al. (1989) considered a latency period before treatment exceeding
29 10 hours and a blood formate level >500 mg/L as predictive of possible permanent sequelae. Liu
30 et al. (1998) in their examination of medical records of 50 patients treated for methanol
31 poisoning over a 10-year period found that: (1) deceased patients had a higher mean blood
32 methanol level than survivors; and (2) initial arterial pH levels <7.0 (i.e., severe metabolic
33 acidosis). Coma or seizure was also associated with higher mortality upon hospital admission.

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

1 ([Sefidbakht et al., 2007](#)). Of 634 forensic autopsies carried out in Turkey during 1992-2003,
2 18 deaths appeared to be related to methanol poisoning ([Azmak, 2006](#)). Brain edema and focal
3 necrosis of the optic nerve were among various sequelae noted. Dethlefs and colleagues ([Naraqi](#)
4 [et al., 1979](#); [Dethlefs and Naraqi, 1978](#)) described permanent ocular damage in 8/24 males who
5 ingested methanol in Papua New Guinea.

6 In summary, most cases of accidental/intentional methanol poisoning reveal a common
7 set of symptoms, many of which are likely to be presented upon hospital admission. See Section
8 4.1.1 for a list of common symptoms.

APPENDIX D. RFC DERIVATION OPTIONS

D.1. Benchmark Dose Modeling Summary

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

D.1.1. Evaluation of Model Fit

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

12 For each continuous endpoint (brain weight and VDR), BMDS continuous models¹ were
13 fitted to the data using the maximum likelihood method. Model fit was assessed by a series of
14 tests as follows. For each model, first the homogeneity of the variances was tested using a
15 likelihood ratio test (BMDS Test 2). If Test 2 was not rejected (χ^2 p -value ≥ 0.10), the model was
16 fitted to the data assuming constant variance. If Test 2 was rejected (χ^2 p -value < 0.10), the
17 variance was modeled as a power function of the mean, and the variance model was tested for
18 adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using either
19 constant variance or modeled variance, models for the mean response were tested for adequacy
20 of fit using a likelihood ratio test (BMDS Test 4, with χ^2 p -value < 0.10 indicating inadequate
21 fit). Other factors were also used to assess the model fit, such as scaled residuals, visual fit, and
22 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

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

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

7 In the application of the BMD approach, continuous models in EPA’s BMDS, version 2.2
8 ([U.S. EPA, 2011a](#)), were fit to data sets for decreased brain weight in male rats exposed
9 throughout gestation and the postnatal period to 6 weeks and male rats exposed during gestation
10 on days 7–17 only ([NEDO, 1987](#)). Although there remains uncertainty surrounding the
11 identification of the proximate teratogen of importance (methanol, formaldehyde, or formate),
12 the dose metrics chosen for the derivation of RfCs were based on blood methanol levels. This
13 decision was primarily based on evidence that the toxic moiety is not likely to be the formate
14 metabolite of methanol ([NTP-CERHR, 2004](#)) and evidence that levels of the formaldehyde
15 metabolite following methanol maternal and/or neonate exposure would be much lower in the
16 fetus and neonate than in adults. While recent in vitro evidence indicates that formaldehyde is
17 more embryotoxic than methanol and formate, the high reactivity of formaldehyde would
18 significantly limit its transport from maternal to fetal blood, and the capacity for the metabolism
19 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

20 As discussed in Section 5.1.2.1, brain weight is susceptible to both the level and duration
21 of exposure suggesting that a dose metric that incorporates a time component would be the most
22 appropriate metric to use. For these reasons and because it is more typically used in internal-
23 dose-based assessments and better reflects total exposure within a given day, daily AUC
24 (measured for 22-hour exposure/day) was chosen as the most appropriate dose metric for
25 modeling the effects of methanol exposure on brain weights in rats exposed throughout gestation
26 and continuing into the F₁ generation.

27 As is discussed in Section 5.1.3.2.2, the additional routes of exposure to the pups in this
28 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

^aAUC 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.)

^bExposed throughout gestation and F₁ generation. Values are means ± SDD

^c*p* < 0.01, ^d*p* < 0.001, as calculated by the authors.

Source: 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 SDD 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

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

1 mean SD change and a 5% change relative to estimated control mean were considered. All
2 models were fit using restrictions and option settings suggested in the EPA BMD Technical
3 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](#))**

4 A summary of the results most relevant to the development of a POD using the BMD
5 approach (BMD, BMDL, and model fit statistics) for decreased brain weight at 6 weeks in male
6 rats exposed to methanol throughout gestation and continuing into the F₁ generation, with a
7 BMR of 1 control mean SD ([NEDO, 1987](#)), is provided in Table D-2. Model fit and was
8 determined by statistics (AIC and χ^2 residuals of individual dose groups) and visual inspection,
9 as recommended by EPA ([U.S. EPA, 2012a](#)). There is a 5.1-fold range of BMDL estimates from
10 adequately fitting models, indicating considerable model dependence. In addition, the fit of the
11 Hill and more complex Exponential models are better than the other models in the dose region of
12 interest as indicated by a lower scaled residual at the dose group closest to the BMD (0.18 and
13 0.16 versus -1.4) and by visual inspection. In accordance with EPA BMD Technical Guidance
14 ([U.S. EPA, 2012a](#)), the BMDL from the Hill model (bolded), is selected as the most appropriate
15 basis for an RfC derivation because it results in the lowest BMDL from among a broad range of
16 BMDLs and provides a superior fit in the low dose region nearest the BMD. The detailed results
17 of the Hill model run, including text and a plot (Figure D-1) are shown after Table D-2. The
18 BMDL_{1SD} was determined to be 858 mg-hr/L using the 95% lower confidence limit of the dose-
19 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	5469.53	4410.68	0.1385	-201.13	-1.39
2nd degree Polynomial	5469.53	4410.68	0.1385	-201.13	-1.39
3rd degree Polynomial	5469.53	4410.68	0.1385	-201.13	-1.39
Power	5469.53	4410.68	0.1385	-201.13	-1.39
Hill ^b	<u>1730.35</u>	<u>858.04</u>	<u>0.5920</u>	<u>-202.79</u>	<u>0.179</u>
Exponential 2	5159.24	4118.16	0.1573	-201.38	-1.336
Exponential 3	5159.24	4118.16	0.1573	-201.38	-1.336
Exponential 4	1802.01	997.71	0.5513	-202.72	0.163
Exponential 5	1802.01	997.71	0.5513	-202.72	0.163

^aThe 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, 2011a](#)) and model options and restrictions suggested by EPA BMD technical guidance ([U.S. EPA, 2012a](#)).

^bAIC = 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^2 d 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.

Source: NEDO ([1987](#)).

```

1  =====
2  Hill Model. (Version: 2.16; Date: 04/06/2011)
3  Input Data File: C:/USEPA/BMDS220/Data/Methanol/hil_NEDOrat-6wk-male_Hil-
4  ConstantVariance-BMR1Std-Restrict.(d)
5  Gnuplot Plotting File: C:/USEPA/BMDS220/Data/Methanol/hil_NEDOrat-6wk-male_Hil-
6  ConstantVariance-BMR1Std-Restrict.plt
7  Tue Mar 27 08:42:04 2012
8  =====
9
10 BMDS Model Run
11 ~~~~~
12
13 The form of the response function is:
14
15  $Y[\text{dose}] = \text{intercept} + v \cdot \text{dose}^n / (k^n + \text{dose}^n)$ 
16
17
18 Dependent variable = Mean
19 Independent variable = Dose
20 rho is set to 0
21 Power parameter restricted to be greater than 1
22 A constant variance model is fit
23
24 Total number of dose groups = 4
25 Total number of records with missing values = 0
26 Maximum number of iterations = 250
27 Relative Function Convergence has been set to: 1e-008
28 Parameter Convergence has been set to: 1e-008
29
30
31
32 Default Initial Parameter Values
33 alpha = 0.00539333
34 rho = 0 Specified
35 intercept = 1.78
36 v = -0.26
37 n = 0.698151
38 k = 5889.18
39
40
41 Asymptotic Correlation Matrix of Parameter Estimates
42
43 (***) The model parameter(s) -rho -n have been estimated at a boundary
44 point, or have been specified by the user, and do not appear in the
45 correlation matrix )
46
47
48 alpha intercept v k
49
50 alpha 1 1.7e-008 2.5e-008 -4e-008
51
52 intercept 1.7e-008 1 0.24 -0.62
53
54 v 2.5e-008 0.24 1 -0.85
55
56 k -4e-008 -0.62 -0.85 1
57
58
59
60 Parameter Estimates
61
62 95.0% Wald Confidence Interval
63 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
64 alpha 0.00498218 0.00100655 0.00300938 0.00695499
65 intercept 1.77449 0.0177456 1.73971 1.80927

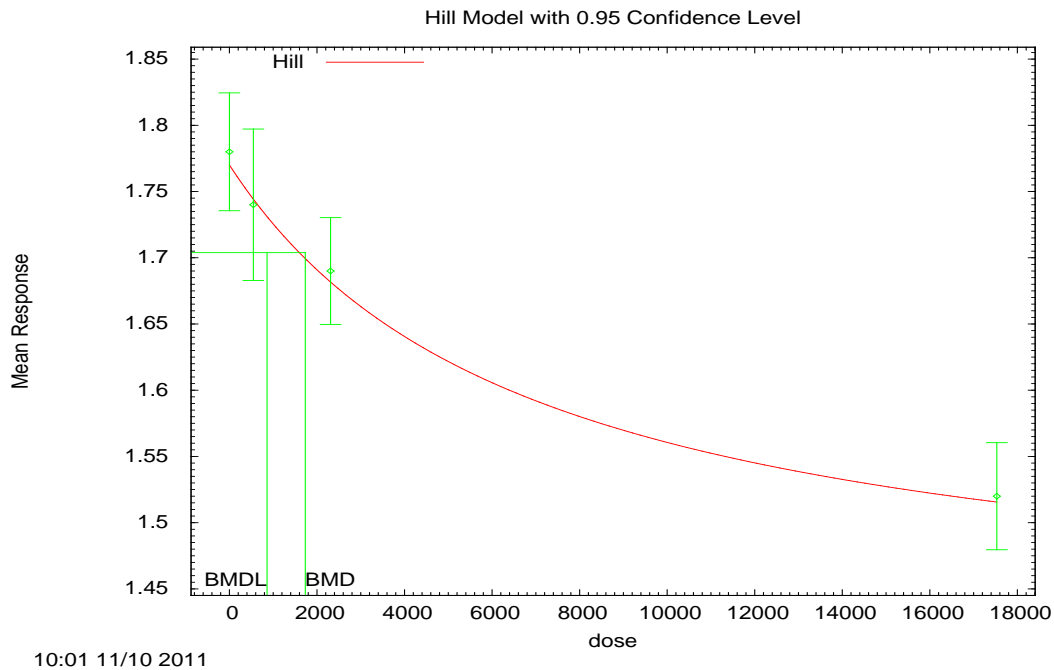
```

```

1  v -0.3555 0.0666435 -0.486119 -0.224881
2  n 1 NA
3  k 6984.58 4505.13 -1845.31 15814.5
4
5  NA - Indicates that this parameter has hit a bound
6  implied by some inequality constraint and thus
7  has no standard error.
8
9
10
11  Table of Data and Estimated Values of Interest
12
13  Dose N Obs Mean Est Mean Obs Std Dev Est Std Dev Scaled Res.
14  -----
15
16  0 12 1.78 1.77 0.07 0.0706 0.27
17  547 12 1.74 1.75 0.09 0.0706 -0.425
18  2308 11 1.69 1.69 0.06 0.0706 0.179
19  1.753e+004 14 1.52 1.52 0.07 0.0706 -0.0151
20
21
22
23  Model Descriptions for likelihoods calculated
24
25
26  Model A1:  $Y_{ij} = \mu(i) + e(ij)$ 
27   $\text{Var}\{e(ij)\} = \sigma^2$ 
28
29  Model A2:  $Y_{ij} = \mu(i) + e(ij)$ 
30   $\text{Var}\{e(ij)\} = \sigma(i)^2$ 
31
32  Model A3:  $Y_{ij} = \mu(i) + e(ij)$ 
33   $\text{Var}\{e(ij)\} = \sigma^2$ 
34  Model A3 uses any fixed variance parameters that
35  were specified by the user
36
37  Model R:  $Y_i = \mu + e(i)$ 
38   $\text{Var}\{e(i)\} = \sigma^2$ 
39
40
41  Likelihoods of Interest
42
43  Model Log(likelihood) # Param's AIC
44  A1 105.539862 5 -201.079724
45  A2 106.570724 8 -197.141449
46  A3 105.539862 5 -201.079724
47  fitted 105.396232 4 -202.792465
48  R 77.428662 2 -150.857324
49
50
51  Explanation of Tests
52
53  Test 1: Do responses and/or variances differ among Dose levels?
54  (A2 vs. R)
55  Test 2: Are Variances Homogeneous? (A1 vs A2)
56  Test 3: Are variances adequately modeled? (A2 vs. A3)
57  Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
58  (Note: When rho=0 the results of Test 3 and Test 2 will be the same.)
59
60  Tests of Interest
61
62  Test -2*log(Likelihood Ratio) Test df p-value
63
64  Test 1 58.2841 6 <.0001
65  Test 2 2.06173 3 0.5597

```


1 Test 3 2.06173 3 0.5597
 2 Test 4 0.287259 1 0.592
 3
 4 The p-value for Test 1 is less than .05. There appears to be a
 5 difference between response and/or variances among the dose levels
 6 It seems appropriate to model the data
 7
 8 The p-value for Test 2 is greater than .1. A homogeneous variance
 9 model appears to be appropriate here
 10
 11
 12 The p-value for Test 3 is greater than .1. The modeled variance appears
 13 to be appropriate here
 14
 15 The p-value for Test 4 is greater than .1. The model chosen seems
 16 to adequately describe the data
 17
 18
 19 Benchmark Dose Computation
 20
 21 Specified effect = 1
 22
 23 Risk Type = Estimated standard deviations from the control mean
 24
 25 Confidence level = 0.95
 26
 27 BMD = 1730.35
 28
 29 BMDL = 858.038



Source: 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](#)).

1 A summary of the results most relevant to the development of a POD using the BMD
2 approach (BMD, BMDL, and model fit statistics) for decreased brain weight at 6 weeks in male
3 rats exposed to methanol throughout gestation and continuing into the F₁ generation, with a
4 BMR of 0.05 change relative to estimated control mean, is provided in Table D-3. Model fit was
5 determined by statistics (AIC and χ^2 residuals of individual dose groups) and visual inspection,
6 as recommended by the EPA BMD Technical Guidance ([U.S. EPA, 2012a](#)). There is a 4.7-fold
7 range of BMDL estimates from adequately fitting models, indicating considerable model
8 dependence. In addition, the fit of the Hill and more complex Exponential models are better than
9 the other models in the dose region of interest as indicated by a lower scaled residual at the dose
10 group closest to the BMD (0.18 and 0.16 versus -1.4) and visual inspection. In accordance with
11 EPA BMD Technical Guidance ([U.S. EPA, 2012a](#)), the BMDL from the Hill model (bolded), is
12 selected as the most appropriate basis for an RfC derivation because it results in the lowest
13 BMDL from among a broad range of BMDLs and provides a superior fit in the low dose region
14 nearest the BMD. Output from the Hill model, including text and plot (Figure D-2), is shown
15 after Table D-3. The BMDL₀₅ was determined to be 1,183 mg-hr/L, using the 95% lower
16 confidence limit of the dose-response curve expressed in terms of the AUC above background for
17 methanol in blood.

Table D-3 Comparison of BMD₀₅ 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	<u>2,322.94</u>	<u>1,182.99</u>	<u>0.5920</u>	<u>-202.79</u>	<u>0.179</u>
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

^aThe BMDL is the 95% lower confidence limit on the AUC estimated to decrease brain weight by 5% using BMDS 2.2 ([U.S. EPA, 2011a](#)) and model options and restrictions suggested by EPA BMD Technical Guidance ([U.S. EPA, 2012a](#)).

^bAIC = 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^2 d 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.

Source: NEDO ([1987](#))

```

1  =====
2  Hill Model. (Version: 2.16; Date: 04/06/2011)
3  Input Data File: C:/USEPA/BMDS220/Data/Methanol/hil_NEDOrat-6wk-male_Hil-
4  ConstantVariance-BMR05-Restrict.(d)
5  Gnuplot Plotting File: C:/USEPA/BMDS220/Data/Methanol/hil_NEDOrat-6wk-male_Hil-
6  ConstantVariance-BMR05-Restrict.plt
7  Tue Mar 27 10:57:37 2012
8  =====
9
10 BMDS Model Run
11 ~~~~~
12
13 The form of the response function is:
14
15  $Y[\text{dose}] = \text{intercept} + v \cdot \text{dose}^n / (k^n + \text{dose}^n)$ 
16
17
18 Dependent variable = Mean
19 Independent variable = Dose
20 rho is set to 0
21 Power parameter restricted to be greater than 1
22 A constant variance model is fit
23
24 Total number of dose groups = 4
25 Total number of records with missing values = 0
26 Maximum number of iterations = 250
27 Relative Function Convergence has been set to: 1e-008
28 Parameter Convergence has been set to: 1e-008
29
30
31
32 Default Initial Parameter Values
33 alpha = 0.00539333
34 rho = 0 Specified
35 intercept = 1.78
36 v = -0.26
37 n = 0.698151
38 k = 5889.18
39
40
41 Asymptotic Correlation Matrix of Parameter Estimates
42
43 (***) The model parameter(s) -rho -n have been estimated at a boundary
44 point, or have been specified by the user, and do not appear in the
45 correlation matrix )
46
47
48 alpha intercept v k
49
50 alpha 1 1.7e-008 2.5e-008 -4e-008
51
52 intercept 1.7e-008 1 0.24 -0.62
53
54 v 2.5e-008 0.24 1 -0.85
55
56 k -4e-008 -0.62 -0.85 1
57
58
59
60 Parameter Estimates
61
62 95.0% Wald Confidence Interval
63 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
64 alpha 0.00498218 0.00100655 0.00300938 0.00695499
65 intercept 1.77449 0.0177456 1.73971 1.80927

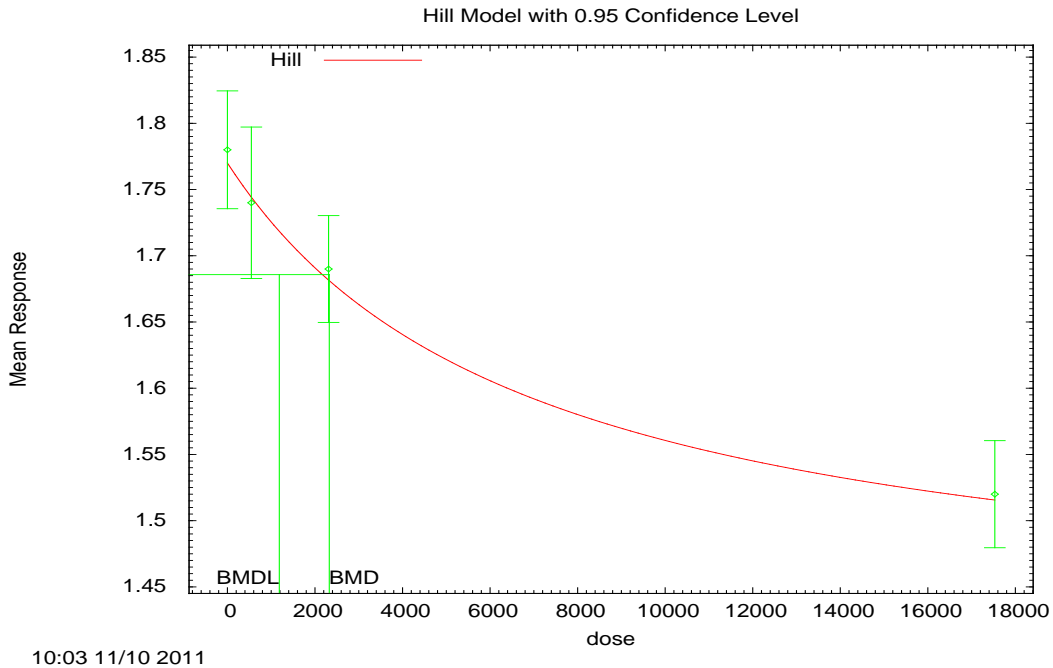
```

```

1 v -0.3555 0.0666435 -0.486119 -0.224881
2 n 1 NA
3 k 6984.58 4505.13 -1845.31 15814.5
4
5 NA - Indicates that this parameter has hit a bound
6 implied by some inequality constraint and thus
7 has no standard error.
8
9
10
11 Table of Data and Estimated Values of Interest
12
13 Dose N Obs Mean Est Mean Obs Std Dev Est Std Dev Scaled Res.
14 -----
15
16 0 12 1.78 1.77 0.07 0.0706 0.27
17 547 12 1.74 1.75 0.09 0.0706 -0.425
18 2308 11 1.69 1.69 0.06 0.0706 0.179
19 1.753e+004 14 1.52 1.52 0.07 0.0706 -0.0151
20
21
22
23 Model Descriptions for likelihoods calculated
24
25
26 Model A1:  $Y_{ij} = \mu(i) + e(ij)$ 
27  $\text{Var}\{e(ij)\} = \sigma^2$ 
28
29 Model A2:  $Y_{ij} = \mu(i) + e(ij)$ 
30  $\text{Var}\{e(ij)\} = \sigma(i)^2$ 
31
32 Model A3:  $Y_{ij} = \mu(i) + e(ij)$ 
33  $\text{Var}\{e(ij)\} = \sigma^2$ 
34 Model A3 uses any fixed variance parameters that
35 were specified by the user
36
37 Model R:  $Y_i = \mu + e(i)$ 
38  $\text{Var}\{e(i)\} = \sigma^2$ 
39
40
41 Likelihoods of Interest
42
43 Model Log(likelihood) # Param's AIC
44 A1 105.539862 5 -201.079724
45 A2 106.570724 8 -197.141449
46 A3 105.539862 5 -201.079724
47 fitted 105.396232 4 -202.792465
48 R 77.428662 2 -150.857324
49
50
51 Explanation of Tests
52
53 Test 1: Do responses and/or variances differ among Dose levels?
54 (A2 vs. R)
55 Test 2: Are Variances Homogeneous? (A1 vs A2)
56 Test 3: Are variances adequately modeled? (A2 vs. A3)
57 Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
58 (Note: When  $\rho=0$  the results of Test 3 and Test 2 will be the same.)
59
60 Tests of Interest
61
62 Test  $-2 \cdot \log(\text{Likelihood Ratio})$  Test df p-value
63
64 Test 1 58.2841 6 <.0001
65 Test 2 2.06173 3 0.5597

```

1 Test 3 2.06173 3 0.5597
 2 Test 4 0.287259 1 0.592
 3
 4 The p-value for Test 1 is less than .05. There appears to be a
 5 difference between response and/or variances among the dose levels
 6 It seems appropriate to model the data
 7
 8 The p-value for Test 2 is greater than .1. A homogeneous variance
 9 model appears to be appropriate here
 10
 11
 12 The p-value for Test 3 is greater than .1. The modeled variance appears
 13 to be appropriate here
 14
 15 The p-value for Test 4 is greater than .1. The model chosen seems
 16 to adequately describe the data
 17
 18
 19 Benchmark Dose Computation
 20
 21 Specified effect = 0.05
 22
 23 Risk Type = Relative risk
 24
 25 Confidence level = 0.95
 26
 27 BMD = 2322.94
 28
 29 BMDL = 1182.99



Source: 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, F₁ generation inhalational study.

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

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

5 The first step in the current analysis is to convert the inhalation doses, given as ppm
6 values from the studies, to an internal dose surrogate or dose metric using the EPA PBPK model
7 (see Appendix B). Predicted C_{\max} values for methanol in the blood of rats, with and without
8 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).

^bExposed throughout gestation. Values are means ± SD

^c $p < 0.01$, as calculated by the authors.

Source: NEDO (1987).

9 The BMD technical guidance (U.S. EPA, 2012a) suggests that in the absence of
10 knowledge as to what level of response to consider adverse, a change in the mean equal to
11 1 control SD from the control mean can be used as a BMR for continuous endpoints. However, it
12 has been suggested that other BMRs, such as 5% change relative to estimated control mean, are
13 also appropriate when performing BMD analyses on fetal weight change as a developmental
14 endpoint (Kavlock et al., 1995). Therefore, in this assessment, both a 1 control mean SD change
15 and a 5% change relative to estimated control mean were considered. All models were fit using
16 restrictions and option settings suggested in the EPA's BMD Technical Guidance Document
17 (U.S. EPA, 2012a).

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

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

1 weeks in male rats exposed to methanol during gestation from days 7–17, with a BMR of 1
2 control mean S.D, is provided in Table D-5. Male brain weight responses were chosen because
3 they resulted in lower BMD and BMDL estimates than female responses (data not shown).
4 Model fit was determined by statistics (AIC and χ^2 residuals of individual dose groups) and
5 visual inspection, as recommended by EPA ([U.S. EPA, 2012a](#)). The Polynomial and Power
6 models reduced to Linear model and returned identical modeling results. There is a greater than
7 5-fold range of BMDL estimates from adequately fitting models, indicating considerable model
8 dependence. In addition, the fit of the Hill and Exponential 4 and 5 models are better than the
9 other models in the dose region of interest as indicated by a lower scaled residual at the dose
10 group closest to the BMD (~ 0.09 versus ~ 0.3) and visual inspection. In accordance with EPA
11 BMD Technical Guidance ([U.S. EPA, 2012a](#)), the BMDL from the Exponential 4 and 5 models
12 (bolded), is selected as the most appropriate basis for an RfC derivation because it results in the
13 lowest BMDL from among a broad range of BMDLs and provides a superior fit in the low dose
14 region nearest the BMD. Output from the Exponential 4 model, including text and plot
15 (Figure D-3), is shown after Table D-5. The BMDL_{1SD} was determined to be 115 mg/L, using the
16 95% lower confidence limit of the dose-response curve expressed in terms of the C_{max} above
17 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	<i>p</i> -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

^aThe BMDL is the 95% lower confidence limit on the C_{max} estimated to decrease brain weight by 1 control mean SD using BMDS 2.1.1.1 ([U.S. EPA, 2009](#)) and model options and restrictions suggested by EPA BMD technical guidance ([U.S. EPA, 2012a](#)).

^bAIC = 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^2 d 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.

Source: NEDO ([1987](#))

```

1  =====
2      Exponential Model. (Version: 1.7; Date: 12/10/2009)
3      Input Data File: C:/USEPA/BMDS220/Data/Methanol/exp_NEDOrat-Gest-Cmax-Std_Exp-
4      ModelVariance-BMR1Std-Down.(d)
5      Gnuplot Plotting File:
6          Tue Mar 27 12:45:12 2012
7  =====
8
9      BMDS Model Run
10 ~~~~~
11
12     The form of the response function by Model:
13     Model 2: Y[dose] = a * exp{sign * b * dose}
14     Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
15     Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
16     Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]
17
18     Note: Y[dose] is the median response for exposure = dose;
19     sign = +1 for increasing trend in data;
20     sign = -1 for decreasing trend.
21
22     Model 2 is nested within Models 3 and 4.
23     Model 3 is nested within Model 5.
24     Model 4 is nested within Model 5.
25
26
27     Dependent variable = Mean
28     Independent variable = Dose
29     Data are assumed to be distributed: normally
30     Variance Model: exp(lnalpha +rho *ln(Y[dose]))
31     The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)
32
33     Total number of dose groups = 4
34     Total number of records with missing values = 0
35     Maximum number of iterations = 250
36     Relative Function Convergence has been set to: 1e-008
37     Parameter Convergence has been set to: 1e-008
38
39     MLE solution provided: Exact
40
41
42     Initial Parameter Values
43
44     Variable Model 4
45     -----
46     lnalpha 7.32457
47     rho -18.5236
48     a 2.1105
49     b 0.000507001
50     c 0.816778
51     d 1
52
53
54
55     Parameter Estimates
56
57     Variable Model 4
58     -----
59     lnalpha 6.99305
60     rho -18.0776
61     a 2.00632
62     b 0.000758964
63     c 0.891583
64     d 1
65

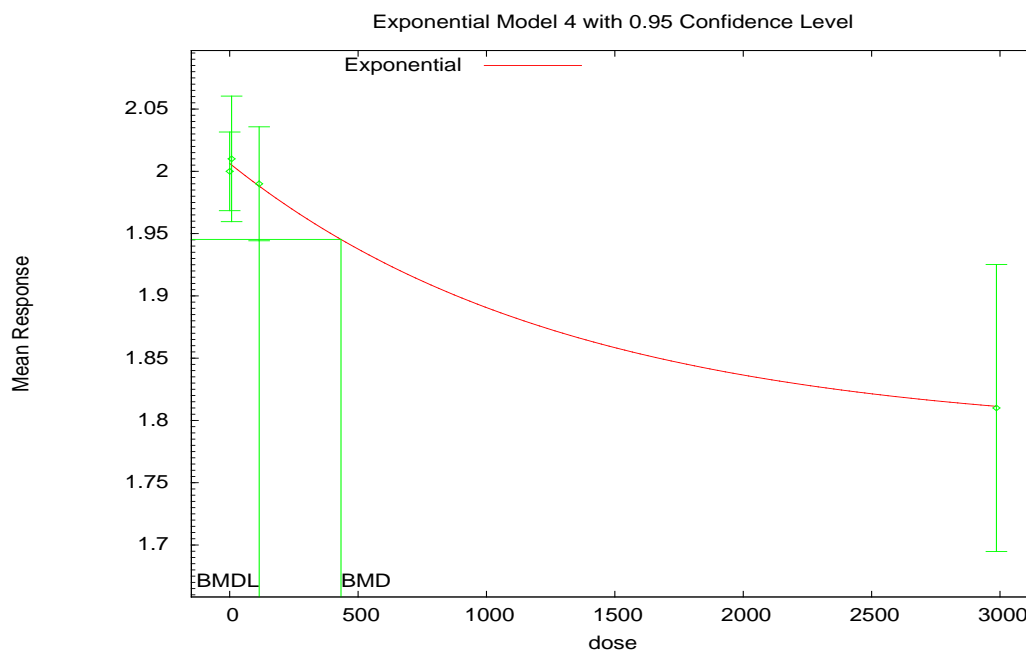
```

```

1
2 Table of Stats From Input Data
3
4 Dose N Obs Mean Obs Std Dev
5 -----
6 0 11 2 0.047
7 7.41 11 2.01 0.075
8 114.6 12 1.99 0.072
9 2986 10 1.81 0.161
10
11
12 Estimated Values of Interest
13
14 Dose Est Mean Est Std Scaled Residual
15 -----
16 0 2.006 0.06098 -0.3437
17 7.41 2.005 0.06132 0.2651
18 114.6 1.988 0.06619 0.09421
19 2986 1.811 0.1536 -0.02792
20
21
22
23 Other models for which likelihoods are calculated:
24
25 Model A1:  $Y_{ij} = \mu(i) + e_{ij}$ 
26  $\text{Var}\{e_{ij}\} = \sigma^2$ 
27
28 Model A2:  $Y_{ij} = \mu(i) + e_{ij}$ 
29  $\text{Var}\{e_{ij}\} = \sigma(i)^2$ 
30
31 Model A3:  $Y_{ij} = \mu(i) + e_{ij}$ 
32  $\text{Var}\{e_{ij}\} = \exp(\alpha + \log(\text{mean}(i))) * \rho$ 
33
34 Model R:  $Y_{ij} = \mu + e(i)$ 
35  $\text{Var}\{e_{ij}\} = \sigma^2$ 
36
37
38 Likelihoods of Interest
39
40 Model Log(likelihood) DF AIC
41 -----
42 A1 83.20596 5 -156.4119
43 A2 92.06049 8 -168.121
44 A3 90.61606 6 -169.2321
45 R 70.76186 2 -137.5237
46 4 90.79294 5 -171.5859
47
48
49 Additive constant for all log-likelihoods = -40.43. This constant added to the
50 above values gives the log-likelihood including the term that does not
51 depend on the model parameters.
52
53
54 Explanation of Tests
55
56 Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
57 Test 2: Are Variances Homogeneous? (A2 vs. A1)
58 Test 3: Are variances adequately modeled? (A2 vs. A3)
59
60 Test 6a: Does Model 4 fit the data? (A3 vs 4)
61
62
63
64
65

```

1 Tests of Interest
2
3 Test -2*log(Likelihood Ratio) D. F. p-value
4 -----
5 Test 1 42.6 6 < 0.0001
6 Test 2 17.71 3 0.000505
7 Test 3 2.889 2 0.2359
8 Test 6a -0.3538 1 N/A
9
10
11 The p-value for Test 1 is less than .05. There appears to be a
12 difference between response and/or variances among the dose
13 levels, it seems appropriate to model the data.
14
15 The p-value for Test 2 is less than .1. A non-homogeneous
16 variance model appears to be appropriate.
17
18 The p-value for Test 3 is greater than .1. The modeled
19 variance appears to be appropriate here.
20
21 The p-value for Test 6a is less than .1. Model 4 may not adequately
22 describe the data; you may want to consider another model.
23
24
25 Benchmark Dose Computations:
26
27 Specified Effect = 1.000000
28
29 Risk Type = Estimated standard deviations from control
30
31 Confidence Level = 0.950000
32
33 BMD = 433.456
34
35 BMDL = 114.856



11:45 03/27 2012

Source: 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)

1 A summary of the results most relevant to the development of a POD using the BMD
 2 approach (BMD, BMDL, and model fit statistics) for decreased brain weight at 8 weeks in male
 3 rats exposed to methanol during gestation from days 7 to 17, with a BMR of 0.05 change relative
 4 to estimated control mean, is provided in Table D-6. Model fit was determined by statistics (AIC
 5 and χ^2 residuals of individual dose groups) and visual inspection, as recommended by EPA
 6 ([2012a](#)). Modeling considerations and uncertainties for this data set were discussed in C.1.2.1
 7 and, as was done for the BMR of 1 SD, the lowest BMDL was chosen for use in the RfC
 8 derivation ([NEDO, 1987](#)), which in this case was the BMDL₀₅ of 119.51 mg methanol/L in blood
 9 estimated by the Exponential 5 model. Results from the Exponential 5 model, including text and
 10 plot (see Figure D-4), are shown after Table D-6.

Table D-6 Comparison of BMD₀₅ 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 degree Polynomial	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

^aThe 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, 2011a](#)) and model options and restrictions suggested by EPA BMD Technical Guidance ([2012a](#)).

^cAIC = 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^2 d 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.

Source: NEDO ([1987](#)).

```

1 =====
2 Exponential Model. (Version: 1.7; Date: 12/10/2009)
3 Input Data File: C:/USEPA/BMDS220/Data/Methanol/exp_NEDOrat-Gest-Cmax-Std_Exp-
4 ModelVariance-BMR05-Down.(d)
5 Gnuplot Plotting File:
6 Tue Mar 27 15:30:45 2012
7 =====
8
9 BMDS Model Run
10 ~~~~~
11
12 The form of the response function by Model:
13 Model 2: Y[dose] = a * exp{sign * b * dose}
14 Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
15 Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
16 Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]
17
18 Note: Y[dose] is the median response for exposure = dose;
19 sign = +1 for increasing trend in data;
20 sign = -1 for decreasing trend.
21
22 Model 2 is nested within Models 3 and 4.
23 Model 3 is nested within Model 5.
24 Model 4 is nested within Model 5.
25
26
27 Dependent variable = Mean
28 Independent variable = Dose
29 Data are assumed to be distributed: normally
30 Variance Model: exp(lnalpha +rho *ln(Y[dose]))
31 The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)
32
33 Total number of dose groups = 4
34 Total number of records with missing values = 0
35 Maximum number of iterations = 250
36 Relative Function Convergence has been set to: 1e-008
37 Parameter Convergence has been set to: 1e-008
38
39 MLE solution provided: Exact
40
41
42 Initial Parameter Values
43
44 Variable Model 5
45 -----
46 lnalpha 7.32457
47 rho -18.5236
48 a 2.1105
49 b 0.000507001
50 c 0.816778
51 d 1
52
53
54
55 Parameter Estimates
56
57 Variable Model 5
58 -----
59 lnalpha 6.99305
60 rho -18.0776
61 a 2.00632
62 b 0.000758964
63 c 0.891583
64 d 1
65

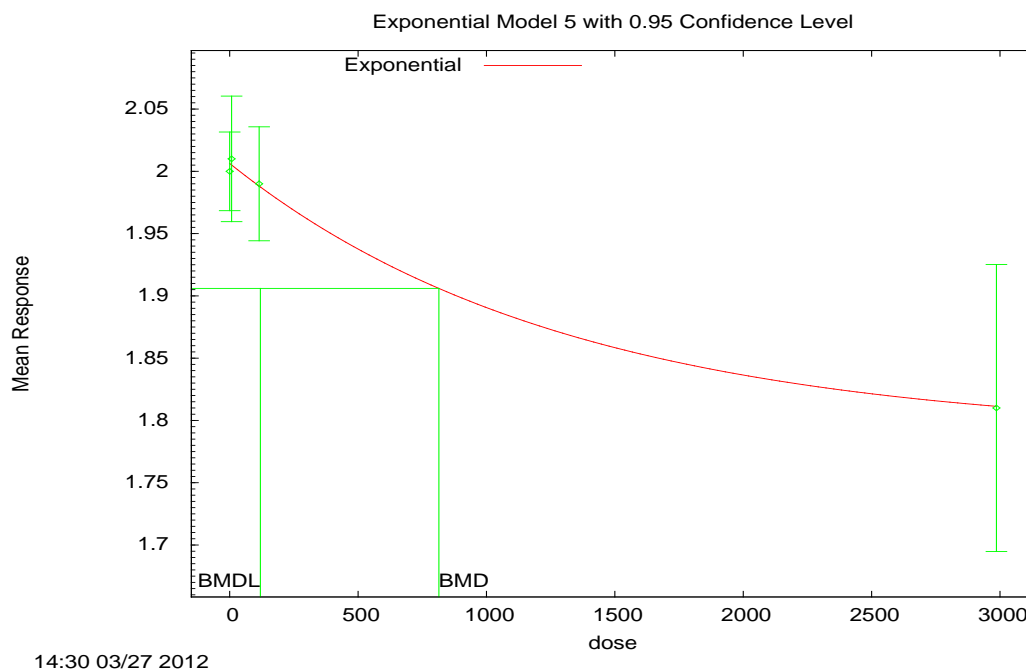
```

```

1
2 Table of Stats From Input Data
3
4 Dose N Obs Mean Obs Std Dev
5 -----
6 0 11 2 0.047
7 7.41 11 2.01 0.075
8 114.6 12 1.99 0.072
9 2986 10 1.81 0.161
10
11
12 Estimated Values of Interest
13
14 Dose Est Mean Est Std Scaled Residual
15 -----
16 0 2.006 0.06098 -0.3437
17 7.41 2.005 0.06132 0.2651
18 114.6 1.988 0.06619 0.09421
19 2986 1.811 0.1536 -0.02792
20
21
22
23 Other models for which likelihoods are calculated:
24
25 Model A1:  $Y_{ij} = \mu(i) + e_{ij}$ 
26  $\text{Var}\{e_{ij}\} = \sigma^2$ 
27
28 Model A2:  $Y_{ij} = \mu(i) + e_{ij}$ 
29  $\text{Var}\{e_{ij}\} = \sigma(i)^2$ 
30
31 Model A3:  $Y_{ij} = \mu(i) + e_{ij}$ 
32  $\text{Var}\{e_{ij}\} = \exp(\alpha + \log(\text{mean}(i))) * \rho$ 
33
34 Model R:  $Y_{ij} = \mu + e(i)$ 
35  $\text{Var}\{e_{ij}\} = \sigma^2$ 
36
37
38 Likelihoods of Interest
39
40 Model Log(likelihood) DF AIC
41 -----
42 A1 83.20596 5 -156.4119
43 A2 92.06049 8 -168.121
44 A3 90.61606 6 -169.2321
45 R 70.76186 2 -137.5237
46 5 90.79294 5 -171.5859
47
48
49 Additive constant for all log-likelihoods = -40.43. This constant added to the
50 above values gives the log-likelihood including the term that does not
51 depend on the model parameters.
52
53
54 Explanation of Tests
55
56 Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
57 Test 2: Are Variances Homogeneous? (A2 vs. A1)
58 Test 3: Are variances adequately modeled? (A2 vs. A3)
59
60 Test 7a: Does Model 5 fit the data? (A3 vs 5)
61
62
63 Tests of Interest
64
65 Test  $-2 * \log(\text{Likelihood Ratio})$  D. F. p-value

```


1 -----
2 Test 1 42.6 6 < 0.0001
3 Test 2 17.71 3 0.000505
4 Test 3 2.889 2 0.2359
5 Test 7a -0.3538 1 N/A
6
7
8 The p-value for Test 1 is less than .05. There appears to be a
9 difference between response and/or variances among the dose
10 levels, it seems appropriate to model the data.
11
12 The p-value for Test 2 is less than .1. A non-homogeneous
13 variance model appears to be appropriate.
14
15 The p-value for Test 3 is greater than .1. The modeled
16 variance appears to be appropriate here.
17
18 The p-value for Test 7a is less than .1. Model 5 may not adequately
19 describe the data; you may want to consider another model.
20
21
22 Benchmark Dose Computations:
23
24 Specified Effect = 0.050000
25
26 Risk Type = Relative deviation
27
28 Confidence Level = 0.950000
29
30 BMD = 814.763
31
32 BMDL = 119.505



Source: 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](#))

1 For the purposes of deriving an RfC for methanol from developmental endpoints using
 2 the BMD method and mouse data, cervical rib incidence data were evaluated from Rogers et al.
 3 ([1993b](#)). In this paper, Rogers et al. ([1993b](#)) also utilized a BMD methodology, examining the
 4 dosimetric threshold for cervical ribs and other developmental impacts by applying a log-logistic
 5 maximum likelihood model to the dose-response data. Using air exposure concentrations (ppm)
 6 as their dose metric, a value for the lower 95% confidence limit on the benchmark dose for 5%
 7 additional risk in mice was 305 ppm (400 mg/m³), using the log-logistic model. Although the
 8 teratology portion of the NEDO study ([1987](#)) also reported increases in cervical rib incidence in
 9 Sprague-Dawley rats, the Rogers et al. ([1993b](#)) study was chosen for dose-response modeling
 10 because effects were seen at lower doses, it was peer-reviewed and published in the open
 11 literature, and data on individual animals were available for a more statistically robust analysis
 12 utilizing nested models available in BMDS 2.2 ([U.S. EPA, 2011a](#)).

1 As described in Section 5.1.2.1, because exposure was during gestation only and due to
 2 the small critical gestational window for cervical rib abnormalities, C_{max} of methanol in blood
 3 (mg/L) is chosen as the appropriate internal dose metric. Because the critical window for
 4 methanol induction of cervical rib malformations in CD-1 mice is between GD6 and GD7
 5 ([Rogers and Mole, 1997](#); [Rogers et al., 1993a](#)), the measured C_{max} plasma methanol levels for
 6 gestation day 6 from the Rogers study are used with background levels (1.6 g/L) subtracted. C_{max}
 7 values for methanol in the blood of mice are summarized in Table D-7. These C_{max} values are
 8 then used as the dose metric for the BMD analysis of the litter-specific cervical rib response. The
 9 overall cervical rib/litter (%) reported by Rogers et al. ([1993b](#)) is shown in Table D-7, but litter-
 10 specific response data from this study (170 litters) obtained from John Rogers (personal
 11 communication) was used for the nested BMD analysis. Due to high mortality, the high
 12 (15,000 ppm) dose group (5 litters) was excluded from this analysis. The individual animal
 13 response data for the four dose groups are displayed below in the text output files for the
 14 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

^aReported C_{max} background levels of 1.6 mg/L were subtracted from reported C_{max} values.

Source: Rogers et al. ([1993b](#))

15 A 10% BMR level is the value typically calculated for comparisons across chemicals and
 16 endpoints for dichotomous responses because this level is near the low end of the observable
 17 range for many types of toxicity studies. However, from a statistical standpoint most
 18 reproductive and developmental studies involve a large enough sample size to support a 5%
 19 BMR for determination of a POD ([U.S. EPA, 2012a](#); [Allen et al., 1994a](#)). Rogers et al. ([1993b](#))
 20 utilized a 5% added risk for the BMR in the original study. This assessment utilizes both a 10%
 21 and 5% extra risk level as a BMR for the determination of a POD.³ The nested suite of models
 22 available in BMDS 2.2 ([U.S. EPA, 2011a](#)) was used to model the cervical rib data. In general,

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.

1 data from developmental toxicity studies are best modeled using nested models, as these models
 2 account for any intralitter correlation (i.e., the tendency of littermates to respond similarly to one
 3 another, relative to other litters in a dose group). All models were fit using restrictions and option
 4 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

5 A summary of the results most relevant to the development of a POD using the BMD
 6 approach (BMD, BMDL, and model fit statistics) for increased incidence of cervical rib in mice
 7 exposed to methanol during gestation from days 6 to 15, with a BMR of 0.10 extra risk, is
 8 provided in Table D-8. Model fit was determined by statistics (AIC and χ^2 residuals of individual
 9 dose groups) and visual inspection, as recommended by U.S. EPA ([U.S. EPA, 2012a](#)). The best
 10 model fit to these data (from visual inspection and comparison of AIC values) was obtained
 11 using the Nested Logistic (NLogistic) model. The textual and graphic (see Figure D-5) output
 12 from this model follows Table D-8. The BMDL₁₀ was determined to be 90.9972 mg/L using the
 13 95% lower confidence limit of the dose-response curve expressed in terms of the C_{max} for
 14 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	1047.37	0.5395
NCTR	223.55	111.78	0.2705	1050.32	0.5640
Rai and Van Ryzin	233.61	116.81	0.2625	1052.14	0.6043

^aC_{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](#)).

^bModel choice based on adequate p value (> 0.1), visual inspection, low AIC, and low (absolute) scaled residual.

^cAIC = 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^2 d 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.

Source: Rogers et al. ([1993b](#)).

```

1  =====
2  NLogistic Model. (Version: 2.15; Date: 10/28/2009)
3  Input Data File: C:/Documents and
4  Settings/llowe/Desktop/ROGERS_CMAX_BMD/ROGERS_CMAX_BMD10/NLog_CR_10. (d)
5  Fri Dec 16 10:48:13 2011
6  =====
7
8  BMDS Model Run
9  ~~~~~
10
11  The probability function is:
12
13
14  Prob. = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/
15  [1+exp (-beta-theta2*Rij-rho*log (Dose))],
16
17  where Rij is the litter specific covariate.
18
19  Restrict Power rho >= 1.
20
21
22
23
24  Total number of observations = 166
25  Total number of records with missing values = 0
26  Total number of parameters in model = 9
27  Total number of specified parameters = 0
28
29
30  Maximum number of iterations = 250
31  Relative Function Convergence has been set to: 1e-008
32  Parameter Convergence has been set to: 1e-008
33
34
35
36  Default Initial Parameter Values
37  alpha = 0.302379
38  beta = -7.2579
39  theta1 = 0
40  theta2 = 0
41  rho = 1
42  phi1 = 0.214334
43  phi2 = 0.304943
44  phi3 = 0.220179
45  phi4 = 0.370088
46
47
48
49  Parameter Estimates
50
51  Variable Estimate Std. Err.
52  alpha 0.127131 *
53  beta -4.62736 *
54  theta1 0.0297845 *
55  theta2 -0.467856 *
56  rho 1 *
57  phi1 0.203691 *
58  phi2 0.305429 *
59  phi3 0.212663 *
60  phi4 0.363199 *
61
62  * - Indicates that this value is not calculated.
63
64  Log-likelihood: -515.686 AIC: 1047.37
65

```

1	Litter Data						
2	Lit.-Spec. Litter Scaled						
3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18
19	20	21	22	23	24	25	26
27	28	29	30	31	32	33	34
35	36	37	38	39	40	41	42
43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58
59	60	61	62	63	64	65	
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	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	

1	0.0000	8.0000	0.365	8	2.923	1	-0.9066
2							
3	61.4000	1.0000	0.387	1	0.387	0	-0.7951
4	61.4000	1.0000	0.387	1	0.387	0	-0.7951
5	61.4000	2.0000	0.342	2	0.684	2	1.7177
6	61.4000	2.0000	0.342	2	0.684	0	-0.8919
7	61.4000	3.0000	0.317	3	0.952	1	0.0472
8	61.4000	3.0000	0.317	3	0.952	3	2.0021
9	61.4000	3.0000	0.317	3	0.952	1	0.0472
10	61.4000	3.0000	0.317	3	0.952	2	1.0246
11	61.4000	4.0000	0.310	4	1.240	3	1.3743
12	61.4000	4.0000	0.310	4	1.240	0	-0.9685
13	61.4000	5.0000	0.316	5	1.578	0	-1.0189
14	61.4000	5.0000	0.316	5	1.578	0	-1.0189
15	61.4000	5.0000	0.316	5	1.578	1	-0.3733
16	61.4000	5.0000	0.316	5	1.578	4	1.5633
17	61.4000	5.0000	0.316	5	1.578	0	-1.0189
18	61.4000	6.0000	0.330	6	1.981	3	0.5566
19	61.4000	6.0000	0.330	6	1.981	2	0.0105
20	61.4000	7.0000	0.350	7	2.453	2	-0.2131
21	61.4000	7.0000	0.350	7	2.453	2	-0.2131
22	61.4000	7.0000	0.350	7	2.453	3	0.2577
23	61.4000	7.0000	0.350	7	2.453	0	-1.1545
24	61.4000	7.0000	0.350	7	2.453	2	-0.2131
25	61.4000	7.0000	0.350	7	2.453	2	-0.2131
26	61.4000	8.0000	0.374	8	2.994	2	-0.4101
27	61.4000	8.0000	0.374	8	2.994	8	2.0644
28	61.4000	8.0000	0.374	8	2.994	0	-1.2350
29							
30	485.4000	2.0000	0.716	2	1.432	2	0.8091
31	485.4000	3.0000	0.638	3	1.915	3	1.0920
32	485.4000	4.0000	0.564	4	2.258	1	-0.9912
33	485.4000	4.0000	0.564	4	2.258	2	-0.2032
34	485.4000	5.0000	0.503	5	2.517	5	1.6327
35	485.4000	5.0000	0.503	5	2.517	1	-0.9972
36	485.4000	5.0000	0.503	5	2.517	3	0.3178
37	485.4000	5.0000	0.503	5	2.517	3	0.3178
38	485.4000	6.0000	0.460	6	2.763	2	-0.4350
39	485.4000	6.0000	0.460	6	2.763	5	1.2756
40	485.4000	6.0000	0.460	6	2.763	2	-0.4350
41	485.4000	6.0000	0.460	6	2.763	3	0.1352
42	485.4000	6.0000	0.460	6	2.763	2	-0.4350
43	485.4000	6.0000	0.460	6	2.763	0	-1.5754
44	485.4000	6.0000	0.460	6	2.763	4	0.7054
45	485.4000	6.0000	0.460	6	2.763	0	-1.5754
46	485.4000	6.0000	0.460	6	2.763	5	1.2756
47	485.4000	6.0000	0.460	6	2.763	2	-0.4350
48	485.4000	6.0000	0.460	6	2.763	4	0.7054
49	485.4000	6.0000	0.460	6	2.763	3	0.1352
50	485.4000	6.0000	0.460	6	2.763	6	1.8458
51	485.4000	6.0000	0.460	6	2.763	3	0.1352
52	485.4000	6.0000	0.460	6	2.763	5	1.2756
53	485.4000	6.0000	0.460	6	2.763	3	0.1352
54	485.4000	7.0000	0.437	7	3.057	4	0.4762
55	485.4000	7.0000	0.437	7	3.057	5	0.9813
56	485.4000	7.0000	0.437	7	3.057	0	-1.5443
57	485.4000	7.0000	0.437	7	3.057	5	0.9813
58	485.4000	7.0000	0.437	7	3.057	1	-1.0392
59	485.4000	7.0000	0.437	7	3.057	4	0.4762
60	485.4000	7.0000	0.437	7	3.057	3	-0.0289
61	485.4000	7.0000	0.437	7	3.057	4	0.4762
62	485.4000	7.0000	0.437	7	3.057	1	-1.0392
63	485.4000	7.0000	0.437	7	3.057	3	-0.0289
64	485.4000	7.0000	0.437	7	3.057	3	-0.0289
65	485.4000	7.0000	0.437	7	3.057	1	-1.0392

```

1  485.4000 8.0000 0.430 8 3.436 7 1.6134
2  485.4000 8.0000 0.430 8 3.436 5 0.7079
3  485.4000 8.0000 0.430 8 3.436 0 -1.5558
4  485.4000 9.0000 0.435 9 3.915 0 -1.6016
5  485.4000 9.0000 0.435 9 3.915 6 0.8530
6
7  2124.4000 1.0000 0.940 1 0.940 1 0.2530
8  2124.4000 1.0000 0.940 1 0.940 1 0.2530
9  2124.4000 1.0000 0.940 1 0.940 1 0.2530
10 2124.4000 2.0000 0.911 2 1.822 2 0.3783
11 2124.4000 2.0000 0.911 2 1.822 1 -1.7500
12 2124.4000 3.0000 0.872 3 2.615 3 0.5058
13 2124.4000 3.0000 0.872 3 2.615 3 0.5058
14 2124.4000 3.0000 0.872 3 2.615 1 -2.1218
15 2124.4000 3.0000 0.872 3 2.615 1 -2.1218
16 2124.4000 4.0000 0.820 4 3.282 4 0.6473
17 2124.4000 4.0000 0.820 4 3.282 4 0.6473
18 2124.4000 4.0000 0.820 4 3.282 2 -1.1551
19 2124.4000 4.0000 0.820 4 3.282 4 0.6473
20 2124.4000 4.0000 0.820 4 3.282 4 0.6473
21 2124.4000 4.0000 0.820 4 3.282 3 -0.2539
22 2124.4000 4.0000 0.820 4 3.282 4 0.6473
23 2124.4000 5.0000 0.759 5 3.795 1 -1.8656
24 2124.4000 5.0000 0.759 5 3.795 5 0.8047
25 2124.4000 5.0000 0.759 5 3.795 5 0.8047
26 2124.4000 5.0000 0.759 5 3.795 4 0.1371
27 2124.4000 5.0000 0.759 5 3.795 4 0.1371
28 2124.4000 5.0000 0.759 5 3.795 3 -0.5305
29 2124.4000 6.0000 0.692 6 4.153 5 0.4466
30 2124.4000 6.0000 0.692 6 4.153 6 0.9736
31 2124.4000 6.0000 0.692 6 4.153 6 0.9736
32 2124.4000 6.0000 0.692 6 4.153 3 -0.6074
33 2124.4000 6.0000 0.692 6 4.153 6 0.9736
34 2124.4000 6.0000 0.692 6 4.153 2 -1.1344
35 2124.4000 6.0000 0.692 6 4.153 4 -0.0804
36 2124.4000 6.0000 0.692 6 4.153 0 -2.1885
37 2124.4000 6.0000 0.692 6 4.153 5 0.4466
38 2124.4000 6.0000 0.692 6 4.153 0 -2.1885
39 2124.4000 6.0000 0.692 6 4.153 5 0.4466
40 2124.4000 6.0000 0.692 6 4.153 4 -0.0804
41 2124.4000 7.0000 0.628 7 4.396 5 0.2650
42 2124.4000 7.0000 0.628 7 4.396 5 0.2650
43 2124.4000 7.0000 0.628 7 4.396 7 1.1421
44 2124.4000 7.0000 0.628 7 4.396 6 0.7036
45 2124.4000 7.0000 0.628 7 4.396 7 1.1421
46 2124.4000 8.0000 0.575 8 4.598 0 -1.7470
47
48

```

```

49 Combine litters with adjacent levels of the litter-specific covariate
50 within dose groups until the expected count exceeds 3.0, to help improve
51 the fit of the X^2 statistic to chi-square.
52

```

```

53
54 Grouped Data
55

```

```

56
57 Mean Scaled

```

```

58 Dose Lit.-Spec. Cov. Expected Observed Residual
59 -----
60 0.0000 1.0000 0.314 0 -0.6101
61 0.0000 2.0000 1.867 3 0.8381
62 0.0000 3.0000 2.598 2 -0.3532
63 0.0000 4.0000 3.940 2 -0.8870
64 0.0000 5.0000 4.141 2 -0.9178
65 0.0000 5.0000 4.141 3 -0.4891

```


1	0.0000	5.0000	4.141	2	-0.9178
2	0.0000	5.0000	1.380	1	-0.2824
3	0.0000	6.0000	3.670	5	0.5865
4	0.0000	6.0000	3.670	6	1.0275
5	0.0000	6.0000	3.670	3	-0.2955
6	0.0000	6.0000	3.670	8	1.9094
7	0.0000	6.0000	3.670	4	0.1455
8	0.0000	6.0000	3.670	4	0.1455
9	0.0000	6.0000	3.670	7	1.4685
10	0.0000	6.0000	3.670	0	-1.6184
11	0.0000	7.0000	4.699	6	0.4941
12	0.0000	7.0000	4.699	3	-0.6450
13	0.0000	7.0000	4.699	3	-0.6450
14	0.0000	7.0000	4.699	7	0.8738
15	0.0000	7.0000	4.699	6	0.4941
16	0.0000	7.0000	4.699	0	-1.7840
17	0.0000	7.0000	2.349	2	-0.1876
18	0.0000	8.0000	5.847	6	0.0512
19	0.0000	8.0000	5.847	11	1.7178
20	0.0000	8.0000	2.923	1	-0.9066
21					
22	61.4000	1.0000	0.775	0	-1.1245
23	61.4000	2.0000	1.367	2	0.5840
24	61.4000	3.0000	3.807	7	1.5606
25	61.4000	4.0000	2.480	3	0.2870
26	61.4000	5.0000	3.157	0	-1.4409
27	61.4000	5.0000	3.157	5	0.8414
28	61.4000	5.0000	1.578	0	-1.0189
29	61.4000	6.0000	3.962	5	0.4010
30	61.4000	7.0000	4.905	4	-0.3013
31	61.4000	7.0000	4.905	3	-0.6342
32	61.4000	7.0000	4.905	4	-0.3013
33	61.4000	8.0000	5.989	10	1.1697
34	61.4000	8.0000	2.994	0	-1.2350
35					
36	485.4000	2.0000	1.432	2	0.8091
37	485.4000	3.0000	1.915	3	1.0920
38	485.4000	4.0000	4.516	3	-0.8446
39	485.4000	5.0000	5.033	6	0.4494
40	485.4000	5.0000	5.033	6	0.4494
41	485.4000	6.0000	5.526	7	0.5944
42	485.4000	6.0000	5.526	5	-0.2120
43	485.4000	6.0000	5.526	2	-1.4216
44	485.4000	6.0000	5.526	4	-0.6152
45	485.4000	6.0000	5.526	7	0.5944
46	485.4000	6.0000	5.526	7	0.5944
47	485.4000	6.0000	5.526	9	1.4008
48	485.4000	6.0000	5.526	8	0.9976
49	485.4000	7.0000	3.057	4	0.4762
50	485.4000	7.0000	3.057	5	0.9813
51	485.4000	7.0000	3.057	0	-1.5443
52	485.4000	7.0000	3.057	5	0.9813
53	485.4000	7.0000	3.057	1	-1.0392
54	485.4000	7.0000	3.057	4	0.4762
55	485.4000	7.0000	3.057	3	-0.0289
56	485.4000	7.0000	3.057	4	0.4762
57	485.4000	7.0000	3.057	1	-1.0392
58	485.4000	7.0000	3.057	3	-0.0289
59	485.4000	7.0000	3.057	3	-0.0289
60	485.4000	7.0000	3.057	1	-1.0392
61	485.4000	8.0000	3.436	7	1.6134
62	485.4000	8.0000	3.436	5	0.7079
63	485.4000	8.0000	3.436	0	-1.5558
64	485.4000	9.0000	3.915	0	-1.6016
65	485.4000	9.0000	3.915	6	0.8530

1
2 2124.4000 1.0000 2.820 3 0.4382
3 2124.4000 2.0000 3.645 3 -0.9699
4 2124.4000 3.0000 5.230 6 0.7153
5 2124.4000 3.0000 5.230 2 -3.0007
6 2124.4000 4.0000 3.282 4 0.6473
7 2124.4000 4.0000 3.282 4 0.6473
8 2124.4000 4.0000 3.282 2 -1.1551
9 2124.4000 4.0000 3.282 4 0.6473
10 2124.4000 4.0000 3.282 4 0.6473
11 2124.4000 4.0000 3.282 3 -0.2539
12 2124.4000 4.0000 3.282 4 0.6473
13 2124.4000 5.0000 3.795 1 -1.8656
14 2124.4000 5.0000 3.795 5 0.8047
15 2124.4000 5.0000 3.795 5 0.8047
16 2124.4000 5.0000 3.795 4 0.1371
17 2124.4000 5.0000 3.795 4 0.1371
18 2124.4000 5.0000 3.795 3 -0.5305
19 2124.4000 6.0000 4.153 5 0.4466
20 2124.4000 6.0000 4.153 6 0.9736
21 2124.4000 6.0000 4.153 6 0.9736
22 2124.4000 6.0000 4.153 3 -0.6074
23 2124.4000 6.0000 4.153 6 0.9736
24 2124.4000 6.0000 4.153 2 -1.1344
25 2124.4000 6.0000 4.153 4 -0.0804
26 2124.4000 6.0000 4.153 0 -2.1885
27 2124.4000 6.0000 4.153 5 0.4466
28 2124.4000 6.0000 4.153 0 -2.1885
29 2124.4000 6.0000 4.153 5 0.4466
30 2124.4000 6.0000 4.153 4 -0.0804
31 2124.4000 7.0000 4.396 5 0.2650
32 2124.4000 7.0000 4.396 5 0.2650
33 2124.4000 7.0000 4.396 7 1.1421
34 2124.4000 7.0000 4.396 6 0.7036
35 2124.4000 7.0000 4.396 7 1.1421
36 2124.4000 8.0000 4.598 0 -1.7470

37
38 Chi-square = 101.30 DF = 96 P-value = 0.3359
39

40
41 To calculate the BMD and BMDL, the litter specific covariate is fixed
42 at the mean litter specific covariate of all the data: 5.379518
43

44 Benchmark Dose Computation
45

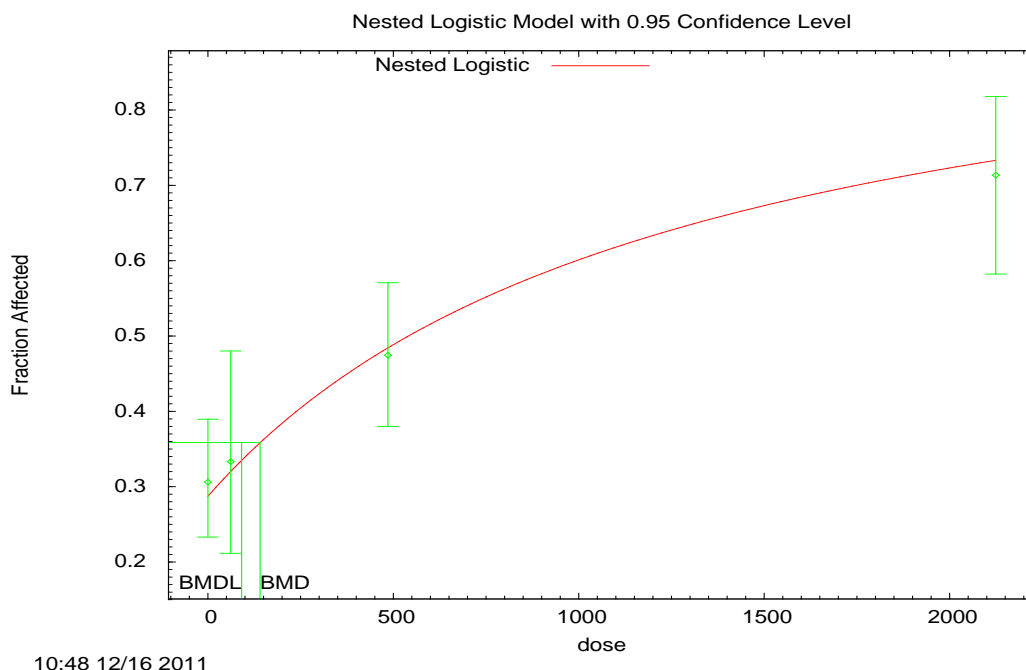
46 Specified effect = 0.1
47

48 Risk Type = Extra risk
49

50 Confidence level = 0.95
51

52 BMD = 140.749
53

54 BMDL = 90.9972
55
56



Source: 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

1 A summary of the results most relevant to the development of a POD using the BMD
 2 approach (BMD, BMDL, and model fit statistics) for increased incidence of cervical rib in mice
 3 exposed to methanol during gestation from days 6 to 15, with a BMR of 0.05 extra risk, is
 4 provided in Table D-9. Model fit was determined by statistics (AIC and χ^2 residuals of individual
 5 dose groups) and visual inspection, as recommended by U.S. EPA ([2012a](#)). The best model fit to
 6 these data (from visual inspection and comparison of AIC values) was obtained using the
 7 NLogistic model. The text and graphic (see Figure D-6) output from this model follow
 8 Table D-6. The BMDL₀₅ was determined to be 43.1039 mg/L using the 95% lower confidence
 9 limit of the dose-response curve expressed in terms of the C_{\max} for methanol in blood ([Rogers et](#)
 10 [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	<i>p</i> -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](#)).

^bModel choice based on adequate *p* value (> 0.1), visual inspection, low AIC, and low (absolute) scaled residual.

^cAIC = 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^2 d 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.

Source: Rogers et al. ([1993b](#)).

```

1 =====
2     NLogistic Model. (Version: 2.15; Date: 10/28/2009)
3     Input Data File: C:/Documents and
4 Settings/llowe/Desktop/ROGERS_CMAX_BMD/ROGERS_CMAX_BMD05/NLog_CR_5. (d)
5             Fri Dec 16 10:56:05 2011
6 =====
7
8     BMDS Model Run
9 ~~~~~
10
11    The probability function is:
12
13
14    Prob. = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/
15
16    [1+exp (-beta-theta2*Rij-rho*log (Dose))],
17
18    where Rij is the litter specific covariate.
19
20    Restrict Power rho >= 1.
21
22
23
24    Total number of observations = 166
25    Total number of records with missing values = 0
26    Total number of parameters in model = 9
27    Total number of specified parameters = 0
28
29
30    Maximum number of iterations = 250
31    Relative Function Convergence has been set to: 1e-008
32    Parameter Convergence has been set to: 1e-008
33
34
35
36    Default Initial Parameter Values
37    alpha = 0.302379
38    beta = -7.2579
39    theta1 = 0
40    theta2 = 0
41    rho = 1
42    phi1 = 0.214334
43    phi2 = 0.304943
44    phi3 = 0.220179
45    phi4 = 0.370088
46
47
48
49    Parameter Estimates
50
51    Variable Estimate Std. Err.
52    alpha 0.127131 *
53    beta -4.62736 *
54    theta1 0.0297845 *
55    theta2 -0.467856 *
56    rho 1 *
57    phi1 0.203691 *
58    phi2 0.305429 *
59    phi3 0.212663 *
60    phi4 0.363199 *
61
62    * - Indicates that this value is not calculated.
63
64    Log-likelihood: -515.686 AIC: 1047.37
65

```

1	Litter Data						
2	Lit.-Spec. Litter Scaled						
3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18
19	20	21	22	23	24	25	26
27	28	29	30	31	32	33	34
35	36	37	38	39	40	41	42
43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58
59	60	61	62	63	64	65	
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	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	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	

1	0.0000	8.0000	0.365	8	2.923	1	-0.9066
2							
3	61.4000	1.0000	0.387	1	0.387	0	-0.7951
4	61.4000	1.0000	0.387	1	0.387	0	-0.7951
5	61.4000	2.0000	0.342	2	0.684	2	1.7177
6	61.4000	2.0000	0.342	2	0.684	0	-0.8919
7	61.4000	3.0000	0.317	3	0.952	1	0.0472
8	61.4000	3.0000	0.317	3	0.952	3	2.0021
9	61.4000	3.0000	0.317	3	0.952	1	0.0472
10	61.4000	3.0000	0.317	3	0.952	2	1.0246
11	61.4000	4.0000	0.310	4	1.240	3	1.3743
12	61.4000	4.0000	0.310	4	1.240	0	-0.9685
13	61.4000	5.0000	0.316	5	1.578	0	-1.0189
14	61.4000	5.0000	0.316	5	1.578	0	-1.0189
15	61.4000	5.0000	0.316	5	1.578	1	-0.3733
16	61.4000	5.0000	0.316	5	1.578	4	1.5633
17	61.4000	5.0000	0.316	5	1.578	0	-1.0189
18	61.4000	6.0000	0.330	6	1.981	3	0.5566
19	61.4000	6.0000	0.330	6	1.981	2	0.0105
20	61.4000	7.0000	0.350	7	2.453	2	-0.2131
21	61.4000	7.0000	0.350	7	2.453	2	-0.2131
22	61.4000	7.0000	0.350	7	2.453	3	0.2577
23	61.4000	7.0000	0.350	7	2.453	0	-1.1545
24	61.4000	7.0000	0.350	7	2.453	2	-0.2131
25	61.4000	7.0000	0.350	7	2.453	2	-0.2131
26	61.4000	8.0000	0.374	8	2.994	2	-0.4101
27	61.4000	8.0000	0.374	8	2.994	8	2.0644
28	61.4000	8.0000	0.374	8	2.994	0	-1.2350
29							
30	485.4000	2.0000	0.716	2	1.432	2	0.8091
31	485.4000	3.0000	0.638	3	1.915	3	1.0920
32	485.4000	4.0000	0.564	4	2.258	1	-0.9912
33	485.4000	4.0000	0.564	4	2.258	2	-0.2032
34	485.4000	5.0000	0.503	5	2.517	5	1.6327
35	485.4000	5.0000	0.503	5	2.517	1	-0.9972
36	485.4000	5.0000	0.503	5	2.517	3	0.3178
37	485.4000	5.0000	0.503	5	2.517	3	0.3178
38	485.4000	6.0000	0.460	6	2.763	2	-0.4350
39	485.4000	6.0000	0.460	6	2.763	5	1.2756
40	485.4000	6.0000	0.460	6	2.763	2	-0.4350
41	485.4000	6.0000	0.460	6	2.763	3	0.1352
42	485.4000	6.0000	0.460	6	2.763	2	-0.4350
43	485.4000	6.0000	0.460	6	2.763	0	-1.5754
44	485.4000	6.0000	0.460	6	2.763	4	0.7054
45	485.4000	6.0000	0.460	6	2.763	0	-1.5754
46	485.4000	6.0000	0.460	6	2.763	5	1.2756
47	485.4000	6.0000	0.460	6	2.763	2	-0.4350
48	485.4000	6.0000	0.460	6	2.763	4	0.7054
49	485.4000	6.0000	0.460	6	2.763	3	0.1352
50	485.4000	6.0000	0.460	6	2.763	6	1.8458
51	485.4000	6.0000	0.460	6	2.763	3	0.1352
52	485.4000	6.0000	0.460	6	2.763	5	1.2756
53	485.4000	6.0000	0.460	6	2.763	3	0.1352
54	485.4000	7.0000	0.437	7	3.057	4	0.4762
55	485.4000	7.0000	0.437	7	3.057	5	0.9813
56	485.4000	7.0000	0.437	7	3.057	0	-1.5443
57	485.4000	7.0000	0.437	7	3.057	5	0.9813
58	485.4000	7.0000	0.437	7	3.057	1	-1.0392
59	485.4000	7.0000	0.437	7	3.057	4	0.4762
60	485.4000	7.0000	0.437	7	3.057	3	-0.0289
61	485.4000	7.0000	0.437	7	3.057	4	0.4762
62	485.4000	7.0000	0.437	7	3.057	1	-1.0392
63	485.4000	7.0000	0.437	7	3.057	3	-0.0289
64	485.4000	7.0000	0.437	7	3.057	3	-0.0289
65	485.4000	7.0000	0.437	7	3.057	1	-1.0392

```

1  485.4000 8.0000 0.430 8 3.436 7 1.6134
2  485.4000 8.0000 0.430 8 3.436 5 0.7079
3  485.4000 8.0000 0.430 8 3.436 0 -1.5558
4  485.4000 9.0000 0.435 9 3.915 0 -1.6016
5  485.4000 9.0000 0.435 9 3.915 6 0.8530
6
7  2124.4000 1.0000 0.940 1 0.940 1 0.2530
8  2124.4000 1.0000 0.940 1 0.940 1 0.2530
9  2124.4000 1.0000 0.940 1 0.940 1 0.2530
10 2124.4000 2.0000 0.911 2 1.822 2 0.3783
11 2124.4000 2.0000 0.911 2 1.822 1 -1.7500
12 2124.4000 3.0000 0.872 3 2.615 3 0.5058
13 2124.4000 3.0000 0.872 3 2.615 3 0.5058
14 2124.4000 3.0000 0.872 3 2.615 1 -2.1218
15 2124.4000 3.0000 0.872 3 2.615 1 -2.1218
16 2124.4000 4.0000 0.820 4 3.282 4 0.6473
17 2124.4000 4.0000 0.820 4 3.282 4 0.6473
18 2124.4000 4.0000 0.820 4 3.282 2 -1.1551
19 2124.4000 4.0000 0.820 4 3.282 4 0.6473
20 2124.4000 4.0000 0.820 4 3.282 4 0.6473
21 2124.4000 4.0000 0.820 4 3.282 3 -0.2539
22 2124.4000 4.0000 0.820 4 3.282 4 0.6473
23 2124.4000 5.0000 0.759 5 3.795 1 -1.8656
24 2124.4000 5.0000 0.759 5 3.795 5 0.8047
25 2124.4000 5.0000 0.759 5 3.795 5 0.8047
26 2124.4000 5.0000 0.759 5 3.795 4 0.1371
27 2124.4000 5.0000 0.759 5 3.795 4 0.1371
28 2124.4000 5.0000 0.759 5 3.795 3 -0.5305
29 2124.4000 6.0000 0.692 6 4.153 5 0.4466
30 2124.4000 6.0000 0.692 6 4.153 6 0.9736
31 2124.4000 6.0000 0.692 6 4.153 6 0.9736
32 2124.4000 6.0000 0.692 6 4.153 3 -0.6074
33 2124.4000 6.0000 0.692 6 4.153 6 0.9736
34 2124.4000 6.0000 0.692 6 4.153 2 -1.1344
35 2124.4000 6.0000 0.692 6 4.153 4 -0.0804
36 2124.4000 6.0000 0.692 6 4.153 0 -2.1885
37 2124.4000 6.0000 0.692 6 4.153 5 0.4466
38 2124.4000 6.0000 0.692 6 4.153 0 -2.1885
39 2124.4000 6.0000 0.692 6 4.153 5 0.4466
40 2124.4000 6.0000 0.692 6 4.153 4 -0.0804
41 2124.4000 7.0000 0.628 7 4.396 5 0.2650
42 2124.4000 7.0000 0.628 7 4.396 5 0.2650
43 2124.4000 7.0000 0.628 7 4.396 7 1.1421
44 2124.4000 7.0000 0.628 7 4.396 6 0.7036
45 2124.4000 7.0000 0.628 7 4.396 7 1.1421
46 2124.4000 8.0000 0.575 8 4.598 0 -1.7470
47
48

```

```

49 Combine litters with adjacent levels of the litter-specific covariate
50 within dose groups until the expected count exceeds 3.0, to help improve
51 the fit of the X^2 statistic to chi-square.
52

```

```

53
54 Grouped Data
55

```

```

56
57 Mean Scaled

```

```

58 Dose Lit.-Spec. Cov. Expected Observed Residual
59 -----

```

```

60 0.0000 1.0000 0.314 0 -0.6101
61 0.0000 2.0000 1.867 3 0.8381
62 0.0000 3.0000 2.598 2 -0.3532
63 0.0000 4.0000 3.940 2 -0.8870
64 0.0000 5.0000 4.141 2 -0.9178
65 0.0000 5.0000 4.141 3 -0.4891

```


1	0.0000	5.0000	4.141	2	-0.9178
2	0.0000	5.0000	1.380	1	-0.2824
3	0.0000	6.0000	3.670	5	0.5865
4	0.0000	6.0000	3.670	6	1.0275
5	0.0000	6.0000	3.670	3	-0.2955
6	0.0000	6.0000	3.670	8	1.9094
7	0.0000	6.0000	3.670	4	0.1455
8	0.0000	6.0000	3.670	4	0.1455
9	0.0000	6.0000	3.670	7	1.4685
10	0.0000	6.0000	3.670	0	-1.6184
11	0.0000	7.0000	4.699	6	0.4941
12	0.0000	7.0000	4.699	3	-0.6450
13	0.0000	7.0000	4.699	3	-0.6450
14	0.0000	7.0000	4.699	7	0.8738
15	0.0000	7.0000	4.699	6	0.4941
16	0.0000	7.0000	4.699	0	-1.7840
17	0.0000	7.0000	2.349	2	-0.1876
18	0.0000	8.0000	5.847	6	0.0512
19	0.0000	8.0000	5.847	11	1.7178
20	0.0000	8.0000	2.923	1	-0.9066
21					
22	61.4000	1.0000	0.775	0	-1.1245
23	61.4000	2.0000	1.367	2	0.5840
24	61.4000	3.0000	3.807	7	1.5606
25	61.4000	4.0000	2.480	3	0.2870
26	61.4000	5.0000	3.157	0	-1.4409
27	61.4000	5.0000	3.157	5	0.8414
28	61.4000	5.0000	1.578	0	-1.0189
29	61.4000	6.0000	3.962	5	0.4010
30	61.4000	7.0000	4.905	4	-0.3013
31	61.4000	7.0000	4.905	3	-0.6342
32	61.4000	7.0000	4.905	4	-0.3013
33	61.4000	8.0000	5.989	10	1.1697
34	61.4000	8.0000	2.994	0	-1.2350
35					
36	485.4000	2.0000	1.432	2	0.8091
37	485.4000	3.0000	1.915	3	1.0920
38	485.4000	4.0000	4.516	3	-0.8446
39	485.4000	5.0000	5.033	6	0.4494
40	485.4000	5.0000	5.033	6	0.4494
41	485.4000	6.0000	5.526	7	0.5944
42	485.4000	6.0000	5.526	5	-0.2120
43	485.4000	6.0000	5.526	2	-1.4216
44	485.4000	6.0000	5.526	4	-0.6152
45	485.4000	6.0000	5.526	7	0.5944
46	485.4000	6.0000	5.526	7	0.5944
47	485.4000	6.0000	5.526	9	1.4008
48	485.4000	6.0000	5.526	8	0.9976
49	485.4000	7.0000	3.057	4	0.4762
50	485.4000	7.0000	3.057	5	0.9813
51	485.4000	7.0000	3.057	0	-1.5443
52	485.4000	7.0000	3.057	5	0.9813
53	485.4000	7.0000	3.057	1	-1.0392
54	485.4000	7.0000	3.057	4	0.4762
55	485.4000	7.0000	3.057	3	-0.0289
56	485.4000	7.0000	3.057	4	0.4762
57	485.4000	7.0000	3.057	1	-1.0392
58	485.4000	7.0000	3.057	3	-0.0289
59	485.4000	7.0000	3.057	3	-0.0289
60	485.4000	7.0000	3.057	1	-1.0392
61	485.4000	8.0000	3.436	7	1.6134
62	485.4000	8.0000	3.436	5	0.7079
63	485.4000	8.0000	3.436	0	-1.5558
64	485.4000	9.0000	3.915	0	-1.6016
65	485.4000	9.0000	3.915	6	0.8530

1
2 2124.4000 1.0000 2.820 3 0.4382
3 2124.4000 2.0000 3.645 3 -0.9699
4 2124.4000 3.0000 5.230 6 0.7153
5 2124.4000 3.0000 5.230 2 -3.0007
6 2124.4000 4.0000 3.282 4 0.6473
7 2124.4000 4.0000 3.282 4 0.6473
8 2124.4000 4.0000 3.282 2 -1.1551
9 2124.4000 4.0000 3.282 4 0.6473
10 2124.4000 4.0000 3.282 4 0.6473
11 2124.4000 4.0000 3.282 3 -0.2539
12 2124.4000 4.0000 3.282 4 0.6473
13 2124.4000 5.0000 3.795 1 -1.8656
14 2124.4000 5.0000 3.795 5 0.8047
15 2124.4000 5.0000 3.795 5 0.8047
16 2124.4000 5.0000 3.795 4 0.1371
17 2124.4000 5.0000 3.795 4 0.1371
18 2124.4000 5.0000 3.795 3 -0.5305
19 2124.4000 6.0000 4.153 5 0.4466
20 2124.4000 6.0000 4.153 6 0.9736
21 2124.4000 6.0000 4.153 6 0.9736
22 2124.4000 6.0000 4.153 3 -0.6074
23 2124.4000 6.0000 4.153 6 0.9736
24 2124.4000 6.0000 4.153 2 -1.1344
25 2124.4000 6.0000 4.153 4 -0.0804
26 2124.4000 6.0000 4.153 0 -2.1885
27 2124.4000 6.0000 4.153 5 0.4466
28 2124.4000 6.0000 4.153 0 -2.1885
29 2124.4000 6.0000 4.153 5 0.4466
30 2124.4000 6.0000 4.153 4 -0.0804
31 2124.4000 7.0000 4.396 5 0.2650
32 2124.4000 7.0000 4.396 5 0.2650
33 2124.4000 7.0000 4.396 7 1.1421
34 2124.4000 7.0000 4.396 6 0.7036
35 2124.4000 7.0000 4.396 7 1.1421
36 2124.4000 8.0000 4.598 0 -1.7470

37
38 Chi-square = 101.30 DF = 96 P-value = 0.3359
39

40
41 To calculate the BMD and BMDL, the litter specific covariate is fixed
42 at the mean litter specific covariate of all the data: 5.379518
43

44 Benchmark Dose Computation
45

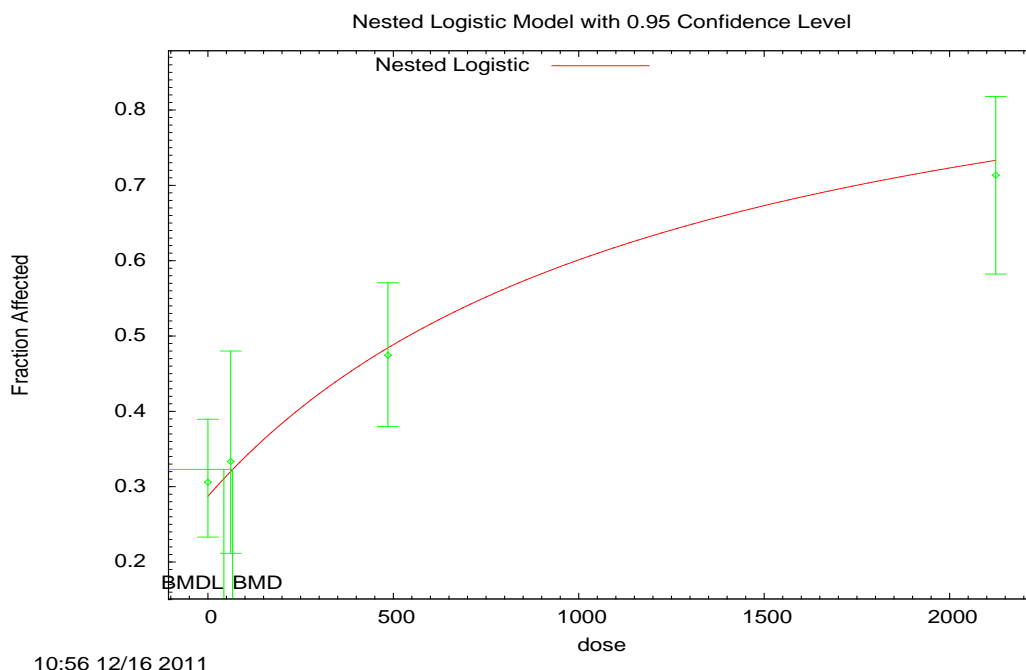
46 Specified effect = 0.05
47

48 Risk Type = Extra risk
49

50 Confidence level = 0.95
51

52 BMD = 66.6706
53

54 BMDL = 43.1039
55



Source: 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)

1 The BMD approach was utilized in the derivation of potential chronic inhalation
 2 reference values from effects seen in monkeys due to prenatal methanol exposure. Deficits in
 3 VDR were evaluated from Burbacher et al. ([1999a](#); [1999b](#)). In the application of the BMD
 4 approach, continuous models in EPA's BMDS 2.2 were fit to the data set for increased latency in
 5 VDR in neonatal monkeys. The maximum blood methanol values (C_{max}) above background
 6 estimated using the PK model described in Appendix B were used as the dose metric.

7 The VDR test, which assesses time (from birth) it takes for an infant to grasp for a
 8 brightly colored object containing an applesauce-covered nipple, is a measure of sensorimotor
 9 development. Beginning at 2 weeks after birth, infants were tested 5 times/day, 4 days/week.
 10 Performance on that test, measured as age from birth at achievement of test criterion (successful
 11 object retrieval on 8/10 consecutive trials over 2 testing sessions), was reduced in all treated male
 12 infants. The times (days after birth) to achieve the criteria for the VDR test in means \pm SD were
 13 23.7 \pm 8.3 (n = 3), 32.4 \pm 9.2 (n = 5), 42.7 \pm 13.9 (n = 3), and 40.5 \pm 17.7 (n = 2) days for males
 14 and 34.2 \pm 4.0 (n = 5), 33.0 \pm 5.8 (n = 4), 27.6 \pm 6.0 (n = 5), and 40.0 \pm 10.6 (n = 7) days for

1 females in the control to 1,800 ppm groups, respectively. As discussed in Section 4.4.2, this type
 2 of response data is sometimes adjusted to account for premature births by subtracting time (days)
 3 premature from the time (days from birth) needed to meet the test criteria ([Wilson and Cradock,](#)
 4 [2004](#)). When this type of adjustment is applied, the times (days after birth or, if shorter, days
 5 after control mean gestation length) to achieve the criteria for VDR test in means \pm SD were
 6 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
 7 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 =
 8 7) days for females in the control to 1,800 ppm groups, respectively. When these data were
 9 modeled within BMDS 2.1.1 ([U.S. EPA, 2009](#)), there was no significant difference between
 10 unadjusted responses and/or variances among the dose levels (indicating lack of a dose-response)
 11 for males and females combined ($p = 0.244$), for males only ($p = 0.321$) and for males only with
 12 the high-dose group excluded ($p = 0.182$), or for adjusted responses of males and females
 13 combined ($p = 0.12$), males only ($p = 0.448$) and males only with the high-dose group excluded
 14 ($p = 0.586$).⁴ The only data that offered a significant dose-response trend was that for unadjusted
 15 ($p = 0.0265$) and adjusted ($p = 0.009$) female responses, largely because of the much larger
 16 overall sample size across dose groups for females versus males (21 females versus 13 males).
 17 However, the model fits for the adjusted female response data were unacceptable. Only the
 18 unadjusted female VDR response data (Table D-10) offered both a dose-response trend and
 19 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

^aFrom Burbacher et al. ([1999a](#)) and Burbacher et al. [[1999b](#)], Table 2].

^bEstimated from the two-compartment PK monkey model described in Appendix B.

^cData reported in means \pm standard deviation.

20 The BMD technical guidance ([U.S. EPA, 2012a](#)) suggests that in the absence of
 21 knowledge as to what level of response to consider adverse, a change in the mean equal to
 22 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 .

1 of the results most relevant to the development of a POD using the BMD approach (BMD,
 2 BMDL, and model fit statistics) for increased latency of VDR in female neonatal monkeys
 3 exposed to methanol with a BMR of 1 control mean SD is provided in Table D-11. Model fit was
 4 determined by statistics (AIC and χ^2 residuals of individual dose groups) and visual inspection,
 5 as recommended by EPA (2012a). The Power model returned a lower AIC than the other
 6 models.⁵ The text and graphic (see Figure D-7) output from this model follows Table D-10. The
 7 BMDL_{1SD} was determined to be 19.59 mg/L, using the 95% lower confidence limit of the dose-
 8 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

^aC_{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).

^bModel choice based on adequate *p* value (> 0.1), visual inspection, low AIC, and low (absolute) scaled residual.

^cAIC = 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^2 d 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.

Source: 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.

```

1 =====
2     Power Model. (Version: 2.16; Date: 10/28/2009)
3     Input Data File: C:/USEPA/BMDS220/Data/pow_monkey_Pow-ModelVariance-BMR1Std-
4 Restrict. (d)
5     Gnuplot Plotting File: C:/USEPA/BMDS220/Data/pow_monkey_Pow-ModelVariance-BMR1Std-
6 Restrict.plt
7
8           Wed Nov 16 11:02:04 2011
9
10    =====
11    BMDS Model Run
12    ~~~~~
13    The form of the response function is:
14
15    Y[dose] = control + slope * dose^power
16
17
18    Dependent variable = Mean
19    Independent variable = Dose
20    The power is restricted to be greater than or equal to 1
21    The variance is to be modeled as Var (i) = exp (lalpha + log (mean (i)) * rho)
22
23    Total number of dose groups = 4
24    Total number of records with missing values = 0
25    Maximum number of iterations = 250
26    Relative Function Convergence has been set to: 1e-008
27    Parameter Convergence has been set to: 1e-008
28
29
30
31    Default Initial Parameter Values
32    lalpha = 4.05748
33    rho = 0
34    control = 27.6
35    slope = 3.85161
36    power = 0.320501
37
38
39    Asymptotic Correlation Matrix of Parameter Estimates
40
41    ( *** The model parameter (s) -power
42    have been estimated at a boundary point, or have been specified by the user,
43    and do not appear in the correlation matrix )
44
45    lalpha rho control slope
46
47    lalpha 1 -1 -0.29 0.6
48
49    rho -1 1 0.27 -0.6
50
51    control -0.29 0.27 1 -0.37
52
53    slope 0.6 -0.6 -0.37 1
54
55
56
57    Parameter Estimates
58
59    95.0% Wald Confidence Interval
60    Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
61    lalpha -13.0645 12.1112 -36.8021 10.673
62    rho 4.77979 3.44065 -1.96376 11.5233
63    control 31.5 1.4819 28.5955 34.4045
64    slope 2.57903e-028 1.21193e-028 2.03691e-029 4.95437e-028
65    power 18 NA

```

```

1
2 NA - Indicates that this parameter has hit a bound
3 implied by some inequality constraint and thus
4 has no standard error.
5
6
7
8 Table of Data and Estimated Values of Interest
9
10 Dose N Obs Mean Est Mean Obs Std Dev Est Std Dev Scaled Res.
11 -----
12
13 0 5 34.2 31.5 4 5.54 1.09
14 2.87 4 33 31.5 5.8 5.54 0.541
15 10.4 5 27.6 31.5 6 5.54 -1.57
16 38.4 7 40 40 10.6 9.81 7.35e-008
17
18
19
20 Model Descriptions for likelihoods calculated
21
22
23 Model A1:  $Y_{ij} = \mu(i) + e(ij)$ 
24  $\text{Var}\{e(ij)\} = \sigma^2$ 
25
26 Model A2:  $Y_{ij} = \mu(i) + e(ij)$ 
27  $\text{Var}\{e(ij)\} = \sigma(i)^2$ 
28
29 Model A3:  $Y_{ij} = \mu(i) + e(ij)$ 
30  $\text{Var}\{e(ij)\} = \exp(\alpha + \rho \ln(\mu(i)))$ 
31 Model A3 uses any fixed variance parameters that
32 were specified by the user
33
34 Model R:  $Y_i = \mu + e(i)$ 
35  $\text{Var}\{e(i)\} = \sigma^2$ 
36
37
38 Likelihoods of Interest
39
40 Model Log (likelihood) # Param's AIC
41 A1 -50.884765 5 111.769529
42 A2 -47.717070 8 111.434139
43 A3 -49.215263 6 110.430526
44 fitted -50.466380 4 108.932759
45 R -54.905426 2 113.810852
46
47
48 Explanation of Tests
49
50 Test 1: Do responses and/or variances differ among Dose levels?
51 (A2 vs. R)
52 Test 2: Are Variances Homogeneous? (A1 vs A2)
53 Test 3: Are variances adequately modeled? (A2 vs. A3)
54 Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
55 (Note: When  $\rho=0$  the results of Test 3 and Test 2 will be the same.)
56
57 Tests of Interest
58
59 Test  $-2 \cdot \log(\text{Likelihood Ratio})$  Test df p-value
60
61 Test 1 14.3767 6 0.0257
62 Test 2 6.33539 3 0.09639
63 Test 3 2.99639 2 0.2235
64 Test 4 2.50223 2 0.2862
65

```

1 The p-value for Test 1 is less than .05. There appears to be a
2 difference between response and/or variances among the dose levels
3 It seems appropriate to model the data
4
5 The p-value for Test 2 is less than .1. A non-homogeneous variance
6 model appears to be appropriate
7
8 The p-value for Test 3 is greater than .1. The modeled variance appears
9 to be appropriate here
10
11 The p-value for Test 4 is greater than .1. The model chosen seems
12 to adequately describe the data
13
14

15 Benchmark Dose Computation

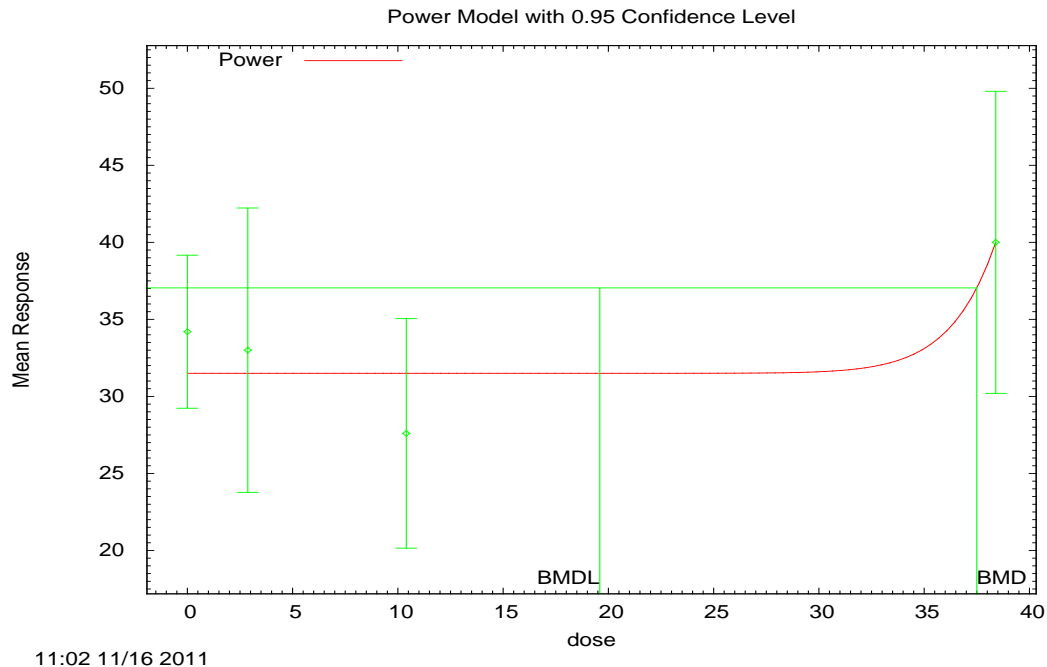
16 Specified effect = 1

17 Risk Type = Estimated standard deviations from the control mean

18 Confidence level = 0.95

19 BMD = 37.4993

20 BMDL = 19.5918



Source: 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.

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