

US EPA ARCHIVE DOCUMENT

APPENDIX

CASE STUDIES TO ACCOMPANY DATA DERIVED EXTRAPOLATION FACTOR DOCUMENT

This document is an Appendix to the draft document on Data Derived Extrapolation Factors. It presents examples where the availability of data for given chemicals support the derivation of non-default values for components of uncertainty factors. Each of the chemicals examined has an existing IRIS file and/or U.S. EPA Program Office risk assessment, although the derivation of DDEFs contained in these examples may not be found in the those documents. The intent of this Case Study document is to present examples that instruct the calculation of DDEF values; Reference values derived in these case studies should not be used in place of values found in IRIS or Program Office risk assessments.

A consistent format, below, has been developed and applied to the case studies. However, different components of case studies are more extensively described for some chemicals for others. This discrepancy is due to chemical-specific data sets, which may be more informative for some areas than others.

1. Summary

This section communicates the current assessment(s) (e.g., IRIS, PPRTV, RED, PMN/existing chemical, IPCS) and reference values. It includes the individual and composite UFs, and indicates how the DDEF was applied.

2. Hazard Identification and Dose Response

This section lists the key studies, identifying the principal study and critical effect including supplemental studies which might help to inform the decision. It describes the methods to characterize the dose response relationship and models or data describing response as a function of internal (target tissue) concentration. It also includes information on the mode/mechanism of action. It is not intended to be a compendium of data; rather it should communicate the information necessary to serve as a basis for the case study.

3. Basis for DDEF

This section provides the rationale for developing a DDEF rather than relying on default values. It identified models or data available for evaluation, describes the basis for selection of DDEF method and highlights the connection between the measure of dose and adverse outcome.

4. DDEF Derivation

This section presents the computation of the DDEF value. It should be presented in a level of detail so that the reader can follow the derivation. Comparison to other possible values for the UF component should be presented.

Each of the case studies illustrates different principles described in the Draft Document. Table 1 provides a summary of the principles illustrated in each. Note that the Agency does not yet have experience with deriving a DDEF for intra-species extrapolation with regard to TD. As such, no case study for that component is provided here.

1 Table 1. DDEF Case Study Chemicals and Issues
 2

Data Derived Extrapolation Factor	Chemical	Other Principles or Issues
Animal to Human, Toxicokinetics (UF _{AK})*	Ethylene glycol Monobutyl Ether (EGBE)	Choice among dose metrics, PBPK modeling, BMD applied to internal, not external doses
	Vinyl chloride	MOA analysis to identify dose metric, internal dose of reactive metabolite in liver chosen as basis for toxicokinetic equivalency between species
Animal to Human, Toxicodynamics (UF _{AD})	Ethylene glycol Monobutyl Ether (EGBE)	<i>In vivo</i> toxicity results identified effects to quantify in both species <i>in vitro</i>
	Dimethyl arsenic acid (DMA)	Mode of action analysis, use of <i>in vitro</i> data, use of genomics data
	N-methyl carbamate pesticides	Application of a DDEF to a common mechanism group of chemicals, use of DDEF approach to identify data gaps
Within Human Variability, Toxicokinetics (UF _{HK})	Boron	Clearance mechanism identified, surrogate measures of clearance employed for quantitation
	Methyl mercury	Toxicokinetic model developed using human data from dietary exposures, choice of dose metric from among several surrogates

3
 4 * The IRIS Assessments for these chemicals calculate a HED based on the results from PBPK Modeling. This draft
 5 document guides the development of a DDEF that results in the same human external dose, when adjusted for
 6 interspecies differences in dosimetry. The DDEF concept applied to interspecies differences in dosimetry makes
 7 explicit the magnitude of species differences, whereas methods that calculate a Human Equivalent Dose or
 8 Concentration do not make that calculation explicit.

1 INTERSPECIES ADJUSTMENT FOR TOXICOKINETICS

3 Ethylene Glycol Monobutyl Ether (EGBE)—UF_{AK} Case Study

5 Summary

7 Ethylene glycol monobutyl ether (EGBE; 2-butoxy ethanol) has an established RfD of
8 0.5 mg/kg-d on the U.S. EPA's Integrated Risk information System (IRIS) database (U.S. EPA,
9 1999). This value is based on the results of a subchronic drinking water study in mice and rats
10 where changes in mean corpuscular volume (MCV) were determined to be the critical effect.

12 As described below, the IRIS RfD is based on a PBPK modeling approach that identified
13 an HED of 5.1 mg/kg-d. To this value, a combined uncertainty factor of 10 was applied, which
14 comprised a single UF value of 10 for variation in sensitivity within the human population
15 (UF_H). The UF_{AK} was reduced to 1 on the basis of the dosimetry adjustments for TK, and the
16 UF_{AD} was reduced to 1 based on the relatively greater sensitivity of the rat compared to the
17 human (as discussed in the EGBE DDEF_{AD} case study presented later in this appendix).

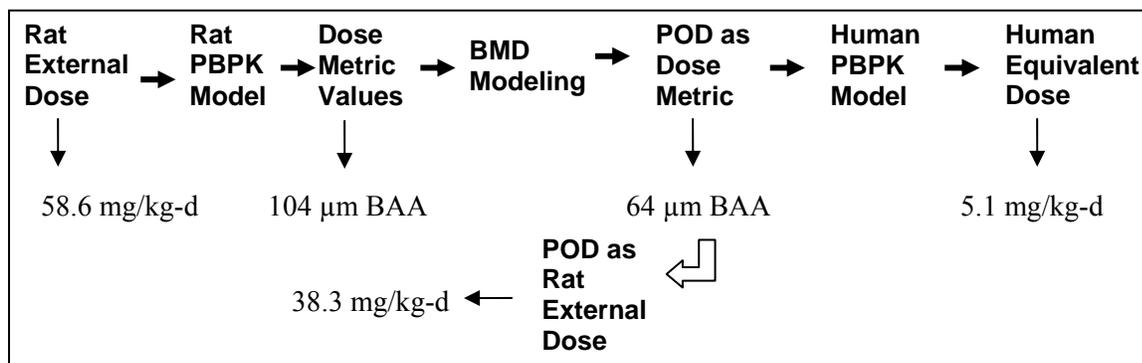
19 Hazard Identification and Dose Response

21 No chronic oral studies are currently available for EGBE; there are only two
22 subchronic 91-day drinking water studies in rats and mice (NTP, 1993). Based on a
23 comparison of NOAELs and LOAELs for hematologic and liver effects, rats are clearly
24 more sensitive than mice. Hematologic and hepatocellular changes were noted in both sexes
25 of rats. In female rats, both hematologic and hepatocellular changes were noted at the low-
26 dose level (58.6 mg/kg-d using water consumption rates and body weights measured during
27 the last week of exposure to estimate dose). Only hepatocellular cytoplasmic changes were
28 observed in low-dose male rats (54.9 mg/kg-d). However, these hepatocellular changes
29 were judged to represent adaptation to a subclinical level of hemolysis produced at this dose.
30 A comparison of the MCV and red blood cell count results for both male and female rats
31 was performed via BMD analysis, which demonstrated that female rats are more sensitive to
32 the effects of EGBE than are males. For this reason, dose-response information on the
33 hematologic effects in female rats was selected as the basis for the oral RfD.

35 Based on extensive review of the literature, hematologic effects appear to be the most
36 sensitive of the adverse effects observed in laboratory animals exposed to EGBE; hematologic
37 effects (e.g., hemoglobinuria) have also been documented in worker populations exposed to
38 technical grade EGBE (Cellosolve) and following ingestion of cleaning products containing
39 EGBE (U.S. EPA, 1999). The LOAEL (58.6 mg/kg-d) used as a starting point in the
40 development of the IRIS RfD is for subchronic MCV changes in female rats (NTP, 1993).

42 In the IRIS assessment, the dose-response relationship was developed by combining
43 PBPK modeling with BMD analysis (Figure 1).

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Figure 1. Analysis Approach for Developing the Human Equivalent Dose with Values Shown

Basis for DDEF

C_{max} for the EGBE metabolite 2-butoxyacetic acid (BAA) in arterial blood of female rats following oral exposure was estimated using the PBPK model of Corley et al. (1994) as modified by Corley et al. (1997). BAA levels, rather than EGBE levels, were deemed appropriate measures of exposure based on the findings of Carpenter et al. (1956), which demonstrated *in vitro* that concentrations of 0.1% BAA induced hemolysis; whereas, hemolysis was not induced by EGBE until concentrations of approximately 2.5% were reached. PBPK modeling was used to translate each of the external doses to dose metrics, C_{max} and AUC. The comparison of the resulting dose metric values to observed erythrocyte responses demonstrated a better correlation for C_{max} than for AUC. Further, the choice of C_{max} as the most appropriate dose metric was confirmed by data that compared erythrocyte effects from gavage and drinking water exposures to similar doses (Ghanayem et al., 1987; Medinsky et al., 1990).

Next, benchmark dose modeling was applied to the results of the PBPK modeling. The results of this analysis indicated that a C_{max} of 64 μM BAA in arterial blood flow is the BMDL_{05} for increased mean MCV in female rats. In comparison, the BAA C_{max} for the LOAEL is 104 μM BAA in arterial blood flow.

The C_{max} value for BAA of 64 μM in arterial blood flow was chosen as the POD for interspecies extrapolation. Next, a human PBPK model was employed to translate this level of the dose metric to an HED of 5.1 mg/kg-d.

DDEF Derivation

The PBPK modeling approach described above was used as the basis for the development of the DDEF. In addition to the data presented in the IRIS file, the rat PBPK model was run to identify that an external dose of 38.3 mg/kg-d in the rat would produce this BMDL_{05} concentration (64 μM) of the BAA metabolite (Dr Richard Corley, personal communication, 2006). The human PBPK model was run to identify the external dose that produced the same level of the dose metric (C_{max} of 64 μM); this value of the dose metric was produced by an

1 external dose of 5.1 mg/kg-d in the human. The C_{\max} dose metric was used as the basis for
2 human equivalence.

3
4 An alternative approach for deriving the DDEF illustrated in this case study would be to
5 use the ratio of rat:human external doses derived from the PBPK model to derive $DDEF_{AK}$ (see
6 Eq. 1, Section 3.2.2.2). Using the data described above, a comparison of the external doses
7 would yield a $DDEF_{AK}$ of $38.3 \text{ mg/kg-d} \div 5.1 \text{ mg/kg-d} = 7.5$. The 7.5-fold DDEF would replace
8 the default UF_{AK} 3-fold factor.

9
10 Applying the $DDEF_{A-TK}$ 7.5-fold* to the rat external dose (38.3 mg/kg-d) yields an HED
11 of 5.1 mg/kg-d. This HED is identical to that derived in the IRIS assessment (U.S. EPA, 1999).

12
13 The above methods can also be compared to the default methodology that is based on
14 body weight scaling (U.S. EPA, 2006). Using a study-specific female rat body weight of 0.188
15 kg, and a default human body weight of 70 kg, these values would result in a default dosimetric
16 adjustment factor (DAF) = $(0.188 \div 70)^{1/4} = 0.23$. The DAF is multiplied by the animal external
17 dose (38.3 mg/kg-d) to yield an HED of 8.7 mg/kg-d, and an apparent value of $38.8 \div 8.7 = 4.4$
18 for the UF_{AK} . The 4.4-fold factor would replace the default 3-fold UF_{AK} . In this case study, the
19 body weight scaling default approach yields a slightly higher RfD than the data derived
20 approaches described above. Furthermore, this approach makes use of quantitative TK data for
21 EGBE.

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23 (*)Note: for EGBE, interspecies toxicodynamic data exist, and have been used in the IRIS
24 assessment to replace the default factor of 3.2 for UF_{AD} with a value of 1. This case study only
25 focused on TK; please see the EGBE $DDEF_{AD}$ case study in this appendix.

26 References

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13 Vinyl Chloride—UF_{AK} Case Study

14 Summary

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16 Vinyl chloride (VC) has an established oral Reference Dose of 3 E-3 (0.003) mg/kg-d in
17 the current Integrated Risk Information System file (U.S. EPA, 2000). The point of departure for
18 liver toxicity is a NOAEL value of 0.13 mg/kg-d from a chronic feeding study in male Wistar
19 rats. PBPK modeling was used to develop a human equivalent dose of 0.09 mg/kg-d, obviating
20 the uncertainty factor for interspecies toxicokinetics. The total UF of 30 comprises an
21 uncertainty factor of 3 to cover toxicodynamic differences between species and a default value of
22 10 for intraspecies variability. Species differences in dosimetry were determined on the basis of
23 external doses required to produce the same level of the toxicologically active metabolite in the
24 critical organ (liver). This case study demonstrates the application of data to inform interspecies
25 toxicokinetic extrapolation (UF_{AK}).
26
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28 Hazard Identification and Dose-Response

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30 Liver was selected as the critical target organ of vinyl chloride in humans and
31 experimental animals. There is a strong epidemiological basis for liver effects in humans.
32 Studies involving workers in the polyvinyl chloride plastics industry from several countries have
33 demonstrated a significant relationship between VC inhalation exposure and liver cancer. While
34 there may be limited evidence suggestive of a risk for other non-liver tumors (e.g., leukemia,
35 brain, lung, pancreas, mammary) “vinylchloride is not likely to be associated strongly with
36 cancers other than liver in humans” (U.S. EPA, 2000). Other noncancer effects noted in
37 epidemiologic investigations include impaired liver function and biochemical and histological
38 evidence of liver damage and focal hepatocellular hyperplasia. Pulmonary function appeared
39 unimpaired, and no solid evidence of teratogenicity in humans has been identified.
40
41

42 Limited data are available for inhalation studies in animals. Data are available from a
43 12-month inhalation study that support liver as the critical organ. Bi et al. (1985) exposed Wistar
44 rats to 0, 10, 100, or 3,000 ppm VC for 6 hours/day, 6 days/week. Cellular alterations,

1 degeneration and necrosis were observed in the seminiferous tubules of the testes, with a
2 NOAEL in the 10 ppm exposure group. This same exposure was the LOAEL for liver effects,
3 characterized only as liver weight changes.
4

5 Like the inhalation results, studies conducted via the oral route identify the liver as the
6 critical target organ. Til et al. (1983, 1991) reported the results of two-year rodent bioassays
7 with VC in feed. Groups of 100 or 50 male and female Wistar rats were exposed to 0, 0.014,
8 0.13, or 1.3 mg/kg-d in feed for only 4 hours/day to minimize volatilization. The VC content of
9 feed was measured before and after feeding to control for volatilization. Multiple hepatic effects
10 were noted including several that were deemed neoplastic or preneoplastic. The pathologists
11 were able to delineate and determine incidences for two effects not thought to represent
12 neoplastic or preneoplastic changes. Liver cell polymorphisms and proliferative bile duct
13 epithelium cysts served as the basis for identifying the liver as the critical target tissue for non
14 cancer effects in the chronic bioassay. These same changes were observed in a second study, but
15 the doses employed in that study (Feron et al., 1981) were higher than those employed by Til and
16 coworkers. Because of a lack of confidence in the outcome from a benchmark dose modeling
17 approach based on external dose, a traditional (NOAEL) approach to dose-response evaluation
18 for events not associated with carcinogenicity was used. The point of departure for species
19 extrapolation was the NOAEL of 0.13 mg/kg-d for liver cell polymorphisms and bile duct cysts.
20

21 The initial process in the mode of action appears to be the formation of reactive and
22 short-lived metabolites that achieve only low steady-state concentrations. These are thought to
23 be responsible for the toxic effects of VC (Bolt, 1978). Experiments that manipulated the
24 longevity of CYP-derived metabolites demonstrated an inverse relationship between metabolite
25 longevity and protein and nucleotide binding (Guengerich et al., 1981). Thus, the metabolism of
26 VC to reactive intermediates was demonstrated to be a critical determinant of toxicity. Because
27 of the short-lived nature of the metabolite(s), a measure of their concentration in the target tissue
28 (liver, the site of their formation) was deemed the appropriate dose metric for quantitative
29 application. This concept and approach has also been applied to methylene chloride (Andersen
30 et al., 1987), and chloroform (ILSI, 1997).
31

32 Basis for DDEF 33

34 The liver was determined to be the target organ, and evidence indicated that formation of
35 a reactive metabolite was likely responsible for toxicity. Benchmark dose modeling of external
36 doses failed. Because of this, the POD for extrapolation was determined as the study NOAEL
37 for liver effects, 0.13 mg/kg-d. Consistent with guidance for RfC derivation, PBPK modeling of
38 the formation of the active metabolite in liver was used as the basis for determining an equivalent
39 human dose. PBPK models were developed for rats and humans and used to extrapolate
40 dosimetry between species (Clewell et al., 1995a,b), were subjected to an external peer review
41 and deemed sufficient for quantitative reliance. The NOAEL dose (0.13 mg/kg-d) was converted
42 into the dose metric for VC—the amount of metabolite in the liver (AML), with units of
43 concentration mg/liter of liver.
44

DDEF Derivation

Importantly for dose extrapolation, these models demonstrated a linear relationship between applied dose and the dose metric (AML) up to doses approximating 25 mg/kg-d. This allowed linear interpolation to be used to identify levels of external doses associated with specific amounts of the internal dose, rather than specific iterations via PBPK modeling. The rat NOAEL dose of 0.13 mg/kg-d produced AML at a value of 3.0 mg/L (Figure 2). This level of internal exposure in the 70-kg human was determined to result from a drinking water exposure of 0.09 mg/kg-d (the human equivalent dose). Thus, doses of 0.13 mg/kg-d and 0.09 mg/kg-d in the rat and human, respectively, are toxicokinetically equivalent.

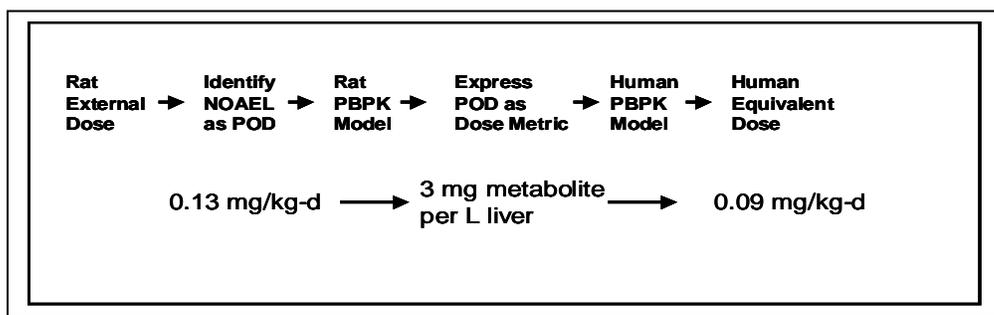


Figure 2. Schematic for dose extrapolation for vinyl chloride. The POD was defined as the external dose in the rat, with PBPK Modeling of the rat. PBPK modeling of the dose metric translated dose from units of applied dose to units of tissue concentration, representing the dose metric. PBPK modeling in the human identified the human equivalent dose, the dose producing the same level of the dose metric in the rat study at the POD.

The IRIS file for VC used the human equivalent dose as the POD, to which UFs are applied. An alternative approach illustrated in this case study to deriving the data derived extrapolation factor for interspecies toxicokinetic differences would be to use the ratio of rat and human external doses resulting in the same level of target tissue exposure at the POD.

Using the data described above (see Section 3.2.2.2), these data indicate a DDEF value for interspecies toxicokinetic extrapolation of $0.13 \div 0.09 = 1.44$. Combined with the default value of 3 for toxicodynamics, a DDEF for interspecies extrapolation (UF_A) would be $1.44 * 3 = 4.32$, in place of the default value of 10. By applying the DDEF calculated above (4.32-fold) and the 10-fold intraspecies UF to the animal NOAEL (0.13 mg/kg-d), the RfD is $0.13 \text{ mg/kg-d} \div 43.2 = 0.003 \text{ mg/kg-d}$. This is the same value that was calculated for the IRIS assessment, which was expressed as the HED/UF, or $0.09 \text{ mg/kg-d} / 30$ (U.S. EPA, 2000).

The above methods can also be compared to the default methodology that is based on body weight scaling (U.S. EPA, 2006). Using a default value of 0.462 kg for adult Wistar rats (U.S. EPA, 1988) and applying the body weight scaling approach (U.S. EPA, 2006) to the NOAEL of 0.13 mg/kg-d, a HED can be calculated as:

1 0.13 mg/kg-d * 0.462 kg = 0.060 mg
2 0.06 mg * (70kg ÷ 0.462kg)^{3/4} =
3 0.06 mg * 43.19 = 2.59 mg
4 2.59 mg ÷ 70 kg = 0.037 mg/kg-d
5

6 In this case study, the body weight scaling default approach yields a slightly lower RfD
7 than the data derived approaches described above. Furthermore, this approach makes use of
8 quantitative data on VC.

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13 INTERSPECIES ADJUSTMENT FOR TOXICODYNAMICS

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15 Ethylene Glycol Monobutyl Ether (EGBE)— UF_{AD} Case Study

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17 Summary

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19 Ethylene glycol monobutyl ether (EGBE; AKA 2-butoxy ethanol) has an established oral
20 reference dose of 0.5 mg/kg-d in the U.S. EPA IRIS database. This value is based on the results
21 of a subchronic drinking water study in mice and rats, where changes in mean corpuscular
22 volume (MCV) were determined to be the critical effect (U.S. EPA, 1999a). Derivation of the
23 HED of 5.1 mg/kg-d is detailed in the EGBE TK case study earlier in this appendix and in the
24 IRIS file for EGBE (U.S. EPA, 1999a,b).

25

26 A total UF of 10 was applied to this HED to derive the RfD. An UF of 10 was used to
27 account for variation in sensitivity within the human population (UF_H) and UF_A was reduced to 1
28 on the basis of dosimetry adjustments to account for TK (discussed in EGBE case study earlier in
29 this appendix) and the relatively greater sensitivity of the rat compared to the human for TD. *In*
30 *this case study, issues associated with EGBE TD are described and an alternative approach to*
31 *DDEF derivation based on the use of in vitro data are described.*

32

33 Hazard Identification and Dose Response

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35 Based on extensive review of the literature, hematologic effects appear to be the most
36 sensitive of the adverse effects observed in laboratory animals exposed to EGBE. Hematologic
37 effects (e.g., hemoglobinuria) have also been documented in worker populations exposed to
38 technical grade EGBE (Cellosolve) and following ingestion of cleaning products containing
39 EGBE (U.S. EPA, 1999a,b).

40

41 Key events in the proposed mode of action include:

42

- 43 • Oxidative metabolism to butoxyacetic acid (BAA)

- 1 • Erythrocyte swelling and lysis (probably preceded by an increase in osmotic fragility and
2 loss of deformability)
- 3 • Decreased erythrocyte count, hemoglobin, and hematocrit, and in response, increased
4 production of immature erythrocytes (reticulocytes) by the bone marrow
5

6 Carpenter et al. (1956) incubated erythrocytes from rats, mice, rabbits, monkeys, dogs,
7 humans and guinea pigs with 0.1% butoxyacetic acid (BAA). Results demonstrating maximum
8 time without hemolysis were 35-40, 40-45, 60-90, 103-120, 147-268 and 360 minutes,
9 respectively. These data indicate that guinea pigs and humans are more resistant and that rats
10 and mice may be more sensitive to the hemolytic effects of BAA. Studies with Cellosolve and
11 BAA demonstrated that RBC were much more sensitive to BAA than Cellosolve. These results
12 led Carpenter et al. (1956) to speculate that the BAA metabolite of Cellosolve was responsible
13 for hemolysis.

14 In a direct comparison of the effects of BAA on rat and human erythrocytes, Udden and
15 Patton (1994) devised a study using filtration, phase contrast light microscopy and routine
16 hematologic methods. RBC were obtained from healthy adults via venipuncture and from 9-11
17 week old male Fischer 344 rats via cardiac puncture. Rat RBCs were incubated with 0.2 and 2.0
18 mM BAA; human erythrocytes were incubated with 2.0 mM BAA only. Rat RBC demonstrated
19 30% hemolysis after incubation with 2.0 mM BAA for 4 hours, and 4% hemolysis after
20 incubation with 0.2 mM BAA for 6 hours or longer. The 4% measure was “mild,” and the data
21 were not shown. Incubation of human RBC with 2.0 mM BAA for 4 hours resulted in no
22 increase in hemolysis over the background (control) level of 1%. Histologic evaluations of rat,
23 but not human RBC preparations post-exposure demonstrated erythrocyte “ghosts,” which are
24 cell membranes from lysed cells. These incubation conditions resulted in increases in mean
25 corpuscular volume (MCV) in rat RBC incubated with 0.2 mM BAA, but not human RBC
26 incubated with 2.0 mM BAA. The increase in MCV is indicative of swelling of RBCs, an event
27 which leads to fragility.
28

29 Finally, these investigators examined the deformability of RBC by pumping them
30 through a narrow-diameter filter and monitoring an increase in inflow pressure. The results were
31 presented graphically, as pressure versus time plots. The logic behind this study was that while
32 RBC normally can deform to pass through tight spaces (like in capillaries), RBC in which
33 swelling has been induced and in which other mechanisms may lead to increased membrane
34 rigidity, the passage through the membrane pores will be limited, resulting in an increase in
35 inflow pressure. Rat erythrocytes demonstrated a roughly tripled pressure when incubated with
36 2.0 mM BAA for 4 hours compared to controls, and pressure was nearly doubled in rat RBC
37 incubated with 0.2 mM BAA for 4 hours. In contrast, pressure differences between human RBC
38 incubated in the absence of BAA and in the presence of 2.0 mM BAA for 4 hours were not
39 distinguishable. These authors indicated that their findings that human RBCs were less sensitive
40 *in vitro* to the hemolytic effects of BAA were consistent with multiple other findings.
41

42 Ghanayem and Sullivan (1993) also performed a species comparison of BAA-induced
43 RBC effects *in vitro*. An advantage of this study is the application of pairwise statistical design
44 to determine the effect of treatment over control. Male Fischer 344 rats 15 weeks of age were
45 used, and human blood was drawn from adult male donors. BAA (2.0 mM incubated up to

1 4 hours) produced only slight, but not statistically significant alterations of hematological
2 parameters: hematocrit was increased 2-4% and MCV was increased less than 4% above vehicle
3 controls. The authors concluded that their results demonstrated that humans were “minimally
4 sensitive” to the *in vitro* effects of BAA. In comparison, RBC from rats were deemed “relatively
5 susceptible” to the effects of BAA. A graphic presentation of data demonstrated that in rats,
6 MCV and hematocrit were increased nearly 60% when exposed to 2.0 mM BAA.

7 8 Basis for DDEF

9
10 Several data sets are available in which the responsiveness of rat and human blood at 2
11 mM can be compared. *However, comparison of effect data for TD is most appropriately*
12 *accomplished by comparing the different concentrations resulting in the same response.*

13
14 Hemolytic effects were observed in rat erythrocytes exposed to BAA at concentrations as
15 low as 0.5 mM. In contrast, hemolytic effects were not observed in human blood exposed to 4
16 mM BAA, and only slight (but significant) hemolytic effects were observed at 8 mM. The IRIS
17 file indicates that these differences approximate 15-fold (8 mM/0.5 mM) difference in
18 sensitivity—with humans being less sensitive than rats.

19 20 DDEF Derivation

21
22 One potential approach would be to derive an adjustment factor of 0.06 for UF_{A-TD}
23 (0.5 mM/8 mM = 0.06). This approach requires the assumption that the TD differences observed
24 *in vitro* would be approximated *in vivo*. Uncertainty regarding this issue prompted NCEA to
25 take a different approach. In the IRIS assessment, the UF value for UF_{A-TD} was established at 1;
26 because: “...a fractional component of the UF_A was considered. However, the *in vivo* relative
27 insensitivity of humans cannot be quantified at this time. Thus, a value of 1 was used to account
28 for pharmacodynamic differences between rats and humans.” (U.S. EPA, 1999b).

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31
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8
9

10 **Dimethyl Arsenic Acid (DMA)—UF_{AD} Case Study**

11

12 1. Summary

13

14 Dimethyl arsenic acid (DMA), also known as cacodylic acid, is an herbicide used on
15 primarily cotton and turf. DMA is also a urinary metabolite in most mammals, including
16 humans, following direct exposure to inorganic arsenic. The Office of Pesticide Programs, in
17 collaboration with NHEERL scientists, developed a non-linear mode of action assessment for
18 DMA for the development of rat bladder tumors (U.S. EPA, 2006). The MOA and dose-
19 response assessments were developed using the MOA and Human Relevance Frameworks. The
20 information provided by the mode of action analysis also provides the basis for the chronic RfD
21 and the respective UFs in the Registration Eligibility Decision (RED) for cacodylic acid. Instead
22 of the default 10-fold factor to extrapolation from animal to human, OPP has reduced the TD
23 component of UF_A to 1X. This case study describes the determination of the 3X factor which
24 was used to account for UF_{A-TK}. The default UF_H of 10X factor was also applied leading to a
25 composite factor of 30X.

26

27 2. Hazard Identification and Dose-Response

28

29 In rat carcinogenicity studies, oral exposure to DMA^V leads to bladder tumors. The
30 overall weight of the evidence provides convincing support for a non-linear mode of action for
31 DMA^V-induced carcinogenesis in rodents. The key events include:

32

- 33 • Reductive metabolism of DMA^V to DMA^{III}.
- 34 • DMA^{III} causes urothelial cytotoxicity. Regenerative cell proliferation then ensues in
35 order to replace dead urothelial cells. The amount of cell killing is a function of the
36 severity of the cytotoxicity which is related to the amount of DMA^{III} present. The
37 amount of DMA^{III} is dependent on the conversion of DMA^V to DMA^{III}.
- 38 • Sustained cytotoxicity leads to regenerative cell proliferation which, in turn, ultimately
39 leads to hyperplasia and bladder tumors.

40

41 To obtain a tumor via the proliferation/replication genetic error process, induced cell
42 proliferation needs to be persistent. There is convincing experimental evidence to indicate that
43 this is the case for the rat bladder. There is a clear association of DMA^V treatment and cell

1 killing/regenerative proliferation and bladder tumors. The amount of proliferation would be a
2 function of the amount of cell killing since the tissue will undergo regenerative proliferation in
3 response to cell killing. As the severity of cytotoxicity increases with increasing levels of DMA^V
4 (DMA^{III}), regenerative proliferation is the rate limiting step for tumor formation, even though the
5 product is chromosome mutations. Thus, a tumor dose-response curve would be influenced by
6 the induced cell proliferation curve, even though chromosomal mutations may be an output.
7 DMA^V-induced tumors would only be produced at treatment durations and dose levels that result
8 in significant cell killing and regenerative cell proliferation in the urothelium of the bladder.
9 Experimental data are available to support the coincidence of key events at similar concentration
10 levels. The levels of DMA^{III} in the urine of rats treated with 100 ppm DMA^V range from
11 0.5-5.0uM. The LC₅₀ values for DMA^{III} in rat and human urinary epithelial cells *in vitro* are
12 0.5-0.8uM. There is a significant increase in chromosome aberrations in human lymphocytes *in*
13 *vitro* at about 1.35 μM DMA^{III}. At 100 ppm, there is significant cell killing and regenerative
14 proliferation in female rat bladders. It appears that chromosomal mutations, cytotoxicity and cell
15 proliferation can potentially occur concurrently at 100 ppm DMA^V, the tumorigenic dose in
16 female rats via the feed.

17
18 Among the several key events, all of which are necessary for tumor formation, cell
19 proliferation has been used for deriving a point of departure because it is needed for increasing
20 the likelihood of chromosome mutation formation and for the perpetuation of genetic errors, as
21 well as for hyperplasia. A BMDL₁₀ value (0.43 mg/kg bw/day) is the basis for the point of
22 departure in deriving reference dose or a margin of exposure. This approach is considered public
23 health protective because a BMDL₁₀ of 0.43 mg/kg bw/day is approximately an order of
24 magnitude lower than the dose (~0.7 mg/kg bw/day or 10 ppm) that resulted in a 1.5-fold
25 nonstatistical increase in cell proliferation after 10 weeks of exposure to DMA^V and about two
26 orders of magnitude lower than the dose (~9.4 mg/kg bw/day) resulting in neoplasia in the
27 feeding studies.

28 29 3. Basis for DDEF

30
31 In the 2006 DMA risk assessment, instead of the default 10-fold factor to extrapolate
32 from animal to human, OPP has reduced the TD component of UF_A to 1X. A 3X factor was
33 used to account for UF_{A-TK}. UF_{A-TD} was reduced to 1X given that at a similar dose at the target
34 site (i.e., bladder urothelial), that humans and rats are expected to respond pharmacodynamically
35 similar. This case is built on a combination of information:

- 36
37 • Chemical-specific *in vitro* data from Cohen et al. (2002) which show that human and rats
38 cell respond similarly to exposure to DMA^{III}—the LC₅₀s for cytotoxicity in human and
39 rat epithelial cells were very similar (0.8 μM and 0.5 μM, respectively).
- 40
41 • There are microarray support (Sen et al., 2005). Qualitatively the genes up-regulated in
42 human urinary bladder epithelial (UROtsa) are similar to those up-regulated in rat urinary
43 bladder epithelial cells (MYP3) exposed to DMA^V *in vitro*. In this study, the rat cell line
was quantitatively more sensitive compared to the human cell line.

- 1 • General information on the development and function of the bladder along with incidence
2 of bladder tumors in human populations qualitatively supports the animal mode of action
3 in humans.
4

5 There are known pharmacokinetic differences between rats and humans. These
6 pharmacokinetic differences include: sequestration of DMA^{III} by rat hemoglobin which results in
7 a long retention time in the rat compared to humans or mice, and the increased urinary output of
8 TMAO in rats compared to humans. Because of uncertainties regarding quantifying the tissue
9 dose in humans using rat data, and in the absence of a fully developed PBPK model at the time
10 of the risk assessment, an inter-species extrapolation factor of 3X was applied. Pharmacokinetic
11 analyses indicate that for similar chronic low level exposures, rats would take longer to achieve
12 steady state concentrations of DMA^V and metabolites in target tissue compared to humans and
13 that for a given exposure target tissue concentrations would be elevated for a longer time after
14 exposure ceased in the rat because rat hemoglobin acts as a slow release storage depot. Note that
15 half-life in the rat for DMA^V appears to correlate with erythrocyte half-life, indicating that the
16 binding to hemoglobin is not readily reversible. There are, however, uncertainties regarding the
17 quantitative differences between rat and human which prevents further reduction of the inter-
18 species factor.
19

20 4. DDEF Derivation

21

22 The TD component of UF_A was reduced to 1X based on the bullets above in combination
23 with the animal MOA and implementation of the Human Relevance Framework. Together, the
24 weight of the evidence provides a strong case for TD equivalence between rats and humans. A
25 3X factor was used to account for UF_{A-TK}. The standard UF_H of 10X factor was also applied
26 leading to a composite factor of 30X
27

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29

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42
43

1 ***N*-methyl Carbamate Pesticides (NMCs)—UF_{AD} Case Study**

2 3 Summary

4
5 The Office of Pesticide Programs released its revised cumulative risk assessment for the
6 *N*-methyl carbamates (NMCs) in 2007 (U.S. EPA, 2007). As required under the Food Quality
7 Protection Act (FQPA, 1996), a cumulative risk assessment incorporates exposures from
8 multiple pathways (i.e., food, drinking water, and residential/non-occupational exposure to
9 pesticides in air, or on soil, grass, and indoor surfaces) for those chemicals with a common
10 mechanism of toxicity. A cumulative risk assessment begins with the identification of a group of
11 chemicals, called a Common Mechanism Group (CMG), which induces a common toxic effect
12 by a common mechanism of toxicity. The NMCs were considered to be a CMG due to their
13 common inhibitory actions on acetylcholinesterase, an enzyme that is normally required for
14 ending cholinergic transmission in the nervous system.

15
16 This case study describes a sensitivity analysis conducted in the risk characterization
17 phase of the revised CRA using a DDEF approach. TD equivalence for animal to human
18 extrapolation (UF_{A-TD} = 1X) was assumed for several chemicals for which human data were not
19 available.

20 21 Hazard Identification and Dose Response

22
23 The NMCs were established as a CMG by U.S. EPA in 2001 (U.S. EPA, 2001) based on
24 their similar structural characteristics and shared ability to inhibit acetylcholinesterase (AChE)
25 by carbamylation of the serine hydroxyl group located in the active site of the enzyme. When
26 AChE is inhibited, acetylcholine accumulates and results in cholinergic toxicity, due to
27 continuous stimulation of cholinergic receptors throughout the central and peripheral nervous
28 systems that innervate virtually every organ in the body. An important aspect of NMC toxicity is
29 the rapid nature of the onset and recovery of effects; following maximal inhibition of
30 cholinesterase (typically between 15 and 45 minutes), recovery occurs rapidly (minutes to
31 hours).

32
33 Inhibition of AChE is considered the first and critical step in the toxicity of NMCs.
34 Human health monitoring has capitalized on the availability of blood cholinesterase
35 measurements, and these have been widely accepted as a marker of exposure. However, since
36 the brain AChE may be considered more as the critical target site, data on inhibition of brain
37 AChE are obviously only available using laboratory animals. Brain AChE data have been
38 widely used as a point of departure for risk assessment.

39
40 U.S. EPA used the relative potency factor (RPF) method to determine the combined risk
41 associated with exposure to NMCs. Briefly, the RPF approach uses an index chemical as the
42 point of reference for comparing the toxicity of the NMC pesticides. RPFs are calculated as the
43 ratio of the toxic potency of a given chemical to that of the index chemical and are used to
44 convert exposures of all chemicals in the group into exposure equivalents of the index chemical.
45 Because of its high quality dose response data for all routes of exposure, as well as high quality

1 time-to-recovery data, U.S. EPA selected oxamyl as the index chemical for standardizing the
 2 toxic potencies and calculating relative potency factors for each NMC pesticide.

3
 4 Basis for a DDEF

5
 6 In the single chemical assessments for most NMCs, a default 10X factor for animal-to-
 7 human extrapolation was used. For three NMCs (aldicarb, methomy and oxamyl), however,
 8 there are studies in human subjects that were determined by U.S. EPA, after considering the
 9 advice of the Human Studies Review Board, to be ethically and scientifically acceptable for use
 10 in risk assessment. These studies were used to derive the chemical-specific inter-species
 11 extrapolation factor for these three chemicals. Table 2 summarizes the dose-response and time-
 12 course modeling data for critical rat and human studies for these three NMCs.

13
 14 Table 2. NMC CRA: Inter-species Extrapolation Factors and Corresponding Rat and Human
 15 BMD_{10S} and BMDL_{10S}
 16

Chemical	Rat						Human			Inter-species UF
	Brain			RBC			RBC			
	BMD ₁₀ (mg/kg)	BMDL ₁₀ (mg/kg)	¹ / ₂ life (hrs)	BMD ₁₀ (mg/kg)	BMDL ₁₀ (mg/kg)	¹ / ₂ life (hrs)	BMD ₁₀ (mg/kg)	BMDL ₁₀ (mg/kg)	¹ / ₂ life (hrs)	
Aldicarb	F = 0.048 M = 0.056	F = 0.035 M = 0.035	1.5	0.031	0.020	1.1	0.016	0.013	1.7	2X
Methomyl	0.486	0.331	1.0	0.204	0.112	0.8	0.040	0.028	1.6	5X
Oxamyl	F = 0.145 M = 0.185	F = 0.111 M = 0.143	0.9	0.278	0.158	0.8	0.083	0.068	2.4	3X

17
 18
 19 With regards to the UF_{AK}, NMCs have similar metabolic profiles across species. NMCs
 20 do not require activation; the parent compound is an active AChE inhibitor. Although some
 21 metabolites of NMCs have been shown to be active as well, none have been shown to be more
 22 potent than the parent chemical. Thus, metabolism is considered to be a detoxification process.
 23 As such, species differences in tissue dosimetry are likely correlated with differences in body
 24 weight to the ³/₄ power and are also consistent with a 3X factor to account for inter-species
 25 differences in toxicokinetics (U.S. EPA, 2006).

26
 27 The mechanism of toxic action of NMCs is reproducible across a range of species,
 28 including rodents and humans. In addition, the AChE enzyme in humans and rats has similar
 29 function and structure (see reviews by Radic and Taylor, 2006; Sultatos, 2006). The half-life to

1 recovery values¹ for rats and humans provided in Table 2 range from approximately 1 to 2 hours
2 and demonstrate the similarity of the half-lives of the two species. Based on this information,
3 given a similar dose or concentration at the target site, it is likely that human and rat AChE
4 would respond similarly. This understanding can inform the interspecies DDEF (UF_{AD}).
5

6 DDEF Derivation

7

8 For the CRA, toxic potencies for the NMCs were determined using brain AChE
9 inhibition measured at peak inhibition following gavage exposures in rats. The Agency used an
10 exponential dose-time-response model to develop benchmark dose estimates at a level estimated
11 to result in 10% brain cholinesterase inhibition (i.e., a benchmark dose or BMD_{10}) to estimate
12 relative potency. Ratios comparing doses (administered) that produce the same magnitude of
13 effect may then be derived. Using the data in Table 2, and dividing the BMD_{10-rat} by the
14 $BMD_{10-human}$ for RBD cholinesterase inhibition:
15

16 Aldicarb: $0.031 \div 0.016 = 1.9 \approx 2$

17 Methomyl: $0.204 \div 0.040 = 5.1 \approx 5$

18 Oxamyl: $0.278 \div 0.083 = 3.3 \approx 3$
19

20 This analysis showed that the ratio of the BMDs for rat/human ranges from 2 to 5 for
21 these NMCs. This range would tend to support the DDEF approach described here to reduce the
22 standard 10X inter-species factor to 3X. The concentration of toxicant at the active site *in vivo* is
23 controlled by toxicokinetic processes. The available data described TD processes (enzyme
24 regeneration) and indicated that rats and humans were very similar in this area. The remaining
25 three-fold (default) value for UFA addresses species differences in toxicokinetics.
26

27 It may be possible to use *in vitro* studies using human and rat tissues and human and rat
28 AChE to test this hypothesis. In other words, it may be possible to use *in vitro* studies to
29 demonstrate toxicodynamic equivalence between rats and humans. If these data were available
30 and they showed toxicodynamic equivalence, the Agency could reduce the inter-species factor
31 for those NMCs without human toxicity studies to 3X. Because of the lack of these *in vitro*
32 studies, the Agency does not believe it appropriate at this time to further refine the standard 10X
33 factor for inter-species extrapolation. Instead, the Agency has used the DDEF approach as a
34 sensitivity analysis in its risk characterization.
35

36 In this sensitivity analysis, the 10X inter-species factor was reduced to 3X based on the
37 assumption of toxicodynamic equivalence for carbaryl, carbofuran, and formetanate HCl. These
38 three NMCs were identified since they were shown in the CRA to contribute a large portion of
39 the estimated human exposure to the cumulative risk to this group. The results of this sensitivity
40 analysis for the food exposure assessment are shown below in Table 3. The Agency has used a
41 probabilistic approach to the food exposure assessment. The estimated exposures of the NMC as

¹ Recovery half-life differs from elimination half-life. While elimination half-life is not an acceptable basis for calculation of a DDEF for *toxicokinetic* components, enzyme regeneration measurements (the subject of the present analysis) are also expressed in half-life values. This usage refers to regeneration of enzymatic activity or de novo synthesis of additional enzyme (protein). In this instance, "half-life" measures are an adequate basis for derivation of DDEF values for *toxicodynamic* events.

1 a group (i.e., oxamyl equivalents) and the margins of exposure (MOE) at the 99.9th percentile are
 2 shown here. The target MOE is 10 or higher for intra-species extrapolation. The table compared
 3 the MOEs when using the standard UF to those obtained with the modified UF_A.

6 Table 3. Sensitivity Analyses NMC Cumulative Food Assessment: DDEF Approach for Inter-
 7 species UF

Age Group		Estimated Exposure at the 99.9 th Percentile (mg/kg oxamyl equivalents)	MOE at the 99.9 th Percentile	Percentile at Which Target of 10 is Reached
Baseline CRA	Children 1-2	0.0229	7.9	99.848 th
	Children 3-5	0.0209	8.6	99.870 th
DDEF Approach for Inter-species UF	Children 1-2	0.0183	9.8	99.896 th
	Children 3-5	0.0171	10.5	N/A

11 Review of this table shows that:

- 13 • Using the standard 10X inter-species factor for all NMCs without human data, the
 14 (baseline) MOEs at the 99.9th percentile of exposure are less than 10 for children 1-2 and
 15 3-5 years. Furthermore, they reach the target of 10 at the 99.848th and 99.870th
 16 percentiles of exposures.
- 17 • When considering an alternative approach to the inter-species factor which assumes a 3X
 18 inter-species factor for carbaryl, carbofuran, and formetanate HCl, the MOEs at 99.9th
 19 percentile of exposure increase to 9.8 and 10.5 for children 1-2 and 3-5 years—a 20%
 20 increase in MOEs. The exposure for the younger age group reaches the target MOE of
 21 10 at the 99.896th percentile of exposures.

23 This sensitivity analysis suggests an opportunity where additional data would provide a
 24 substantial improvement in the refinement of the CRA.

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16
17

18 **INTRASPECIES ADJUSTMENT FOR TOXICOKINETICS—** 19 **(UF_{HK})**

21 **Boron and Compounds**

23 Summary

25 In the U.S. EPA IRIS file for Boron and Compounds (posted 2004), an RfD value of 2X
26 10⁻¹ mg/kg-d has been developed based on the critical developmental effect of decreased fetal
27 weights (U.S. EPA, 2004). The point of departure for the derivation of this value is the BMD L₀₅
28 value of 10.3 mg/kg-d. The BMD L₀₅ is based on the combined results of two studies chosen for
29 the derivation of the RfD (Heindel et al., 1992; Price et al., 1994, 1996). Using data from rats
30 (Vaziri et al., 2001) and humans (Pahl et al., 2001), a mathematical model was applied to the TK
31 component of the interspecies extrapolation factor to address interspecies toxicokinetics. The
32 interspecies toxicokinetic adjustment factor was 3.3. An intra-human kinetic adjustment factor
33 of 2.0 was estimated from three studies (Dunlop, 1981; Krutzén et al., 1992; Sturgiss et al.,
34 1996), using glomerular filtration rate as a surrogate for boron clearance. The remaining
35 uncertainty in the RfD derivation was from toxicodynamics. Inter-species and intra-human
36 toxicodynamic uncertainty were assigned the default value of 3.16. The product of all the
37 adjustment and sub-factors served as the total adjustment factor of 66. The RfD was derived by
38 dividing the BMDL₀₅ of 10.3 mg/kg-d by the adjustment factor and rounding to one digit. This
39 case study demonstrates the use of data to develop an intraspecies toxicokinetics extrapolation
40 (UF_{H-TK}). Specifically, it demonstrates how an intra-human kinetic adjustment factor of 2.0 was
41 estimated from three studies (Dunlop, 1981; Krutzén et al., 1992; Sturgiss et al., 1996), using
42 glomerular filtration rate as a surrogate for boron clearance.
43

1 Hazard Identification and Dose-Response

2
3 Oral animal studies have identified the testes and the developing fetus as the two most
4 sensitive targets of boron toxicity in multiple species (U.S. EPA, 2004). Testicular effects
5 include reduced organ weight and organ:body weight ratio, atrophy, degeneration of the
6 spermatogenic epithelium, impaired spermatogenesis, reduced fertility, and sterility. The
7 mechanism of action for boron's effect on the testes is not known, but the available data suggest
8 an effect on the Sertoli cell. Developmental effects following oral exposure to boron have been
9 reported in mice, rabbits, and rats and include high prenatal mortality; reduced fetal body weight;
10 and malformations and variations of the eyes, CNS, cardiovascular system, and axial skeleton.
11 Similarities in the NOAEL values for the reproductive toxicity studies and quality control issues
12 complicated the choosing of testicular effects as the critical effect.

13
14 Developmental effects (decreased fetal weights) are considered the critical effect. The
15 studies by Price et al. (1994, 1996), NTP (1990) and Heindel et al. (1992) in rats were chosen as
16 critical developmental studies because they were well-conducted studies of a sensitive endpoint
17 that identified both a NOAEL and LOAEL. Rats were more sensitive than mice and rabbits,
18 which were also studied for developmental toxicity.

19
20 The POD was determined by BMD modeling. BMD evaluation of multiple
21 developmental endpoints identified decreased fetal body weight as the most suitable endpoint.
22 Two studies (Heindel et al, 1992; Price et al., 1994, 1996) provided data on fetal body weight
23 and the results were combined for BMD evaluation. The benchmark response (BMR) level for
24 mean fetal weight was chosen to be the BMD L₀₅ value of 10.3 mg/kg-d.

25
26 No data are available to identify a mode of action, but boron is absorbed, distributed and
27 eliminated unchanged in urine. It is not metabolized, so some measure of exposure to parent
28 compound should serve as the basis for dose (exposure) expression.

29 Basis for DDEF

30
31
32 Following administration, boron is rapidly absorbed and distributed throughout the body.
33 It distributes with total body water; concentrations in all tissues examined were similar. Bone
34 and fat tissues represent outliers—boron seems to accumulate in bone and the low water content
35 of adipose tissue reduces boron distribution to fat.

36
37 Given the relatively uniform distribution of boron to the tissues and that the majority of
38 the compound is excreted quickly, the likelihood for sequestration of boron by a given tissue is
39 minimal. Although there are no direct measurements of fetal boron concentrations, boron
40 concentrations in the fetus should be the same as in the mother because boron is freely diffusible
41 across biological membranes and will rapidly and evenly equilibrate in all body water
42 compartments. As the boron RfD is based on developmental effects observed in rats, the most
43 relevant kinetic data are those pertaining to pregnant rats and pregnant humans. There are
44 insufficient data to compare plasma boron in rats and humans at the same exposure levels.
45 Therefore, boron clearance is used as an estimator of internal dose.

1 Since boron is not metabolized, clearance from blood and tissues is via urinary
 2 elimination. Data demonstrate that boron is a small, uncharged molecule, with no protein
 3 binding. Evidence from human dialysis studies indicates clearance is via passive diffusion.
 4 These (and other) data identify passive renal mechanisms as those most governing boron
 5 clearance. For that reason, the most appropriate renal measurement for boron clearance is the
 6 glomerular filtration rate (GFR). Several studies have characterized the variability of GFR
 7 among pregnant humans (Table 4). The application of these data describing variance of GFR
 8 among pregnant humans serves as the basis for estimating human interindividual differences in
 9 internal exposure.

10 DDEF Derivation

13 For the assessment of intraspecies toxicokinetic variability, glomerular filtration rate
 14 (GFR) is used as a surrogate for boron clearance. Although the study of Pahl et al. (2001)
 15 provides an estimate of boron clearance variability in pregnant women, the data are judged to be
 16 inadequate for this purpose. As boron clearance is largely a function of GFR, the larger more
 17 certain data base on GFR and its variability among humans is used to estimate boron clearance
 18 variability. Because the measured boron clearances in the rat and human kinetic studies were
 19 less than GFR, tubular reabsorption could be contributing to the variability of boron clearance in
 20 the population. Variability in these factors, however, is judged to be minor in comparison to the
 21 variability in GFR.

22
 23 Table 4. Measures of GFR Variability Among Pregnant Women
 24

Study	Mean GFR(mL/min)	Standard Deviation
Dunlop	150.5 ^a	17.6
Krutzen	195 ^b	32
Sturgiss	138.9 ^c	26.1

25
 26 ^a Serially-averaged observations across three time periods (16, 26, and 36 weeks) for 25 pregnant women.

27 ^b Third trimester values for 13 pregnant women.

28 ^c Serially-averaged observations across two time periods (early and late pregnancy) for 21 pregnant women (basal
 29 index plus basal control individuals).

30
 31
 32 GFR data have been used previously in the context of the boron RfD by Dourson et al.
 33 (1998), who proposed the ratio of the mean GFR to the GFR value 2 standard deviations (SD)
 34 below the general population mean (mean/[mean - 2 SD]) as the metric for the intraspecies
 35 toxicokinetic adjustment factor. This approach is referred to as the sigma method, which is a
 36 common term used for statistical methods using multiple standard deviations to establish
 37 “acceptable” lower bounds. For the derivation of AF_{HK}, the sigma method is modified by using
 38 3 SD as the reduction factor for establishing the lower bound (i.e., mean GFR - 3 SD). The basic
 39 formula modified from Dourson et al. (1998) for AF_{HK} is:
 40

$$GFR_{AVG}^{AFHK} = GFR_{AVG} - 3SD_{GFR}$$

where GFR_{AVG} and SD_{GFR} are the mean and standard deviation of the GFR (mL/min) for the general healthy population of pregnant women. The use of three standard deviations rather than two (as in Dourson et al., 1998) is based on a statistical analysis of the published GFR data, with more consideration being given to the full range of GFR values likely to be found in the population of pregnant women. In the aggregate, the data suggest that a lower bound GFR 2 SD below the mean does not provide adequate coverage of the susceptible sub-population (those pregnant women experiencing or predisposed to preeclampsia). While no conclusive information exists from controlled-dose studies in humans, it may be possible that the variability in boron clearance might be greater than GFR variability. Therefore, AF_{HK} must also account for any residual uncertainty in using GFR as a surrogate.

Three studies (Dunlop, 1981; Krutzén et al., 1992; Sturgiss et al., 1996) were found to address GFR variability in pregnant women. Dunlop (1981) assessed GFR for 25 women at three different time points during pregnancy (16, 26, and 36 weeks) and again after delivery. In this study (Dunlop, 1981) GFR was measured as inulin clearance and the overall average and standard deviation was 150.5 and 17.6 mL/min, respectively. Sturgiss et al. (1996) performed a similar assessment of GFR (also using inulin clearance) for 21 women in early (12-19 weeks) and late (30-35 weeks) pregnancy and again at 15-25 weeks post partum and found a mean GFR of 138.9 mL/min with a standard deviation of 26.1 mL/min. Krutzén et al. (1992) evaluated GFR during pregnancy for four different groups of women (13 normal healthy women, 16 diabetic women, 8 hypertensive women, and 12 women diagnosed with preeclampsia) by using iohexol clearance in the second and third trimester and again 6-12 months post partum. In this study (Krutzen et al, 1992) the third trimester mean GFR and standard deviation for the healthy women was reported as 195 and 32 mL/min, respectively. In general, the GFR values reported in this study are much higher than those reported by Dunlop (1981) and Sturgiss et al. (1996). The reason for this discrepancy is not known. The GFRs from these studies and the results of the sigma method value calculations for UF_{H-TK} are shown in Table 5.

Table 5. Sigma-method value calculation for UF HK^a

Study	Mean GFR (SD) (mL/min)	Mean GFR – (3SD)	Sigma-Method Value
Dunlop	150.5 (17.6) ^b	97.7	1.54
Krutzen	195 (32) ^c	99	1.97
Sturgiss	138.9 (26.1) ^d	60.6	2.29
Averages	161.5	85.8	1.93

^a Mean GFR ÷ (Mean GFR - 3 SD).

^b Serially-averaged observations across three time periods (16, 26, and 36 weeks) for 25 pregnant women.

^c Third trimester values for 13 pregnant women.

^d Serially-averaged observations across two time periods (early and late pregnancy) for 21 pregnant women (basal index plus basal control individuals).

1 Considering the Krutzén et al. (1992) results in the context of the sigma method, a
2 reduction of 2 SD from the healthy population mean to establish the lower bound (which results
3 in a GFR slightly higher than the mean of the preeclamptic GFR), would appear to be
4 insufficient for adequate coverage of the susceptible population. Thus the use of 3 SD below the
5 healthy GFR mean gives coverage in the sensitive subpopulation to about 1 SD below the mean
6 preeclamptic GFR.
7

8 As no single study is considered to be definitive for assessment of population GFR
9 variability, UF_{HK} is determined from the average of the individual sigma-method values for each
10 of the three studies (Table 4). The mean GFR and standard deviation values in Table 5 are based
11 on average GFR across the entire gestational period, except for the Krutzén et al. (1992)
12 estimate, which was for the third trimester only. The average sigma-method value from the three
13 studies is 1.93. Considering a small residual uncertainty in the use of GFR as a surrogate for
14 boron clearance, the average sigma-method value of 1.93 is rounded upward to 2.0 and
15 established as the value for UF_{HK} .
16

17 By virtue of their lower GFR, pregnant women diagnosed with preeclampsia could be
18 considered to be a sensitive subpopulation, at least toxicokinetically. Toxicodynamic sensitivity
19 is presumably independent of toxicokinetic sensitivity. The onset of preeclampsia generally
20 occurs after the week 20 of pregnancy and is characterized by acute hypertension, often
21 accompanied by edema and proteinuria. Women with preeclampsia are at increased risk for
22 premature separation of the placenta from the uterus and acute renal failure, among other adverse
23 health effects. The fetus may become hypoxic and is at increased risk of low birth weight or
24 perinatal death.
25

26 The ~2-fold intraspecies variability factor derived from three standard deviations below
27 the mean of three studies for pregnancy GFR (mean = 161.5 mL/min; mean - 3 SD = 85.8) is
28 considered preferable for providing adequate coverage to women predisposed to adverse birth
29 outcomes due to renal complications. Therefore, the default value of 3.16 for UF_{H-TK} was
30 replaced with a data-derived adjustment factor of 2.0.
31

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33

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23
24

25 **Methylmercury (MeHg)**

26
27

27 Summary

28
29

30 Methylmercury (MeHg) has a reference dose (RfD) of 1×10^{-4} mg/kg-d placed on U.S.
31 EPA's IRIS database in 2001. No Toxicological Review is available for MeHg, but the
32 underlying data and interpretations are published in the *Water Quality Criterion for the*
33 *Protection of Human Health: Methylmercury* (U.S. EPA, 2001a).

34
35

36 Multiple RfDs were calculated from BMDL₀₅ values for various endpoints reported in
37 three epidemiological studies measuring neurobehavioral deficits in children *exposed in utero*.
38 Composite uncertainty factors of 10 were used in all calculations. This included a default 3 fold
39 factor for human toxicodynamic variability and uncertainty and a three fold factor for human
40 toxicokinetic variability and uncertainty. This latter UF_{HK} was based on published analyses of
human toxicokinetic data.

1 Hazard Identification and Dose Response

2
3 MeHg can produce a variety of toxicities depending on the dose. These range from
4 seizures and death to subtle neurobehavioral changes humans exposed *in utero*. The choice of
5 studies, critical effects, model, and point of departure choices were informed by an NRC
6 advisory report and a subsequent review by an independent scientific panel.
7

8 Mercury is methylated in soils and sediments by microorganisms and is bioaccumulated
9 through aquatic food webs. It can reach relatively high concentrations (1 ppm or more) in
10 predatory fish and sea mammals consumed by humans and wildlife. MeHg is absorbed readily
11 from the human gut and is transported through the body, crossing both the blood/brain and
12 placental barriers. Human studies from environmental exposures (fish consumption) were
13 available and served to define the POD. Neurobehavioral effects were observed in two studies of
14 children exposed *in utero* from maternal consumption of seafood (Faroes and New Zealand).
15 The Faroe Islands study was a longitudinal study of about 900 mother-infant pairs (Grandjean et
16 al., 1997). The main independent variable was cord-blood mercury; maternal-hair mercury was
17 also measured as was child hair mercury. At 7 years of age, children were tested on a variety of
18 tasks designed to assess function in specific behavioral domains. In the New Zealand study
19 (Kjellstrom et al., 1996a,b), increased maternal hair mercury was associated with decreased
20 scores on standard IQ tests in 6 year old children. No effects were reported in a third such study
21 in the Seychelles Islands, but these data were also included in the modeling (NRC, 2000).
22

23 No mode of action for methylmercury has been established.
24

25 Test responses of children in three large studies (Faroe Islands, Seychelles and New
26 Zealand) were coupled with measured or calculated MeHg cord blood concentrations for the
27 dose-response analysis. Benchmark dose (BMD) analysis was applied to the results from
28 multiple individual neurobehavioral tests (e.g., Boston Naming Test, Continuous Performance
29 Test, California Verbal Learning Test. Data were modeled using a K-power model with $K \geq 1$;
30 $K = 1$ generally gave the best fit. These data were continuous in exposure and effect. An
31 abnormal response was defined as one falling into the lowest 5% of test responses ($P_0 = 0.05$).
32 The benchmark response (BMR) was set at 0.05, based on the external review committee's
33 advice that the combination of BMR and P_0 were within the observed range of responses and
34 were, in fact, typical for these types of measurements. $BMDL_{05}$ values of 46 to 79 ppb MeHg in
35 fetal cord blood were chosen as the points of departure for RfD calculation.
36

37 There is a correlation between maternal-blood mercury concentrations and fetal-blood
38 mercury concentrations. A review of results from 21 studies demonstrated that the ratio of
39 concentrations (fetal:maternal) is typically higher than 1, with overall mean values supporting a
40 ratio close to 1.7. Ratios for the 5th and 95th percentiles of cord:maternal Hg approximate 0.85
41 and 3.5, respectively. Based on the advice of an NRC panel U.S. EPA chose not to make a
42 numerical adjustment between cord-blood and maternal-blood mercury in calculating the RfD.
43 The relationship between cord-blood and maternal-blood mercury was discussed as an area of
44 variability and uncertainty, during UF derivation. Conversion of cord blood measurements to
45 estimated ingested maternal dose used a one-compartment model with fixed parameters, as
46 advised by the scientific reviews.

1 Twenty-four RfDs were calculated using various BMDL_{05S} but with the same dose
2 conversion and a composite uncertainty factor of 10. These calculations resulted in one RfD =
3 0.2 µg/kg bw /day, three RfD = 0.05 µg/kg bw /day, and twenty RfD = 0.1 µg/kg bw /day (or 1x
4 10⁻⁴ mg/kg-d).

5 6 Basis for DDEF 7

8 A PBPK model and a one-compartment model for pregnant women were used to examine
9 the relationship between ingested doses of MeHg and maternal blood levels. To estimate human
10 interindividual variability (of MeHg concentrations in maternal blood to ingested MeHg dose),
11 the most deterministic (sensitive) parameters of the TK model were identified and varied. Model
12 results demonstrated that external doses required to produce maternal blood concentrations of 1
13 ppm varied up to three-fold. This value (3) served as a non-default value for UF_{HK}; the TD
14 component (UF_{HD} was left at a default value of 3 and the overall intraspecies UF value was 10.
15

16 DDEF derivation 17

18 Multiple measures of MeHg exposure are available from several human studies. For
19 MeHg hair and blood are considered more appropriate than urine, particularly for longer term
20 exposure. The toxicity evaluated was induced during gestation, at a time when MeHg exposure
21 to the fetus' developing brain via the placental blood supply. Thus MeHg originated from the
22 maternal blood circulation. Both a PBPK model and a one-compartment model were used to
23 assess variables in fetal MeHg exposure. Independent of model type, ability to estimate maternal
24 hair concentrations required at least two more parameters (blood to hair transfer and hair growth
25 rate) than required to estimate maternal blood concentrations. In addition, because the U.S. EPA
26 set cord blood concentrations to equal maternal blood concentrations, the model was able to
27 predict fetal cord blood concentrations with less uncertainty than maternal hair concentrations.
28 Largely for these reasons, blood MeHg concentrations were selected as the most appropriate
29 dose metric.
30

31 The U.S. EPA characterized human toxicokinetic variability as differences in external
32 (ingested) doses of MeHg that resulted in the same concentration of MeHg in maternal blood.
33 The concentration selected for analysis was one that was relevant to the BMDL₀₅ for the
34 neurobehavioral effects—namely 1 ppm. This concentration is about 12 to 20 times higher than
35 the concentrations serving as the POD, but the choice may be based in part on increasing model
36 uncertainty when predicting concentrations lower than 1 ppm. An evaluation of the uncertainty
37 and variability in model parameters was conducted in three studies (Stern, 1997; Swartout and
38 Rice, 2000; Clewell et al., 1999) to identify the extent to which the external (ingested) dose
39 might vary when compared to a fixed maternal hair or blood concentration.
40

41 Results from the Stern (1997) analysis were available in the original publication, whereas
42 specific predictions of values at given percentiles for the Swartout and Rice (2000) and Clewell
43 et al. (1999) studies required additional model exercises by the original authors; these analyses
44 were published by NRC (2000). All data used in the models were from human studies. The
45 analysis demonstrated the ratio of external (ingested) doses (in µg/kg/day) that resulted in the
46 same blood concentration; the ratio was selected as that resulting from the comparison of the

1 dose at the 50th percentile of the distribution to the dose at the 1st percentile of the distribution.
 2 These percentiles of the distribution represented the “general” human and the ‘sensitive’ human,
 3 respectively. Because the dose at the 1st percentile of the distribution will be lower than the dose
 4 at the 50th percentile of the distribution, the ratio will have a value greater than 1.0.
 5

6 Table 6 presents the ratios developed (external dose at the 50th percentile / external dose
 7 at the 1st percentile) for each of the three studies. Considering TK variability as described by the
 8 ratio of external doses at the specified percentiles of the distribution, values for blood and hair
 9 ranged from 1.7 to 3.3. Maximum values were 3.3 and 3.0 for hair and blood, respectively. The
 10 U.S. EPA’s IRIS entry for MeHg states, “Using maternal blood as the starting point, the
 11 consolidated range from the three analyses is 1.7 to 3.0.” On this basis, a value of 3 was chosen
 12 to serve as the numerical replacement for the default value of the portion of the UF related to
 13 intraspecies TK variability. As no data were available to address intraspecies differences in
 14 susceptibility (TD), this portion of the UF was left at the default value of 3. Together these
 15 values for the components of UF_H combine to equal a value of 10, which was characterized as a
 16 “hybrid” value, comprising values based on default methodology and on data.
 17
 18

19 Table 6. Comparison of Results from Three Analyses of the Interindividual Variability in the
 20 Ingested Dose of MeHg Corresponding to a Given Maternal-Hair or Blood Hg Concentration.
 21 This is Table 3-1 from NRC (2000).
 22

Study	Maternal Medium	50 th Percentile ^a (µg/kg-d)	50 th Percentile/ 5 th Percentile ^b	50 th Percentile/ 1 st Percentile ^c
Stern (1997)	Hair	0.03 – 0.05 ^d (mean = 0.04)	1.8-2.4 (mean = 2.1)	2.3-3.3 (mean = 2.7)
	Blood	0.01	1.5-2.2 (mean = 1.8)	1.7-3.0 (mean = 2.4)
Swartout and Rice (2000)	Hair	0.08	2.2	Data not reported
	Blood ^e	0.02	2.1	2.8
Clewell et al. (1999)	Hair	0.08	1.5	1.8
	Blood ^f	0.07	1.4	1.7

23
 24 ^aPredicted 50th percentile of the ingested dose of methylmercury that corresponds to 1 ppm Hg in hair or 1 ppb in
 25 blood.
 26 ^bRatio of 50th percentile of ingested dose of methylmercury that corresponds to 1 ppm Hg in hair or 1 ppb in blood
 27 to the 5th percentile.
 28 ^cRatio of 50th percentile of ingested dose of methylmercury that corresponds to 1 ppm Hg in hair or 1 ppb in blood to
 29 the 1st percentile.
 30 ^dRange reflects minimum and maximum values among eight alternative analyses.
 31 ^eData from J. Swartout, U.S. Environmental Protection Agency, personal commun.; June 9, 2000.
 32 ^fData from H.J. Clewell, ICF Consulting, personal commun.; April 19, 2000.
 33
 34

1 Acknowledgment: Some passages in this document were taken from the U.S. EPA's IRIS entry
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