Methyl Iodide (Iodomethane)

RISK CHARACTERIZATION DOCUMENT FOR INHALATION EXPOSURE

Appendices to Volume I (Health Risk Assessment)

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Appendices to Volume I:

Appendix A. Review of Physiologically Based Pharmacokinetic Model for Human Equivalent Concentration

Appendix B. Calculations

Appendix C. U.S. Environmental Protection Agency Risk Assessment

Appendix A. Review of Physiologically Based Pharmacokinetic Model For Human Equivalent Concentration

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I. INTRODUCTION

This review on acute Human Equivalent Concentrations (HECs) of methyl iodide (MeI) derived from Physiologically Based Pharmacokinetic (PBPK) modeling pertains to three sets of endpoints: 1) fetal death in rabbits from maternal exposure, 2) nasal olfactory epithelial degeneration in rats, and 3) neurotoxicity in rats. Data and discussions already presented in Volume I are briefly mentioned but not repeated here in detail.

For deriving the HECs, the PBPK model is expected to account for interspecies pharmacokinetic differences. A diagram of the basic model taken directly from Arysta (2007) is included in Figure A-1. The same basic PBPK model structure is used for all three endpoints but the rat model contains an enhanced nose compartment for simulating the nasal endpoint HECs. It is also used for the neurotoxicity endpoint. The same basic model and endpoints are also used by U.S. Environmental Protection Agency (USEPA). However, the final HECs presented in this review differ from USEPA (2007) due to different modeling baselines and dose metrics. They are highlighted herein.

The iterative PBPK model runs presented in this review were conducted by Arysta (2008a, b, c, d, e, f). To avoid confusion, instead of the term "bystander" used in the Arysta modeling reports that was applicable to both "bystander" and "resident" in Volume I and II, the term "General Population" is used in this Appendix for anyone who does not receive occupational exposure as being a part of the work task associated with the MeI use. This review also includes discussions of occupational HECs that consider a worker's 16-hour MeI exposure as a member of the general public in addition to the 8-hour work exposure in the Arysta model. They are termed as "8-hour occupational HEC" or "8-hour HEC" in short.

In this review document, sources for detailed information on the Arysta model are cited. The rabbit and rat model codes were initially submitted by Arysta (2007). The source codes and model input and output for the specific model iterations presented in this review are in Arysta (2008a, b, c, d, e, f). Additional model description can be found in Sweeney *et al.* (2009) which became publicly available after the DPR model review. Further information related to supporting data for the model construct and application previously submitted to DPR are also published within an entire issue of Inhalation Toxicology (2009 issue 6, volume 21); many of these articles are added to the citations in Volume I for additional sources of information. For ease of cross reference between this review and Volume I, the same literature citation designation is used throughout both documents. This may mean, for example, a publication is designated as "b" while it is "a" for the same authors in the same year appearing later in sequence or not be used at all in this review.

II. FETAL DEATH IN RABBIT

Aspects of the modeling framework that are important to establishing the HEC specifically for the fetal death endpoint include input parameters and model validation and the dose metric(s) for the HEC. Two key issues in selecting the dose metric for the HEC are the exposure duration and

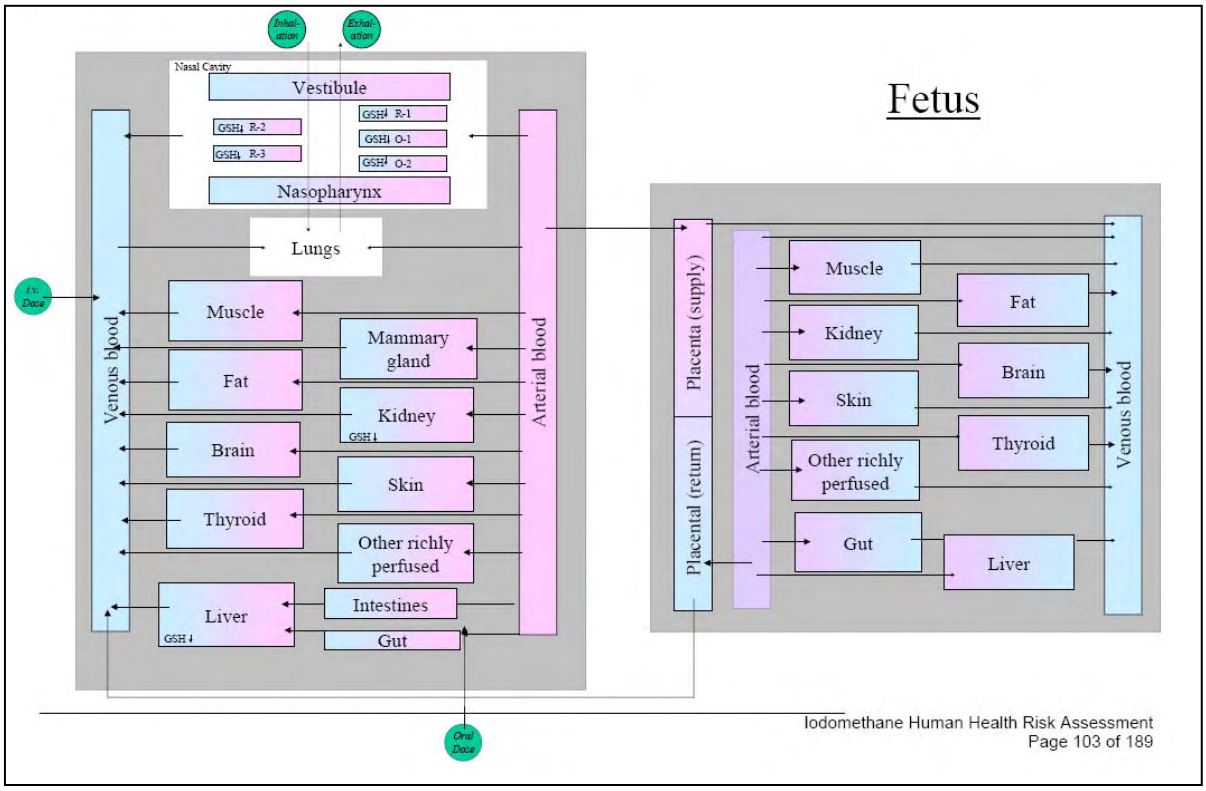


Figure A-1. Diagram of MeI PBPK model. Taken directly from Arysta (2007).

frequency associated with the NOEL and the considerations of possible mode of actions (MOAs). These essential determinants are discussed before the final presentation of the HEC.

II.A. Model and Parameters

The rabbit model was reviewed by USEPA (2007) for establishing HECs corresponding to a NOEL of 10 ppm, 5-fold higher than the 2 ppm established by DPR. The model description, codes, and parameters are in Arysta (2007) and Mileson *et al.* (2007). They are subsequently published in open literature (Sweeney *et al*., 2009). This section provides only a very brief description of the model, mainly as a backdrop for focusing on some key issues that could significantly impact the modeling outcome.

Comparison of model output to the experimentally measured values is used to calibrate and adjust input variables for model fit. The iodide level in blood is designated as "plasma" in all Arysta model runs, while it is referred to as "serum" in toxicity studies. To simplify, "serum" is used in this review for the model output. Key determining variables involved in the initial model fit to data from NaI exposures in the study by Morris *et al.* (2004) (see: Volume I, Table 50 and Section III.J.2.) are: placental and maternal uptake, urinary elimination rate, and maternal and fetal iodide transfer (Mileson *et al*., 2007). The model documentation stated that further parameter adjustments (unspecified) to fit data from the MeI study by Sloter (2005a, b) compromised the fit to the Morris data. Mileson *et al.* (2007) further speculated that model parameters that may be iodide concentration-dependent (e.g., transfer between maternal and fetal blood) may need dose-dependent correction but this adjustment was not carried out due to the modeler's conclusion that the current model provided a reasonable fit.

The specific input parameters and simulation patterns highlighted below are identified for their importance to biological considerations in establishing the HEC and their potential to significantly impact the model outcome. Validation against measured data is included in these discussions.

II.A.1. Alveolar Ventilation Rate (QAC)

<u>Rabbit simulation</u>

The Arysta pregnant rabbit HEC model initially used the QAC of 12 L-hr/kg$^{3/4}$ from non-pregnant rabbits. This is only approximately 70% of the pulmonary ventilation rate measured by DeLorme (2004) for non-pregnant females during 18.5 ppm MeI exposure. On the other hand, a higher QAC of 20 L-hr/kg$^{3/4}$ for GD20-30 rabbits was used for comparing model output to experimental data from the Sloter studies (Sloter 2005a, b) in which rabbits received MeI on GD23-26. The justification for the use of a lower QAC in single day simulation is as a "health protective" or "conservative" input for the HEC determination (Mileson *et al*., 2007) and for targeting an early pregnancy stage (Mileson, 2008). Nevertheless, DPR considers it more important that a biologically valid parameter is used in PBPK modeling.

The illustration of the model fit to the data from the Sloter studies is provided by Mileson *et al.* (2007) and given in Figure A-2a (a single exposure in 24 hours) and A-2b (4 repeated daily MeI exposures). These figures are taken directly from Figures 4a, b in Mileson *et al.* (2007). The model showed reasonable fit to the 3 data points for fetal serum levels within 24 hours but underestimated the maternal data, especially at hour 3. The fit for both maternal and fetal data became poorer as time progressed (Figure A-2b). No experimental data are available for model validation beyond a few days. The poor model fit in time indicates increasing uncertainty for extending the model beyond 24 hours, especially for accommodating the assumption by Mileson *et al.* (2007) that the NOEL should represent a single-day incremental exposure after the steady state of fetal blood iodide is reached on day 13. This issue on defining a single-day HEC corresponding to the NOEL is discussed in Section II.C.1.

Table A-1 presents the model output at 20 ppm MeI exposure but using the non-pregnant 12 L-hr/kg$^{3/4}$ QAC. As expected, the lower QAC results in further deviation from the experimental measurements. During the first 24 hours, the maternal serum iodide concentrations were only 32 - 53% of the measured values. The fetal concentrations were lower at hour 3 (48% of measured values) and hour 6 (66% of measured values), but caught up with the measured values at hour 24. Thus, the use of lower QAC would significantly impact the HEC especially when maternal serum iodide is used as the dose metric. A comparison of HECs is available in Table A-10.

After being informed of the above concerns by DPR, Arysta provided a revised model simulation using the QAC of 20 L-hr/kg$^{3/4}$ in September 2008 (Arysta, 2008e). This model output became available when DPR's model review was near its completion and the majority of model presentations from the lower QAC runs were already in place, and only at 2 ppm. DPR did not consider it necessary to delay the review process by requesting further model runs from Arysta (i.e., at 20 ppm) nor invest substantial resources to re-do a different set of basic model behavior illustrations. The value of QAC is clearly labeled for all data presented in this review. It should be emphasized that the DPR preferred 20 L-hr/kg$^{3/4}$ QAC is used for generating model runs for DPR's final HECs for this endpoint. They are presented in Section II.D.

The lower QAC was used in the final single-day HEC modeled by USEPA (USEPA, 2007; Mileson, 2008) at the NOEL of 10 ppm. Model output at 20 ppm using 20 L-hr/kg$^{3/4}$ QAC was recently provided by Rodriguez (2009) as a part of USEPA's comments to the March 2009 draft of this DPR model review. The graphic comparisons of model fit at both QACs are presented in Figure A-3, taken directly from Rodriguez (2009). The modeled maternal serum iodide was 67 - 91% of the measured values in the 24 hours. The modeled fetal serum iodide was 95% of measured level at hour 3, but exceeded the measured levels by 31% at hour 6 and 81.3% at hour 24. Thus, the higher estimation of fetal serum iodide profile would be a concern if it is used as the dose metric for HEC determination. While data are not directly available for any AUC comparison, the use of lower QAC might be somewhat justified for USEPA's HEC based on the fetal serum iodide dose metric because of the better model fit than at the higher QAC (Rodriguez, 2009). However, the issue remains regarding the physiological incongruity of using a low non-pregnant QAC for the window of vulnerability during GD23-26. Apparently the inability to achieve a good fit at both maternal and fetal serum iodide levels is beyond the adjustment of QAC parameter and is further evident from the standpoint of fetal-to-maternal (F/M) iodide ratio as presented in the next section.

Figure A-2a

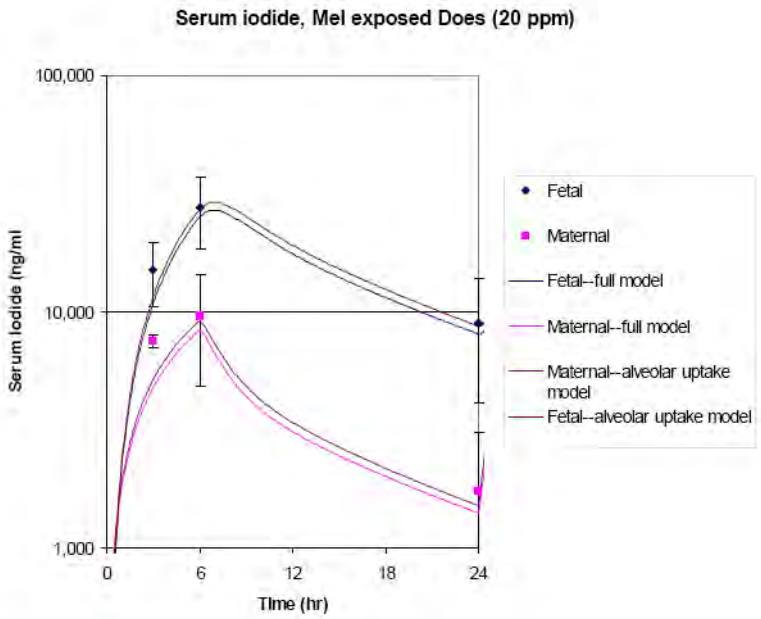


Figure A-2b

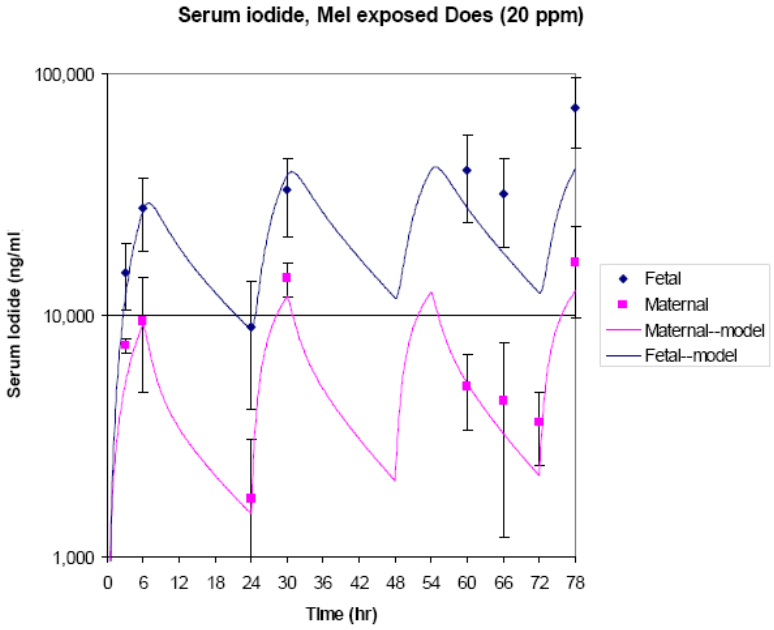
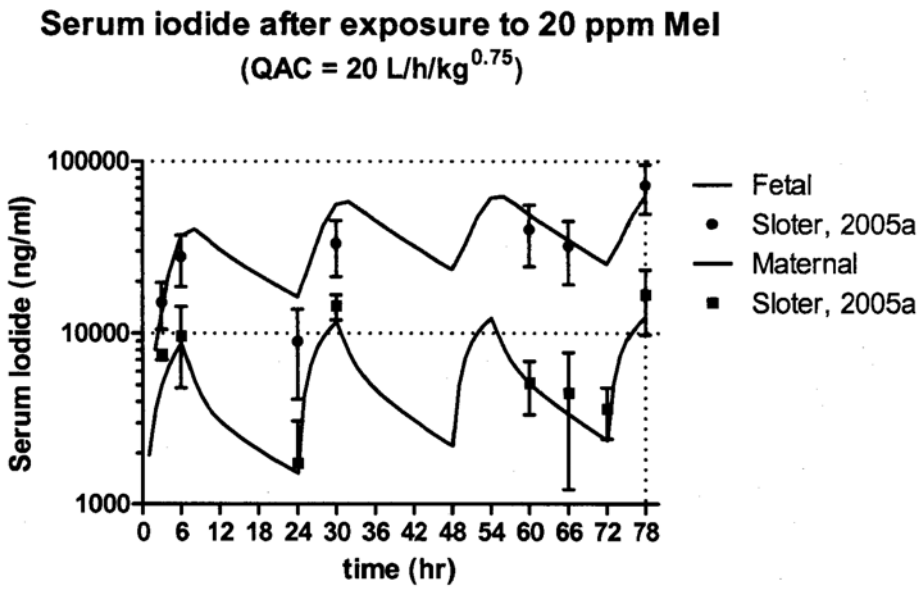


Figure A-2. Modeled versus measured iodide in rabbits (Taken from Mileson *et al*., 2007, Figure 4a and 4b). The measured data are from Sloter, 2005a. The "full model" in 4a includes both alveolar and nasal MeI uptakes. Lines: simulations; Symbols: experimental data. The QAC for this simulation is 20 L-hr/kg$^{3/4}$. Using the lower QAC of 12 L-hr/kg$^{3/4}$ would result in much poorer model fit than demonstrated here (see Table A-1).



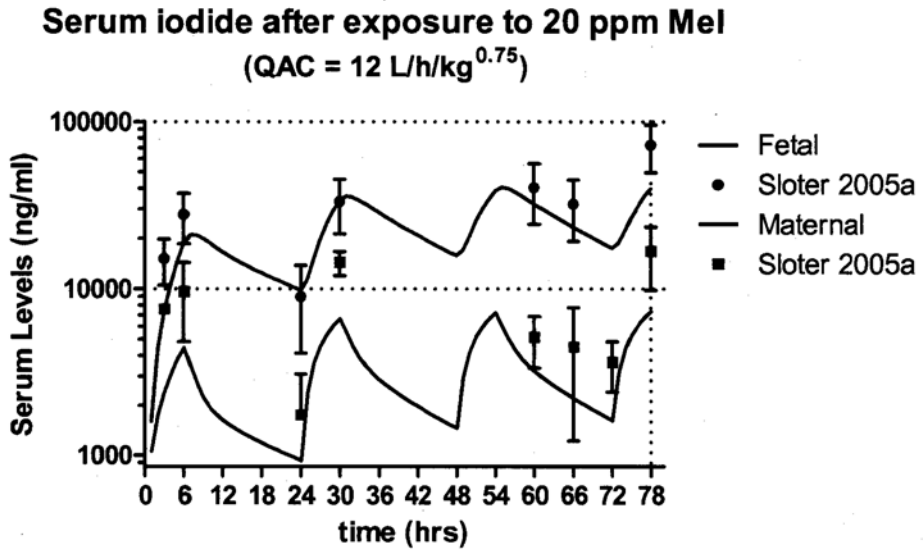


Figure A-3. Modeled rabbit maternal and fetal iodide at QAC 12 and 20 L-hr/kg$^{3/4}$ (Taken from Rodriguez, 2009, Figure 1). The measured data (points) were from Sloter, 2005a. QAC at 20 L-hr/kg$^{3/4}$ was used in the top simulation (lines) while 12 L-hr/kg$^{3/4}$ was used in the bottom simulation (lines).

Table A-1. Modeled versus measured rabbit serum iodide concentrations after repeated 20 ppm MeI (6 hour/day) exposure.[a]

Hour	Modeled[b] (mg/L)		F/M	Measured[c] (mg/L)		F/M
	Maternal	Fetal		Maternal	Fetal	
3	2.39	7.22	3.0	7.50 ± 0.49	15.10 ± 4.62	2.0
6	4.41	18.45	4.2	9.57 ± 4.75	27.80 ± 9.25	2.9
24	0.92	9.77	10.6	1.74 ± 1.34	8.96 ± 4.83	5.2
30	6.55	33.33	5.1	14.30 ± 2.36	33.20 ± 11.90	2.3
60	3.19	32.09	10.1	5.11 ± 1.76	40.10 ± 15.70	7.9
66	2.19	23.35	10.7	4.47 ± 3.25	32.00 ± 11.90	7.2
72	1.61	17.50	10.9	3.61 ± 1.20	-	-
78	7.28	39.67	5.5	16.60 ± 6.80	72.60 ± 23.20	4.4

a/ The beginning of MeI treatment is hour zero. "F/M" is the fetal-to-maternal ratio.

b/ The QAC for model output was 12 L-hr/kg$^{3/4}$ for a non-pregnant rabbit. A separate simulation by Rodriguez (2009) showed that changing the QAC to 20 L-hr/kg$^{3/4}$ did not significantly alter the modeled F/M ratios.

c/ The corresponding measured values are from a Sloter study (Sloter, 2005b) in which pregnant rabbits received 20 ppm exposure starting on GD23. The maternal and fetal levels from another Sloter study at 25 ppm MeI exposure, 6 hr/day (Sloter, 2005a, data in Table A-4) are: 17.2 and 37.1 mg/L at hour 30 and 26.4 and 70.6 mg/L at hour 78.

Human Simulation

A QAC of 16.5 L-hr/kg$^{3/4}$ is used in simulating exposure scenarios for pregnant women. It was noted that a very slightly lower QAC of 16.4 L-hr/kg$^{3/4}$ was used by USEPA in their HEC simulation (Rodriguez, 2009). In the Arysta model, the minute volume for the 24-hour MeI exposure is 567 L/hr (tidal volume at 630 ml/breath, 15 breath/minute). This rate is close to the 0.5 m^3/hr recommended in the USEPA Exposure Factors Handbook (EFH) (USEPA, 1997) for short-term sedentary activities. While USEPA used the same breathing rate for modeling its occupational exposure scenarios, DPR's current default breathing rate is 833 L/hr. This default is lower than the 1.0 m^3/hr given in the EFH for light activities (USEPA, 1997). Subsequent to the above considerations, Arysta provided revised model simulations by changing the tidal volume (TVol) from 630 to 925 ml/breath to match the 1.47-fold higher DPR default breathing rate of 833 L/hr (Arysta, 2008e). The revised model outputs are presented in Section II.D. and used in this review for establishing DPR's final occupational HECs.

One question exists in how the higher breathing rate for workers is incorporated into the model. Rodriguez (2009) opined that the 1.47-fold change should instead be reflected on the QAC which resulted in an 8-hour occupational HEC of 0.5 ppm based on the maternal serum iodide AUC dose metric and the NOEL of 2 ppm in rabbits. However, this "8-hour only" HEC did not

account for the 16 hours of additional exposure for these workers as a member of the general public at the HEC of 0.24 ppm.

II.A.2. Maternal versus Fetal Iodide Levels

The transfer between maternal and fetal iodide was one of the three key factors that determined the maternal and fetal serum iodide profile (Sweeney *et al.*, 2009). The other two factors were placental and mammary uptake and urinary elimination rate. In the model, CLTRANS1C denotes placenta to fetus transfer and CLTRANS2C denotes fetus to placenta transfer. General illustrative model outputs for both rabbits and humans are presented in Table A-2 and discussed below in the context of their respective simulations.

Rabbit Simulation

The rabbit model used CLTRANS1C of 0.07 L/hr-kg$^{0.75}$ and CLTRANS2C of 0.015 L/hr-kg$^{0.75}$. The resultant fetal-to-maternal serum iodide ratios (F/M) are compared to the two sets of experimental data.

The first comparison is to the measurements by Sloter (2005b) after 20 ppm MeI exposure. As shown in Table A-1, at the same MeI exposure level, the modeled F/M ratios are higher than the experimental data at all time points. A separate simulation by Rodriguez (2009) showed that changing the QAC to 20 L-hr/kg$^{3/4}$ did not significantly alter the modeled F/M ratios.

The second comparison is to the measurements from a NaI study by Morris *et al.* (2004). Although these data are used for model adjustment, the resultant F/M ratios at 2 and 10 ppm MeI exposure (Table A-2) show significant overestimation of the relative distribution of iodide to the fetal blood. The blood F/M ratios between 2 - 24 hours after a single i.v. injection of NaI on GD25 from Morris *et al.* (2004) are 2.6-5.8 at 0.75 mg/kg and 1.6-2.8 at 10 mg/kg (Table A-6). The ratios from model output during the similar period are 3.1-8.2 at 2 ppm MeI and 3.4-10.0 at 10 ppm MeI exposure (Table A-2). Although the NaI study and MeI simulation differ in many respects (NaI given via i.v. versus MeI given via inhalation), the F/M comparison is possible since the NaI dose range in the Morris study was designed to encompass the range of iodide from toxicologically relevant MeI exposure levels. In this case, the range of total amount of iodide in the simulated 2-10 ppm MeI exposure is 1.5-7.6 mg/kg/day MeI, or 1.3-6.8 mg/kg/day iodide at 100% MeI inhalation absorption. The comparison is of value because it is based on the level of iodide in circulation and not the external NaI or MeI dose.

No other experimental data are available for similar validation comparisons. The general pattern is a higher simulated F/M ratio than the results from the two model calibration studies, even within the first 24 hours of MeI exposure. This indicates that a more holistic adjustment of transfer rates and other input parameters within the model construct would be desirable especially if fetal serum iodide is used as the dose metric for the HEC.

Table A-2. Modeled fetal-to-maternal serum iodide (F/M) ratio in rabbits and humans exposed to MeI.[a]

Hour	Modeled[a] – Rabbits						Modeled[a] – Humans		
	2 ppm			10 ppm			3.7 ppm		
	M	F	F/M	M	F	F/M	M	F	F/M
3	0.16	0.50	**3.1**	0.94	3.20	**3.4**	0.53	0.53	**1.0**
6	0.21	0.91	**4.4**	1.57	6.98	**4.5**	0.95	0.95	**1.0**
24	0.06	0.50	**8.2**	0.41	4.14	**10.0**	2.20	2.06	**0.9**
30	0.29	1.44	**5.0**	2.72	14.2	**5.2**	1.36	1.40	**1.0**
60	0.20	1.63	**8.1**	1.53	15.5	**10.2**	0.11	0.17	**1.6**
66	0.18	1.47	**8.0**	1.13	11.9	**10.5**	0.071	0.13	**1.8**
72	0.17	1.33	**8.0**	0.89	9.40	**10.6**	0.049	0.097	**2.0**
78	0.42	2.33	**5.5**	3.38	19.7	**5.8**	0.037	0.078	**2.1**
96	0.20	1.63	**8.1**	0.93	9.94	**10.6**	0.021	0.049	**2.4**

a/ Data from Mileson *et al*., 2007. "M" and "F" are mg/L iodide concentrations in maternal and fetal serum, respectively. The rabbit simulation represents daily 6 hours of MeI exposure for 4 days using the lower QAC of 12 L-hr/kg$^{3/4}$. The human simulation represents a single 24-hour MeI exposure.

Human Simulation

The ratio of cord-to-maternal blood iodide concentration was reported in an earlier study by Cottino *et al.* (1972) with 18 women who received iodomethylsparteine through i.v. injection at termed delivery. The time between the injection and delivery ranged from 15 minutes to 48 hours, and the paired cord-to-maternal blood iodide concentration ranged from 0.5 to 3.4. More recently, Rayburn *et al.* (2007) conducted a survey on plasma iodide concentration of paired maternal and cord blood at delivery (Table 51 in Volume I). These data are subsequently published in the open literature (Rayburn *et al*., 2008). The surveyed subjects did not have any documented exposure to any test substances nor povidone iodine. The results are summarized in Table A-3. The average cord-to-maternal blood iodide ratio of 1.2 from this study was used to adjust the CLTRANS1C (placenta to fetus) and CLTRANS2C (fetus to placenta) parameters to 0.15 and 0.12 L/hr-kg$^{0.75}$ respectively in the model (Mileson *et al*., 2007; Barton, 2007). Mileson *et al.* (2007) considered this 1.2 ratio as "conservative" compared to using the pre-term ratio of 0.9. In this consideration, Arysta regarded the 0.9 ratio as a possibly better alternative for representing the end-of-first-trimester stage which they assumed to be the window of vulnerability to MeI toxicity and thus the target for their HEC simulation. However, it is DPR's view that even if the end of first trimester is the only target period for fetal death from MeI

exposure, data are unavailable for determining how well iodide levels collected from deliveries during gestation week 29 – 36 may represent the early gestation stage when excess iodide is introduced through exposure to MeI. The related issue of fetal stage at the weight of 0.27 kg is a subject of separate discussion in Section II.A.4.

Table A-3. Plasma iodide concentration in human maternal and cord blood at delivery.[a]

Delivery	N	Blood Iodide (µg/dL)		Cord-to-Maternal	
		Maternal	Cord	Ratio	Range
Pre-term (29-36 week)	29[b]	1.6±0.4	1.4±0.5	0.9±0.4	0.35 – 2.11
Term (37-41 week)	92	1.5±0.5	1.7±0.7	1.3±0.8	0.35 – 5.4
All subjects	121[b]	1.5±0.7	1.6±0.7	1.2±0.7	0.35 – 5.4

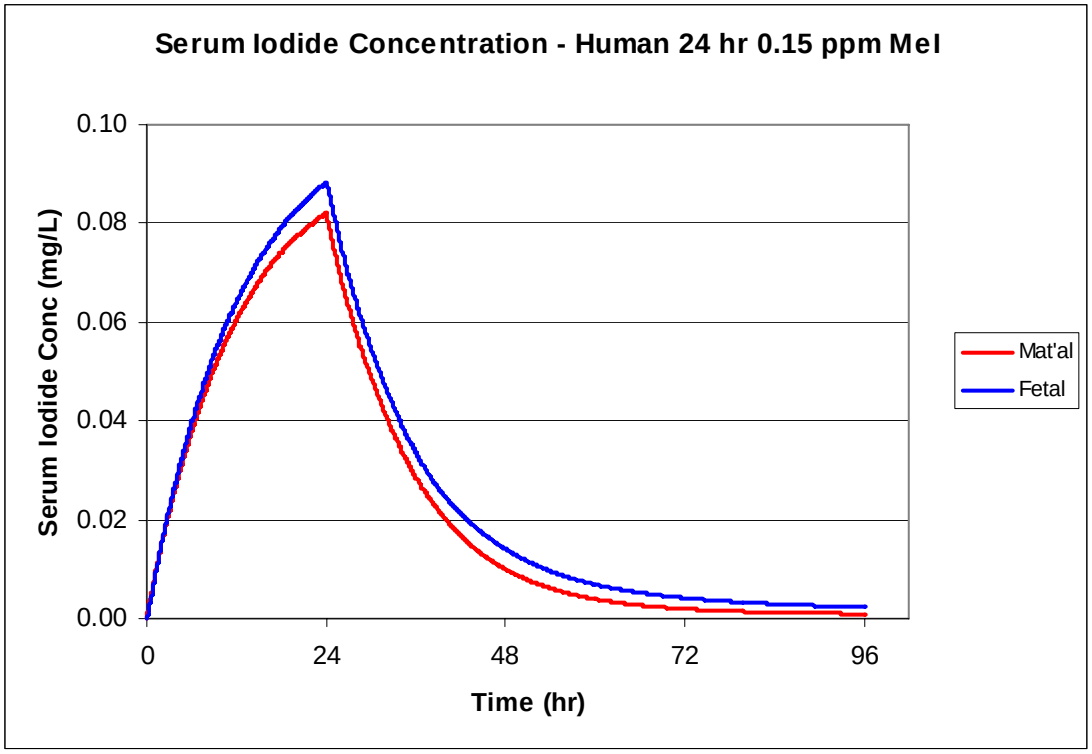
a/ Data as reported by Rayburn *et al.* (2007).

b/ Excludes 2 data pairs both at gestation week 33 delivery. One pair with extreme cord blood concentration suspected as contamination, resulted in a cord-to-maternal ratio of 255.9. The other with high maternal blood concentration reported as requiring antiarrhythmic therapy, resulted in a cord-to-maternal ratio of 0.41. They are not included in the statistical analysis.

Another important observation regarding the F/M ratio is that it is dynamic, not a fixed value. This can be demonstrated in two scenarios: by comparing simulation results from two MeI exposure levels and by following the time of diminishing serum iodide concentrations after MeI exposure. Figure A-4 illustrates the first scenario at 0.15 and 3.4 ppm MeI exposure levels. These levels are parts of a large set of simulations from Arysta (2008c) and are used here only for illustration purposes. While the fetal iodide concentration is consistently higher than the maternal level at 0.15 ppm MeI exposure, this relationship is reversed near the peak concentration at 3.4 ppm MeI.

Table A-2 illustrates the second scenario at 3.7 ppm MeI exposure. The F/M ratio gradually increases with decreasing serum iodide level after the end of 24 hours of exposure, from 1.0 within hour 24 to 2.1 - 2.4 by hour 78 - 96. The higher simulated F/M ratio is of interest because the corresponding serum iodide levels are down at the range reported by Rayburn *et al.* (2007) but with 2-fold higher F/M ratio in the simulation output. The discrepancy raises uncertainty about how well either the Rayburn study or the model can describe the dynamics of iodide distribution between maternal and fetal compartments after MeI exposure.

Overall, data in both rabbits and humans indicate greater uncertainty at the fetal compartment level. In the rabbit model, the F/M ratios did not compare well with measured data at 20 ppm MeI. Nor did they compare well with data from the only available NaI study. In the human model, it is uncertain how well data from the Rayburn study can adequately represent the modeled MeI exposure scenarios, specifically regarding the unmatched gestation stage (i.e., applying data from beyond gestation week 29 to model the end of the first trimester stage) and the iodide exposure status (i.e., applying data from non-iodide exposure conditions to substantially high excess iodide exposure scenarios). The simulation shows that the ratio is not constant after MeI exposure and can be much higher than the targeted ratio at a comparable range of serum iodide concentrations in the Rayburn study.



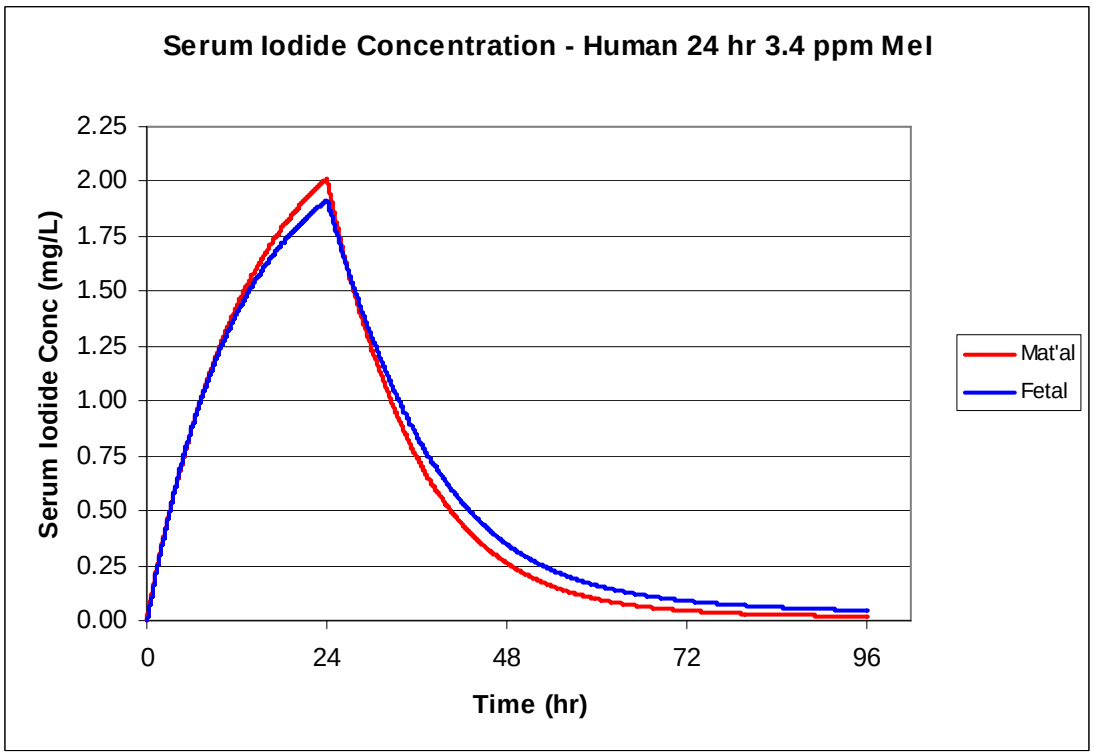


Figure A-4. Modeled human serum iodide at 0.15 and 3.4 ppm MeI for 24 hours. Data from Arysta (2008c).

II.A.3. Fetal Thyroid Iodide Level

Arysta used data from the Morris *et al.* (2004) rabbit study with NaI as starting points for modeling iodide disposition variables. The stated model assumption was that when blood kinetics are accurately predicted, tissue-specific parameters are sufficiently accurate without needing further adjustment (Mileson *et al*., 2007). Although the modeling focuses on the serum MeI and iodide profiles, iodide distribution to fetal thyroid is of specific interest because fetal thyroid is the target tissue of the Arysta proposed mode of action (MOA) (Arysta, 2007). However, since no single MOA for fetal death can be clearly supported (Section II.B.), the following discussion is mainly on the concerns for the extremely high iodide accumulation in this biologically relevant tissue, and not necessarily for considerations that would directly influence the HEC dose metric selection.

The Morris study showed that within 24 hours of NaI injection on GD25, fetal thyroid did not accumulate iodide as compared to levels in fetal trachea. In fact, at 0.75 and 10 mg/kg NaI, fetal thyroid iodide level is substantially lower than the maternal level (Table A-6 and Figure A-11). Thus, it is surprising that the model predicted a 4.2 mg/L peak iodide level in fetal thyroid follicles at the end of the 6 hour MeI exposure, a level that is 217-fold higher than the maternal level at 0.02 mg/L (Figure A-5). The fetal-to-maternal ratio is further increased to 513-fold after 18 hours of no exposure. Regarding the ratio of fetal to maternal iodide in rabbit thyroid follicles, model output from different sets of runs indicated that compared to the ratio of 217 at the end of 6-hour of 2 ppm MeI exposure, the ratio is 37 at 20 ppm MeI (maternal 0.4 mg/L, fetal 14.9 mg/L) and 68 at 10 ppm (maternal 0.14 mg/L, fetal 9.8 mg/L) MeI. Thus, within the 2 to 20 ppm MeI range, the iodide concentrations in fetal thyroid follicles are substantially higher than the maternal, contrary to the lack of fetal thyroid iodide accumulation reported in the Morris study from NaI iv injection. Further comparison to rabbit data cannot be made due to the difference in dosing regimen and the NaI versus MeI exposure.

The human model shows an even more distinct pattern of iodide accumulation to the fetal thyroid follicles and at a much higher level than rabbits (Figure A-6). For a single 24-hour exposure to 0.15 – 3.4 ppm MeI, the fetal thyroid iodide saturates at 208 mg/L. Unlike the decline in rabbits after the exposure (Figure A-5), this level remains unchanged in human fetal thyroid after MeI exposure, for at least up to hour 96; i.e., 3 days after the exposure. This plateau is 50-fold above the already seemingly high modeled rabbit fetal thyroid level at the end of 6-hour exposure. Within the human model, the fetal plateau is 1,800-fold higher than the maternal level at the end of the 24-hour exposure to 0.15 ppm MeI.

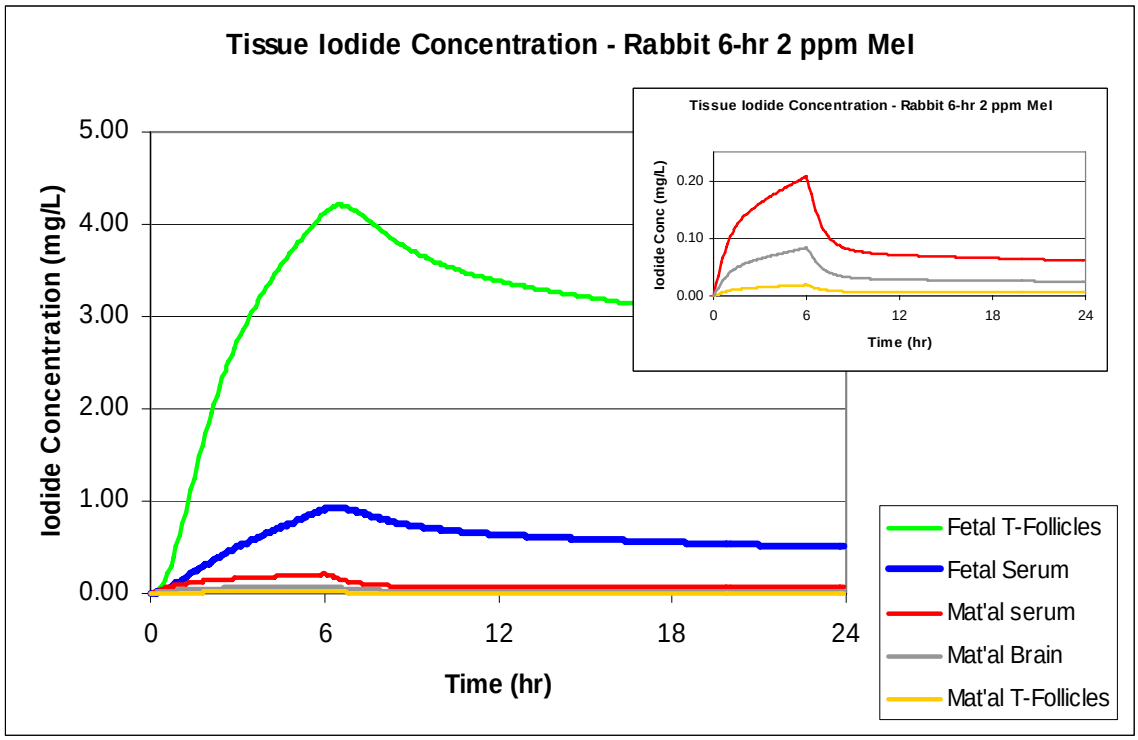


Figure A-5. Modeled tissue iodide in rabbits at 2 ppm MeI for 6 hours (simulation using the lower QAC of 12 L-hr/kg$^{3/4}$). Data from Arysta (2008c). "T-follicles": thyroid follicles. The reduced figure at upper right corner magnified the maternal patterns. The maternal profiles are enlarged at the upper right box.

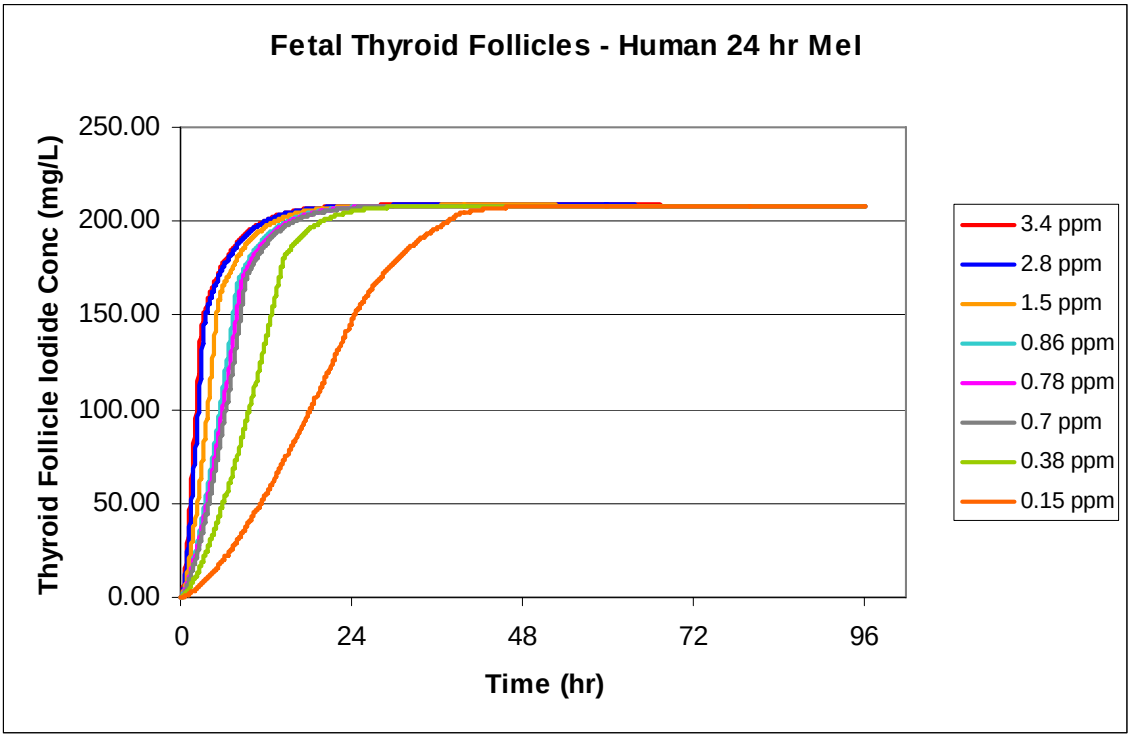


Figure A-6. Modeled iodide in human fetal thyroid follicles at 0.15 - 3.4 ppm MeI. Data from Arysta (2008c).

The high predicted level of iodide in human fetal thyroid can be a serious concern. On the one hand, its high simulated level may merely indicate the need to further adjust the model construct or parameters. The model documentation mentioned that the lack of modeling stability in predicting fetal thyroid iodide resulted in abandoning the initial attempt to use it as the dose metric for HEC determination (Arysta, 2007). On the other hand, although no tissue distribution data from MeI exposure are available for validation, these high levels could have some likelihood of reality since the model description stated that the iodide transfer rates were derived based on the fit to human fetal thyroid iodide levels as used in the models for perchlorate risk assessment (Gargas *et al*., 2005).

Further investigate may be needed for applying the basic human iodide submodel used for assessing the thyroid inhibition by perchlorate to scenarios of excess iodide from MeI exposures. This is desirable even if merely to ensure that the higher level of iodide sequestered into fetal thyroid would not significantly impact the dose metric selection for the HEC modeling. More importantly, if such high fetal thyroid iodide is indeed reflective of human consequence from MeI exposure, its human fetal health implication would be of great concern at the range of 0.15 – 3.4 ppm MeI demonstrated in Figure A-6. In fact, by applying USEPA's default uncertainty factor of 30 to their acute HEC of 4.5 ppm MeI (for 50% GSH depletion in nasal tissue) (USEPA, 2007), the acute RfC would be 0.15 ppm, exactly the level illustrated in Figure A-6.

II.A.4. Human Fetal Stage

The human model is represented by a maternal body weight of 61.1 kg and a fetal weight of 0.27 kg (i.e., a single fetus at maternal weight fraction "VFETC"of 0.0044). The model targets the stage of fetal thyroid ontogeny (Mileson, 2008). Human free T4 and T3 in cord blood begin to increase in gestation week (GW) 12, and the iodide uptake to follicular cells begins to increase in GW18-20 (Howdeshell, 2002). However, fetal thyroid vulnerability should not be limited only to the onset of its ontogeny. The development of iodide autoregulation takes place much later, during GW36-40 (Fuse, 1996; Howdeshell, 2002). Thus, human fetal thyroid vulnerability would not be limited to the end of the first trimester, even when the model is used in the context of the MOA proposed by Arysta (2007), i.e., excess fetal iodide perturbs fetal thyroid function as in a typical Wolf-Chaikoff effect. Instead, the period of vulnerability would extend to any time before the full development of thyroid autoregulation (Fisher and Klein, 1981).

It is also important to consider the impact of MeI exposure at a higher VFETC for the gestation period beyond the end of the first trimester because there is no compelling evidence for a single proposed MOA. In this case, assuming the applicability of the low normalized sensitivity coefficient[1] for VFETC (given as "<0.05" in Table 11 of Mileson *et al*., 2007), a 5- to 10-fold higher value for the VFETC (from 0.27 kg, or 0.6 pounds, to 3 to 6 pound fetuses) for later gestation period may still be significant. A more precise estimate on the effect of VFETC was provided in the Arysta comments to DPR March 2009 draft Volume I (Arysta, 2009). A 10-fold

[1] Normalized sensitivity coefficient (SC) is the change to the predicted dose metrics from changes in model parameter values. It is calculated as the ratio of fractional change in the model prediction to 1% change in the model parameter.

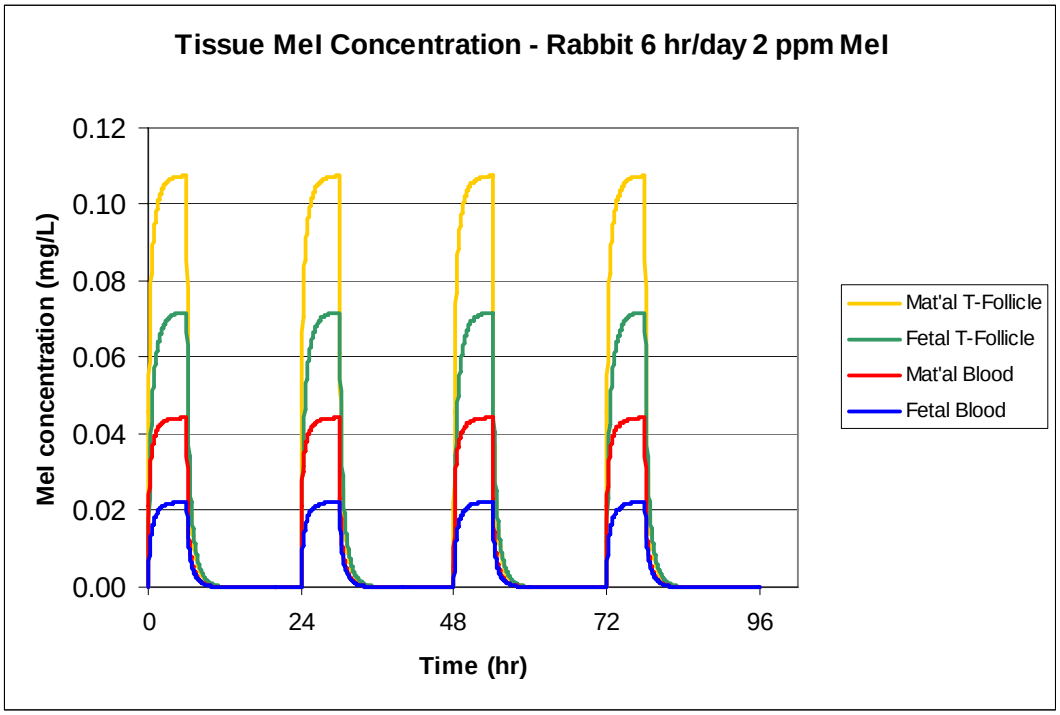
higher VFETC would result in decreased iodide level by 12% in the maternal but 42% in the fetal serum at 0.24 ppm MeI exposure. Given the uncertainties regarding the choice of fetal stage in modeling, its huge impact on the fetal dose metric would also support the use the maternal instead of fetal serum iodide dose metric in HEC determination.

II.A.5. Time Course Profile

Patterns from multiple days of exposure for the dose metrics of interest provide the necessary context for simulating single-day HECs. The general patterns of blood and thyroid distribution of MeI and iodide in rabbits after multiple days of 2 ppm MeI exposure (6 hr/day) are illustrated in Figure A-7 for MeI and Figure A-8 for iodide. Figure A-7 shows that the concentration of MeI diminishes after the 6 hours of exposure without a day-to-day accumulation. Figure A-8 shows that the rise of serum iodide from the first 6 hours of exposure does not return to the baseline by the end of 24 hours. Simulations for humans also follow the similar pattern. Corresponding comparison of maternal and fetal output in rabbits (i.e., comparing blood to blood, thyroid to thyroid) shows that the maternal is higher for the MeI profiles whereas the fetal is higher for the iodide profiles.

II.A.6. Summary

Available data for adjusting the input variables are limited, with experimental data only available at approximately 10-fold above the modeled 2 ppm and with high variability. The fundamental issue concerning modeling fetal death based on surviving fetal data remains unresolved. When compared to available experimental data, the model output shows some discrepancy within the first 24 hours of exposure and to a greater extent beyond the one-day period. In general, the model output shows greater iodide in rabbit fetal serum relative to the maternal level than experimentally reported. This may indicate a greater uncertainty for using rabbit fetal serum iodide as dose metric for HECs. Other questions remain for some biological considerations (e.g., fetal stages) and simulation outcomes (e.g., extremely high fetal thyroid iodide level). Overall, maternal iodide dose metric carries greater confidence than the fetal which is more subjected to unresolved model uncertainties. Similar conclusion was reached by the modeler (Sweeney *et al*., 2009). The dose metric selection is discussed latter, following the MOA discussions.



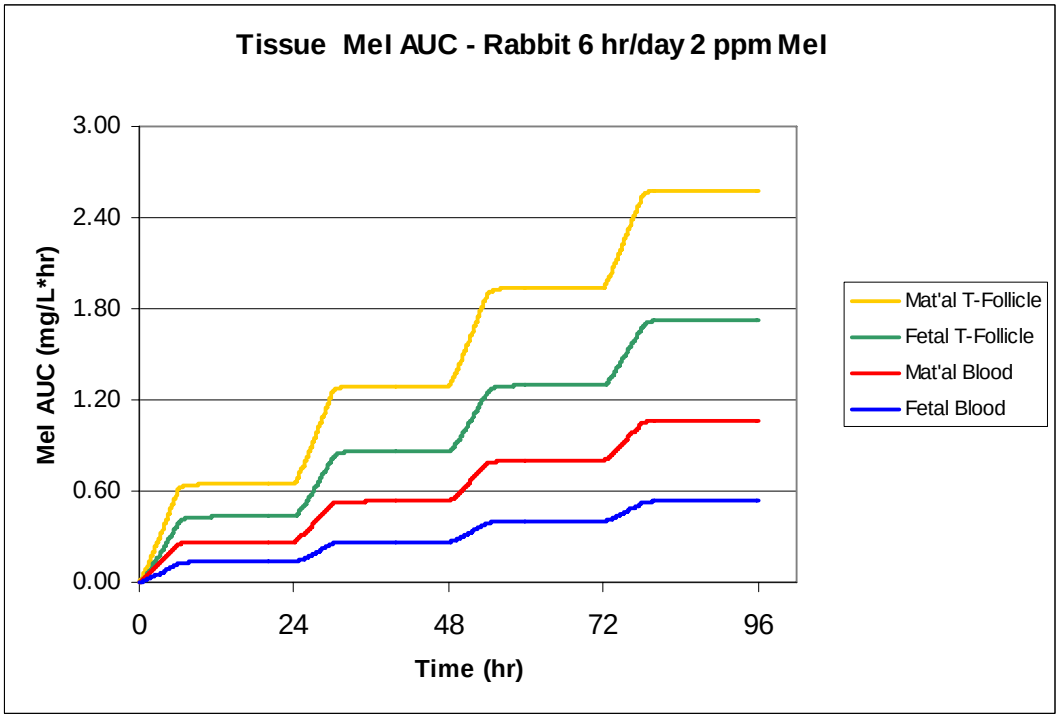
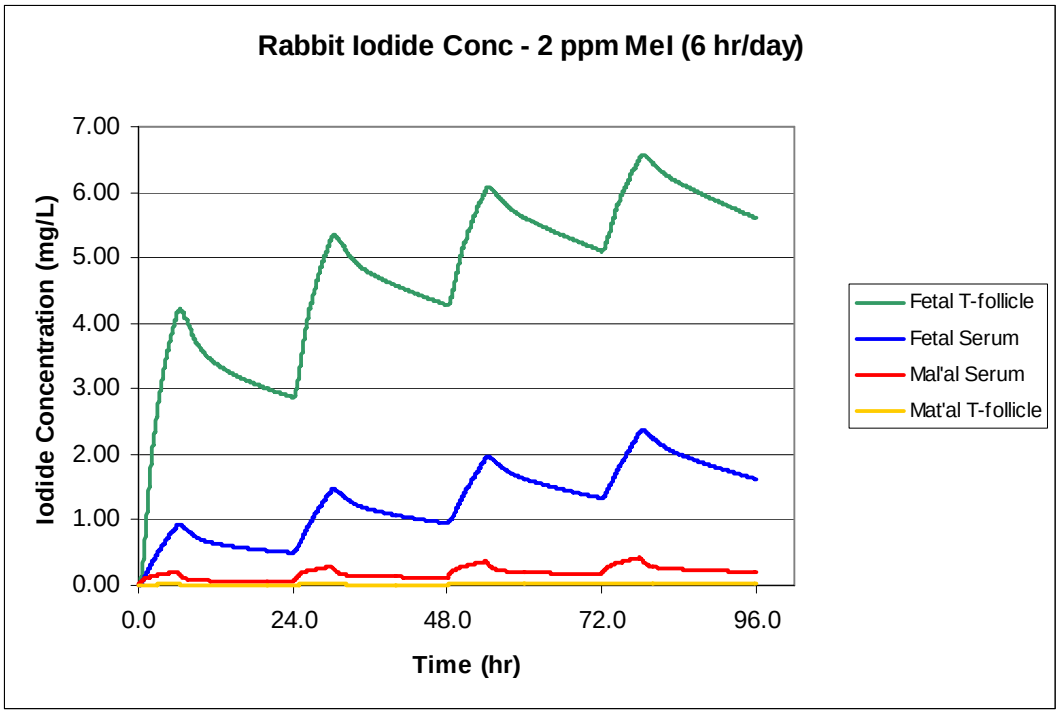


Figure A-7. Modeled rabbit MeI concentrations and AUC (area under the curve) from 2 ppm MeI exposure, 6 hours/day. Simulated at the lower QAC of 12 L-hr/kg$^{3/4}$. Data from Mileson *et al.* (2007). "T-follicle": Thyroid follicle.



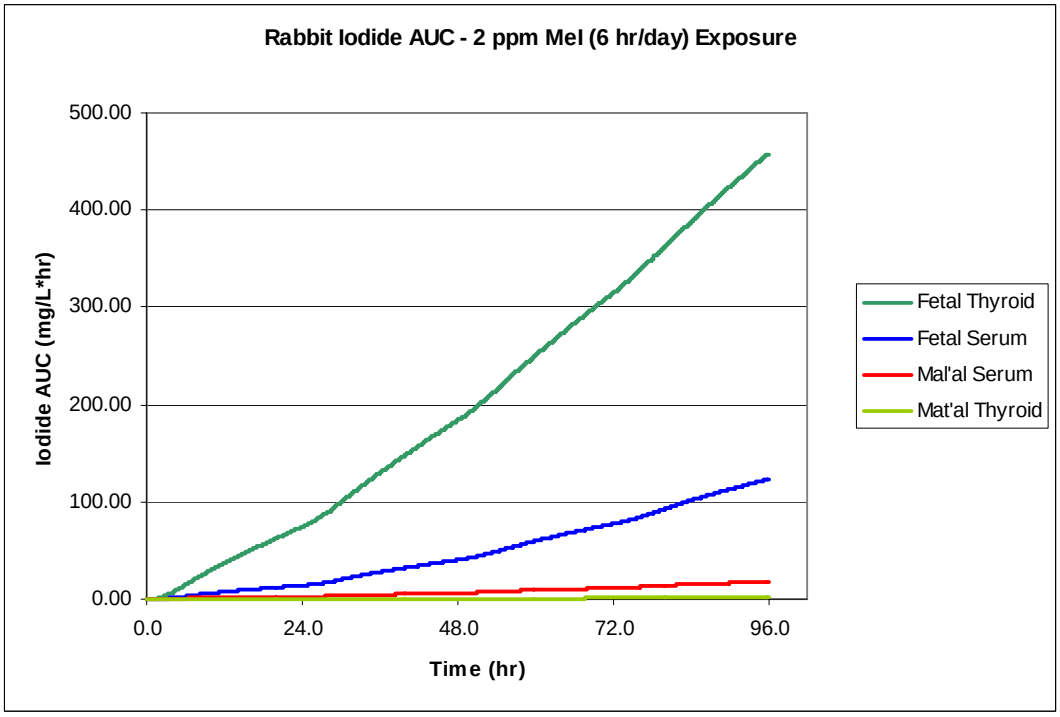


Figure A-8. Modeled rabbit iodide concentrations and AUC (area under the curve) from 2 ppm MeI exposure, 6 hours/day. Simulated at the lower QAC of 12 L-hr/kg$^{3/4}$. Data from Mileson *et al.* (2007). "T-follicle": Thyroid follicle.

II.B. Mode of Action

For MeI, the most consistent observations in laboratory animals following exposure are: elevated iodide and lowered GSH in tissues and in blood, increased hemoglobin S-methylcysteine adduct, and elevated maternal and fetal cholesterol levels. DPR explored four respective possible MOAs: fetal thyroid perturbation from excess iodide, GSH depletion, direct alkylation, and altered cholesterol homeostasis. These observations can be parts of key events within more than one MOA leading to fetal death. On the other hand, USEPA presented only fetal thyroid perturbation as the MOA in their risk assessment (USEPA, 2007). Kirman *et al.* (2009) investigated 4 possible MOAs: DNA methylation, GSH depletion, thyroid hormone modulation and reproductive hormone modulation. Of these, fetal thyroid perturbation was considered by Kirman *et al.* (Arysta) as most consistent with the available data based on the unique pattern of fetal rabbit iodide accumulation as compared to other species, including human.

The International Programme on Chemical Safety (IPCS) framework for MOA evaluation of non-cancer effects for human relevance includes 1) key events, 2) concordance of dose-response relationship, 3) temporal association, 4) strength, consistency, and specificity of association of toxicological response with key events, and 5) biological plausibility and coherence (Boobis *et al*., 2008). In the following discussion, DPR organized the data slightly differently due to data limitations and gaps and also to facilitate comparison to other investigated MOAs. Of the many data limitations, dose-response data (IPCS item 2) are lacking at the dose range of interest. Some of the data for comparing maternal and fetal thyroid effects showed wide variability with small sample size. The concordance data (e.g., thyroid stimulating hormone or TSH, serum iodide levels, GSH depletion) are only available on certain pre-selected time points and dose levels (e.g., companion studies with NaI are at different iodide equivalence levels than the amount of iodide in MeI). Studies designed for PBPK modeling used high concentrations of MeI (20 and 25 ppm) that may or may not inform the pharmacokinetics at the 2 ppm NOEL. The limited fetal death data in the companion studies with NaI makes it difficult to relate to the MeI fetal endpoint. Since all fetal data are collected from fetuses that survived, up to GD 29, relating these data to dead fetuses requires an unlikely assumption that they are representative of the latter, even within the same litter, for which the MOA is to apply. Therefore, in this section, predominant observations are presented in the context of their strength (IPCS item 4) for supporting the possible MOA under each discussion, and their pertinence to the dose metric selection for modeling the HECs.

II.B.1. Fetal Thyroid Perturbation from Excess Iodide

Both USEPA (USEPA, 2007) and Kirman *et al.* (2009) considered the only plausible MOA for fetal death is fetal thyroid function perturbation from accumulation of excess iodide, and thereby support the use of fetal serum iodide level for HEC dose metric. DPR detailed evaluation of the available data showed two periods of concern for fetal death: immediately after two 6-hour exposures on GD 23-24 or GD 25-26 (30 hours from the first exposure), and on GD 29 after 4 or more days of exposure up to GD 28 (Nemec, 2002d and 2003; Sloter, 2005a and b). While fetal

thyroid perturbation may be involved in the latter, there is no clear evidence that it is the sole or immediate MOA for fetal death detected during the GD 23-26 period. The incidences for as little as 2 exposures, while not statistically significant, are biologically relevant to identify this period as the window of vulnerability and the outcome of an acute exposure to MeI (Mileson *et al*., 2009).

Key events

For MOA, the proposed key events include MeI rapid transformation to iodide, and subsequent differential distribution to the maternal and fetal compartments. Fetal thyroid perturbation is the ultimate step that results in fetal death.

Biological Plausibility

This MOA is biologically plausible for MeI toxicity. Iodide is a metabolite of MeI and the thyroid is one of the predominant target tissues after MeI exposure. Depending on the duration and frequency of MeI exposure, the manifestation of thyroid toxicity reported in laboratory animals includes thyroid weight increase, thyroid histopathological changes that include follicular adenoma and carcinoma, and changes in hormone levels especially serum TSH (Volume I, Section III.TOXICOLOGY PROFILE). Elevated iodide levels were measured in the serum after MeI exposure. The window of fetal vulnerability to MeI in rabbits on GD 23 through GD 26 determined by Nemec (2003) and Nemec *et al*. (2009) coincided with active period of fetal thyroid function.

The issue is whether the death of rabbit fetuses is through the MOA of excess iodide specifically to the fetus, or related and influenced by maternal toxicity. If the fetal thyroid effect is the direct cause of fetal death, fetal serum iodide is an appropriate dose metric for PBPK modeling. The fetal death associated with amiodarone, an iodide-containing antiarrhythmic drug structurally similar to thyroid hormone, in pregnant rabbits and rats, has been mentioned as a support for the role of iodide in fetal death (USEPA, 2007; Mileson *et al*., 2009). However, DPR's review of the literature found that amiodarone is a multi-faceted agent with thyroid and extrathyroid effects (Kennedy *et al*., 1989), which do not provide definitive support for only iodide involvement. Two aspects of this MOA, temporal correspondence and species specificity, are discussed in the following sections. It is DPR's position that convincing evidence is lacking for supporting this MOA as the sole or immediate MOA for the endpoint, and thus additional MOA and dose metrics need to be considered.

Temporal Correspondence

The temporal relationship between thyroid effects and fetal death and its comparison between MeI and iodide exposures are examined using data for GD 23 through GD 26 from Sloter (2005a and b). Detailed descriptions of these studies are presented under Volume I, Section III.G.2. Rabbit - Inhalation. Briefly, the first study (Sloter, 2005a) was designed to examine the MOA for fetal toxicity by MeI by collecting data on thyroid status, GSH and iodide levels, hemoglobin adduct,

fetal survival, and histopathology (Volume I, Table 36 to 41). The second study (Sloter, 2005b) included a NaI treatment group for comparison to determine the role of iodide in the toxicity (Volume I, Table 42 to 48). Pertinent results from these tables, with a focus on GD 23 to GD 26, are repeated in Table A-4 and A-5, as well as shown in Figure A-9 and A-10 for ease of discussion. While there are limitations in these sets of data due to difference in MeI concentration (25 ppm versus 20 ppm), wide variations of values, and absence of information at <20 ppm, they are sufficient for this discussion.

Rabbit Thyroid and Fetal Death from MeI

Comparison of maternal and fetal TSH data after MeI exposure shows greater consistency in maternal than in fetal TSH patterns after 2 days of MeI exposure, suggesting a role for maternal effects in the MOA. The maternal TSH level is significantly increased, as early as after 2 days of exposure (GD 23 to GD 24) - the first time point of evaluation (Table A-4 and A-5). Its persistence onto day 4 (from GD 23 to GD 26) of exposure was observed only with MeI at 25 ppm (Table A-4). On the other hand, the average TSH levels in the fetuses are somewhat lower than the controls during GD 23-24, but greatly increased in the GD 23-26 groups. Figure A-9 showed that the increase for fetal TSH was most evident between GD 25 and 26 to a peak level of 5.1 ng/ml, which declined only slightly (4.4 ng/ml) by GD 29. This high persistent fetal TSH from 4 days of MeI exposure and over the 3 days of recovery period (GD 26 to GD 29) raises a concern on potential for post-natal developmental toxicity in the surviving fetus. A more detailed discussion related to this concern is presented in Volume I Section V.C.1.a. Pre- and Post-natal Sensitivity.

There is a lack of causal relationship between fetal thyroid perturbation and fetal death, within the GD 23 and 24 period, after MeI exposure, to support this MOA. Histopathology in the fetal thyroid included decreased colloid, follicular epithelial vacuolation and follicular hypertrophy (Table A-4 and A-5). In Sloter (2005a) at 25 ppm MeI, higher incidences were reported in the 4-day than the 2-day exposure groups, yet the “% fetus/litter” of late resorption immediately after the end of MeI exposure were the same at these corresponding time points (i.e., 5.7% after 2 days and 5.2% after 4 days of MeI exposure) (Table A-4). The fact that these thyroid histopathologic findings are from fetuses that survived to a given examination time point when death to some fetuses had already occurred, makes it more difficult to establish any relationship between these two endpoints.

Table A-4. Rabbit maternal and fetal thyroid status reported by Sloter (2005a).[a]

Effects	MeI (6 hr/day) on GD 23-24		MeI (6 hr/day) on GD 23-26	
	0 ppm	25 ppm	0 ppm	25 ppm
Maternal				
TSH, ng/mL	0.73 ± 0.13	0.99 ± 0.32*	0.77 ± 0.11	1.05 ± 0.30*
T3, μg/dL	0.190 ± 0.021	0.144 ± 0.030*	0.154 ± 0.025	0.122 ± 0.026*
T4, μg/dL	1.9 ± 0.5	1.4 ± 0.5	1.8 ± 0.5	1.6 ± 0.6
Serum iodide, mg/L (range)	0.027 - <0.1	17.2±4.0[b] (9.8-22.7)	<0.02 - <0.1	26.4±19.5[b] (10.2-78.0)
Fetal[c]				
TSH, ng/mL	1.70 ± 0.23	1.47 ± 0.42	1.37 ± 0.27	9.71 ± 8.94*
T3, μg/dL	0.007 ± 0.0028	0.003 ± 0.003*	0.011 ± 0.005	0.012 ± 0.006
T4, μg/dL	0.09 ±0.07	0.05 ± 0.10	0.26 ± 0.13	0.06 ± 0.07*
Thyroid colloid decrease[d]	0/79 (0%)	10/73 (14%)	0/99 (0%)	72/81 (89%)
Thyroid follicular hypertrophy[e]	0/79 (0%)	27/73 (37%)	0/99 (0%)	76/81 (94%)
Serum iodide, mg/L	0.18 (0.12-0.26)	37.1±12.0[b] (21.6-55.9)	<0.1 - 0.165	70.6±27.8[b] (33.5-128.0)
Fetal Late Resorption immediately after the 2 or 4 days of exposure				
% Fetus/Litter	1.8±3.9	5.7±9.2	1.7±3.7	5.2±7.6
% Litter Affected	2/10 (20%)	3/9 (33.3%)	2/10 (20%)	4/10 (40%)

a/ * for statistical significance at $p<0.05$. T4 is reported as bound T4. Animals were killed immediately after the last MeI dosing.

b/ not including the duplicates for one sample.

c/ TSH, T3, and T4 measurements were from pooled samples of multiple surviving fetuses per litter.

d/ mild to severe grade in males, minimal to moderate grade in females.

e/ minimal to moderate in males, minimal to mild grade in females.

Table A-5. Rabbit maternal and fetal thyroid status and survival reported by Sloter (2005b).[a]

Effects	GD 23-24			GD 23-26		
	0 ppm	20 ppm MeI	iv NaI	0 ppm	20 ppm MeI	iv NaI
Maternal						
TSH, ng/mL	0.46±0.11	0.62±0.04*	0.62±0.08*	0.58±0.24	0.58±0.15	0.60±0.07
T3, μg/dL	0.173 ±0.016	0.158 ±0.020	0.133 ±0.022*	0.122 ±0.024	0.114 ±0.025	0.123 ±0.016
T4, μg/dL	1.43±0.42	1.44±0.46	1.15±0.71	0.60±0.38	0.84±0.89	0.61±0.66
Thyroid hyper-trophy	-	-	-	0/5 (0%)	2/5 (40%)[b]	0/5 (0%)
Thyroid colloid depletion	-	-	-	0/5 (0%)	3/5 (60%)[b]	1/5 (20%)[b]
Serum iodide[c], mg/L (range)	-	14.3±2.4 (12.9-18.7)	6.29±0.53 (5.75-6.75)	0.0052-0.049	16.6±6.8 (11.1-29.6)	9.63±11.0 (3.33-29.2)
Fetal[d]						
TSH, ng/mL	1.9±0.5	1.7±0.4	2.0±0.6	1.9±0.9	5.1±1.5*	5.2±2.9*
T3, μg/dL	0.010 ±0.002	0.014 ±0.005	0.008 ±0.002	0.015 ±0.003	0.027 ±0.013	0.016 ±0.002
T4, μg/dL	0.05±0.04	0.08±0.05	0.03±0.03	0.06±0.04	0.03±0.05	0.01±0.01
Thyroid hyper-trophy	0/20 (0%)	16/20 (80%)[b]	12/20 (60%)[b]	0/20 (0%)	16/20 (80%)[e]	18/20 (90%)[e]
Thyroid colloid depletion	0/20 (0%)	1/20 (5%)[b]	0/20 (0%)	0/20 (0%)	13/20 (65%)[f]	17/20 (85%)[g]
Serum iodide[c], mg/L	-	33.2±11.9 (14.8-46.4)	23.0±6.32 (15.4-30.3)	0.124-0.217	72.6±23.2 (53.9-110)	35.9±17.2 (14.4-55.3)
Fetal Late Resorption assessed on GD 29						
% Fetus/Litter	-	-	-	4.7±6.48	50.4 ±28.09*	6.8±10.9
Litter Affected	-	-	-	2/5 (40%)	4/4 (100%)	2/5 (40%)
Fetal Viability on GD 29						
% Fetus/Litter	-	-	-	93.1±10.1	46.8±29.3*	87.5±7.05

a/ * for statistical significance at $p<0.05$. T4 is reported as bound T4. Except for fetal late resorption, all maternal and fetal data were from animals killed immediately after the last MeI dosing; fetal data were from live fetuses that survived the treatment.

- The inhalation dose at 20 ppm MeI (6 hr/day) is estimated as 15 mg MeI/kg/day (Nemec, 2002d), or 13.4 mg iodide/kg/day [=15 x (127/142)].
- The dose for NaI consists of four 15 minutes infusions of NaI in 6 hrs, at a total of 81.2 μmole/doe/day. This is 12.2 mg (=149.89 x 0.0812) sodium iodide/day, or 3.04 mg/kg/day at a body weight of 4 kg, or 2.57 mg iodide/kg/day [= 3.04 x (127/150)].

b/ All in grade "minimal".

c/ Samples taken immediately after the end of the last MeI exposure.

d/ TSH, T3, and T4 measurements were from pooled samples of multiple fetuses per litter.

e/ Approximately half of them as grade "minimal", half of them grade "mild".

f/ 6/20 in grade "minimal", 1/20 in grade "mild", 6/20 in grade "moderate".

g/ 7/20 in grade "minimal", 4/20 in grade "mild", 6/20 in grade "moderate".

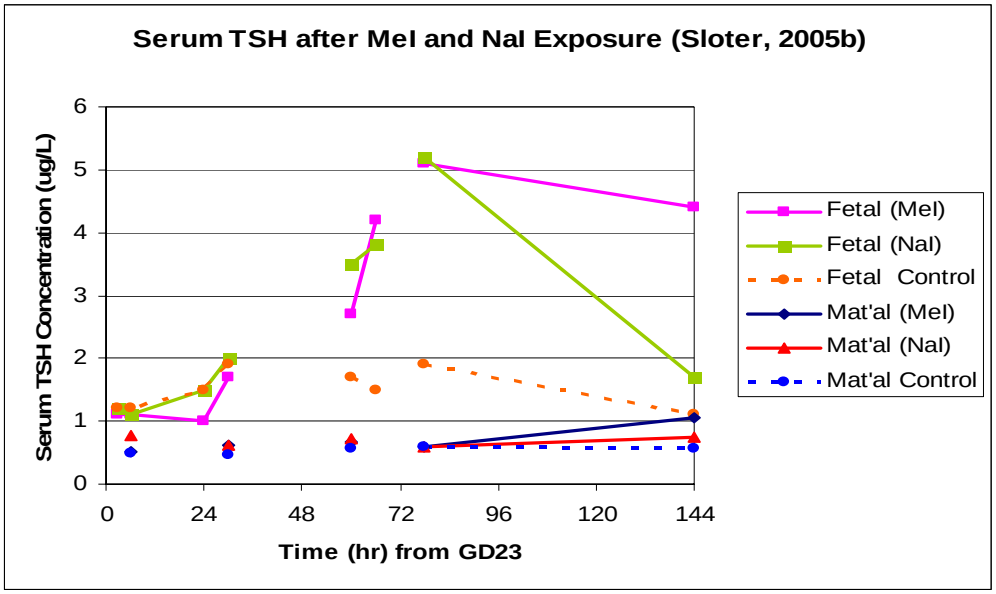


Figure A-9. Rabbit maternal and fetal serum TSH after MeI and NaI exposure. Data from Sloter, 2005b. Rabbits received 6-hr/day inhalation exposure to 20 ppm MeI at hrs 0-6 24-30, 48-54, 72-78; or 81.2 µmoles of NaI/day through 4 i.v. injection (total 3.04 mg NaI/kg) within each daily 6 hours treatment period. Data gaps are indicated by the disconnected lines. Hour 72=GD 26.

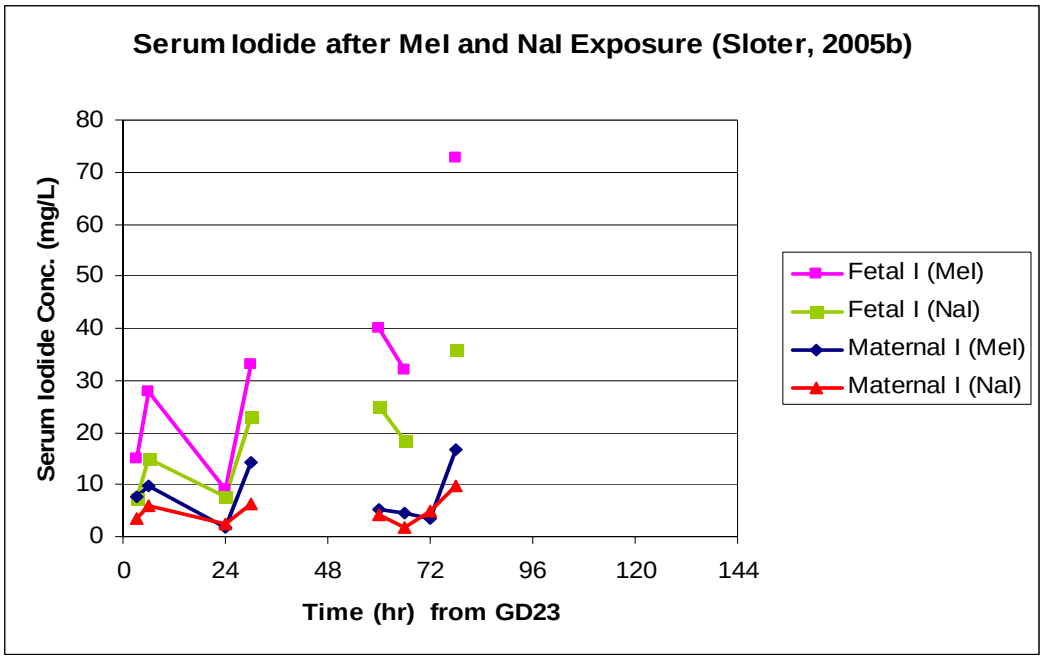


Figure A-10. Rabbit maternal and fetal serum iodide after MeI and NaI exposure. Data from Sloter, 2005b. Rabbits received 6-hr/day inhalation exposure to 20 ppm MeI at hrs 0-6 24-30, 48-54, 72-78; or 81.2 µmoles of NaI/day through 4 i.v. injection (total 3.04 mg NaI/kg) within each daily 6 hours treatment period. Data gaps are indicated by the disconnected lines. The iodide level in the controls cannot be effectively presented in the same graph because of their markedly lower levels (maternal at 5.18-48.6 µg/L, fetal at 124-217 µg/L). The same X-axis scale as in Figure A-9 is used for ease of data comparison. The gaps are due to missing data points. Hour 72=GD 26.

Rabbit Thyroid and Fetal Death – MeI versus NaI

Data from MeI and NaI treatments in rabbits provide further analysis on the involvement of iodide in fetal death. The comparisons show that fetal thyroid perturbation through excess iodide is not the sole MOA for fetal death. Effects of high fetal serum iodide on fetal thyroid may be second in sequence, after an initial MOA that causes fetal death from MeI. In Sloter (2005b), the estimated iodide dose for MeI (13.4 mg/kg/day) is 6-fold higher than that for NaI (2.58 mg/kg/day) exposure, assuming 100% uptake and rapid and complete breakdown. Yet, both MeI and NaI treatments resulted in comparable incidence and severity on fetal thyroid histopathology (Table A-5, more detailed in Volume I, Table 48). With the higher iodide exposure in the MeI group, significantly increased fetal late resorption on GD 29 (2 days after the last exposure on GD 26) and reduced fetal viability was seen only in the MeI group, but not with the NaI group (Table A-5).

Data from the second study by Sloter (2005b), as presented in Figure, provide the pattern of maternal and fetal iodide distribution after 20 ppm MeI inhalation exposure at 6 hours/day (15 mg MeI/kg/day, 13.4 mg iodide/kg/day) and 4 NaI i.v. injections within 6 hours/day (total of 3.04 mg NaI/kg/day, 2.58 mg iodide/kg/day) is shown (Sloter, 2005b). Within the conditions of the experimental protocol, the time course pattern of serum iodide from both treatments appears similar in the peaks and valleys corresponding to the 6 hours per day treatment schedule (Figure A-10). While the calculated iodide based on the reported dose for the NaI group is 20% that for MeI group, the data showed much higher iodide levels than expected for the NaI group. The average serum iodide levels for all GD periods from NaI exposure are 75% and 61% of those from MeI exposure in the maternal and fetal blood, respectively (Volume I, Table 43 and 44). The fetal-to-maternal serum iodide ratio varies widely, ranging from 2 (3 hours after start of GD 23 exposure to MeI) to 10.7 (12 hours after the 6 hours of NaI exposure from GD 23-25).

The fetal late resorption data (Sloter, 2005b) after MeI exposure showed high variability with only 4 - 5 litters per group (Table A-5). The incidence (% fetus/litter) at 20 ppm MeI after only 4 days of exposure (GD23-26) is high (50.4% versus 4.7% in the controls), while no deviation from the controls can be found for NaI treatment (6.8%). Yet, the fetal serum iodide is only 2-fold higher in the MeI compared to the NaI group. Assuming a linear relationship in serum iodide level between 10 and 20 ppm MeI exposures, the fetal serum iodide equivalent level at the LOEL of 10 ppm MeI for fetal death could be the same or lower than the serum iodide from NaI intravenous infusion from this study. The much lower fetal death (6.8%) from NaI treatment in this study versus the 11.1% for fetal death at 10 ppm MeI (Volume I, Table 34a) would indicate that excess fetal iodide is likely not the only contributing factor to fetal resorption or death from MeI within this time frame. However, data limitations (e.g., different dosing regimen for MeI and NaI treatment, limited data measurement time points) preclude further conclusion.

In addition, an iodide effect on fetal TSH levels does not seem to be associated with fetal death. As shown with the GD 23-26 exposure group, fetal death was higher from MeI than NaI exposure; yet, fetal TSH levels measured on GD 26 were not different between the two treatments (Table A-5). Fetal death was already evident after 2 days (Table A-4, immediately after GD 23-24 versus GD 23-26 exposure to MeI) while the markedly higher fetal TSH from MeI exposure was not evident until measured after GD 26 exposure (Table A-4 and A-5).

Rabbit Serum Iodide – Maternal versus Fetal

Analysis of the iodide data shows that maternal serum iodide may be an appropriate dose metric, if maternal thyroid effect is one of the key events for fetal death. A single day tissue distribution profile of iodide in rabbits from NaI treatment is available in Morris *et al.* (2004; Volume I, Table 50). Although the iodide in fetal plasma was not reported in the study for comparison, maternal data showed close resemblance between blood and plasma, indicating these two expressions may be interchangeable. In the calculation of F/M thyroid ratios, values for fetal thyroid+trachea (Volume I, Table 50) are used since iodide level in this fraction represents that in the thyroid alone (Thrall *et al*., 2009b). The F/M ratios for iodide in the blood are higher than those for the thyroid, and are generally higher at the low NaI level (Table A-6).

Table A-6. Rabbit fetal-to-maternal iodide ratio (F/M) in thyroid and blood.[a]

Hours after injection	Thyroid F/M		Blood F/M	
	Low NaI	High NaI	Low NaI	High NaI
0.5	1.38	0.83	2.22	0.65
1.0	1.60	0.69	2.69	0.91
2.0	0.95	1.15	4.11	1.55
4.0	0.86	0.89	2.64	2.33
6.0	0.78	0.94	5.71	2.59
12.0	0.51	0.62	4.17	2.83
24.0	0.18	0.42	5.80	2.71

a/ Data from Morris *et al*., 2004. Pregnant rabbits received a single i.v. injection of $Na^{131}I$ at "Low NaI" level of 0.75 mg/kg or "High NaI" level of 10 mg/kg on GD 25.

The corresponding fetal tissue iodide distributions from the study, along with the maternal thyroid and blood concentrations are presented in Figure A-11a and Figure A-11b for the two NaI treatments. At both NaI treatment levels, the highest maternal iodide concentration is in the thyroid. The pattern of iodide distribution to the thyroid is different between the does and the fetuses. The maternal and fetal thyroid iodide both reached approximately the same level within 2 hours, and remain more or less steady until 6 hours. Thereafter, the maternal thyroid continues to accumulate iodide but not the fetal thyroid. At the end of 24 hours, the concentration in the maternal thyroid was higher than the blood by 270-fold with 0.75 mg NaI/kg treatment and 70-fold with 10 mg NaI/kg treatment. The high maternal thyroid iodide pattern on GD 25 appears to correspond to the TSH profile shown in Table A-5 for NaI and MeI exposures by Sloter (2005b), i.e. increased maternal TSH at the earliest measurement, i.e., immediately after the second of a 2-day NaI exposure on GD 23-24. However, fetal TSH level did not change during this time. If maternal thyroid effect is indeed a key event, maternal serum iodide can be a reasonable marker of MeI exposure as long as it is a valid indicator of effects to the thyroid. In the absence of a clear MOA to direct attention to a specific target organ, maternal serum iodide is still the default dose metric for modeling the exposure.

Contrary to the pattern of maternal iodide distribution, the highest fetal iodide concentration is in the stomach contents and the lowest in the blood, with the levels at other measured sites (trachea, thyroid+trachea, amniotic fluid) all higher than in the blood. This higher iodide in fetal tissues raises a concern for potential damage from excess iodide in tissues besides thyroid and may contribute toward MeI-induced fetal death immediately after the second 6-hr MeI exposure.

Figure A-11a

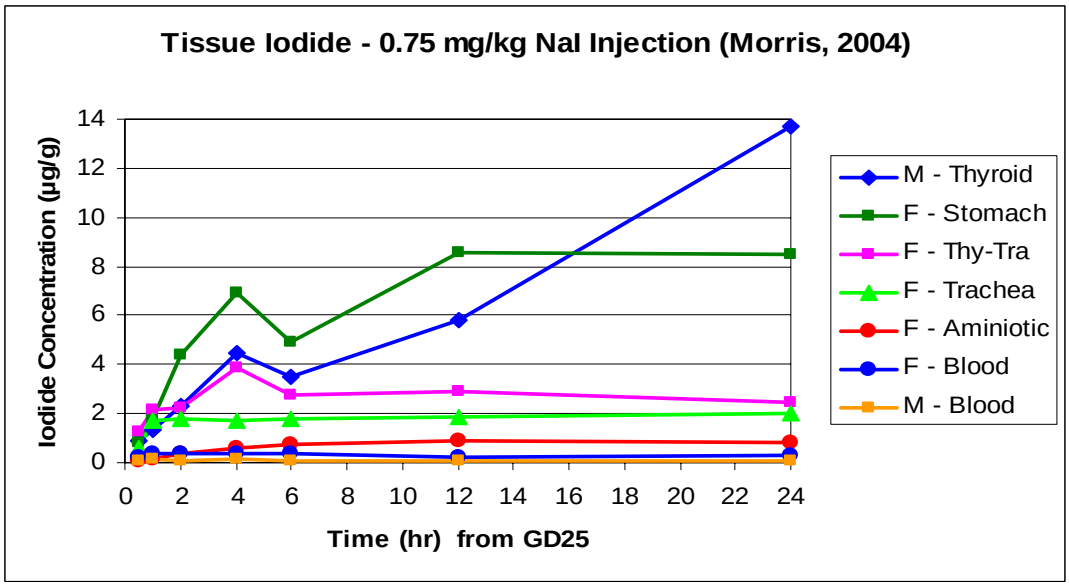


Figure A-11b

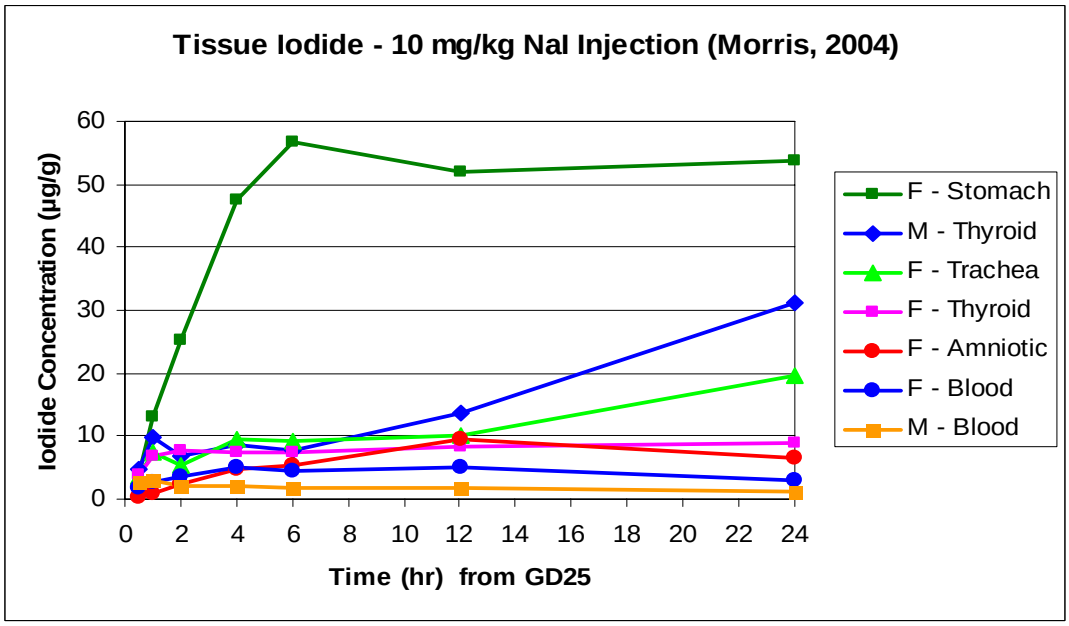


Figure A-11. Rabbit fetal tissue iodide after a bolus injection of NaI at 0.75 mg/kg (Figure A-11a) or 10 mg/kg (Figure A-11b). Data from Morris *et al*., 2004. Maternal concentration in blood and thyroid are included as reference levels. Data at time zero are not presented because of its relatively low iodide level.

Rat Fetal Death from MeI

Both USEPA (2007) and Mileson *et al.* (2009) indicated that this proposed MOA for rabbits should also be applicable to rats. If supported by data, this interspecies relevance could strengthen the validity of the proposed MOA. Since the onset of active fetal thyroid function in rats occurs on GD 17-18 (Morreale de Escobar *et al*., 2004; Howdeshell, 2002), USEPA (2007) and Mileson *et al.* (2009) speculated that the same window of fetal vulnerability temporally associated with fetal thyroid ontogeny could be demonstrated had the developmental toxicity study in rats by Nemec (2002c) been extended beyond GD 17. Cited for support is decrease in number of live births (Volume I Table 33; Nemec, 2002a and b) from the 2-generation reproductive toxicity study where the pregnant rats were exposed to MeI up to GD 20. However, it should be noted that the Nemec (2002c) study submitted to DPR showed MeI dosing to GD 19, not on GD 17 as indicated by USEPA (2007) and Mileson *et al.* (2009). In this study, no significant fetal death was found on GD 20 at MeI up to 60 ppm (81 mg/kg/day), the highest dose tested. Furthermore, an examination of the survival data at and shortly after birth showed higher rat pup survival (Volume I, Table 33) than for rabbits (Volume I, Table 34a). At the highest dose of 50 ppm MeI (68 mg/kg/day MeI), the number of live pup/litter at birth was reduced by 12% (12.5 versus 14.2 pup/litter) and pup survival to day one was reduced by 15% (82.6% versus 98.0%) (Volume I, Table 33; Nemec, 2002a and b). These decreases are much lower than the 41% reduction in rabbits (3.6 versus 6.1 viable fetus/litter; Volme I, Table 34a; Nemec, 2002d) in rabbits at the highest tested dose of 20 ppm (15 mg/kg/day) (Nemec, 2002d), a dose level approximately 4-fold lower than in rats. Thus, it is not obvious how the existing data could have supported the speculation of USEPA (2007) and Mileson *et al.* (2009). Additional data from available studies on MeI and iodide pertinent to the interspecies sensitivity comparisons between rats and rabbits are presented in the following section.

Species Specificity

A key feature of the thyroid perturbation as the sole MOA for MeI-induced fetal death proposed by Kirman *et al.* (2009) is that fetal rabbits are uniquely sensitive to excess iodide because (1) the fetal rabbit thyroid lacks the autoregulatory capacity to limit the effects of excess iodide (Price and Sherwin, 1986), (2) the fetal rabbit concentrates more iodide relative to the doe (Logothetopoulos and Scott, 1956), (3) higher postnatal death in rabbits than other species (Arrington *et al*., 1965), and (4) human conceptus does not highly concentrate iodide relative to the maternal circulation (Sloter *et al*., 2009; Rayburn *et al.*, 2007). However, DPR considers the data from cited studies as insufficient to establish such species specificity for iodide toxicity in the rabbit and the rationale is presented below.

Fetal Thyroid Iodide Accumulation – Autoregulatory Capacity

Price and Sherwin (1986) studied the *in vitro* uptake and efflux of radiolabeled iodide using thyroid slices (5 to 15 mg/slice) from New Zealand white pregnant rabbits (26 to 30 days pregnant) and the intact lobes (2 to 5 mg) from their fetuses. After 90 minutes of incubation, fetal preparation showed a 9-fold higher tissue-to-medium iodide ratio (65±1.6) compared to that (7.1±1.6) for the maternal slices. Preincubation of slices with NaI (30 µM) for 90 minutes resulted in lower maternal ratio (4.9±1.2), suggesting lower uptake into the tissues due to

autoregulatory control. The fetal ratio (62±3.6) was similar to the ratio without preincubation. ^{125}I efflux from prelabel slides showed slower efflux with fetal tissue (0.026±0.004 min^{-1}) compared to maternal tissue (0.074±0.010 min^{-1}). Based on these results, the authors concluded that the fetal thyroid lacked an autoregulatory mechanism to prevent excess iodide uptake. DPR does not consider the limited *in vitro* data in this single study sufficient to define the presence or absence of autoregulatory capability in fetal rabbits *in vivo*. Moreover, this 9-fold factor was not evident from the Morris *et al.* (2004) study with NaI given by intravenous injection to GD 25 pregnant rabbits. The fetal/maternal thyroid iodide ratios were highly variable, likely due to the low levels in the fetal thyroid and the dynamic nature between these maternal and fetal compartments (Table A-6).

Fetal Iodide Accumulation – Maternal and Fetal

In a study by Logothetopoulos and Scott (1956), pregnant guinea pigs (GD 36 to 65), rabbits (GD 12 to 30), and rats (GD 20 to 21) were injected with ^{131}I after pretreated with propylthiouracil to prevent binding of iodide to the thyroid. They showed active placental transport of iodide that could be inhibited by thiocyanate existed in guinea pigs, rabbits, and rats. Due to the limited time course of study to characterize the dynamics of circulating iodide, the authors cautioned that their conclusion was based on the assumption of steady level of maternal serum iodide and specific activity equilibrium across placenta at the point of study after subcutaneous injection of carrier free ^{131}I. The authors stated that iodide concentrating mechanism might not exist in rats during GD 20-21, the only gestation days tested, because the fetal-to-maternal plasma iodide ratios ranged from 1.2-1.5. In comparison, the data suggested fetal accumulation of iodide in rabbits because the fetal-to-maternal iodide ratios were greater than 1 (ranged approximately from 1.5 to 2.6, n=3, from graph) on GD 24 and GD 26 (range from 1.2 to 3.3, n=5). However, the authors also stated that two rabbits have the fetal-to-maternal iodide ratio of less than 1, indicating wide variability. Further observations on the contribution of rabbit fetal iodide accumulation to the fetal rabbit sensitivity to MeI are presented in Section **II.C.1.**

Post-natal Death from Iodide – Rabbit versus Other Species

Toxicity data on prenatal iodide exposure and postnatal death in rabbits and other species are limited. In Arrington *et al.* (1965), rabbits, rats, hamster, and swine were exposed to iodide using different protocols. For this discussion, only the data for rats and rabbits are pertinent. Pregnant Dutch and New Zealand white rabbits were exposed to 250 to 1000 ppm iodine in the diet for 2, 5 or 10 days before parturition. At the 250 ppm or 500 ppm levels for 2 days before parturition, there was only 30% pup survival in 3 days or 4 weeks postpartum, compared to 91% for control. At 500 or 1000 ppm given for more than 2 days, the survival for either periods were: 3% (500 ppm for 5 days), 0% (500 ppm for 10 days), and 4% (1000 ppm for 5 days). For Long-Evans rats, 2500 ppm iodide resulted in "less than 10% of the young survived 3 days," but it was not clear when the exposure occurred. This seemed to be a separate experiment than the one with female rats exposed to 2500 ppm iodide beginning 24 hours after parturition. There was increased average parturition time (control 1.8 hours; treated 3.7 hours, excluding the 4 females which failed to complete parturition in 24 hours). Based on estimated doses of 90 mg iodide/kg/day for rabbits, and 150 mg iodide/kg/day for rats for 2500 ppm, the authors concluded

that rabbits are more sensitive than rats to iodide. The authors also suggested that the actual iodide exposure of rabbits might have been higher due to coprophagy. It is DPR's view that the results from this study are inadequate to support a definitive conclusion about species differences for iodide due to different protocols used, lack of detailed information about food consumption and the rat experimental design, and coprophagy in rabbits. Moreover, the results examined postnatal pup survival and thus do not contribute toward the MOA of MeI-induced fetal death. Instead, the postnatal death raises the concern for the potential neurodevelopmental effects from excess iodide that has not been thoroughly investigated as the only studies on rabbit fetuses were those where the experiments were terminated up to GD 29.

Iodide Levels in Human – Maternal and Fetal

Kirman *et al.* (2009) concluded that the human conceptus does not accumulate iodide when fetal and maternal iodide levels from two human studies are compared to those from the rabbit studies. The average human umbilical cord-to-maternal plasma iodide ratio was 0.9 - 1.3 for pregnant women with no known history of excess iodide intake (Volume I, Table 51; Rayburn *et al.*, 2007). This ratio was said to be similar to values Kirman *et al.* (2009) calculated from women given iodomethylsparteine from Cottino *et al.* (1972). The average cord/maternal ratios were 0.6 to 1.0 for three time intervals (0.25 to 1 hour, 1.5 to 3 hours, and 4 to 9 hours). On the other hand, Kirman *et al.* (2009) calculated an 8 to 10-fold higher iodide levels in the fetal serum than in the maternal serum for untreated rabbits from the Sloter studies. As support, Kirman *et al.* (2009) cited the information from a review by Roti *et al.* (1983), which indicated higher iodide levels in the sheep fetus (4 to 5 folds) and rabbit fetus (5 to 9 folds) blood compared to their respective maternal blood.

It should be noted that both maternal and fetal iodide levels in the non-treated rabbits from the Sloter studies are relatively low and most were below the detection limit (Volume I, Table 39, 40, and 43). Thus, there is some uncertainty in the calculations of these levels. Furthermore, any comparisons using average values alone obscured the wide variability in background iodide levels. In the Rayburn *et al.* (2007) study, the maternal plasma and cord plasma iodide levels ranged from 0.3 to 5.6 µg/dL and 0.3 to 4.5 µg/dL, respectively (Volume I, Table 51; Rayburn *et al.*, 2007). The untreated human cord/maternal plasma iodide ratios ranged from 0.35 to 5.4, with ratios at or greater than 2 in 12 of 121 set of ratios. In the study by Cottino *et al.* (1972), 18 pregnant women received iodomethylsparteine through i.v. injection during labor and delivery with maternal and cord blood samples collected from each for a single time point after injection. The cord/maternal serum iodide appeared to increase with time: 0.5 to 0.6 (0.25 hours to 1 hour, n=6), 0.7 to 0.9 (1.5 to 3 hours, n=5), and 1.0 to 3.4 (4 to 4.5 hours, n=3). The ratios for later time points (7, 9, 24, and 48 hours) were about 1, but there was only one subject for each time point. Thus, existing data do not allow a definitive determination of whether or not the human conceptus can accumulate iodide. The higher iodide accumulation in rabbit fetuses as concluded by Kirman *et al.* (2009) has an important implication on the final HEC for fetal death in PBPK model. It should be noted that the higher iodide accumulation in rabbit fetus compare to human fetus, if true, could support the use of maternal serum iodide as the dose metric (discussed in Section II.D.1. General Population HEC).

II.B.2. GSH Depletion

Key Event

Data are lacking for considering the GSH depletion as a key event to fetal death by MeI. Nevertheless, the possibility of an MOA through oxidative stress cannot be ruled out.

Biological Plausibility

GSH reduction is a marker for oxidative stress. Oxidative stress during the pre-natal period has been recognized as one of the MOA for human diseases of developmental origin (Hussain and Ali, 1998; Dani *et al*., 2004; Rossi *et al*., 2006; Luo *et al*., 2006; Wells *et al*., 2009). Maternal oxidative stress can result in fetal pre-programming for diseases and/or heighten susceptibility.

Data on GSH depletion in rabbit maternal and fetal blood and tissues are limited to exposures during GD 23 to 26 and are summarized in Table A-7 (Sloter, 2005a and b). The GSH profile at the site of contact (i.e., maternal olfactory and nasal epithelium) is not included in the table because the issue at hand is systemic toxicity. The time course from the second study (Sloter, 2005b) is available only from 3 hours after the start of MeI exposure on GD 23 to the end of the 6 hours of exposure on GD 24 (Figure A-12). Among the few tissue sites investigated, fetal blood showed the most significant and persistent change (Table A-7). There is no difference in GSH reduction between 20 and 25 ppm MeI exposure. A 38% reduction (or, 62% of the controls) occurred after one 6-hour exposure to 20 ppm MeI (GD 23; Sloter, 2005b). After the second day of exposure (on GD 24), fetal blood GSH was reduced by 45% (or, 55% of the controls) (Sloter, 2005b). A similar pattern was also noted at 25 ppm, with a further decline from 28% reduction after 2 exposures to 43% reduction after 4 exposures (Sloter, 2005a). On the other hand, maternal GSH decline from 1 to 2 exposures lacks consistency. The pattern is detected only in the second study (Sloter, 2005b) while the first study (Sloter, 2005a) shows an apparent recovery from 2 to 4 exposures. These data show that fetal blood GSH reduction is greater than the maternal. In addition, the fetal GSH baseline is approximately 25% lower than the maternal; i.e., average 0.419 mM in fetuses versus 0.548 mM in the does (Table A-7, mean of control means from both studies). Thus, the fetal blood GSH concentration after MeI exposure can be as low as only 47% of the maternal at a given measurement time point (i.e., 0.216 mM/0.460 mM for GD23-26, 25 ppm). This may suggest that fetuses can be at a greater risk for oxidative stress.

The corresponding timing between GSH changes and MeI toxicity can be more closely traced through *in vitro* studies. Glutathione depletion by MeI causes cytotoxicity in cultured neural cells (Davenport *et al*., 1992; Bonnefoi, 1992). Chamberlain *et al.* (1999) demonstrated a steep dose-response relationship in the death of cultured rat cerebellar granule cells, with a 6 hours delayed onset after 5 minutes of exposure to MeI above 0.5 mM MeI[2]. The EC_{50} was reported at 1.6 mM, and a 100% cell death occurred at 3 mM MeI 24 hours after the 5 minute exposure. The role of GSH depletion in cytotoxicity was demonstrated in the enhancement of cell death with GSH synthesis inhibitor BSO and the protection against cell death with antioxidants (e.g.,

[2] 0.5 mM MeI (71 mg/L MeI) is comparable to the molecular concentration of fetal serum iodide (~70 mg/L) after GD 23-26 maternal inhalation exposure to 20-25 ppm MeI (Table 56 and 57).

GSH enhancer IP-GSH, and vitamin E, BHT, and DF). The GSH depletion reached a peak level within 15 minutes after the 5 minutes of exposure (e.g., lowered by approximately 40% immediately after exposure to 1.6 mM MeI). Interestingly, by hour 7, shortly after the 6 hour lag time for cell death, the GSH level returned to the pre-exposure level. In fact, the peak of cell death was not achieved until hour 24, long after the recovery of GSH level.

Table A-7. Rabbit tissue and serum GSH after MeI exposure.[a]

MeI Treatment	Liver GSH (mM)			Kidney GSH (mM)		Blood GSH (mM)			Study
	Control	MeI	%	MeI	%[a]	Control	MeI	%	
Maternal									
GD23-24; 25 ppm	5.91 ±2.3	8.06 ±0.78	136%	1.59 ±0.09	100%	0.599 ±0.12	0.469* ±0.06	78%	Sloter, 2005a
GD23-26; 25 ppm	7.34 ±0.70	6.77 ±1.6	92%	1.86 ±0.36	103%	0.387 ±0.15	0.460 ±0.15	119%	Sloter, 2005a
GD23; 20 ppm	8.00 ±0.89	6.54** ±0.29	82%	-	-	0.565 ±0.097	0.451 ±0.100	80%	Sloter, 2005b
GD23-24; 20 ppm	7.23 ±0.66	7.31 ±0.87	101%	-	-	0.642 ±0.045	0.413** ±0.051	64%	Sloter, 2005b
Fetal									
GD23-24; 25 ppm	2.16 ±0.53	2.37 ±0.13	110%	-	-	0.473 ±0.08	0.338* ±0.12	72%	Sloter, 2005a
GD23-26; 25 ppm	2.22 ±0.29	2.23 ±0.18	100%	-	-	0.382 ±0.09	0.216* ±0.09	57%	Sloter, 2005a
GD23; 20 ppm	2.01 ±0.14	2.05 ±0.16	102%	-	-	0.410 ±0.104	0.253* ±0.098	62%	Sloter, 2005b
GD23-24; 20 ppm	2.27 ±0.18	2.01 ±0.46	89%	-	-	0.411 ±0.115	0.227* ±0.056	55%	Sloter, 2005b

a/ %: Percentage of control values. *, ** for statistical significance at $p < 0.05$; and $p < 0.01$, respectively. Data represents the GSH status from Sloter studies (as indicated) immediately after the last 6-hr exposure (i.e., on GD 23 for one 6-hr exposure, GD 24 for 2 exposures, or GD 26 for 4 exposures).

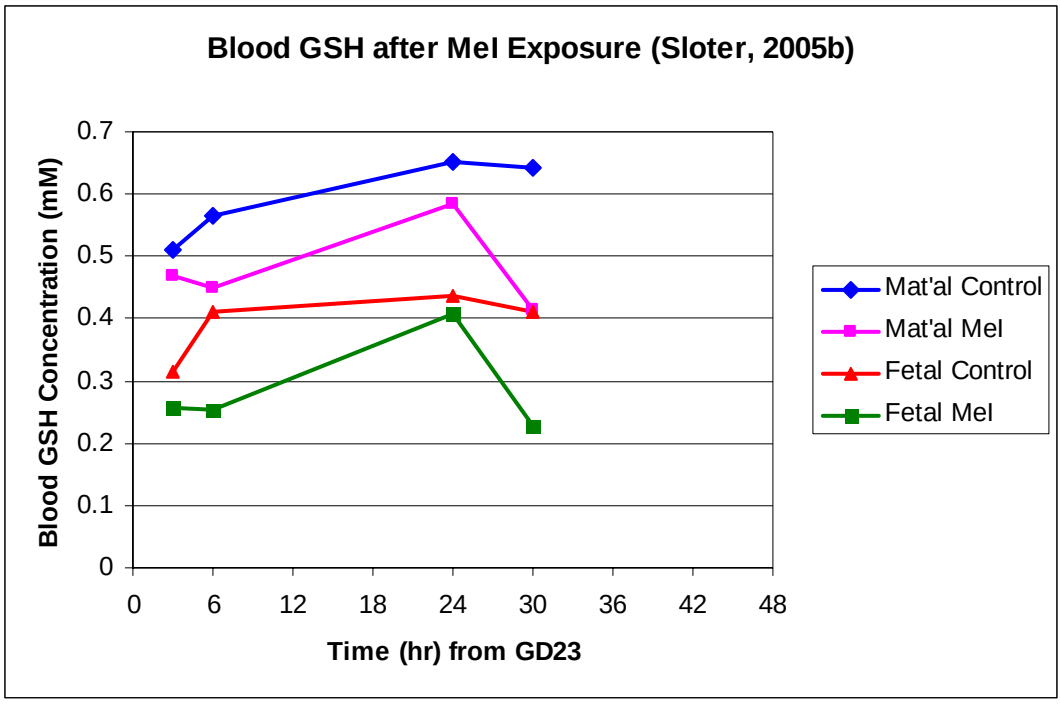


Figure A-12. Rabbit blood GSH level after MeI exposure. Data from Sloter, 2005b. MeI treatment was at 20 ppm, 6 hrs/day, beginning on GD 23 and ending immediately after the second treatment on GD 24 (hour 30). "Mat'al"=Maternal

The aforementioned GSH database may suggest two possible implications with regard to MeI fetal toxicity in rabbits. First, compared to the adult brain, death of neural cells in the fetal brain can be more detrimental and possibly contribute to fetal death. Its delayed onset after a 5 minute exposure in the study with rat cerebellar cells (Chamberlain *et al*., 1999) may suggest that fetal death can occur with a single day exposure, even though the shortest experimental period for the reported rabbit fetal death was after two 6-hour exposures in 30 hours (Nemec, 2002d). Second, the delayed cytotoxicity with respect to the timing for peak and recovery of GSH suggests the potential difficulties in attempting to correlate down stream endpoints such as fetal death with initial markers of oxidative stress such as GSH depletion. These possibilities remain even if the missing results from the Nemec study (2000d) were negative since it was only from a single mid-coronal section of the fetal brain. Since this was likely to be performed on live fetuses, it is also questionable how such data could represent those fetuses that died or were dying. Thus, the possibility of brain abnormalities as a cause of fetal death can not be excluded.

With the limited information available, the possibility remains that oxidative stress could contribute to the death of rabbit fetuses caused by MeI, apart from, or in concert with, toxicity from maternal and/or fetal excess iodide. In the MeI database, maternal and fetal GSH data are not available after MeI exposure for any interspecies sensitivity comparison on fetal toxicity that

might provide further insight into the specific role of GSH depletion in rabbit fetal death. Data are also not available to rule out the possible post-natal toxicity of MeI exposure due to GSH depletion in critical tissues.

II.B.3. Direct Alkylation

Direct alkylation, with adduct as a biomarker of exposure to reactive chemicals or their reactive metabolites, is a possible MOA. Elevated levels of hemoglobin S-methylcysteine adduct, formed by direct alkylation of MeI, have been reported in maternal and fetal blood after MeI exposure in rabbits (Sloter, 2005a and b) and adult rats (Xu *et al.*, 1990; Volume I, Table 9, Himmelstein, 2004).

Data for adduct formation in rabbit maternal and fetal blood reported by the two Sloter studies are summarized in Table A-8. Substantial variability exists, especially in some datasets. The increased adducts in the maternal blood ranged from 134-214% of the controls as a result of 2 to 4 days of MeI exposure. The corresponding adduct in fetal blood is more consistent, i.e., 143-164% of the controls. Although the increased adducts in maternal and fetal blood are similar when compared to their corresponding controls, the increase in the fetal blood is over an already higher (16-24%) background rate such that the concentration of adducts in fetal blood can be as high as 50% (131.1/93.4) above the maternal level. Sloter (2005a and b) stated that the increase in fetal hemoglobin adducts from MeI exposure indicated that some un-reacted parent chemical MeI is distributed to the fetus. Given that no further data are available to separate the possible roles of the parent chemical MeI from iodide in causing rabbit fetal death, both maternal MeI and iodide are valid dose metrics for MeI exposure.

Table A-8. Rabbit maternal and fetal hemoglobin S-methylcysteine adduct after MeI exposure.

MeI Treatment	Hemoglobin S-methylcysteine adduct (nmol/g globin)				Study
	Maternal		Fetal		
	Control	MeI[a]	Control	MeI[a]	
GD23-24; MeI 25 ppm	70.2±33.1[b]	101.5±22.1 (145%)	81.2±20.4	116.3±34.3 (143%)	Sloter, 2005a
GD23-26; MeI 25 ppm	48.4±6.3	103.4±27.8 (214%)	60.1±9.2	98.7±17.5 (164%)	Sloter, 2005a
GD23-26; MeI 20 ppm	69.7±9.3	93.4±5.0 (134%)	86.9±20.1	131.1±11.0 (151%)	Sloter, 2005b

a/ The percentages of the controls are in parenthesis.
b/ The author reported that two rabbits had 2- to -3 fold higher baseline levels than others.

II.B.4. Altered Cholesterol Homeostasis

Altered cholesterol homeostasis may be a key event in the MOA for rabbit fetal death. Baseline rabbit fetal lipid levels were higher than maternal levels, and were significantly elevated after maternal exposure to 25 ppm MeI on GD 23-24 and GD 23-26 (Table A-9, Sloter, 2005a). In the untreated rabbits, the maternal rabbit cholesterol and triglycerides, not HDL or LDL+VLDL, appeared to decline from GD 21 to GD 27 (Volume I, Table 36; Sloter, 2005a). However, the decline was missing with MeI treatment (Table A-9). There are no data for cholesterol levels in fetal or pregnant rats. Change in cholesterol status after MeI exposure has also been reported in adult male and female rats (Volume I, Table 9, Himmelstein, 2004; Table 11 and 12, Kirkpatrick, 2002b) and male dogs (Volume I, Table 28, Harriman, 2004).

Table A-9. Rabbit maternal and fetal serum lipid levels from exposure to 25 ppm MeI during GD 23 -26.[a]

Lipid levels mg/dL	MeI on GD 23-24			MeI on GD 23-26		
	Control	MeI	Increase	Control	MeI	Increase
Maternal						
Cholesterol	10±2.3	13±11.4	30%	8±1.5	8±2.1	0%
Triglyceride	53±31.3	63±26.3	19%	43±13.2	63±31.4	47%
HDL	9.5±1.98	12.8±8.31	35%	10.3±1.23	9.4±2.05	-8%
LDL+VLDL	1±0.7	2±2.2	120%	2±0.8	2±0.8	0%
Fetal						
Cholesterol	176±29.7	205±22.0 ‡	16%	150±11.4	209±45.3 ††	39%
Triglyceride	154±34.1	215±16.3‡‡	40%	137±12.1	202±56.9††	47%
HDL	28.1±3.36	33.3±3.81 ‡‡	19%	27.9±2.09	34.5±2.93††	24%
LDL+VLDL	121±23.8	144±19.7‡	19%	111±10.6	157±43.5 ††	41%

a/ Data from Sloter, 2005a. † Significant, $p < 0.05$; †† Significant, $p < 0.01$, in comparison with Group 6 (of the same gestational age as this treated group). ‡ Significant, $p < 0.05$; ‡‡Significant, $p < 0.01$, in comparison with Group 4 (of the same gestational age as this treated group).

Published literature suggested a potential relationship between cholesterol levels and fetal survival. Zilversmit *et al.* (1972a) reported that maternal serum cholesterol level (triglyceride, VLDL, LDL, or HDL) in pregnant rabbits declined markedly starting approximately GD14, reaching its lowest between GD 21 and GD 23, and remained low till 1 to 2 days after parturition. Pregnant rabbits that received Triton WR 1339 (a plasma triglyceride clearance blocker) injection 14 days prior to insemination and on GD 20 had increased plasma cholesterol as well as fetal resorption and abortion on GD 25. In another study by Zilversmit *et al*. (1972b), 86% of newborn rabbits were found dead from the does fed 0.25% cholesterol (given 100 g diet/day before GD 15 and 200 g diet/day thereafter) and 2.6% fat diet. Fetal death was attributed to the added cholesterol in the diet because a follow up study showed no effects with 2.6% fat diet alone. Fetal resorption between GD 25-30 was 9/58 in the cholesterol treated group (versus 4/99 in the controls). Fetal body weight was significantly lower from does exposed to the high cholesterol diet (Zilversmit *et al*., 1972b). Lower fetal body weight (84.5% of controls) and higher cholesterol levels (40-61% higher triglycerides, LDL, and total cholesterol) were reported at as low as 0.2% cholesterol in the diet, with corresponding changes in placental appearance (Montoudis *et al*., 1999).

II.B.5. Summary

No single predominant MOA or its associated key events(s) leading to fetal death can be determined. The delineation of a MOA for fetal death is compromised by the fact that all fetal data are from those that survived the MeI treatment, an unlikely assumption that they are representative of those that died. Observations from the review of the available data, albeit limited, for evidence on the possible MOAs are summarized below.

- The lack of concordance between fetal thyroid status and fetal death after 2 and 4 days of MeI exposure raises doubt about the extent of the immediate involvement of fetal thyroid status (e.g., 2 exposures within 30 hours) in the MOA for fetal death. In a broader sense, maternal thyroid status appears to correspond better to fetal death, and thus maternal dose metric (MeI or iodide) may be more appropriate than fetal dose metrics. However, no MeI measurements for maternal or fetal tissues are available.

- The lack of concordance between fetal death from MeI and NaI or KI exposures on equal iodide basis suggests that a different MOA than excess fetal iodide alone is likely involved within the narrow (GD 23-26) window of fetal vulnerability. Data from iodide studies also fail to indicate that rabbit fetuses are more sensitive than rats, as is the case for MeI.

- Oxidative stress is a possible MOA for rabbit fetal death based on the observation that MeI exposure results in maternal and fetal GSH depletion in the blood or tissues and the correlation between GSH depletion and cytotoxicity. However, data are insufficient to define the extent of its role. The disadvantage of using MeI as dose metric is that there are no data on its concentrations in maternal or fetal blood for PBPK model validation.

- S-methylcysteine hemoglobin adduct detected after MeI exposure indicates the presence of MeI in the fetus. However, data are not available to further assess the direct role of MeI parent chemical in fetal death.

- Cholesterol homeostasis appears to be important for fetal survival during the late gestation period in rabbits. Although MeI exposure results in significant changes in these parameters, current data are insufficient to quantitatively assess their contribution to fetal death.

- Available information did not show a unique sensitivity of rabbit to iodide. Inadequate data are in studies cited to establish the absence of autoregulatory capacity in the rabbit; greater fetal/maternal iodide ratios in the rabbit than other species, including human; or higher postnatal death in rabbits than other species after iodide exposure.

II.C. Dose Metric

Appropriate selection of dose metric(s) is essential to the application of a PBPK model for establishing the HEC. Considerations for the dose metric determination for the fetal death endpoint include the exposure scenario associated with the NOEL and the model output parameter(s) that best reflect the pattern of human exposure without tending toward underestimation. No candidate target tissue marker beyond blood level of MeI or iodide can be identified without a clearly supported MOA, especially for immediately after the second 6 hr/day exposure (i.e., hour 30).

II.C.1. Single Day 2 ppm NOEL

One key question in modeling the HEC for fetal death is how to derive a single-day HEC based on the 2 ppm NOEL established in a study when pregnant rabbits received MeI exposure during GD6-28. The two possible assumptions for the NOEL are: 1) a single- day exposure, and 2) a single-day incremental exposure. The first assumption was used by USEPA to model HEC at a NOEL of 10 ppm (Barton, 2007). The second assumption is proposed by Mileson *et al.* (2007) for modeling the HEC at the 2 ppm NOEL, i.e., the one-day increment of the area under curve (AUC) for fetal serum iodide after its steady state is achieved on day 13 of repeated exposure (i.e., AUC_{day14} - AUC_{day13}). On the human side of the equation, the AUC is extended over 96 hours to accommodate the iodide clearance subsequent to a 24-hour exposure (i.e., $AUC_{day\ 0\text{-}4}$). Thus, this second assumption can be expressed as "human $AUC_{day0\text{-}4}$ = rabbit [AUC_{day14} - AUC_{day13}]".

In this review, several aspects of MeI toxicity data are considered for determining the most appropriate frequency of exposure for modeling the HEC based on the 2 ppm NOEL. The dilemma is due to the lack of data to determine the dose-response relationship for less than the 23 days (GD6-28) of exposure from which the current 2 ppm NOEL is established. There are no data for estimating what might be the NOEL had pregnant rabbits been exposed to MeI for only a few days. However, it has been demonstrated that an 8 – 9 days of 20 ppm MeI exposure prior to GD22 (i.e., GD6-14 or GD15-22) did not result in pre-natal fetal death (Volume I, Table 55). Thus, there is no support for modeling HEC based on 14 days of MeI exposure (i.e., rabbit [AUC_{day14} - AUC_{day13}]). It has also been demonstrated that during GD23-26, fetal death is evident at hour 30, immediately after the second 6-hour daily exposure to 20 - 25 ppm MeI (Volume I, Table 55; Table A-4 and A-5). Although the incidence was not statistically

significant, it is unequivocally recognized as biologically significant for identify the window of vulnerability. Thus, it is reasonable to model the HEC based on a single- day exposure.

There are also no data for assessing the dose-response relation for 1 - 2 days of MeI exposure and establishing a NOEL other than the current 2 ppm benchmark. The only studies within this duration are conducted at a single MeI concentration 10- to 13-fold higher than 2 ppm. These studies, in which test animals are killed right after the second day of exposure, would also not be sufficient for characterizing the full magnitude of response because enough time has not been given for the manifestation of fetal effects.

Given the lack of data, DPR follows the conventional default for assessing developmental effects and assumes that these effects can occur as a result of a single exposure event within a specific window of vulnerability corresponding to a specific vulnerable developmental stage (USEPA, 1991). This guideline was also acknowledged and followed in Arysta's risk assessment (Mileson *et al*., 2009). In addition to the support from fetal death immediately after the second 6 hours of MeI exposure, support for the single-day exposure assumption is also provided by the profiles on GSH depletion as presented in Section II.B.2. (including Table A-7 and Figure A-12). Significant GSH depletion in fetal blood was detected as early as after one 6-hour 20-ppm exposure. There is no clear evidence that the level of GSH at 62% of controls is further reduced significantly with additional repeated exposure, at least within the available measurement time point, i.e., Table A-7 which shows a range of 55 to 72% of controls after 2- to 4-day of exposure. Moreover, *in vitro* study with neural cell cultures showed a very quick decline of GSH that reaches the maximum depletion 15 minutes after a 5-minute exposure to MeI (Chamberlain *et al*., 1999) while cell death did not begin until 6 hours after the exposure, near the time for full GSH recovery. Thus, for a possible MOA that involves oxidative stress and with delayed onset of cytotoxicity and cell death, a single-day exposure would also be reasonable for HEC determination.

A comparison of critical thresholds for developmental effects from repeated dosing versus a single day exposure during the window of vulnerability was conducted by RIVM of The Netherlands (Van Raaij *et al*., 2009). A "NLR" ratio of a single-day NOAEL ($NOAEL_{single}$) to repeated day $LOAEL_{rep}$ ($NLR = NOAEL_{single}/LOAEL_{rep}$) was used to indicate the difference between the threshold for single- versus multiple-day exposure. Of the 20 chemicals for which data are available for the NLR calculation, 17 showed NLR at or below 2 for fetal resorption endpoint. In two cases, the NLR is less than 1. The authors concluded that the NOAEL for resorption from repeated dosing studies is a relevant POD for setting acute exposure standard.

In summary, with insufficient support for a single predominant MOA within the time frame of 30 or less hours, it is prudent to model the HEC at the 2 ppm NOEL based on a single-day exposure for both rabbits and humans.

II.C.2. HEC Dose Metric

The 8 possible dose metrics are the combinations of maternal or fetal blood levels of MeI or iodide at their peak (or steady state) concentration or area under the curve (AUC).

For the fetal compartment, fetal circulating MeI can be a possible dose metric because its presence is supported by the detection of S-methylcysteine hemoglobin adducts in the fetal blood. However, no experimental data are available for model validation. Fetal circulating iodide is a candidate dose metric because experimental data in rabbits are available. However, the use of this dose metric should be viewed with caution for several reasons. First, all the experimental measurements used for model validation are from fetuses that survived the MeI exposure, a direct opposite outcome to the endpoint targeted for modeling. Secondly, the model predicted iodide F/M (fetal-to-maternal) ratios are generally higher than those experimentally measured in rabbits (Section II.A.2.). This tends to result in underestimation of risk by setting a higher benchmark for modeling the HEC than can be supported. Moreover, the 0.9 - 1.3 cord-to-maternal ratios from the survey by Rayburn *et al.* (2007) (see: Table A-3) has been cited as support for lower fetal iodide load in humans than in rabbits (Mileson *et al*., 2007). However, the range of human cord-to-maternal ratio is wide with 12 of the 121 sets at or above 2 (the highest ratio of 5.4), significantly overlapping the average F/M ratio in rabbits. An earlier study by Cottino *et al.* (1972) also showed a wide blood ratio (0.5 - 3.4) among 18 women who received iodomethylsparteine for 15 minutes to 18 hours at termed delivery. Moreover, the issue of appropriate human fetal stage for modeling the HEC also brought into question about the applicability of Rayburn study data. While this remains a question of interest, it should be remembered that a significantly higher fetal body weight model input parameter would have a much greater impact on the predicted human iodide levels in the fetal than maternal serum, thus proportionally raising the HEC based on fetal serum iodide dose metric. Finally, the baselines for the data on F/M ratio between rabbits and humans are dissimilar. The maternal levels reported in the Rayburn study (2007) are from subjects with no known excess iodide exposure, with the plasma iodide ranging from 0.003 to 0.05 mg/L (or, 0.3 to 5.6 μg/dL as reported). Whereas, the rabbit maternal iodide concentration from MeI exposure is 460 – 4,700 fold higher, ranging from 14 to 26 mg/L (Table A-4 and A-5).

Additional discussion on dynamic changes in the F/M ratio has been presented in Section II.A.2. No other data, including fetal iodide distribution in rats, are available for supporting the argument of wide interspecies sensitivity to excess iodide. Thus, while species specific distribution of iodide to fetus remains a valid pharmacokinetic consideration, fetal serum iodide should not be the definitive dose metric for HEC, especially when it yields higher HECs than all other valid dose metrics and presumes on a MOA that cannot be sufficiently supported.

The use of the maternal MeI or iodide dose metric has the advantage of focusing more on the total internal dose of exposure without the additional uncertainties of further modeling to the fetal distribution level. They are more suitable dose metrics when no single MOA can be supported. The potential disadvantage of the maternal MeI dose metric is that no measurement data are available for model validation. Moreover, its level is only a small fraction of iodide due to its rapid transformation and thus can potentially be more subjected to accuracy concerns for reflecting the pattern of total MeI exposure.

In contrast, USEPA (2007) agreed with Arysta's proposed MOA that fetal thyroid perturbation from excess iodide is the definitive MOA for rabbit fetal death. However, maternal instead of fetal serum iodide was initially chosen by USEPA as the dose metric due to the inadequacy of fetal versus maternal iodide data in humans. Following the completion of a study by Rayburn *et*

al. (2007) that surveyed iodide levels in human maternal and cord blood, USEPA determined that the data on human maternal-to-fetal iodide distribution was sufficient for changing the HEC dose metric from maternal to fetal iodide (Barton, 2007).

The choice of peak concentration versus AUC is an important consideration, especially because the NOEL determined in rabbits consists of only 6 hours of exposure while both the general public and workers are expected to be exposed for 24 hours. Although workers associated with the use of MeI may only receive 8 hours of exposure during work, if they also live within the exposed community, they can be expected to receive further MeI exposure at the ambient level for the remaining 16 hours. The cumulative dose from 6- versus 24-hour MeI exposure durations between rabbits and humans can be accounted for by the AUC dose metric but not by the peak concentration, especially if the steady state of the selected dose metric is reached before the end of the specified exposure duration or the protracted decline after the exposure is to be accounted for.

In conclusion, the overall evidence presented in this and previous sections indicate that maternal iodide dose metric is more reliable compared to the fetal iodide picture for reflecting the maternal MeI exposure status on which the rabbit NOEL was based. Maternal dose metrics are also more reliable than fetal dose metric for interspecies comparisons without contending with the uncertainties of an exclusive MOA based fetal thyroid perturbation from excess iodide, and the apparent higher simulated fetal to maternal serum iodide ratio in rabbits. Finally, the decision for using maternal serum iodide AUC dose metric is supported by the overall conclusion by Sweeney *et al.* (2009). Although coming from somewhat different points of consideration, the model authors stated that the "*… confidence in the PBPK model predictions for the reproductive/development effects of iodide in rabbits is considered moderate using fetal iodide and high using maternal iodide…*".

The HEC should be based on the equivalence AUC between a single 6-hour 2 ppm MeI exposure in rabbits and a single 24-hour MeI exposure in humans (i.e., referred to by Arysta model output as "bystanders"). Workers are expected to continue receiving exposure at the MeI level as a member of the general public after receiving occupational exposure during work hours.

II.D. HECs

The rabbit model uses meidpr2.csl and meidpr2cmd files provided by Arysta (2007). Three sets of HEC simulation runs were conducted in 2008. These include: 1) output for the general population (Arysta's "bystander's") HECs (Arysta, 2008a, b) and their subsequent correction (Arysta, 2008c); 2) output for occupational (Arysta's "worker's") exposure HECs (Arysta, 2008d); and 3) output for using higher rabbit QAC reflective of the GD23-26 window of vulnerability (Arysta, 2008e). HECs are modeled for a single day of exposure to the 2 ppm (6 hours/day) NOEL in rabbits based on fetal death endpoint.

II.D.1. General Population HEC

Although maternal iodide AUC is selected as the dose metric for HEC determination, HECs based on all 8 dose metrics (i.e., permutation of peak concentration or AUC of MeI or iodide in maternal or fetal blood) are presented below for demonstrating their overall impact to the HEC.

The HEC is determined by matching the rabbit and human values of a given dose metric. When the dose metric is based on the peak concentrations, it is taken from immediately after the cessation of exposure (i.e., hour 6 for rabbits, hour 24 for humans) and before their decline thereafter (Figure A-7 and A-8). When the dose metric is based on AUCs, the duration coverage is 24 hours for rabbits and 96 hours for humans to account for the elimination time. Thus, the rabbit 24-hour AUC from a 6-hour exposure is matched to the human 96-hour AUC from a 24-hour exposure. The corresponding graphic depictions are presented later, in Figures A-13 in conjunction with presenting the selected HEC.

The initial sets of HECs were modeled based on a rabbit QAC of 12 L-hr/kg$^{3/4}$. Subsequent to DPR's consideration regarding the biological uncertainties in using model input parameters that are not reflective of the GD23-26 window of vulnerability, Arysta re-submitted a new set of HEC model runs for a single MeI exposure using a QAC of 20 L-hr/kg$^{3/4}$ and the same maternal body weight of 4.1 kg, and fetal weight of 0.046 kg (Arysta, 2008e). The final sets of HECs are presented in Table A-10. The earlier set of HECs based on the low rabbit QAC are included in strikethrough form to demonstrate the impact of the 1.7-fold increase in QAC. The HECs based on iodide profile are proportionally increased while the change is much less based on the MeI profile. The lower sensitivity of MeI output parameters is likely due to the rapid equilibration with tissues (Arysta, 2009). This strengthens the previous conclusion of modeling HEC based on maternal serum iodide instead of the maternal MeI profile.

Table A-10. List of 24-hour HECs for rabbit fetal death endpoint[a]

Dose metric	Maternal MeI	Fetal MeI	Maternal Iodide	Fetal Iodide
Peak	~~2.8~~ 2.9 ppm	~~3.4~~ 4.1 ppm	~~0.38~~ 0.58 ppm	~~1.5~~ 2.4 ppm
AUC	~~0.7~~ 0.73 ppm	~~0.86~~ 1.0 ppm	~~0.15~~ 0.24 ppm	~~0.78~~ 1.3 ppm

a/ The HEC is modeled for the NOEL of 2 ppm (6 hr/day) MeI, using the QAC of 20 L-hr/kg$^{3/4}$ for rabbit late stage pregnancy. The strike-through HECs are modeled for early pregnancy at QAC of 12 L-hr/kg$^{3/4}$, given only for illustration purposes.

Table A-10 shows that within the same output parameters (i.e., serum MeI or iodide), the HECs are generally lower based on maternal than fetal dose metrics. The greatest difference is in modeling for maternal rather than fetal serum iodide. The model input that resulted in a much greater accumulation of iodide from maternal to fetal blood for rabbit than humans contributes significantly to this overall 4- to 5-fold higher HEC. The generally lower HECs based on AUC than peak concentration is reflective of the 6- versus 24-hour exposure between rabbits and humans.

The determination of HEC through matching rabbit and human simulation results can be illustrated in Figure A-13 for two maternal serum iodide dose metrics; i.e., AUC and peak concentration. All the 24-hour MeI levels listed in Table A-10 are included in Figure A-13. In Figure A-13a for maternal serum iodide AUC, the 0.24 ppm HEC is based on the equivalent AUC level between the 96-hour human AUC (at 3.66 mg/L*hr) after a 24- hour exposure to 0.24 ppm MeI (the lowest simulation curve) and the 24-hour rabbit AUC (at 3.72 mg/L*hr) after a 6-hour exposure to 2 ppm MeI (thick black curve). Similarly in Figure A-13b for maternal serum iodide peak concentration, the 0.58 ppm HEC is based on the equivalent peak level between human peak at hour 24 (0.31 mg/L) and rabbit peak at hour 6 (0.31 mg/L).

Figure A-13a

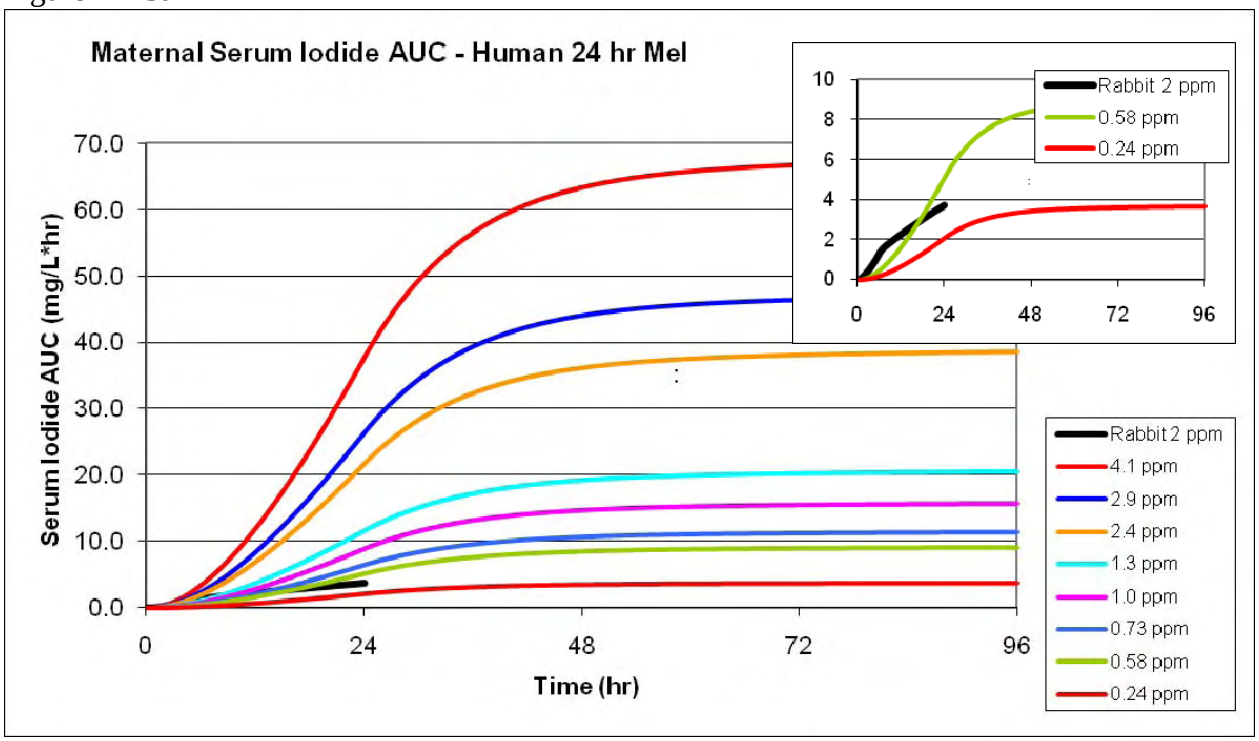


Figure A-13b

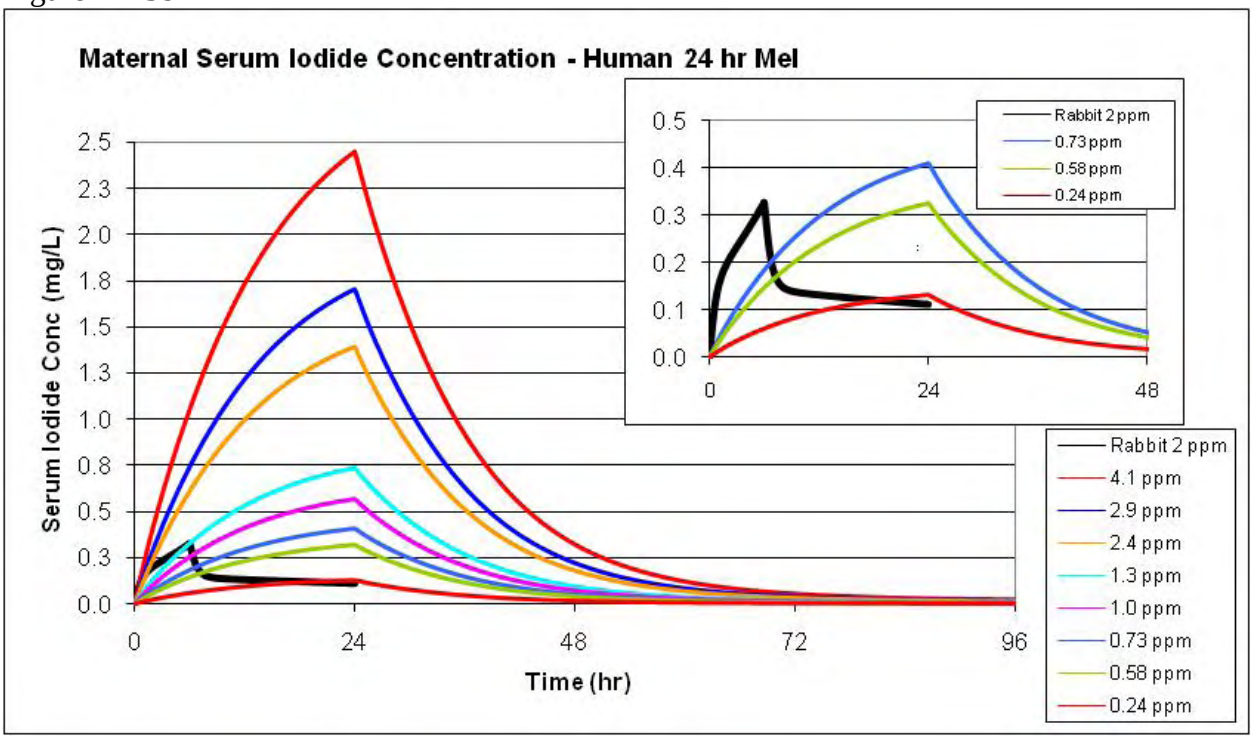


Figure A-13. The 24-hour HEC based on maternal serum iodide dose metrics. Rabbit NOEL for late gestation period (thick black line) is compared to the 24-hour HECs at: a) 0.24 ppm HEC based on AUC, b) 0.58 ppm HEC based on peak concentration. Data from Arysta (2008e). Areas of interest are enlarged in the insets.

An interesting overarching observation regarding the implication of rabbit fetal iodide accumulation on the final HEC for fetal death endpoint is noted here. This apparently unique feature in rabbit fetus versus rats and humans was repeatedly cited by Arysta (Arysta, 2007; Mileson *et al*., 2009; Kirman *et al*., 2009) as support for their fetal thyroid perturbation MOA due to excess iodide from MeI exposure, for presuming greater sensitivity of rabbit than human fetuses to MeI, and consequently for supporting the use of the fetal serum iodide dose metric for establishing HEC. However, these implications bring out many issues for which data are unavailable to resolve. First of all, Mileson *et al.* (2009) characterized the normal rabbit fetal iodide accumulation as 9- to 11- fold higher than the maternal. This was based on serum iodide data from the control groups in the MeI study by Sloter (2005b) (see also Volume I, Table 43). With MeI exposure, this F/M ratio is reduced to approximately 2-fold (Sloter, 2005b; Mileson *et al*., 2009). The physiological significance for this dramatic change to rabbit fetal thyroid at the MeI NOEL is unclear. As to the implication of greater rabbit sensitivity than humans, it is noted that the physiological range of fetal blood iodide is 0.12 - 0.22 mg/L in rabbits (Volume I, Table 43) and 0.014 - 0.017 mg/L in humans (Rayburn *et al*., 2007). This approximately 8- to 16- fold higher rabbit level would indicate a higher requirement for iodide or greater tolerance for its excess, but in itself offers no support that rabbits are thereby “more sensitive” to excess fetal iodide from MeI. If anything, the higher baseline in rabbits might suggest less sensitivity at a given amount of iodide increase from MeI. In this regard, the modeled peak rabbit fetal iodide is 1.4 mg/L at the end of 6-hour exposure to 2 ppm MeI, a 6- to 12-fold above the baseline for rabbit fetuses, but nearly 100-fold above the baseline human cord blood level. On the other hand, at the HEC of 0.24 ppm (Table A-10; based on DPR’s maternal iodide dose metric) the modeled peak fetal iodide of 0.15 mg/L is 10-fold higher than the human baseline. This is within the same ratio of increase over baseline in rabbit fetuses at the NOEL of 2 ppm. If equal multiplier over baseline is of biological significance, this observation could provide additional support for the 0.24 ppm HEC derived from maternal serum iodide dose metric (Table A-10).

MeI at 0.24 ppm represents the HEC at the most pertinent dose metric and is the final 24-hour HEC for assessing the risk of the general public. An ideal portrayal of the total amount of maternal exposure to MeI would include also the portion of MeI that is not yet converted to iodide. However, the MeI level is relatively insignificant since the rate of conversion to iodide is rapid such that the peak level of MeI is 35-fold below the peak iodide level at the end of the 24 hours of exposure.

II.D.2. Occupational HEC

As stated under Section II.A.1, a 567 L/hr (or minute volume of 9.45 L/min) sedentary (or resting) breathing rate is used for modeling the HEC for the general public presented in the previous section. For worker’s occupational exposure, a 1.47-fold higher DPR default breathing rate of 833 L/hr is used to model the 8-hour occupational HEC. This is achieved by Arysta through changing the tidal volume (TVol) from 630 to 925 ml/breath while keeping the same 15 breath/minute.

Based on the same selected HEC dose metric as for the 0.24 ppm 24-hour HEC, the 8-hour occupational HEC provided by Arysta is 0.22 ppm. The relationships between the 8-hour occupational HECs, the rabbit 2 ppm NOEL, and the 24-hour HECs for the general public are

presented in Figure A-14a. An additional day of the 8-hour exposure cycle is included to illustrate the pattern within two 24-hour cycles. The 8-hour occupational HEC curve approximates the curve for the 24-hour HEC shortly beyond hour 24. This indicates that a single-day modeling of AUC can be adequate for the 8-hour HEC determination. Alternatively, Rodriguez (2009) noted that without the additional 16 hours exposure for the worker as a member of the general public the 8-hour HEC is 0.5 ppm.

Although the peak maternal serum iodide concentration is not a chosen dose metric for the HEC, a question may be raised regarding the sufficiency of a single-day simulation for such a scenario. Simulation over multiple 8-hour exposure days showed that the peak concentration increases only by 2% from the second to the third day of exposure (data not shown). Thus, had the peak concentration been selected as the final HEC dose metric, it would be more appropriate to match the peak rabbit iodide concentration to the peak on the second-day peak human 8-hour exposure. As shown in Figure A-14b, this second-day peak was considered in establishing the HEC of 0.35 ppm based on maternal peak serum iodide concentration.

II.D.3. Summary

USEPA derived a 24-hour HEC at 7.4 ppm and an 8-hour occupational HEC at 23 ppm. These are based on the NOEL of 10 ppm (6 hr/day) in rabbits, the dose metric of fetal serum iodide AUC, and sedentary breathing rate of 567 L/hr. DPR's 24-hour HEC is 0.24 ppm and 8-hour occupational HEC is 0.22 ppm. These are based on the NOEL of 2 ppm (6 hr/day) in rabbits, the dose metric of maternal serum iodide AUC, and DPR's default breathing rate of 833 L/hr (i.e., 83% of USEPA's recommended "light activity" rate) for occupational exposures. It is noted that for workers, an additional 16 hours of MeI exposure as members of the general public (or "resident") would increase the maternal serum iodide AUC and exceed the maternal serum iodide benchmark in rabbits at the NOEL of 2 ppm.

Figure A-14a

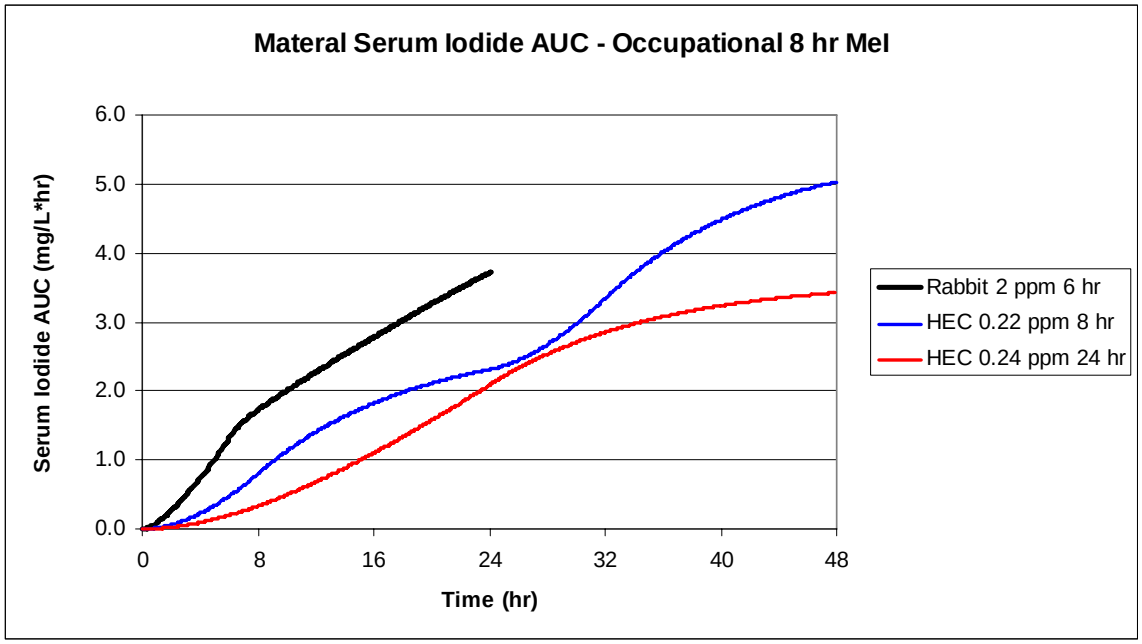


Figure A-14b

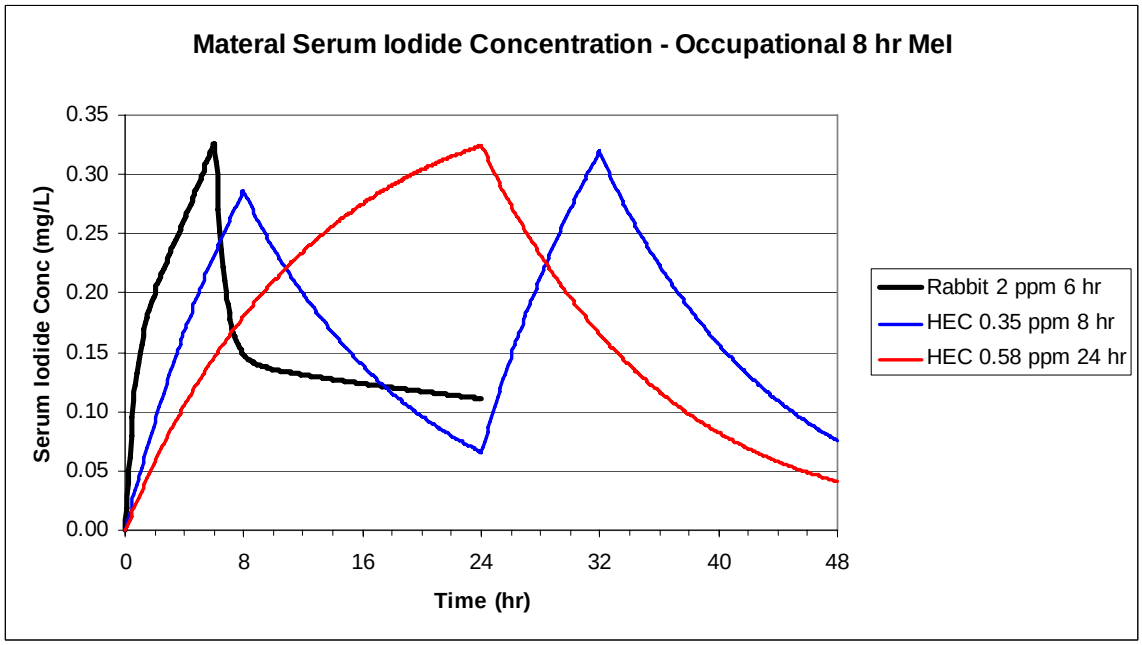


Figure A-14. The 8-hour occupational HEC based on maternal serum iodide dose metrics. Rabbit NOEL for late gestation period (thick black line) is compared to the HECs at: a) 0.22 ppm HEC based on AUC, b) 0.35 ppm HEC based on peak concentration. Data from Arysta (2008e).

III. NASAL EFFECTS

This section presents the HECs for nasal effects observed in rats. The Arysta's nasal model has been reviewed by USEPA (Barton, 2007; USEPA, 2007) for establishing the HECs based on GSH depletion in the olfactory epithelium. The sources for model information are provided in the introductory section of this review. The basic model diagram is presented in Figure A-1. This section provides only a very brief description of the Arysta mei3 model[3] as a backdrop for focusing on some key issues that could significantly impact the modeling outcome. Compared to USEPA, DPR differs on the threshold GSH level for the HEC dose metrics and this difference results in lower HECs. The overall support for GSH depletion as the MOA for the nasal effects was discussed by Kirman *et al.* (2009). Based on the qualitative and quantitative evaluations, the authors concluded that the confidence for this MOA was only medium. Nevertheless, DPR's use of GSH depletion as the dose metric is not dependent on this endpoint as a definitive MOA for toxicity but as an early marker for the protection of olfactory epithelial cells and is consistent with the nasal toxicity observations that define a NOEL at 21 ppm.

III.A. Model and Parameters

Instead of a detailed tissue structure, the nasal olfactory architecture in the MeI model is simplified into a descriptive structure of 4 layers each for the dorsal meatus or ethmoid regions. The model parameter for the width of the olfactory epithelium is designated as WOE. The width of the next layer, the olfactory exchange membrane with lamina propria, is WOX. The values for the WOE and WOX in the HEC model are: 0.008 and 0.005 cm for rats, 0.006 and 0.05 cm for children, and 0.008 and 0.134 cm for adults. The cardiac output in the mei3 model for rat nose and brain HEC simulations (see Section IV) is 2/3 of the minute volume (MINVol, or "breathing rate").

Model validation through comparing simulation output to the measured data in rats by Himmelstein (2004) at 25 and 100 ppm MeI was provided by Arysta (2007). Input parameter adjustments were made to achieve adequate fit to the experimental data, e.g., using lower ventilation rate than measured by DeLorme (2004). Some of these documented adjustments are extensive, e.g., using only 1.5% of the *in vitro* measured GSH conjugation rate for simulating kidney GSH changes and increasing the nasal GSH turnover rate from 0.016/hr measured by Poet and Wu (2004) to 0.19/hr (Arysta, 2007). The graphic presentations of the final output from the Arysta model documentation are reproduced in Figure A-15a (from Figure 9c of Mileson *et al*., 2007) for the nasal olfactory region, and in Figure A-15b (from Figure 8 of Mileson *et al*., 2007) for serum iodide levels. The simulation on nasal GSH depletion tracks the experimental data sufficiently at 25 ppm but not at 100 ppm (Figure A-15a). However, compared to the measured data, the model underestimates the peak serum iodide concentration at 25 ppm (Figure A-15b). This is mainly attributed to the higher model input of rat body weight (0.376 kg) than the lower weight (0.207 kg) of rats used in the Himmelstein study. This issue is pertinent to systemic endpoints and is further discussed in Section IV.A.

[3] The basic model "mei3" was provided to DPR in July 26, 2007. It is understood that this model remains the same for the model output subsequently provided by Arysta in 2008.

Figure A-15a

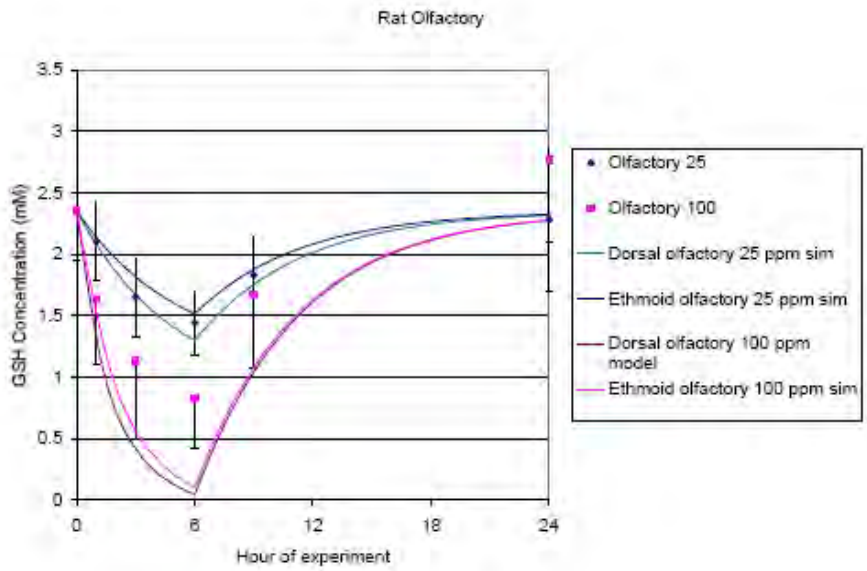


Figure A-15b

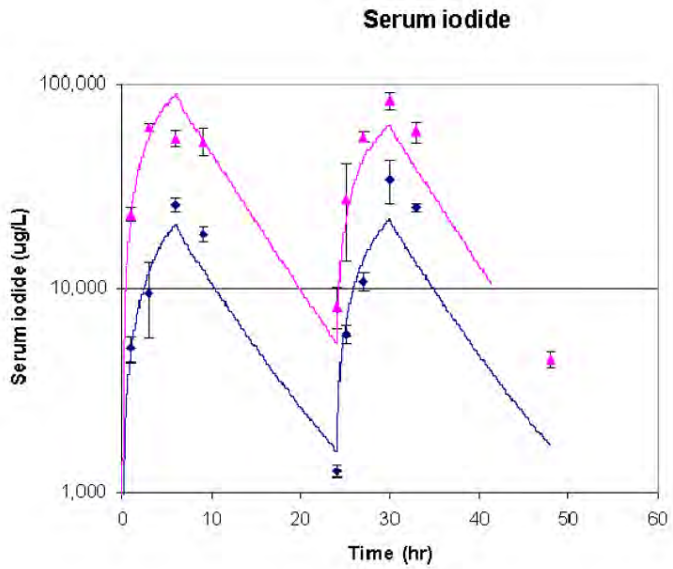


Figure A-15. Modeled versus measured nasal GSH and serum iodide in rats. a) GSH concentrations at dorsal meatus and ethmoid olfactory regions (from Mileson *et al*., 2007, Figure 9c); b) Blood iodide concentrations (from Mileson *et al*., 2007, Figure 8). Lines: simulations; Symbols: experimental data from Himmelstein, 2004 with 6 hr/day exposure at 25 and 100 ppm MeI.

III.B. Mode of Action

The MOA is unclear for nasal effects at the site of MeI contact. Although MeI is shown to directly alkylate macromolecules (e.g., hemoglobin adduct formation), the role of alkylation has not been defined for the nasal effect. Other reactive metabolites may also be formed through biotransformation, e.g., cytochrome P450 oxidation that forms formaldehyde. However, Chamberlain *et al*. (1998a and b) found no evidence for the immediate involvement of formaldehyde from oxidative conversion of MeI. No formaldehyde was detected in the *in vitro* study after incubation of microsomal fractions with MeI in the presence of a NADPH generating system (Chamberlain *et al*., 1998b). No alteration of olfactory effects was noted through pre-treatment with cobalt protoporphyrin IX that depletes hepatic cytochrome P450. The role of oxidative metabolism cannot be readily excluded based on this negative finding alone because the animals were severely compromised at the chosen lethal dose of 60 mg/kg (s.c. injection) cobalt protoporphyrin IX; i.e., reported as hunched with exaggerated piloerection, pinched sides, had slight hepatic midzonal necrosis. Moreover, the 4-hr 100 ppm MeI exposure level may be too high to determine the involvement of oxidative transformation since the nasal histopathology for the pre-treated and non-pre-treated groups was both subjectively described as “marked” in severity.

The available data indicate that localized GSH depletion from conjugation with MeI is likely an early event associated with nasal epithelial degeneration. Reduction in non-protein sulfhydryl (NP-SH) was reported in both the olfactory and respiratory epithelia of adult male rats within 1 hour of exposure to 100 ppm MeI (Chamberlain *et al*., 1998a). However, lesions occurred in the olfactory but not respiratory epithelium, and severity increased from 2 to 4 hours of exposure. The specific sensitivity of olfactory epithelium to MeI is attributed to a higher conjugation rate of cytosolic glutathione S-transferase (GST) in the olfactory than the respiratory tissue (Chamberlain *et al*., 1998b). The involvement of GSH depletion is further confirmed through modulating the olfactory degeneration by pre-treatment to either replenish GSH (with isopropyl ester of GSH) or deplete GSH (with phorone and buthionine sulphoximine). An *in vitro* study by Chamberlain *et al.* (1998b) showed that the MeI-GSH conjugation was catalyzed by the theta class GST and resulted in the formation of S-methyl GSH.

Events subsequent to GSH depletion that result in cell degeneration and cell death are unclear. Given that pre-treatment to deplete GSH enhanced nasal effects, Chamberlain *et al.* (1998a) suggested that GSH depletion through its conjugation with MeI is a part of a detoxification process, not the first step to toxicity. The possible down stream events leading to cellular degeneration may involve subsequent formation of cytotoxic metabolites such as methanethiol, or oxidative stress caused by extensive GSH depletion (Chamberlain *et al*., 1998a and b).

III.C. Dose Metric

Based on the apparent relationship between GSH depletion and cellular degeneration in the olfactory epithelium, DPR concluded that GSH depletion is a likely early event for the nasal effect and agreed with USEPA that the depletion at the dorsal olfactory epithelium can be the dose metric for modeling the nasal effect HECs.

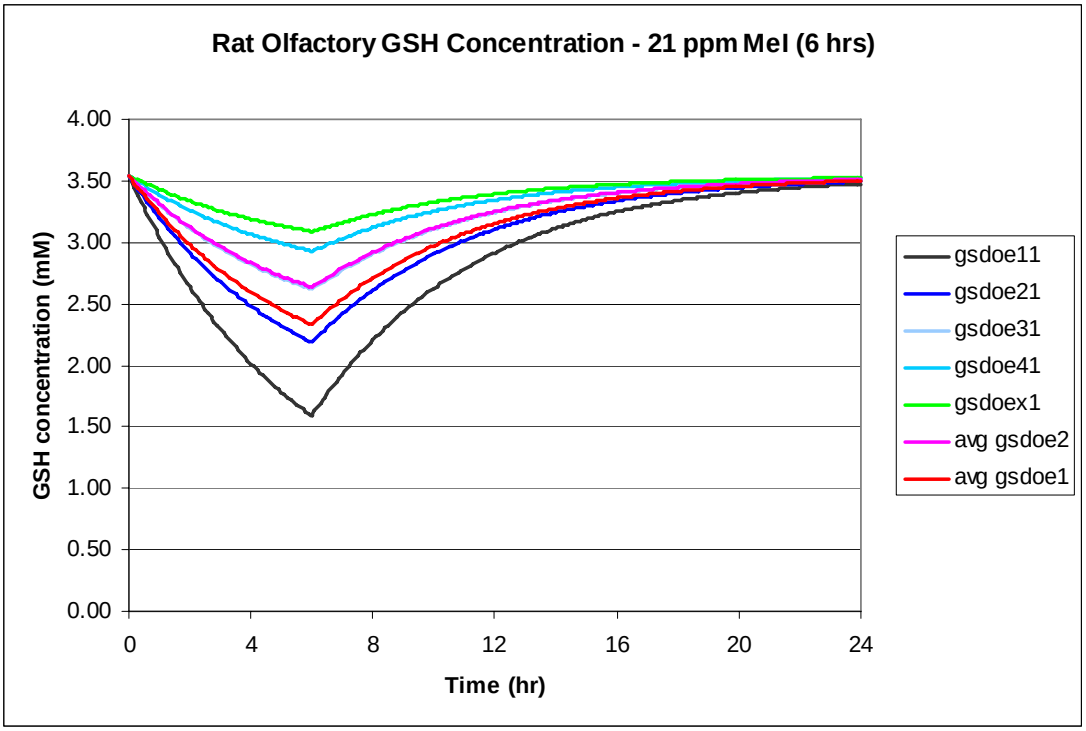
III.C.1. Regional Olfactory GSH

Since the experimental measurements in rats did not differentiate the tissue layers, HEC modeling is limited to interspecies comparison based on the average regional GSH concentrations. The averaging should consist of only the 4 layers of each meatus or ethmoid region and not the innermost blood exchange layer because the interspecies WOX (values given in previous section) are quite different between rats and humans and between human age groups (Barton, 2007). Due to the relatively greater thickness of the basement exchange layer in humans, including this layer in the human HEC model would largely reflect the GSH level at this layer and not at the four outer architectural olfactory epithelial layers that are in closer contact with MeI and that have greater GSH depletion. Figure A-16 shows the modeled GSH level for each of the 4 layers in the dorsal meatus ("gsdoe11, gsdoe 21, gsdoe31, gsdoe41), the basement exchange layer ("gsdoex1"), and the regional 4-layer average for both meatus ("ave gsdoe1") and ethmoid ("ave gsdoe2") regions for rats at the NOEL of 21 ppm exposure for 6 hours (Kirkpatrick, 2002b). Because of the interspecies anatomical differences (i.e., no rat "ethmoid" equivalence in humans), it is reasonable that the GSH dose metric in humans is matched to the dorsal meatus region in rats (i.e., "ave gsdoe1") for the HEC simulation (USEPA, 2007).

III.C.2. Threshold GSH Depletion

The key issue for the HEC dose metric based on nasal effects is the benchmark level of GSH depletion. Without elaboration, USEPA (2007) modeled the HEC based on a 50% depletion benchmark, the same level as proposed by Arysta (Sweeney *et al*., 2005; Sweeney and Gargas, 2005; Arysta, 2006, 2007). In contrast, this DPR review concludes that a benchmark at 25% depletion can better represent a level of no effects. The issue exists because no available study with MeI exposure includes both the measurement of GSH levels and side-by-side histopathological observations at or near the NOEL.

The support provided by Arysta (Sweeney *et al*., 2005; Arysta, 2006, 2007) for the 50% benchmark as equivalent to "no effect level" is mainly based on data for other chemicals and in other target tissues. These data generally include chemicals that require metabolic activation (e.g., esterase activity for ethyl acrylate by Frederick *et al*., 1992; cytochrome P450 activity for naphthalene by Plopper *et al*., 2001 and Phimister *et al*., 2004; Lin *et al*., 2006); observations in cells other than the olfactory epithelium (Clara cells by Plopper *et al*., 2001; Phimister *et al*., 2004); cellular effects that may not be directly indicated by non-protein sulfhydryl depletion (ethyl acrylate by Frederick *et al*., 1992), and toxicity endpoints beyond the initial cellular degeneration and are associated with tumor formation (cell proliferation by propylene oxide by Rios-Blanco *et al*., 2003; Lee *et al*., 2005).



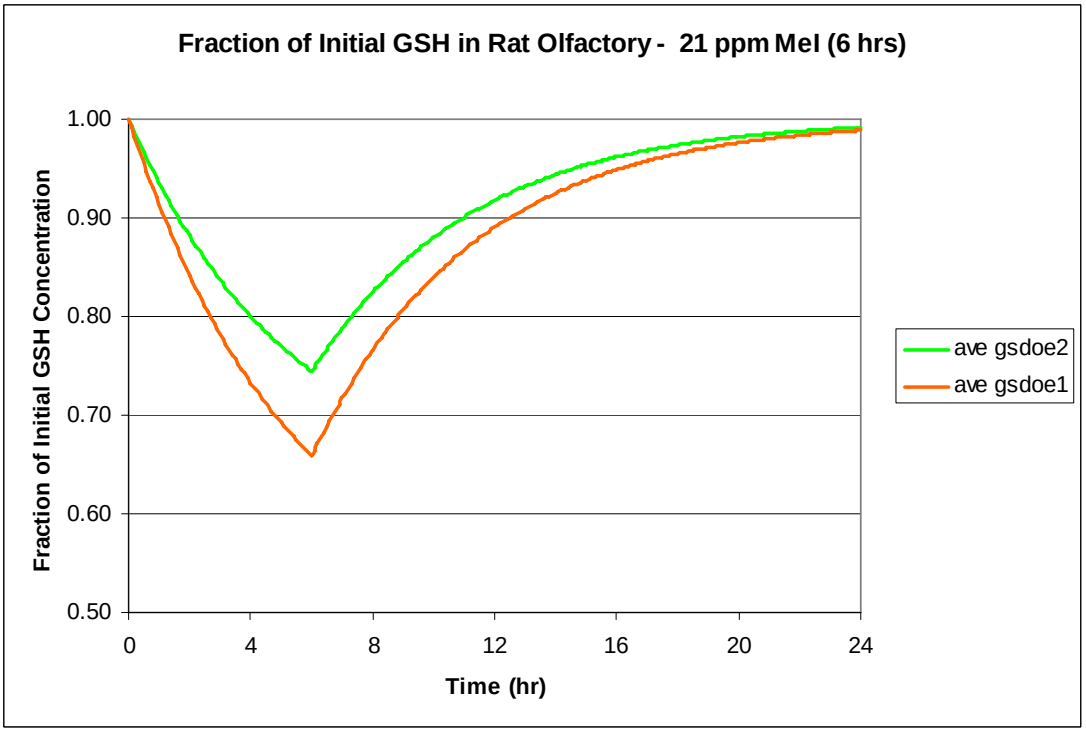


Figure A-16. Modeled rat olfactory GSH at 21 ppm MeI for 6 hours. Top: GSH level. Bottom: Fraction of initial GSH. The "gsdoe11", "gsdoe21", "gsdoe31", and "gsdoe41" were the 4 meatus layers, the "ave gsdoe1" was their average. The "gsdoex1" was the basement exchange layer. The "ave gsdoe2" was the average of the ethmoid layers. Model output from Arysta (2008c).

In addition, three studies were specifically cited in Sweeney *et al.* (2005) as providing the support for the 50% benchmark for MeI. The first citation was a report by Biaglow *et al.* (1986) that investigated the enhancement of therapeutic response of tumor tissues to nitro radiation or chemotherapeutic drugs, i.e., misomidazole and SR-2508. The report mentioned that only 10-20% GSH depletion can cause liver damage but "*spontaneous peroxidative damage… can occur with other*[4] *normal tissue when GSH reaches 50% of contro*l". The second citation was a report by Frederick *et al.* (1992) regarding the application of a PBPK/PD model to oral dosing of ethyl acrylate for estimating its delivery dose associated with forestomach tumorigenicity in rats. Nevertheless, the nasal tissue was not among the 14 sites for which measured metabolic binding of ethyl acrylate to thiol was available for the model. In addition, a pattern of GSH circadian rhythm was attributed to this report. However, no data were presented. The third citation was a report by Plopper *et al.* (2001) which referred to a threshold of 75% depletion of intracellular GSH pool for irreversible organelle changes in mouse Clara cells at the distal conducting airway through conjugation of reactive metabolites of naphthalene after i.v. injection.

DPR concluded that none of these 3 reports provided substantive support for the 50% benchmark in nasal olfactory epithelium. At best, the aforementioned statement by Biaglow *et al.* (1986) would indicate that the 50% depletion is not a clear "no effect" level but a level of "beginning to show effects", i.e., close to a "low-effect" level. A diurnal variation of GSH as much as 50% in some tissues was mentioned as support for the 50% benchmark. However, if this wide diurnal fluctuation is indeed reflective of the baseline pattern of GSH levels in the olfactory epithelium, it can arguably support a modeling benchmark substantially below 50% to account for the possible heightened vulnerability during the low GSH state within a 24-hour period.

More importantly, the evidence for inadequate health protectiveness of 50% GSH depletion as benchmark for establishing the HEC can be found within the MeI database. Three MeI datasets are available to bridge the observation of histopathological tissue damage at 100 ppm to an estimated GSH depletion bordering 50%. The 100 ppm is above the LOEL of 70 ppm from which the 21 ppm NOEL used in nasal HEC modeling was established (Kirkpatrick, 2002b). The first dataset by Himmelstein (2004) provides time-course GSH measurements from 2 days of exposure while the remaining two datasets by Chamberlain *et al.* (1998a) and Reed *et al.* (1995) provide the time-to-effects at 100 ppm.

- Data on nasal GSH levels from the Himmelstein study are summarized in Table A-11. Note that the percentage given in this table is relative to the corresponding controls which fluctuate with time. Thus, they may not reflect the level of changes in GSH concentration from one time point to the next. At 100 ppm, olfactory GSH depletion at hour 1 was 48% on day 1 of exposure and merely 8% on day 2 of exposure. Peak depletion at hour 6 was 74% on day 1 and 56% on day 2.

[4] specified as non-liver tissues (Biaglow et al., 1986)

- The Chamberlain *et al.* (1998a) study showed that slight olfactory epithelium degeneration was evident within 24 hours of a 2-hour exposure (Volume I, Table 10). Marked degeneration with various degrees of exfoliation occurred with a 4-hour exposure. The authors reported that the non-protein sulfhydryls in the olfactory epithelium and the lung were reduced by approximately 60% one hour after the MeI exposure and remained at this level for the 6 hours of exposure. However, graphic interpretation appeared to show a little less reduction at hour 1 than hour 6.

- The Reed *et al.* (1995) study with Wistar-derived albino rats showed undulated appearance at as early as hour 0.5, vacuolation and early degenerative changes at hour 1, clear evidence of destruction and exfoliation at hour 2, degenerated cells attached to damaged epithelium at hour 3, and marked degeneration and damage to transitional epithelium at hour 6 (Volume I, Table 10). The GSH depletion at the earlier time points may be estimated by interpolating the first day data from the Himmelstein study shown in Table A-11; i.e., approximately 24% depletion at hour 0.5 (between 0% and 48% GSH depletion), 48% at hour 1 as reported, and approximately 60% at hour 2 (between 48% and 72%).

Overall, these data show a high degree of consistency. Even excluding the undulated appearance at hour 0.5 with an estimated 22% GSH depletion, and excluding also the generally lower GSH depletion from the second day data from Himmelstein study, data at hour one showing vacuolation and early degenerative cellular changes can be associated with as low as 48% GSH depletion. Considering the variation at the average of 48% depletion (i.e., or 52.4 ± 18.3% of controls), the 50% depletion cannot be taken as representing a “no-effect” benchmark.

Thus, a reasonable approach to establish a “no-effect” benchmark for HECs is simply by estimating the GSH depletion at the NOEL of 21 ppm. Two sets of data are available for this approach. One is from the data reported by Himmelstein (2004) as given in Table A-11. The other is from modeling the GSH level at the NOEL. Table A-11 shows that at 25 ppm MeI, a level slightly above the 21 ppm NOEL, the peak olfactory epithelial GSH depletion at hour 6 is 35 - 42% (i.e., or 65.1 ± 14.0% of the controls for day 1, and 57.8 ± 14.3% for day 2 of MeI exposure). Thus, the expected GSH depletion at 21 ppm would be lower than 35% even without considering its variability. The model output at the 21 ppm NOEL as presented in Figure A-16 shows a 34% average GSH depletion at the meatus region and 25% average depletion at the ethmoid region. Given that the model may slightly underestimate the GSH concentration at the dorsal olfactory region (Figure A-15a), 25% GSH depletion is reasonably close to the upper limit of “no-effect” benchmark.

Table A-11. Average level of GSH in rat nasal epithelium.[a]

Hour	Olfactory Epithelium - % of initial		Respiratory Epithelium - % of initial	
	25 ppm MeI	100 ppm MeI	25 ppm MeI	100 ppm MeI
0(1st dose)	100.0 (0%)	100.0 (0%)	100 (0%)	100.0 (0%)
1	84.8 (15%)	52.4 (48%)	90.6 (9%)	68.6 (31%)
3	63.4 (37%)	27.6 (72%)	48.9 (51%)	17.2 (82%)
6	65.1 (35%)	24.2 (74%)	52.0 (48%)	14.3 (86%)
9	85.6 (14%)	64.1 (36%)	86.8 (13%)	57.0 (43%)
24(2nd dose)	86.9 (13%)	114.3 (-14%)	120.0 (-20%)	157.8 (-58%)
25	102.5 (-3%)	92.3 (8%)	96.6 (3%)	93.1 (7%)
27	63.0 (37%)	55.5 (44%)	51.2 (49%)	54.6 (45%)
30	57.8 (42%)	44.4 (56%)	57.1 (43%)	43.2 (57%)
33	69.2 (31%)	74.6 (25%)	70.8 (29%)	77.4 (23%)
48	125.4 (-25%)	143.6 (-44%)	95.5 (4%)	129.1 (-29%)

a/ Data from Himmelstein, 2004. Rats were exposed to MeI for 6 hours per day for two days. Data presented as average % of the controls. Data expressed as % depletion is given in the parenthesis. Note that the percentage given in this table is relative to the corresponding controls which fluctuate with time. Thus, they may not reflect the level of changes in GSH concentration from one time point to the next.

Two additional observations can be made from data in Table A-11 regarding the pattern of GSH recovery after MeI exposure. First, the on-going GSH replenishment appears to play a significant role in the rapid return to its background level after the end of exposure. This process often surpasses the initial GSH level (as indicated by the negative % of depletion) by hour 24 after a 6-hour exposure. This pattern provides further support for the use of a NOEL established from the repeated dosing study by Kirkpatrick (2002b) to address the risk of acute exposure scenarios. Second, as was pointed out in the review by Sweeney and Gargas (2005), cellular damage through GSH depletion tends to be time-dependent. Thus, considering the prolonged GSH depletion in human 24-hour exposure scenario and the lack of time to recover from day to day, repeated days of exposure may result in a greater severity of cellular damage for which an HEC based on a single day exposure at 25% GSH depletion may not be adequate to protect.

III.D. HECs

The rat model uses mei3.csl and mei3cmd files submitted to DPR by Arysta (2007). Three sets of HEC simulation runs were conducted by Arysta in 2008. These include: 1) output for the general population (Arysta's "bystanders") and occupational (Arysta's "worker's") HECs based on both 25% and 50% GSH depletion (Arysta, 2008b) and their subsequent correction (Arysta, 2008c); 2) output for occupational (Arysta's "worker's") exposure HECs that applied the higher DPR default breathing rate of 833 L/hr but assumed a 40% oral breathing (i.e., only 500 L/hr passes through the nose) (Arysta, 2008d); and 3) repeating output #2 but assumed 0% oral breathing (Arysta, 2008f). HECs are modeled for a single 24-hour day of exposure for the general population and at 8 hours for occupational exposure.

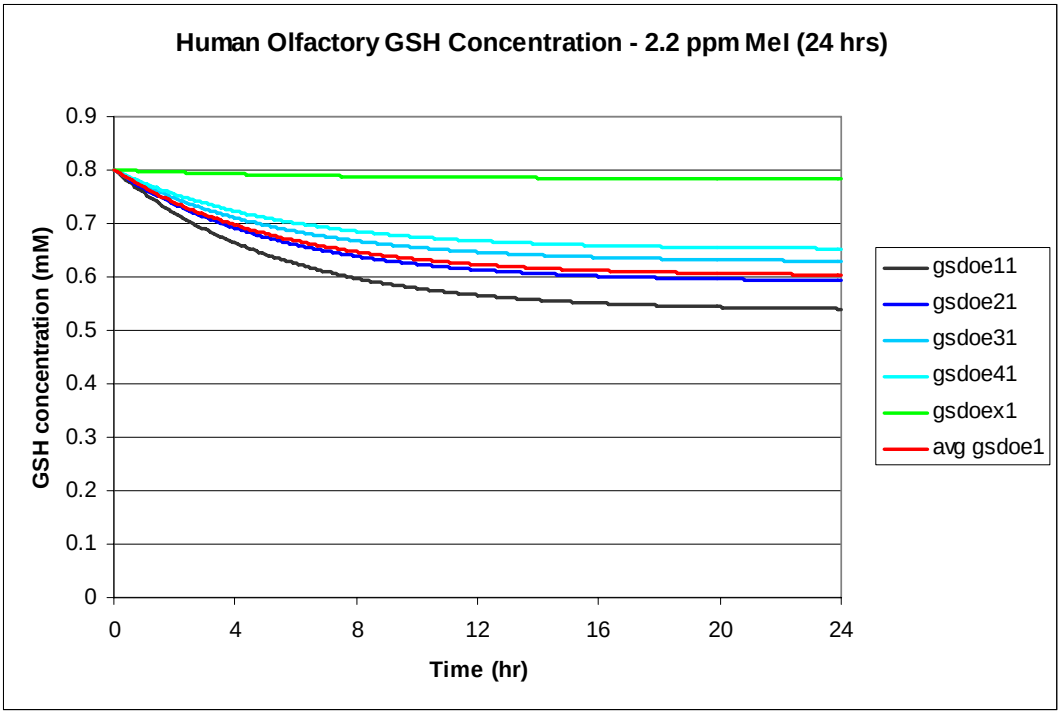
III.D.1. General Population HEC

Based on a 25% olfactory GSH depletion, the HEC is 2.2 ppm for a 24-hour exposure. Using the same model, USEPA (2007) reported that applying input parameters for children at ages of 3 month-old infants, children at 1, 5, 10, and 15 years old did not result in different HECs than for the adults. The simulations for children were reported as using age appropriate body weight and nasal, and ventilation parameters (values not specified in USEPA report). A same WOE thickness of 0.006 cm was used for all ages, but a slightly lower WOX thickness at 0.05 cm was used for infants and young children, while 0.08 cm was used for older children and adults. Arysta came to the same conclusion of no age differences in HEC by assuming a constant TVol/BWt ratio in age-specific model runs (Mileson *et al*., 2009).

The corresponding pattern of GSH depletion is presented in Figure A-17. The highest level of GSH depletion at the outermost olfactory layer gsdoe11 is 32% (or at a fraction of 0.675) of the initial GSH concentration. The level stays below the 35 – 42% GSH depletion reported in rats at 25 ppm, slightly above the NOEL of 21 ppm. The time to a steady state depletion with less than 0.5% change in the GSH level is not reached until beyond hour 14 of exposure. This indicates the importance of the time factor in the overall risk assessment for this endpoint, i.e., increase in exposure duration is expected to result in greater GSH depletion before a steady state depletion is reached.

III.D.2. Occupational HEC

In the nasal HEC model performed by Arysta, DPR's default ventilation rate of 833 L/hr (0.833 m^3/hr) was entered as 925 ml/breath at the same resting 15 breath/min rate. This yields a minute volume of 13.88 L/min, or the target breathing rate of 833 L/hr (versus the 1.5-fold lower resting rate of 9.45 L/min or 567 L/hr). The initial HEC modeling assumes only 60% nasal contribution to the total ventilation at this light activity level (Arysta, 2008d). The resultant 500 L/hr air intake through the nose is lower than the 567 L/hr used for the general population HEC simulation with a 100% nasal contribution.



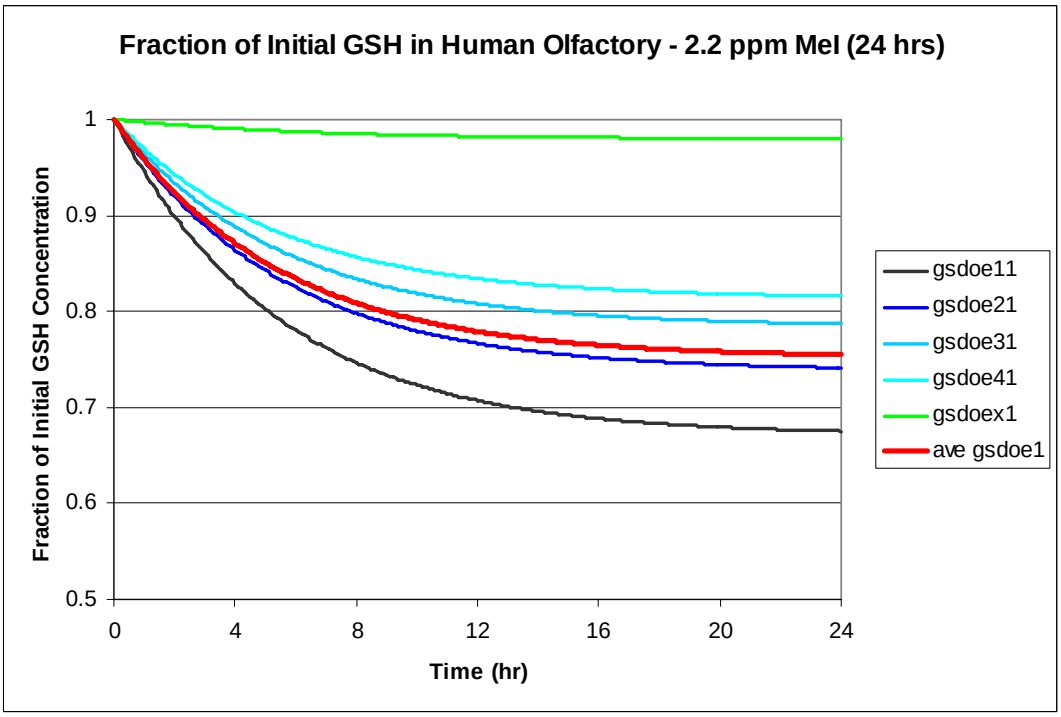


Figure A-17. The 24-hour HEC of 2.2 ppm based on 25% olfactory GSH depletion. Include 4 meatus layers (gsdoe11, gsdoe21, gsdoe31, gsdoe41; with gsdoe11 as the outmost layer), basement exchange layer (gsdoex1), and the 4-layer average in meatus (ave gsdoe1). Data from Arysta (2008b, c).

A study by Wheatley *et al.* (1991) was cited as basis for the 40% oral contribution assumption. However, the associated data for the 5 subjects in this study are not pertinent for the current MeI model because their lowest initial inspiratory flow of 1 L/s (or 60 L/min; 3,600 L/hr) is already substantially higher than the 833 L/hr. Moreover, the authors reported that the interindividual variability was great, showing one of the 5 subjects had 70% nasal contribution at 120 L/min (7,200 L/hr), an inspiratory level more than 8-fold above the DPR rate for the MeI model input. The study concluded that the oronasal partitioning is related to the ventilation rate and the 60% nasal air flow was attributed to a 1940 publication which only designated the condition as "on exercise".

More recently, Bennett *et al.* (2003) reported the average nasal contribution among 11 males and 11 females as 79% in females at 23.5 L/min (1,400 L/hr). The DPR's default of 833 L/hr is still lower than the "20% of maximum physical work level" in this study which showed 80-100% nasal contribution in female subjects. DPR default rate is actually much lower than the USEPA EFH's recommended average light activity rate of 1,300 L/hr for outdoor workers (USEPA, 1997). Thus, a more appropriate adjustment for oral contribution should be closer to 0 - 10%. This issue was not considered in the USEPA's model review for the HECs (USEPA, 2007). Within the nasal model construct, the breathing rate alone did not significantly impact the simulated olfactory GSH level. However, in general, physiological accuracy remains to be desirable in PBPK modeling, especially since the same model is used for the next set of HEC based on systemic uptake of MeI.

Subsequent model simulation using 100% nasal breathing resulted in an 8-hour occupational HEC of 2.8 ppm (Arysta, 2008f). The GSH profile at this HEC is illustrated in Figure A-18. The modeled recovery after the 8-hour exposure is near completion at the end of a 24-hour cycle. However, because the GSH decline has not reached a plateau by the end of 8 hours, greater depletion can be expected from longer exposure at this MeI level. This HEC also does not account for the remaining 16 hours of exposure at the level of the general population. Nevertheless, because of its similarity to the 24-hour HEC of 2.2 ppm, and the ongoing GSH replenishing, the additional 16-hour exposure after work may not significantly impact this occupational HEC.

III.D.3. Summary

The USEPA-derived 24-hour HEC is 4.5 ppm and 8-hour occupational HEC is 5.8 ppm. These are based on 50% average GSH depletion from the 4 architectural layers of the dorsal meatus in the olfactory region. By using the benchmark GSH depletion at 25% instead of 50%, the DPR 24-hour HEC is 2.2 ppm. DPR's 8-hour occupational HEC is 2.8 ppm using DPR's default breathing rate of 833 L/hr (i.e., 83% of USEPA's recommended "light activity" rate) for occupational activities, and assuming 100% nasal breathing.

Several uncertainties are noted. First, the 25% benchmark GSH depletion does not fully account for the data variability in animal studies from which it is established. Furthermore, the HEC is based on the average GSH depletion over 4 olfactory epithelial modeling structures, underestimating the greater depletion at the outermost cell layer that is in immediate contact with MeI. Finally, the GSH depletion has not reached a plateau at the end of an 8-hour occupational

exposure such that further depletion exceeding the 25% no-effect benchmark is expected if the occupational exposure is extended beyond 8 hours at the 2.8 ppm HEC.

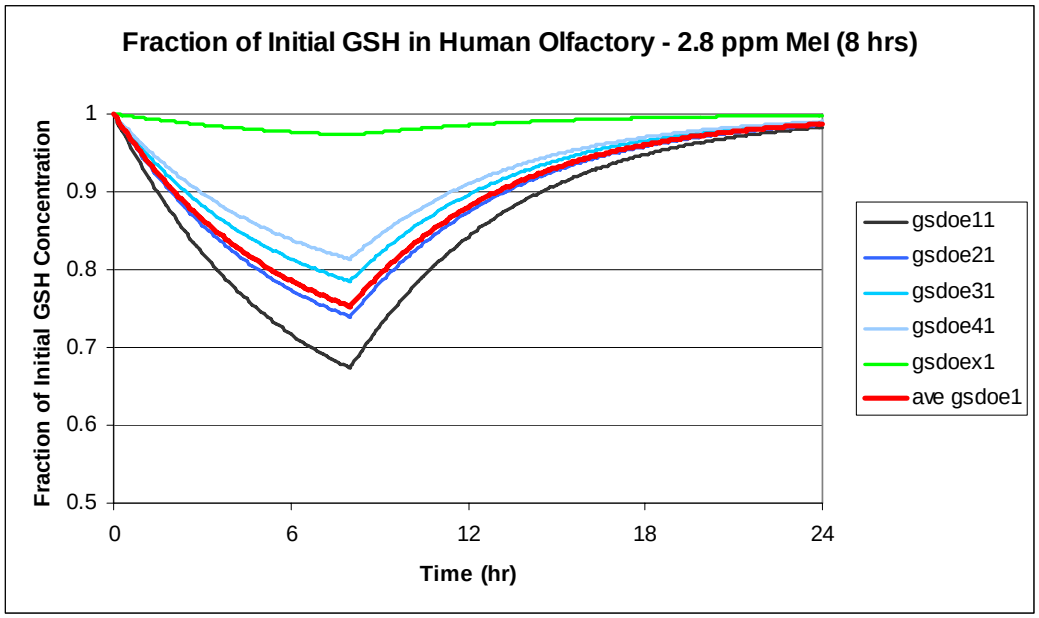


Figure A-18. The 8-hour occupational HEC of 2.8 ppm based on 25% olfactory GSH depletion. During working hours, the breathing rate was 833 L/hr with 0% oral breathing. Include 4 meatus layers (gsdoe11, gsdoe21, gsdoe31, gsdoe41; with gsdoe11 as the outmost layer), basement exchange layer (gsdoex1), and the 4-layer average in meatus (ave gsdoe1). Data from Arysta (2008f).

IV. NEUROTOXICITY

This section presents the establishment of HECs for neurotoxicity following the same format as the nasal effect HEC section. However, the coverage is less extensive due to a general agreement between DPR and USEPA on the selection of the critical NOEL of 27 ppm (Schaefer 2002 and 2003), the limited availability of data on the possible MOA, and the use of same aforementioned rat nose model for HEC simulation. The key difference between the HECs established by DPR and USEPA is in the dose metric selection that resulted in lower HECs. The lack of data to support any possible MOA for neurotoxicity was discussed by Kirman *et al.* (2009). It should be noted that the authors concluded that the confidence is low for supporting the use of peak brain MeI concentration as dose metric for PBPK modeling.

IV.A. Model and Parameters

The same PBPK model used for the HEC for nasal effects in rats is used for establishing the neurotoxicity HEC. Model behavior pertinent to the HEC determination is discussed in this section.

As discussed in Section III. A., within the model construct, simulation for serum iodide level in rats is affected by the input rat body weight. A supplementary model run using a lower rat body weight was provided by Arysta for illustrating its impact on model outcome (Arysta, 2008f). The comparative analysis showed that the MeI profiles in the blood and brain are not significantly different between input body weights of 0.207 kg (closer resembling the "Himmelstein rat") and 0.376 kg (used in rat model). This is likely due to the rapid metabolism of MeI after uptake. However, the iodide profiles in the serum and brain are inversely proportional to the two input body weights; i.e., the 1.8-fold lower body weight (i.e., 0.376/0.207 = 1.8) resulted in 1.8-fold higher iodide concentrations. On the other hand, the 2.5-fold blood-to-brain iodide ratio remained the same.

A more striking difference in the iodide profile is the 5.5-fold increase in the serum and brain iodide peak concentrations when the 6-hour MeI exposure was increased by 1.3-fold, from 21 to 27 ppm. The 0.226 kg for male rat at the 27 ppm dose group from the Schaefer (2002 and 2003) study was used for the HEC simulation. However, this additional 1.66-fold difference in the body weight parameter (i.e., 0.376/0.226 = 1.66) could not proportionally account for the marked increase in the iodide profile. The exact reason for this model behavior cannot be deciphered merely based on the output from these two simulations. This unresolved uncertainty within the model output provided by Arysta further deters the use of iodide profiles for the HEC determination at the NOEL of 27 ppm.

IV.B. Mode of Action

The MOA for MeI neurotoxicity is not known. One possibility is via MeI induced GSH depletion in the brain. In a rat study by Chamberlain *et al.* (1998a), a 20-30% decrease in brain NP-SH was reported at the end of a 6 hour exposure to 100 ppm MeI, with a slightly greater decrease in the forebrain than in cerebellum. The information from *in vitro* investigation showed that GSH depletion by MeI results in cytotoxicity in cultured neural cells. Cell death became

more evident long after the recovery of GSH level (Davenport *et al*., 1992; Bonnefoi, 1992; Chamberlain *et al*., 1999). A MOA via direct effect of MeI or excess iodide on the brain is also possible. Kirman *et al.* (2009) considered the MeI-induced neurological effects reported in the rat study (Schaefer, 2002) as transient and therefore, the likely MOA is via cell membrane disruption via modification of ion currents in neurons as has been demonstrated for organic solvents with general anesthetic properties. There are no MeI or iodide data to support this speculation.

IV.C. Dose Metric

USEPA agreed with Arysta's proposal to use the peak concentration of MeI in the brain as dose metric for neurotoxicity HEC. The argument for the relevance of peak concentration was the assumption that MeI neurotoxicity is similar to the sedative effects of many volatile solvents. However, based on the time lapse between MeI exposure and the changes in neurobehavioral measurements in the Schaefer studies, USEPA cautioned that this assumption could not be substantiated (USEPA, 2007). In reviewing the March 2009 draft of this document, USEPA agreed that the AUC is a more appropriate HEC dose metric (Rodriguez, 2009).

Several limitations for the choice of dose metric associated with the 27 ppm NOEL are noted. First, there are no data to show that the 3 hour time point for administering the battery of neurobehavioral tests represents the time to peak effects. There is also no assurance that the severity of neurotoxicity would not increase had the exposure in rats been extended past 6 hours in the study. Neither is there assurance that the NOEL would remain the same had the rats been exposed for 24 hours.

The model output generally shows that the brain MeI concentration is proportional to the concentration in the blood. By choosing the brain concentration dose metric, USEPA demonstrated that it is also proportional to the exposure between the NOEL (27 ppm) and the LOEL (93 ppm). However, DPR's concerns remain about their use of brain MeI concentration instead of its AUC for the HEC dose metric. Since the simulated steady state brain MeI concentration is reached within 30 minutes of exposure, substantial uncertainties exist with the use of peak blood MeI concentration, i.e., without accounting for the longer duration of sustained MeI in human brain. Furthermore, the peak concentration dose metric is inconsistent with the suggested MOA regarding similarity to the anesthetic or sedative effects of chemical solvents. For these effects, it is necessary that the time-dose factor should be accounted for, instead of the dose factor alone. Interestingly, while peak MeI concentration in the brain was used by USEPA as basis for modeling the HEC, it was stated in USEPA's MeI risk assessment that time-dose relationship as in the "Haber's law" (i.e., $C^n \times t = K$) should be assumed for all systemic effects (USEPA, 2007). The same "Haber's law" was also mentioned in the published MeI risk assessment by Arysta (Mileson *et al*., 2009) as applicable to all systemic effects and yet peak MeI concentration instead of AUC was used for their HEC derivation.

Given that the MOA is unknown, the AUC of MeI in the brain instead of the MeI peak concentration was used by DPR as the dose metric to account for the time-dose consideration. However, the contribution of iodide to neurotoxicity of MeI cannot be ruled out. Nevertheless, given the aforementioned (Section IV.C.1) modeling uncertainties in its disproportionate

increase in rats with increased MeI exposure levels around the NOEL, the dose metric of brain iodide AUC simulated by the model is not suitable here for HEC determination.

IV.D. HECs

Simulation outputs for a single day of exposure at 24 hours for the general population as well as for 8 hours for occupational exposure were provided by Arysta (2008f). However, these modeling data for HECs are generated based on the brain MeI peak concentration dose metric. No simulation of HEC based on the AUC of brain MeI was provided. Thus, the HECs based on the latter dose metric are extrapolated from the existing modeling datasets, including those simulated for establishing nasal HEC, since the same model is used for both endpoints. The rationale for extrapolation is included in the following presentation of HECs.

IV.D.1. General Population HEC

A 24-hour HEC simulation was only provided for 10 ppm MeI based on USEPA's dose metric of peak brain MeI concentration (Arysta, 2008f). Model outputs from rat nasal HEC simulations are additionally used in estimating the HEC based on brain MeI AUC. The 24-hour MeI AUC in the brain at 2.2, 4.5, and 10 ppm are presented in Table A-12.

Table A-12. Modeled human brain MeI AUC at 2.2 to 10 ppm MeI for 24 hours

Simulation Condition	Brain MeI AUC (mg/L* hr)	Reference
Rat; NOEL 27 ppm 6-hour	0.794	Arysta, 2008f
Human; 2.2 ppm 24-hour	0.519	Arysta, 2008b, c
Human; 4.5 ppm 24-hour	1.062	Arysta, 2008b, c
Human; 10 ppm 24-hour	2.370	Arysta, 2008f

These model outputs show a linear relationship between the brain MeI AUC and the MeI air concentration, with the intercept at -0.00167 and the slope at 0.2365. Thus, a 24-hour HEC of 3.4 ppm would yield a brain MeI AUC of 0.794 mg/L*hr, the same AUC determined for the rat in 24 hours from a 6 hour exposure at the NOEL of 27 ppm. Graphic presentations of these simulations are shown in Figure A-19 for both the AUC and peak concentration of brain MeI. It should be noted that the 0.133 mg/L peak MeI concentration in this simulation from Arysta is 30% higher than the 0.1 mg/L presented by USEPA (Barton, 2007; USEPA, 2007). The exact reason for the difference between the two model simulations is unclear. Using the lower peak MeI concentration from USEPA's rat model, the HEC would be lower. This was confirmed by a model run by Rodriguez (2009) showing the HEC at 2.8 ppm, approximately 20% lower than 3.4 ppm. Nevertheless, since the reasons for the difference between Arysta and USEPA's model output remain unknown, the 3.4 ppm is used in DPR risk assessment for the acute neurotoxicity endpoint based on Arysta's data submission accompanied by a set of modeling information.

Figure A-19a

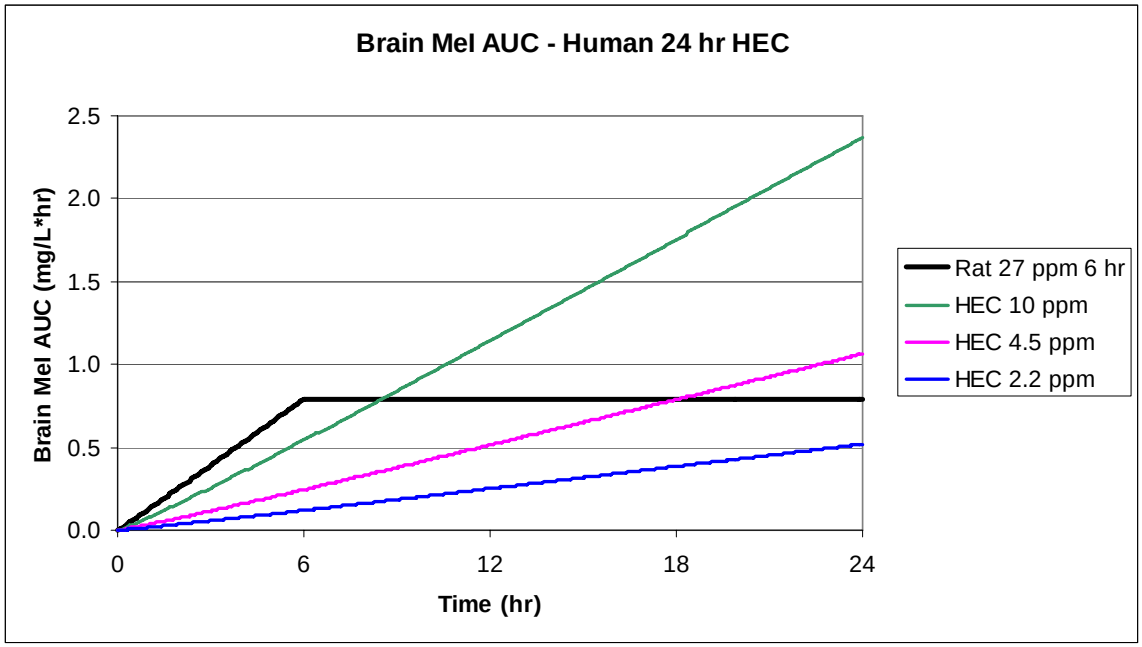


Figure A-19b

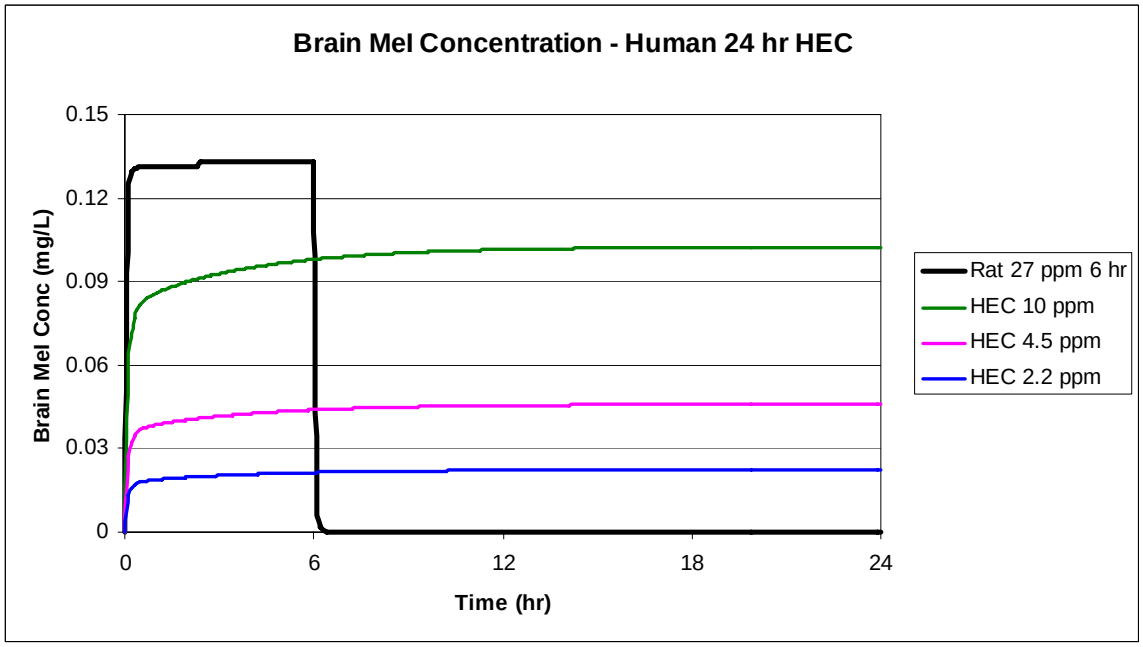


Figure A-19. Modeled 24-hour HEC based on brain MeI AUC. Presented are brain MeI levels of a) AUC, and b) peak concentration. The HEC of 3.4 ppm can be extrapolated from the modeled AUC. Data from Arysta (2008c, f).

The HEC simulations are based on the parameters for a 70 kg adult with breathing rate of 567 L/hr. It was stated by Sweeney (2008) that based on their sensitivity analysis the HEC for a 6 kg child would be similar to the HEC for a 70 kg adult. According to a later publication by Sweeney *et al.* (2009), the same ratio of tidal volume (TVol) to body weight was used for adults and children (Sweeney *et al*., 2009).

IV.D.2. Occupational HEC

An 8-hour HEC simulation was provided for 9.7 ppm MeI (Arysta, 2008f) based on DPR's default breathing rate of 833 L/hr, but with no additional 16-hour exposure after work. Similar to the derivation of 24-hour HEC described in the previous section (Section IV.C.1), a comparison can be made to the available modeling results from other Arysta model outputs at different MeI concentrations. Although this HEC was only modeled for an 8-hour period (Figure A-20), a simulation extended over 24 hours also showed that MeI rapidly declined after the cessation of exposure.

The HEC based on brain MeI AUC for only 8 hours of exposure is 9.7 ppm. This is approximately 3-fold higher than the 24-hour HEC of 3.4 ppm presented in Section IV.C.1, although the latter was modeled at a lower breathing rate of 567 L/hr. Rodriguez (2009) noted that based on USEPA's lower simulated brain MeI profile, the 8-hour HEC would be 7.5 ppm. However, the "8-hour only" HEC of 9.7 ppm from Arysta and 7.5 ppm from USEPA do not take into account the additional 16-hour exposure after work. Thus, it is reasonable to set the worker's 8-hour HEC at 3.4 ppm, the same level for the 24-hour HEC for the general public. This is also approximately one-third of the 9.7 ppm assuming that the 8 hour of exposure is evenly spread out over 24 hours. No other simulations were submitted for further evaluation.

IV.D.3. Summary

The HEC derived by USEPA is 10 ppm for both 24-hour and 8-hour exposures. This is based on the peak concentration of MeI in the brain. Since the peak is reached within 30 minutes of exposure, the difference in the duration of exposure did not impact the USEPA's HEC. By using the brain MeI AUC as dose metric, DPR's HEC for both 24-hour and 8-hour occupational exposure is 3.4 ppm. It is noted that the simulated peak rat brain MeI concentration from Arysta's simulation is 30% higher than the level reported for the USEPA simulation. Using the lower level from the latter would result in a lower 24-hour HEC of 2.8 ppm.

Figure A-20a

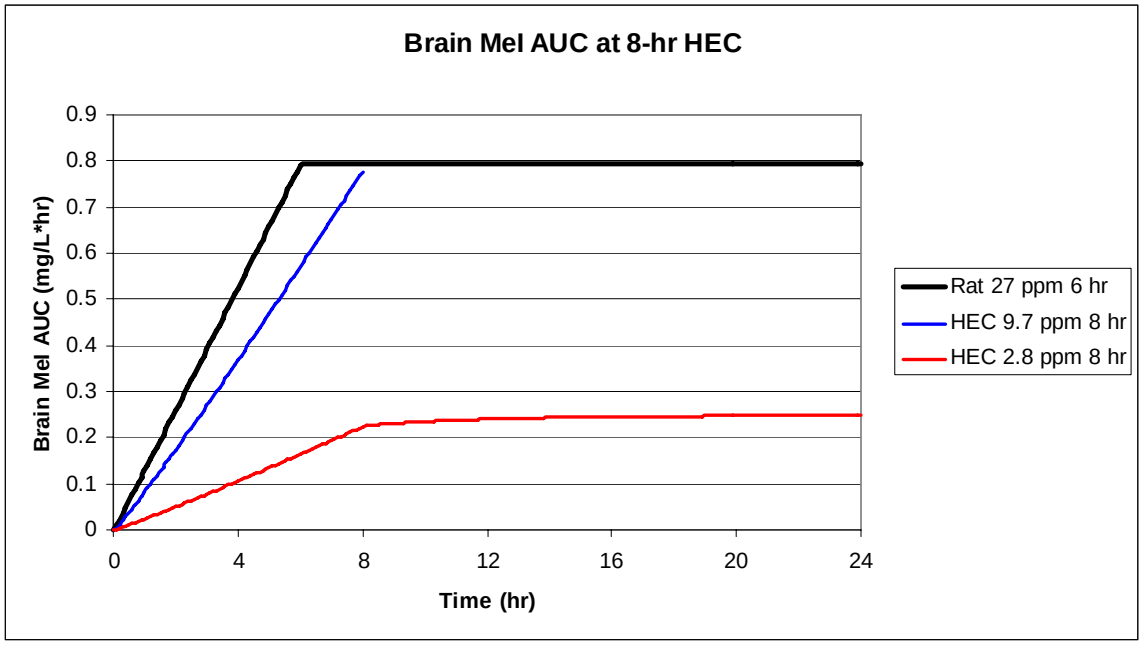


Figure A-20b

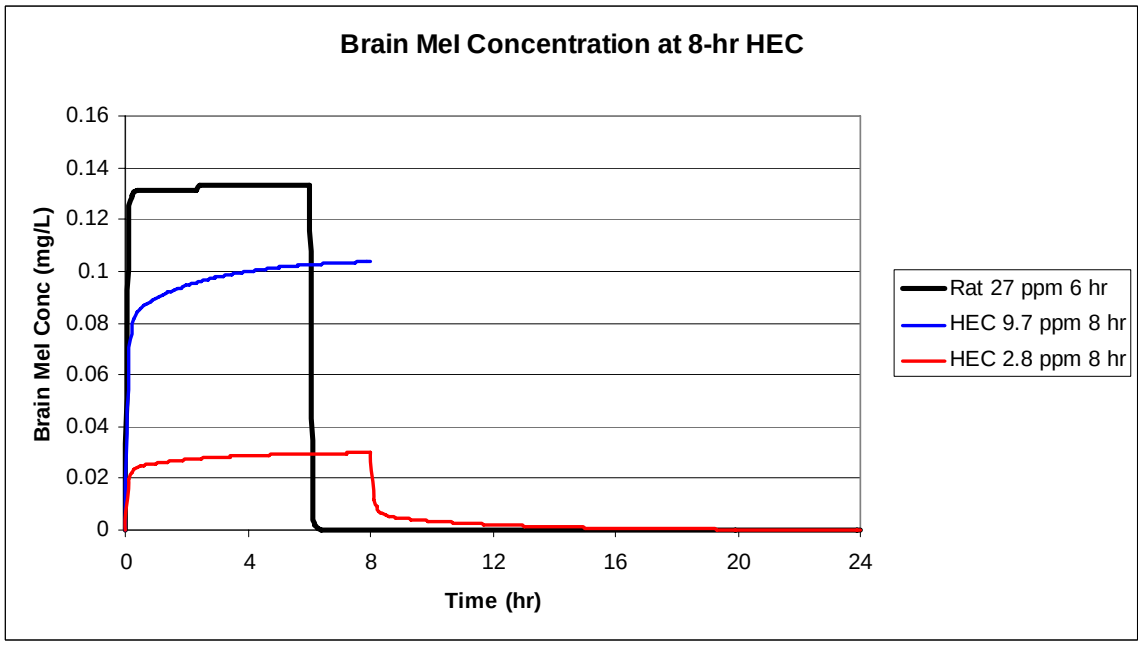


Figure A-20. The 8-hour-only HEC of 9.7 ppm based on brain MeI AUC. Presented are brain MeI levels of a) AUC, and b) peak concentration. Data from the 24-hour simulation of 8-hour exposure at 2.8 ppm MeI (nasal HEC simulation) based on the same breathing rate of 833 L/hr are included for comparison. Data from Arysta (2008f).

V. Summary of All Acute HECs

A summary of MeI HECs established from Section II through IV are presented in Table A-13. These values are provided for calculating the margin of exposure (MOE).

In using toxicity endpoints identified in animal studies, the current approach in health risk assessment takes into account the pharmacokinetic (PK) and pharmacodynamic (PD) differences between species (i.e., PK_{animal}, PD_{animal}) and the variation within human population (i.e., PK_{human}, PD_{human}). The PK_{animal} is accounted for in the PBPK modeling presented in this review. Default values for the remaining 3 factors (i.e., PD_{animal}, PK_{human}, PD_{human}) are then used for setting the health-protective benchmark MOE.

Because MeI perturbs thyroid functions, additional considerations should be given for protecting against neurodevelopmental effects from pre- and post-natal exposures for which toxicity data are lacking (Volume I, Section V.C.1.). Moreover, because MeI is rapidly converted to iodide, the toxicity of excess iodide from MeI should also be considered. Thus, although the lowest 24-hour HEC of 0.24 ppm is only applicable for assessing the risk of exposure for women of child-bearing age, care should be taken in establishing MeI reference concentrations (RfCs) based on the HECs listed in Table A-13 to ensure that the excess iodide from MeI does not exceed health-based standards for all age groups, not limited to women of child-bearing age. The consideration for iodide toxicity also applies in the derivation of RfCs for subchronic and chronic scenarios.

Table A-13. Acute HECs for MeI based on fetal death, nasal effects, and neurotoxicity

Endpoints	Rabbit Fetal Death	Rat Nasal Effect	Rat Neurotoxicity
24-hour HEC[a]	0.24 ppm	2.2 ppm	3.4 ppm
8-hour HEC[b]	0.22 ppm[c]	2.8 ppm[c]	3.4 ppm

a/ The 24-hour HEC used resting breathing rate (BR) of 567 L/min, 1.4x lower than DPR default BR of 833 L/hr for occupational work that is also used for the general public. The 24-hour HEC of 0.24 ppm and 3.4 ppm, but not the nasal HEC of 2.2 ppm, would be lower when DPR's default BR is used.

b/ The 8-hour HEC include the 8-hour exposure during work time and a remaining 16-hour exposure as a member of the general public.

c/ The 8-hour HEC does not include 16 hours of "ambient air" exposure. No model runs are available to assess its impact. However, since the 8-hour and 24-hour HECs are within the same range, the 0.22 ppm can be used for both scenarios.

d/ The 8-hour HEC does not include 16 hours of "ambient air" exposure. However, adding another 16 hours is not likely to significantly affect the HEC.

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Appendix B. Calculations

A. Equation for the conversion of NOEL (ppm) to amortized daily dose (mg/kg/day) for comparison of study NOELs (Table B-1):

Equation 1:

$$\text{ppm} \times \frac{\text{MeI molecular weight}}{\text{Molar volume}} \times \text{Breathing Rate} \times \frac{\text{Exposure hours/day}}{\text{24 hours}} \times \frac{\text{Exposure days/week}}{\text{7 days}} \times \text{AF}$$

where MeI molecular weight=141.95 g/mole, and molar volume=25.1 L/mole

Table B-1. Calculation of mg/kg/day for inhalation toxicity studies.

Studies	Species	Endpoint	NOEL ppm	NOEL mg/L[a]	BR[b] L/kg/day	Hours/ day	Days/ week	NOEL mg/kg/day
Acute Exposure								
Schaefer, 2002	Rat	Neurotoxicity	27	0.15	960	6/24	NA	37
Kirkpatrick, 2002b	Rat	Nasal effect	21	0.12	960	6/24	NA	28
Himmelstein, 2004	Rat	Nasal and thyroid effects	25	0.14	1890[c]	6/24	NA	67
Nemec, 2002c	Rat	Maternal weight effect	60	0.34	960	6/24	NA	81
Nemec, 2002d	Rabbit	Fetal death	2	0.011	540	6/24	NA	1.5
Nemec, 2003	Rabbit	Fetal death	20	0.11	540	6/24	NA	15
Sloter, 2005a	Rabbit	Fetal death, thyroid effect	25	0.14	540	6/24	NA	19
Sloter, 2005b	Rabbit	Fetal death, thyroid effect	20	0.11	540	6/24	NA	15
Subchronic Exposure								
Kirkpatrick 2002b	Rat	Body and liver weight effects	21	0.12	960	6/24	5/7	20
Nemec, 2004	Rat	Systemic, repro-ductive effects	25	0.14	960	6/24	7/7	34
	Rat	systemic effects	25	0.14	960	6/24	5/7	24
Nemec, 2002a	Rat	Reproductive effects	20	0.11	960	6/24	7/7	27
	Rat	Developmental effects	5	0.03	960	6/24	7/7	7
Nemec, 2002c	Rat	Maternal body weight effect	20	0.11	960	6/24	7/7	27
Nemec, 2002d	Rabbit	Maternal body weight effect	10	0.057	540	6/24	7/7	8
Chronic Exposure								
Kirkpatrick, 2005	Rat	Salivary gland metaplasia	5	0.03	960	6/24	5/7	5
	Rat	Nasal and thyroid effects	20	0.11	960	6/24	5/7	19

a/ 1ppm= 0.0056 mg/L (21°C). Inhalation absorption factor (AF) is assumed at 100% (Sved *et al*., 2002).
b/ Default breathing rates (BR) of 960 L/kg/day (rats), and 540 L/kg/day (rabbits) (Zielhuis and van der Kreek, 1979).
c/ BR was experimentally determined.

B. HEC and RfC calculations (Table B-2)

Equation 2: HEC, when not determined by PBPK modeling, is:

$$\text{HEC} = \text{NOEL} \times \frac{\text{Animal Breathing Rate}}{\text{Human Breathing Rate}} \times \frac{\text{Animal Exposure hours/day}}{\text{Human Exposure hours/day}} \times \frac{\text{Animal Exposure days/week}}{\text{Human Exposure days/week}} \times \frac{1}{\text{PK}_{\text{animal}}}$$

where PK_{animal}= default factor of $10^{0.5}$ (actual value used 3.16).

Example: Using 2 ppm as the LED for thyroid tumors (Kirkpatrick, 2005)

HEC = 2 ppm x 0.96/0.28 x 6/8 x 5/7 x $1/10^{0.5}$= 1.2 ppm for worker
HEC = 2 ppm x 0.96/0.28 x 6/24 x 5/7 x $1/10^{0.5}$= 0.39 ppm for bystander and residents

C. Reference concentration (Table B-2)

Equation 3: $$\text{RfC} = \text{HEC} \times \frac{1}{\text{PD}_{\text{animal}}} \times \frac{1}{\text{PK}_{\text{human}} \times \text{PD}_{\text{human}}}$$

where PD_{animal}= default factor of $10^{0.5}$ (rounded to 3), and PK_{human} x PD_{human} = total default uncertainty factor of 10. If an additional uncertainty factor of 10 is applied, the RfCs will be 10-fold lower.

D. Margin of Exposure:

Equation 4: $$\text{MOE} = \frac{\text{HEC}}{\text{Human Exposure}}$$

Example: MOE for thyroid tumor HEC and resident lifetime exposure

MOE= 0.39 ppm HEC/0.003 ppm resident exposure level = 130

E. Iodide levels from MeI exposure:

Equation 5: $$\text{Additional Iodide in MeI (ug/day)} = \text{RfC (ppb)} \times \frac{5.65\ \text{ug/m}^3}{\text{ppb}} \times \text{BR (m}^3\text{/day)} \times \frac{127}{142}$$

When,

1 ppb = 5.65 µg/m^3
BR: age specific breathing rate (8.3 m^3/day for 1-3 years old, 10 m^3/day for 4 - 8 years old; 15 m^3/day for 9 - 13 years old, 17 m^3/day for 14 - 18 years old, and pregnant and lactating females; and 15.2 m^3/day for 19+ years (USEPA, 1997)
(127/142): molecular weight ratio of iodide to MeI

F. Interspecies scaling factor:

Equation 6:

$$\frac{Dose_a x BW_a}{BW_a^{0.75}} = \frac{Dose_h x BW_h}{BW_h^{0.75}} \longrightarrow \frac{Dose_a}{Dose_h} = \frac{BW_h^{0.25}}{BW_a^{0.25}}$$

G. Calculation of human equivalent potency factor

Potency factor using laboratory animal cancer incidence data is calculated using the Multistage Cancer model in the USEPA Benchmark Dose Software (http://www.epa.gov/NCEA/bmds/).

Adjust animal potency factor to human equivalent factor by these equations:

Equation 7:

$$Potency_a x Dose_a = Potency_h x Dose_h \longrightarrow Potency_h = Potency_a x \frac{Dose_a}{Dose_h}$$

Equation 8: (combining Equation 7 and interspecies scaling factor from Equation 6)

$$Potency_h = Potency_a x \frac{BW_h^{0.25}}{BW_a^{0.25}} \longrightarrow Potency_h = Potency_a x (\frac{BW_h}{BW_a})^{0.25}$$

Example: $Potency_a = 5.0 \times 10^{-3}$ mg/kg/day^{-1} (rat) from the BMD analysis for thyroid tumors
Body weight human = 70 kg (default), body weight rat= 0.625 kg

$$Potency_h = 5x10^{-3} \text{ mg/kg/day}^{-1} x(\frac{70}{0.625})^{0.25} = 1.6x10^{-2} \text{ mg/kg/day}^{-1}$$

H. Oncogenic Risk

Equation 9: Risk= Potency factor (human equivalent) x human exposure level

Example: risk for thyroid tumors

Risk = (1.6×10^{-2} mg/kg/day^{-1}) x 5 μg/kg/day= 8×10^{-5}

Equation 10: Unit risk= potency on a “per ppb” basis

Example: unit risk for workers

a) find ? ppm = 1 mg/kg/day since potency factor is expressed as mg/kg/day^{-1}

$$? \text{ ppm x } 5.65\,\text{mg/m}^3 \text{ x } 0.28\,\text{m}^3/\text{kg/day x } \frac{8\,\text{hours/day}}{24\,\text{hours}} \text{ x } \frac{5\,\text{days/week}}{7\,\text{days}} = 1\,\text{mg/kg/day}$$

? = 2.8 ppm

(Similar to Equation 1. In this case, 0.28 m^3/kg/day is human default adult breathing rate)

b) $\frac{2.2x10^{-2}}{2.8\ ppm} = \frac{risk}{1ppb}$ *unit risk* = 6x 10^{-6} per 1 ppb

Example: unit risk for general population

a) find ? ppm = 1 mg/kg/day since potency factor is expressed as mg/kg/day^{-1}

$$? \text{ ppm x } 5.65\,\text{mg/m}^3 \text{ x } 0.28\,\text{m}^3/\text{kg/day x } \frac{24\,\text{hours/day}}{24\,\text{hours}} \text{ x } \frac{7\,\text{days/week}}{7\,\text{days}} = 1\,\text{mg/kg/day}$$

? = 0.63 ppm

(Similar to equation 1. In this case, 0.28 m^3/kg/day is human default adult breathing rate)

b) $\frac{2.2x10^{-2}}{0.63\ ppm} = \frac{risk}{1ppb}$ *unit risk* = 2.5 x 10^{-5} per 1 ppb

<u>Equation 11</u>: Acceptable human exposure level= acceptable risk level ÷ unit risk

Example:

Acceptable worker exposure level= 1 x 10^{-5}÷ 6 x 10^{-6} per 1 ppb = 0.17 ppb

Acceptable general population exposure level= 1 x 10^{-6}÷ 2.5 x 10^{-5} per 1 ppb = 0.04 ppb

Table B-2. Calculation of HECs and RfCs for MeI.

Duration/endpoint	NOEL or LED (ppm)	BR ratio[a]	Hours/ Day ratio[b]	Days/ week ratio[c]	PK factor[d]	HEC (ppm)	RfC UF=30[e] (ppb)
Acute Exposure							
Development effect (Nemec, 2002d)	0.5 (LED_{01})	0.54/0.28	6/8	7/7	$10^{0.5}$	0.23 ppm (workers)	8
		0.54/0.28	6/24	7/7	$10^{0.5}$	0.08 ppm (adult)	3
Nasal effect in rats (Kirkpatrick, 2002b)	21 (NOEL)	0.96/0.28	6/8	7/7	$10^{0.5}$	17.1 (workers)	570
		0.96/0.28	6/24	7/7	$10^{0.5}$	5.7 (non-worker adults)	190
		0.96/0.45	6/24	7/7	$10^{0.5}$	3.5 (children)	118
		0.96/0.59	6/24	7/7	$10^{0.5}$	2.7 (infants)	90
Neurotoxicity in rats (Schaefer, 2002)	12.8 ($LED_{0.36\sigma}$)	0.96/0.28	6/8	7/7	$10^{0.5}$	10.4 (workers)	347
		0.96/0.28	6/24	7/7	$10^{0.5}$	3.5 (non-worker adults)	116
		0.96/0.45	6/24	7/7	$10^{0.5}$	2.2 (children)	72
		0.96/0.59	6/24	7/7	$10^{0.5}$	1.9 (infants)	55
Subchronic Exposure							
Decreased pup body weight rats (Nemec, 2002a)	3.0 ($LED_{0.36\sigma}$)	0.96/0.45	6/24	7/7	$10^{0.5}$	0.51 (children)	17
		0.96/0.59	6/24	7/7	$10^{0.5}$	0.39 (infants)	13
Neurotoxcity in rats (Schaefer, 2002)	4.3 (extrapolated from acute, MF=3)	0.96/0.28	6/8	7/7	$10^{0.5}$	3.5 (workers)	116
		0.96/0.28	6/24	7/7	$10^{0.5}$	1.2 (non-worker adults)	39
		0.96/0.45	6/24	7/7	$10^{0.5}$	0.7 (children)	24
		0.96/0.59	6/24	7/7	$10^{0.5}$	0.5 (infants)	18
Chronic Exposure							
Salivary gland metaplasia in rats (Kirkpatrick, 2005)	3.4 (LED_{05})	0.96/0.28	6/8	5/7	$10^{0.5}$	2.0 (workers)	66
		0.96/0.28	6/24	5/7	$10^{0.5}$	0.66 (non-worker adults)	22
		0.96/0.45	6/24	5/7	$10^{0.5}$	0.41 (children)	14
		0.96/0.59	6/24	5/7	$10^{0.5}$	0.31 (infants)	10
Neurotoxcity in rats (Schaefer, 2002)	1.28 (extrapolated from acute, MF=10)	0.96/0.28	6/8	7/7	$10^{0.5}$	1.0 (workers)	35
		0.96/0.28	6/24	7/7	$10^{0.5}$	0.35 (non-worker adults)	12
		0.96/0.45	6/24	7/7	$10^{0.5}$	0.22 (children)	7
		0.96/0.59	6/24	7/7	$10^{0.5}$	0.16 (infants)	5
Lifetime Exposure							
Thyroid tumors (Kirkpatrick, 2005)	2 (LED_{01})	0.96/0.28	6/8	5/7	$10^{0.5}$	1.2 (workers)	39
		0.96/0.28	6/24	5/7	$10^{0.5}$	0.39 (non-worker adults)	13

a/ For DPR methodology, default breathing rates are: 0.96 m^3/kg/day for rats and 0.54 m^3/kg/day for rabbits (Zielhuis and van der Kreek, 1979); 0.28 m^3/kg/day for adult, 0.45 m^3/kg/day for children 3-5 years old, and 0.59 m^3/kg/day for infants (Andrews and Patterson, 2000).

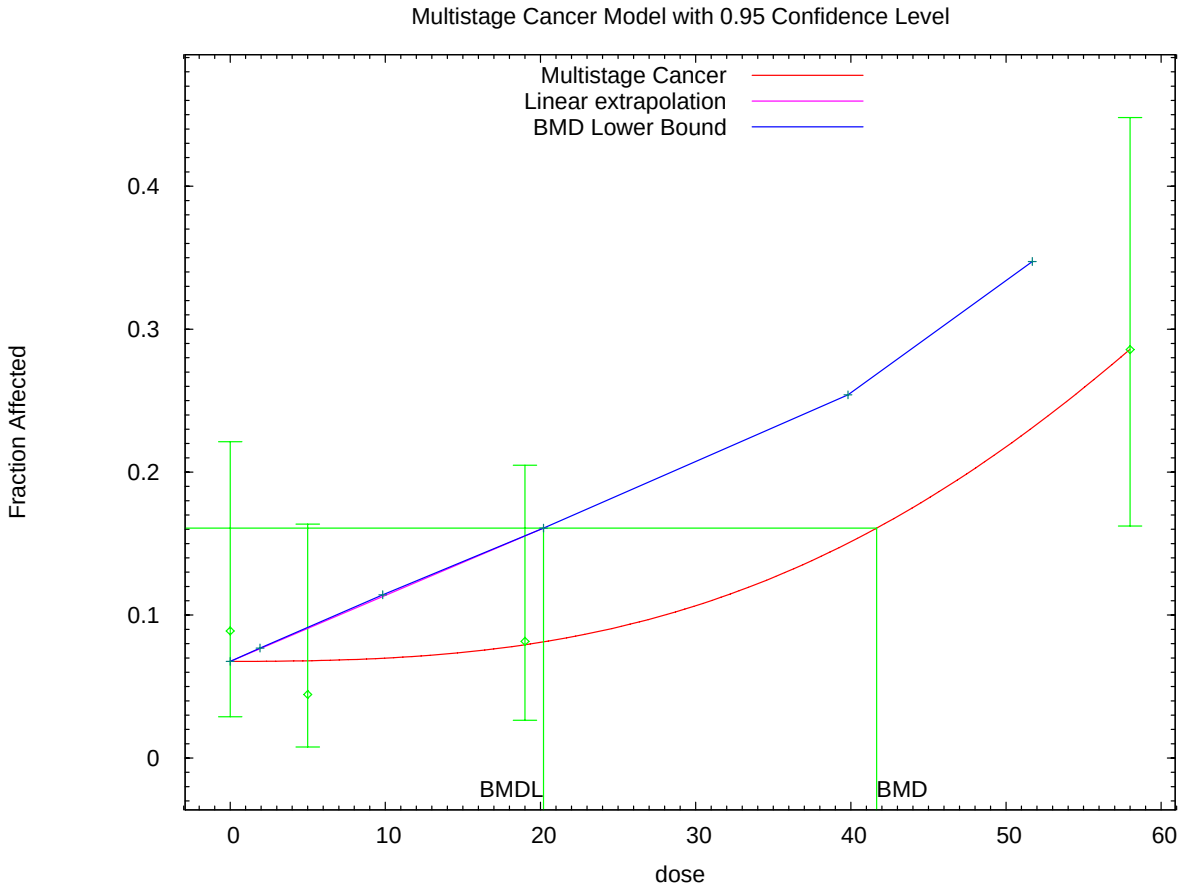
b/ Workers and non-workers are assumed to be exposed for 8 hours/day and 24 hours/day, respectively.

c/ Workers and non-workers are assumed to be exposed daily. For experiments conducted with exposures for 5 days/week, the HECs are reduced (i.e., by a 5/7 factor) because it is reasonable to assume a lower HEC for daily repeated exposure.

d/ PK_{animal} factor=3.16

e/ UF of 30 =3 (pharmacodynamic factor PD_{animal}) x 10 (intraspecies factor PD_{human} x PK_{human}). Note that the total UF for acute RfC is 30 because the interspecies PK difference is accounted for by PBPK modeling. The factor of 3 for PD_{animal} is an approximation of $10^{0.5}$, or 3.16. Thus, the total UF for subchronic, chronic, and lifetime RfCs is 100 if calculated from the NOEL because it includes the default interspecies PK difference (PK_{animal}) of $10^{0.5}$, or 3.16-fold. The actual RfC for fetal death and cancer includes an additional UF of 10-fold, and is thus 10-lower than those indicated in this table.

BMD multistage cancer model output for thyroid tumors in male rats (Table 22, Kirkpatrick, 2005)




```
  10:08 05/18 2009
 ====================================================================
         Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
         Input Data File: C:\USEPA\BMDS2\Temp\tmp13.(d)
         Gnuplot Plotting File:  C:\USEPA\BMDS2\Temp\tmp13.plt
                                                Mon May 18 10:08:18 2009
 ====================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                  -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive

   Dependent variable = affected
   Independent variable = DOSE

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3
```

```
 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 2.22045e-016
 Parameter Convergence has been set to: 1.49012e-008

****  We are sorry but Relative Function and Parameter Convergence are currently
unavailable in this model.  Please keep checking the web sight for model updates which
will eventually incorporate these convergence criterion.  Default values used.  ****

                  Default Initial Parameter Values
                     Background =    0.0625262
                        Beta(1) =            0
                        Beta(2) = 8.05112e-005
                        Beta(3) =            0

            Asymptotic Correlation Matrix of Parameter Estimates
 ( *** The model parameter(s)  -Beta(1) have been estimated at a boundary point, or
have been specified by the user, and do not appear in the correlation matrix )

              Background      Beta(2)      Beta(3)
Background            1        -0.62         0.59
   Beta(2)        -0.62            1           -1
   Beta(3)         0.59           -1            1

                                 Parameter Estimates
                                                        95.0% Wald Confidence Interval
  Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
  Background        0.0675944           *                *                   *
    Beta(1)                0           *                *                   *
    Beta(2)     1.32052e-005           *                *                   *
    Beta(3)     1.14016e-006           *                *                   *

* - Indicates that this value is not calculated.

                        Analysis of Deviance Table
        Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
      Full model        -60.6616         4
    Fitted model        -61.0351         3      0.746884      1          0.3875
   Reduced model        -66.9693         1       12.6153      3        0.005547

            AIC:          128.07

                                  Goodness  of  Fit
                                                                  Scaled
      Dose     Est._Prob.    Expected    Observed     Size       Residual
   ----------------------------------------------------------------------
     0.0000     0.0676         3.042     4.000          45        0.569
     5.0000     0.0680         3.062     2.000          45       -0.628
    19.0000     0.0793         3.884     4.000          49        0.062
    58.0000     0.2860        12.012    12.000          42       -0.004

 Chi^2 = 0.72      d.f. = 1        P-value = 0.3953

    Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
              BMD =        41.6607
             BMDL =        20.1955
             BMDU =        55.3514

Taken together, (20.1955, 55.3514) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor =    0.00495161
```

Appendix C. U.S. Environmental Protection Agency Risk Assessment

This section is a brief comparison between this RCD and the USEPA risk assessment (USEPA, 2007).

1. Human Equivalent Concentration

In the previous version of this comparison (August 2009 draft), for acute exposure, DPR and USEPA both use PBPK modeling to derive the HECs. However there are differences in the selection of the acute fetal death NOEL, MOA, and the dose metrics for PBPK modeling. These differences have been discussed under **IV.A.1. Acute Inhalation Toxicity** and Appendix A. As a result, DPR determined HECs are lower than those of the USEPA. For fetal death, the USEPA HECs are 23 ppm (worker 8 hours) and 7.4 ppm (bystander 24 hours) based on a NOEL of 10 ppm (Nemec, 2002d) and fetal serum iodide AUC after a single day of exposure from PBPK modeling. In comparison, DPR establishes a lower of NOEL at 2 ppm and uses the dose metric of maternal serum iodide AUC after a single day of exposure to derive much lower HECs of 0.22 ppm, and 0.24 ppm, for 8 hours and 24 hours respectively, of adult exposures. For rat olfactory epithelium degeneration (Kirkpatrick, 2002b), both DPR and USEPA establish the same NOEL for the study, but the difference lies in the selection of dose metric. USEPA considers a regional average of 50% GSH depletion as the dose metric, and the HECs are 5.8 ppm (workers) and 4.5 ppm (bystander all ages). In comparison, DPR sets the regional average of 25% GSH depletion, resulting in HECs of 2.8 ppm (workers) and 2.2 ppm (bystander and residents). For both DPR and USEPA, the NOEL for acute neurotoxicity in rats (Schaefer, 2002) is the same at 27 ppm. The difference is the dose metric with peak brain MeI concentration by USEPA and the AUC of brain MeI by DPR, to establish the HECs. The USEPA HEC is 10 ppm (workers and bystanders), while the DPR HEC is 3.4 ppm (all groups).

The differences in the HECs between DPR and USEPA remain as DPR values in **Volume I** are calculated from the benchmark dose, instead of the NOEL, for fetal death and neurotoxicity, and the use of a default factor, instead of PBPK modeling, to account for interspecies pharmacokinetic differences. The revised acute HECs are listed in Table C-1. The largest difference remains those for fetal death where DPR values are about 100-fold lower.

For the calculation of HECs of systemic effects after repeated MeI exposure, USEPA uses a default RGDR of 1, the ratio of the blood:gas partition coefficient of the chemical for the test species to humans ($H_{b/g\ animal}$/ $H_{b/g\ human}$), to account for the pharmacokinetic differences between species (USEPA, 2007 and 1994). An UF of 3 is then applied to account for the PD differences since the interspecies PK differences have presumably been included in the RGDR, as it is an approximation of the PBPK model. DPR has not adopted the use of the USEPA methodology because of the uncertainty involved. In the case of a default RGDR of 1, no quantitative adjustment for interspecies PK from animals to humans has been made. Alternatively, DPR's methodology seeks to make interspecies exposure adjustment only for the "intake" portion of the exposure scheme, and not the "uptake and tissue distribution" to the target site. For this approach, the “intake” is calculated based on the breathing rate (air "intake") on a per body weight basis (as is the common expression for exposure or dose). This calculation is similar to the estimation of exposure through the diet while the "intake" is the amount of food

consumed instead of the air breathed in. DPR recognizes that sufficient data and experience are not yet available for a subsequent adjustment down to the "uptake and tissue distribution" portion of the dose estimation that enables an adequate account for all PK aspects of the interspecies difference in a simple dosimetric equation. Thus, DPR maintains the PK factor of $10^{0.5}$ (Table B-2). Both USEPA and DPR apply a factor of $10^{0.5}$ for interspecies PD differences and a total 10-fold factor for interindividual PK and PD differences in the human population. As shown in Table C-1 for subchronic and chronic exposures, when the test species is the rat, there are only slight differences between these two approaches in the HECs for adults. It is because mathematically, the total PK correction as the product of breathing rate ratio (0.96 $m^3/kg/day_{rat}/0.28\ m^3/kg/day_{human}$) and PK uncertainty factor of 3 equals to 1, the same as the USEPA RGDR default ratio of 1. However, this is not the case for the children and infant HECs. The DPR calculations show much lower values for these groups than for adults. USEPA did not calculate HECs for exposure of young children to MeI.

Table C-1. Comparison of HECs between USEPA and DPR.[a]

Study	USEPA HEC (ppm)		DPR HEC (ppm)			
	Worker	By-stander	Worker	Bystander		
				Adult	Child	Infant
Acute Toxicity						
Fetal death in rabbits (Nemec, 2002d)	**23**	**7.4**	**0.23**	**0.08**	NA	NA
Acute nasal effect in rats (Kirkpatrick, 2002b)	**5.8**	**4.5**	**17.1**	**5.7**	**3.5**	**2.7**
Neurotoxicity in rats (Schaefer, 2002)	**10**	**10**	**10.4**	**3.5**	**2.2**	**1.9**
Subchronic Toxicity						
Reproductive and developmental effect (Nemec, 2002a)	3.75	1.25	**0.51**	**0.39**	NA	NA
Systemic effects (Kirkpatrick, 2002b)	15.75	3.75	NA	NA	NA	NA
Neurotoxicity (Schaefer, 2002)	NA	NA	**3.5**	**1.2**	**0.7**	**0.5**
Chronic Toxicity						
Salivary gland effect (Kirkpatrick, 2005)	3.75	0.89	**2.0**	**0.66**	**0.41**	**0.31**
Neurotoxicity (Schaefer, 2002)	NA	NA	**1.0**	**0.35**	**0.22**	**0.16**

a/ Bolded values are used in the calculation of margins of exposure by DPR. USEPA did not calculate MOEs for repeated exposures; the HECs are included only as a comparison of the RfC methodology. NA=not calculated.

For MeI oncogenicity, DPR determines that both a genotoxic and a non-genotoxic mode of action may be involved in the formation of thyroid tumors in rats. Thus, both a threshold (using MOE) and a nonthreshold (using potency factor) are used to assess human exposure. While USEPA concludes that a genotoxic mechanism can not be excluded, the USEPA considers the formation of this tumor type as a threshold effect with the rat as the most sensitive species.

2. Exposure Assessment

A comparison of DPR's MeI exposure estimates for workers, bystanders, and residents with those in the USEPA risk assessment is provided in Volume II. Both DPR and USEPA estimate acute exposures, but only DPR provides repeated exposure values in the risk assessment. For occupational acute exposures, among the differences are: replicate samplers were considered as a single replicate (DPR) or as two values (USEPA), measured air

concentrations were adjusted (DPR) or not (USEPA) for maximum application rate, and exposure was based on upper-bound value (DPR) versus maximum measured value (USEPA). The end result is that DPR estimates are similar to USEPA for applicators and hole punchers for drip irrigation, but higher values, as much as 3.4-fold, for other workers as shown for tarp monitor for shank injection (Table C-2).

Table C-2. Comparison of acute exposures between USEPA and DPR.[a]

Scenarios	**DPR ppm**	**USEPA ppm**
Workers (No PPE)		
Shallow shank-tarped soil fumigation (broadcast and bedded)		
Applicators (using shanks, 10-12")	1.51	1.03
Shovelmen and Shovelers	1.09	0.76
Tarp Monitors	3.75	1.11
Tarp Hole punchers, cutters, and removers	0.16	0.07
Planters	0.01	0.007
Drip irrigation fumigation (tarped-bed)		
Applicator	0.25	0.24
Hole Puncher	0.02	0.02
Planter	0.01	0.007
Bystanders[b]	**ppm at 30 meters from field**	**ppm at 25 meters from field**
Drip irrigation	0.6	0.24
Shank injection, raised bed	0.3	0.32
Shank injection, flat fume	0.4	0.23

a/ Values from **Volume II.**
b/ 24-hour TWA air concentration.

For bystander exposures, the difference in MeI air concentrations arises from the use of different models and modeling assumptions, and selection of field studies. USEPA uses both the ISCST3 model and the Probabilistic Exposure and Risk model for Fumigants (PERFUM) to evaluate distributional bystander exposure from data derived from fumigation studies conducted in California, Florida, and Michigan, and to estimate the buffer zone distances (USEPA, 2007). DPR estimates the air concentrations using the ISCST3 model using only California data. In modeling, USEPA adopts the whole field, probabilistic approach, while DPR applies the maximum direction approach. A comparison of 24-hour time-weighted average air concentrations (at 30 meters for DPR and 25 meters for USEPA values) for a 40 acre field, shows similar values for raised bed shank injection, but 2 to 3 fold higher for flat fume shank injection and drip irrigation, respectively (Table C-2).

Both USEPA and DPR conclude that MeI would not be expected to contaminate the ground or surface water. USEPA conducts a qualitative drinking water assessment because MeI is water soluble, and may be found in ground water and surface water if the treated soil is exposed when there is rain (USEPA, 2007). Tier II PRZM/EXAMS for surface water and Tier I SCIGROW for ground water are used to estimate MeI concentration in the drinking water. While no residue levels were provided, USEPA concludes that MeI would not be expected to "adversely impact ground water or surface water." The DPR evaluation is presented in **Volume III**. An additional concern for iodide in drinking water is included in the assessment.

3. Risk Characterization

The differences in HECs and exposure estimates result in DPR and USEPA reaching different conclusions regarding the potential risk with MeI acute exposure to workers and bystanders. Using a benchmark of 30 for acceptable risk, the USEPA concludes that MeI registration can be approved with some restrictions on the application rate and field size, and requirements (USEPA, 2007 and 2008). DPR reaches the opposite conclusion with a benchmark of 300 for acute exposure and fetal death endpoint, and a benchmark of 30 for other endpoints and durations.

A more important difference is that DPR recommends a more thorough investigation of the potential pre- and post-natal developmental neurotoxicity. In the absence of sufficient information, the application of an additional uncertainty factor of 10-fold is necessary because of concerns about inadequacy of toxicity testing on young animals and the potential toxicity from additional iodide from MeI exposure. This factor should be applied toward the MOE benchmark, resulting in a higher benchmark of 300 as well as proportionally lower calculated reference concentrations. Under the section for uncertainty factors, USEPA states that MeI is a non-food use pesticide and consequently, it was not subjected to the Food Quality Protection Act (1996) and the 10x FQPA factor did not apply (USEPA, 2007).

With iodide, DPR is concerned with increased body burden from iodide in the drinking water and after MeI inhalation exposure. While iodide contamination of surface water is unlikely, a screening evaluation estimates an upper-bound level of 18 ppm iodide in the ground water. Iodide levels from MeI inhalation exposure are calculated from the RfCs of the acute toxicity endpoints. From both water and air, the calculated iodide intakes under most cases are

higher than established health standards for iodide. Thus, the recommended MeI RfCs for any duration should be much lower, at levels not to exceed 1 ppb, for the protection of young children. On the other hand, the USEPA is apparently only concerned with iodide in the air, as a result of MeI degradation after application. This level was considered to be "lower than those expected to cause toxic effects," but the data to support this statement were not provided in their risk assessment (USEPA, 2007). If an UF of 30 is applied to the USEPA HEC, the RfCs will result in excess iodide exposure much higher than any established health standards (as discussed in Volume I, Section V.C.). For example for the USEPA 24-hr HEC of 7.4 ppm for fetal death endpoint, the RfC is 247 ppb with a corresponding excess iodide body burden of 10,400 µg/day for young children[5]. For the 4.5 ppm 24-hr HEC for nasal effects, the hypothetical RfC is 150 ppb with the corresponding excess iodide for young children at 6,300 µg/day. The NAS tolerable upper limit (2000) and the ATSDR MRL (2004) are 200-300 µg/day, and 113-153 µg/day, respectively, for 1-3 year old.

[5] Total iodide exposure=RfC (ppb) x 5.65 (µg/m^3)/ppb x BR (8.3 m^3/day for 1-3 year old) x 127/142. The ratio of molecular weight of iodide (127) to MeI (142) is used to account for the weight of iodide in MeI, assuming 100% absorption of MeI and conversion to iodide. BR=breathing rate from USEPA (1997).