Methyl Iodide (Iodomethane)

RISK CHARACTERIZATION DOCUMENT FOR INHALATION EXPOSURE

Volume IV – Part 1

Responses to Scientific Review Committee Comments

CH_3I

Department of Pesticide Regulation

California Environmental Protection Agency

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List of Comments

A. Scientific Review Committee Comments on the August 2009 Draft RCD

B. Scientific Review Committee Comments and Errata on the December 2009 Draft RCD and List of Tasks for completion of final RCD

A. Scientific Review Committee on August 2009 Draft RCD (Comments received November 18, 2009)

Appendix 1

Comments of the SRC on DPR's RCD on methyl iodide

Overview

The Scientific Review Committee (SRC) has prepared comments for consideration by DRP to facilitate development of a revised risk assessment for further consideration by the Committee. This document represents the conclusions of the SRC based primarily on the review of the transcript from the meetings on September 24 and 25, 2009. The transcript represented an important source of information. In certain circumstances the information considered by the SRC expands on questions raised reflecting follow-up literature reviewed by committee members, e.g., questions relating to the mechanism of action with respect to carcinogenicity and neurodevelopmental toxicity. There may also be new points raised here by committee members, for instance regarding the conclusions derived from the Department of Pesticide Regulation's presentation and risk characterization document, the testimony of interested parties, written submissions, the primary literature, and information from Arysta.

The SRC does not anticipate receiving a separate text responding to this document. Rather we look forward to a revised document that is responsive to this input. The SRC will limit its review to the revised risk assessment document. We would appreciate receiving a revised document that shows "track changes". The SRC will then be able to more easily determine where DPR has made changes.

It will be important for DPR to also explicitly present the rationale for decisions that differ from the comments provided by the SRC. At the next meeting DPR can highlight the specific ways in which the revised document is responsive to these points. There are major issues to be addressed as determined by the SRC and we look forward to a revised, new document that reflects the addressing of the issues.

Exposure Assessment

General Points

- No measurements of concurrent exposures to chloropicrin (added to MeI) or to other pesticides (that may be used in ways leading to co-exposure with MeI) have been done. The SRC is concerned about the potential additive or multiplicative (synergistic) adverse health effects of such co-exposures; without exposure estimates such effects cannot be assessed appropriately.
- In the assessment, dermal exposure from vapors or liquid MeI is assumed to contribute very little to total delivered dose. Because accidental exposure to liquid due to failures in delivery systems, repair of delivery lines, contact with injected or dripped pesticide, or loading mishaps in the field are each plausible exposure scenarios, this should be taken into account. Moreover, the EPA-approved labeling appears to proscribe glove use to protect against the trapping of vapors. An exposure scenario with direct skin contact to liquid MeI, assuming 100% absorption, should be included as a component of total worker exposure.
- All exposure estimates are based on a scenario of fumigation of a single field. But if several farms are adjacent and conducting simultaneous fumigation, workers, bystanders and residents may be downwind of multiple plumes even if only one field per farm is being fumigated at a given time. This multiple source scenario should be included in exposure estimates of both acute and chronic duration, to better capture the density of California agriculture. Use of applicable methyl bromide data to address such estimates is appropriate. Where relevant, all tables should clearly specify when exposure/risk estimates are based on modeled or measured data. Although buffer zones are sometimes proposed to mitigate this type of situation, simple air-dilution modeling indicates that with a wind speed of 1 m/s and neutral stability, the exposure at a distance of 1000 m from a treated field is close to 30% of the on-field concentration—indicating that there could be significant added exposure to workers in one field if an adjacent field is treated during an overlapping time. A similar situation applies to bystanders who could be significantly impacted by multiple fields when the bystanders are within 1000 m of two or more fields.
- The risk assessment tends to focus on acute exposures for workers and chronic exposures for bystanders. But both groups can have sub-chronic exposures (lasting periods of days) as a result of the longer-term emission from fields. Workers are (in theory) protected from acute exposure by protective equipment. Chronic exposures (extending out to several weeks) for bystanders tend to be rather low, because of the rapid dissipation of MeI. But during the period within a day or so after application there are sub-chronic exposures to both workers and bystanders during which workers are not using protection and for which bystanders are not yet protected by MeI dissipation. The risk assessment has not explicitly addressed these subchronic exposures and whether or how they might have impact.

Worker Exposure Estimates

The worker exposure assessment was based on an empirical interpretation of limited sampling in actual worker exposure situations. The exposure estimates for workers are set to the upper bound from these empirical observations. This approach has a great deal of uncertainty, because it

addresses only the variance observed in a limited set of observations. To the extent that the sampling process fails to capture a full range of conditions that impact exposures—particularly high-end exposure, this approach introduces significant uncertainty about the reliability of these estimates. This adds uncertainty to the exposure assessment that is not discussed or quantified. Among the problems that have been identified in the worker exposure assessment are the following:

- MeI products registered by USEPA are labeled with requirements for respirator use. DPR's calculations of expected exposure for workers assume a protection factor of 0.9, (that the exposure with the mandated protection is only 10% of what would be expected without protection), which even if theoretically attainable, is not an accurate reflection of protection levels likely to be achieved in practice in California agriculture. There was significant testimony about the limitations of respiratory protection. There was even discussion about the use face masks with very limited protection. DPR needs to address this issue realistically and not assume a best case scenario. Occupational health professionals are well aware of the limitations of respirator use in industrial settings let alone field use. It would appear unrealistic to assume continual oversight to ensure effective respirator use.
- Any data on equipment changeout, breakthrough, cleaning and training should be included in the document, since these factors affect the protection factor that realistically can be achieved in real-world applications. Any information from methyl bromide use on compliance with respirator fit testing, training, cartridge change out, etc, could be applied to MeI use scenarios.
- Taking the points above into account, it would be reasonable to apply no more than a 50% attenuation factor as a default respirator-associated value, but bearing in mind that in some scenarios no attenuation at all may be expected. This is most likely for post-application field workers who punch openings in or otherwise handle tarps, as they will not be required to wear respirators according to current labeling.
- Tractor driver exposure estimates were calculated assuming that engineering controls provide 90% protection, but here again it is not clear how frequently the 90% dilution will be achieved in practice. Once again, a default value of 50% would be more reasonable, although in some scenarios this may be even less.
- Worker exposure studies were conducted under relatively low ambient environmental temperature conditions, but during the peak fumigation months temperatures can be quite high. Exposure estimates should explicitly account for this by adjusting exposure estimates for increased volatility of MeI under typical and worst case exposure scenarios. This would be consistent with the approach used for drift and bystander exposure which does take into account a range of environmental conditions. The literature on chemical properties indicates that for volatile compounds such as MeI the vapor pressure (the driving force for volatilization) can often double between 20 and 30 °C.
- Worker risks assume an 8-hour day and 3 months of exposure. This is unlikely to reflect common hourly and seasonal practices. Given that overtime pay does not begin until 10 hours, this is a common minimal shift, with even longer work days likely to apply. In one of the off-site monitoring studies, the actual fumigation took 8 hours; it is highly likely that workers were on site beyond that period (e.g., for set-up and post-application tasks).

Similarly, the optimal fumigation periods for different crops or the same crop with different growing periods could result in a trained applicator working more than 3 months per year by moving from one region of California to another. Margins of exposure should be in place to protect against worst case exposure scenarios.

- Data on the actual, unadjusted exposure concentrations derived from worker studies should be presented without protection factors included. A separate column or columns that explicitly indicate adjustment for varying personal protection factors should be added (see above).
- Integrated sampling times of 5 to 6 hours do not allow evaluation of shorter-term peak exposures that could be toxicologically relevant. This should be taken into account in exposure scenarios.
- There are a number of factors that affect bystander exposure. It is not clear that the field measurements have captured the full range and variability among factors such as tarp type, field conditions, soil amendments, and application methods. Applying a simple mass-balance model to the DPR exposure concentrations indicates that they are reasonable and unlikely to underestimate bystander exposure (See Appendix A). But the SRC believes there can be more exposure variability than is identified in the DPR risk assessment.
- There is no clear effort to address the proximity effect for hole-punchers, who have their faces quite near the venting point. No exposure estimate reduction should be applied to hole punchers and planters since they have no mandated respiratory protection (as already noted above).

Off-Field Exposure Estimates

- It appears that the bystander exposure assessment was carried out with the help of the EPA ISCST3 model. This model requires a soil emission rate as an input. Field volatility of MeI was measured in seven studies in California during a broadcast, flat fume and raised bed, tarped, shallow shank injection of MeI. To obtain this emission rate the model was run for a situation where bystander concentrations were measured for the studies noted above and the model was used to "back-calculate" emissions. There are a number of factors that affect bystander exposure such as tarp type, field conditions, soil amendments, application methods, and it is not clear that the field measurements have captured the full range and variability among these.
- Flux rates used to generate estimates of exposure from MeI drift were back-calculated from modeling the data from the 7 off-field studies. The tables of data from these studies, as presented in Appendix II of Volume II, are not adequate for the SRC's evaluation. For example, no information is provided on sampling intervals, number of samplers including replicates, or total duration of sampling, for instance was sampling done continuously through nighttime and for how many days. Information on temperature, humidity, wind conditions, and atmospheric stability over the duration of the studies is also important to allow for evaluation of the uncertainty in the estimates. Please provide new data tables to address this along with explanatory text. It is important to clarify how the climate conditions used in the modeling, and those expected during application compare to the conditions under which samples were taken. Summary memos should be provided for review with appropriate justification.

- The outputs of the model, the estimated flux or emissions from treated fields should be presented as ranges or distributions rather than point estimates alone. These emission rates are crucial to the calculation of acute and chronic risks for bystanders.
- There did not appear to be an adequate explanation for the higher buffer zone concentrations predicted after drip irrigation, when worker exposures were higher for shank injection applications. It is counter-intuitive that higher concentrations within the application zone could translate to lower concentrations in the buffer zone. This should be addressed explicitly.
- Community exposure, meaning offsite drift beyond the buffer zone, is not measured but is estimated from modeling to be no greater than 0.07 µg/L. DPR assumes that community exposures will be less than or equal to seasonal bystander exposure, at the buffer zone. If, however, multiple farms are fumigating in a community, the community may be affected by more than one plume and as a result experience greater exposure concentrations than are predicted at the buffer zone after a single fumigation. The SRC's effort to make a mass-balance assessment indicates that the 0.07 µg/L is not likely to underestimate exposure and is adequate to capture seasonal variability (See Appendix A). So the SRC recommends that DPR provide a mass-balance diagram to support the recommendation that the community exposure is not likely to exceed 0.07 µg/L.
- Part of the supporting argument given by DPR about community exposure estimates is that the 2-week average concentration of methyl bromide measured in a community (0.046 ug/L) was comparable to the estimated 2 week average MeI concentration estimated modeled at the buffer zone (0.07ug/L) and that the compounds are chemically similar. Basing community exposure to MeI on methyl bromide data, however, may underestimate risk given the different physical chemistry of the two compounds. To use methyl bromide observations, there should at least be a full description of the variability and uncertainty of the measurements.
- Label requirements and buffer zone requirements are in conflict. The USEPA-derived buffer zone for non-worker bystanders <u>including residents</u> is 152 m. Yet the product label states "Do not apply within ¼ mile [402 m] of any occupied sensitive site", which one presumes includes residences. The buffer zones should also specify ¼ mile between a treated field margin and any residence or school. There should be a new explicit analysis of buffer zone requirements after desirable air concentrations are determined in a revised toxicity analysis.

Environmental Fate

- The most critical issue with Volume III concerns the inadequate evaluation of the potential for groundwater exposures. The half-life of MeI in water is relatively long, and the potential for contamination of this vital resource is of major importance for California.
- DPR needs to determine if there are data emerging from Florida and other states where use of MeI is ongoing.
- DPR concluded that groundwater contamination by MeI is unlikely and that groundwater contamination by the iodide anion breakdown product is uncertain. But they also observed that the amount of MeI applied in vulnerable areas (those with high potential for transfer to groundwater) is uncertain and that the amount and timing of water applied

after fumigation is uncertain. They note that these issues cannot be fully addressed until additional field dissipation data are obtained.

- Use of a "water cap" or post-fumigation irrigation may result in an increased risk of groundwater contamination.
- One key discussion that is missing in all of the exposure discussions is a summary of the overall fate or mass balance of MeI when it is applied to a field—something analogous to a pharmacodynamics flow chart. The current discussions of environmental fate are rather qualitative and should be more quantitative.
- It also appears that there are no good data on the rates of MeI degradation in soil and how these vary with climate conditions and soil properties. The degradation estimates are apparently based on a single study in Watsonville. A single study cannot capture the variability in climate conditions and soil properties.
- It would be very useful to provide a fate diagram that illustrates the approximate fractions of a unit mass (1 kg) that follow different transport/transformation pathways—the fraction that evaporates, the fraction that is transformed to the iodide anion, the fraction transformed to other products. Also for the fraction that evaporates it would be useful to have this broken down into the fraction that evaporates within 1 day, 3 day, 1 week and 2 weeks.
- If groundwater contamination has either been documented already or is likely to occur based on application and physical properties of MeI, and taking all of the uncertainties noted above into account, then the risks associated with oral exposure from drinking water, dermal exposure from bathing, and inhalation exposure from showering should each be incorporated into the MeI risk assessment.
- If supplemental air release or dietary exposure is reasonable based on the above uncertainties, additional risk modeling should also take these routes into account.

Metabolism

- Figure 1 in Volume I is inadequate. A more comprehensive figure is required delineating the metabolic pathways for MeI, including identification of intermediates formed prior to the formation of CO_2.
- There does not appear to be a discussion of the relative bond strength between Cl-C, Br-C, and I-C. It is apparent that the I-C bond is weakest and most vulnerable for SN2 reactions that would result in alkylation. The alkylation potential should be addressed at length since it demonstrates an enhanced capability for covalent bond breakage and binding with macromolecules. There is extensive discussion of bond strengths, SN2 reactions, and alkylation in the literature even modest organic chemistry texts. This is a matter of great simplicity with the implication that alkylation will be the key pathway for binding with nucleophiles. This basic organic chemistry should be taken very seriously. This suggests that alkylation is very likely to be a primary mode of chemical binding.

PBPK Model—Application to Computing HECs

General Comments

- The pharmacokinetic model that was used for MeI is very detailed and complicated. Saliently, it introduces both model uncertainty and parameter uncertainty to the overall risk assessment process. Some of the key model parameters included in the PBPK modeling are based on very sparse data; this further increases the uncertainty and limits the reliability of results from the PBPK model. Overall, the data do not appear to be sufficient to allow reliance on PBPK modeling to estimate human equivalent concentrations given the multiple uncertainties present in the model itself, and the questionable assumption made by the modelers that inorganic iodide is the species responsible for causing the adverse effects.
- The only mode of action that seems to have been considered in the pharmacokinetic model is excess serum iodide, but the inter-species differences in the distribution of and responses of humans and rodents to serum iodide have not been fully accounted.
- It is not appropriate to rely on a model-based dose metric for which the mode-of action has not been clearly established. Both MeI and NaI cause increases in TSH and cause thyroid hypertrophy and colloid depletion in the rabbit fetus, but only MeI reduced the percent of viable fetuses/litter, caused post-implantation loss, and increased late resorptions. The DPR document notes (page 139) "lack of concordance between fetal death from MeI and NaI on equal iodide bases suggests a different MOA than excess fetal iodide." In addition, there was insufficient data on serum iodide from MeI exposures (exposures below 20 ppm and time-course measurements) to establish exposure-response relationships and the PBPK model-based simulations did not provide adequate fits to the serum iodide data.
- DPR reduced the 10X factor for interspecies differences to 3 because they relied on the PBPK model to address species differences in pharmacokinetics. However, since the Panel rejects the utility of this model, the 10X factor should be restored.

Dose Metrics and HECs

- The selection of dose metrics and estimations of human equivalent concentration (HEC) is problematic.
- It is uncertain which dose metric is appropriate for the fetal death endpoint. The AUC of the parent MeI in either the mother or the fetus over the latter part of gestation (e.g. days 23-26 in rabbits) is a plausible hypothesis for the measure of the causal agent most likely to be directly related to adverse effects. Of the potentially significant types of effects that appear sensitive to MeI, fetal growth inhibition is likely to be the result of a kind of tax on the metabolic energy available to the fetus to grow and develop and prepare itself for entry into the outside world. As such it could be acting either on the mother or the fetus directly or both.
- Because the number of changes in organ-specific parameters that were needed to simulate GSH depletion in the rat and because there are no human data to test model predictions,

model-based estimates of the HEC for nasal toxicity cannot yet be considered reliable for interspecies or low dose projections.

- Because of the much higher level of GSH in the olfactory epithelium of rats (3.5 mM) compared to humans (0.8 mM), and because conjugation with GSH protects the exposed site from alkylation, humans may be more susceptible than rats to MeI toxicity at that site. Therefore, using GSH depletion of the olfactory epithelium as a dose metric would require a 4-fold adjustment for interspecies susceptibility.
- Because of uncertainties in events subsequent to exposure and the selection of a proper dose metric, and uncertainties in model-based estimates of HECs, extrapolations of animal dose to human dose based on scaling external exposures by body weight$^{3/4}$ should be presented. In addition, an interspecies uncertainty factor of 10 instead of 3 should be used to judge the adequacy of margins of exposure. (A summary of SRC-recommended uncertainty factors is included in Appendix B)

Selection of Critical Toxicological Endpoints for Risk Assessment

- The SRC agrees with DPR's selection of critical endpoints for the assessment of MeI, but recommends that fetal growth restriction, a common toxicological finding in reproductive/developmental studies, should be considered as an additional critical endpoint.
- Effects on cholesterol levels might also be given more attention since they bear relevance for human health. Linear increases in serum cholesterol, including LDLs, are common among rats, dogs, and rabbit fetuses. DPR's MeI document should consider a dose-response analysis and characterization of human risk for cholesterol changes.
- As an overall comment on DPR's approach to the toxicology, there is a greater emphasis on potential effects of excess iodide than on MeI as an alkylating agent, with potential genotoxic and cellular regulation effects that might result from covalent modification of cellular macromolecules.

Dose-Response Analysis for non-cancer endpoints

General Comments

- Most if not all of the dose points treated as "NOELs" are in fact more conservatively LOAELs.
- Because a large number of effects showed elevated responses at the DPR specified NOELs, benchmark dose analyses would be a more appropriate approach to dose response for characterizing health risks of MeI. This approach uses all of the dose-response data and does not simply rely on statistical significance by pair-wise comparisons between small groups of control and exposed animals.
- The point-of-departure for estimating a margin of exposure should be an exposure value slightly below the range of the experimental data. The deviation from no effect should take into account the severity of the endpoint in question: e.g. for fetal death a point of departure based on 1% or less rather than a 5% incidence.

- Dr. Hattis has prepared an analysis that may be helpful for dose-response assessment of fetal growth inhibition, should this endpoint be added to the risk assessment. This may be found at the conclusion of these comments as Appendix C.

Nasal Lesions

The total incidence of nasal lesions in rats (pages 42, 43, and 59) and thyroid lesions in female mice (page 51) needs to be provided and analyzed. The current limitation of the analysis to the subtypes and tissue layers is incomplete and potentially misleading.

Neurotoxicity

- Arysta reports a section on "Neurotoxicology" that actually deals with acute behavioral effects during a single exposure; these reflect anesthetic-like actions typical of lipid-soluble, volatile organohalogens, as well as acute symptomatology such as seizure activity. Notably, the studies designated as "neurotoxicity" have small sample sizes that are inadequate to detect significant differences as large as 25% of the test population; with endpoints such as seizures, the benchmark needs to be clearly below that incidence - even a single animal showing seizures is a worrisome finding, since spontaneous seizure activity never occurs in controls. There is a statement that histopathology did not show any neurotoxic damage but we have not been provided with the actual data. Elsewhere, they show data that indicate a high brain/plasma concentration ratio for MeI, as well as GSH depletion; these are important for neurotoxicity, as described in the next bullets.
- According to DPR personnel, the "neuropathology" consists of a single coronal section from each animal examined at one time point after exposure. If this is so, there are numerous brain regions that would be missed, and examining one section is not necessarily representative. There are standard methods for neuropathology examinations but these do not appear to have been followed; the assessment appears to be only "qualitative," which would overlook many features of neurotoxicity that are critical in safety assessment. See Appendix D.
- Since neurotoxicity typically develops over period of days to weeks, examinations at one time point shortly after exposure are not really definitive in assessing the complex group of endpoints in this category. Further, the test involved only a single exposure, rather than a repeated or chronic exposure that would be more representative of likely human exposures in agricultural communities; neurotoxicants in general produce adverse effects at much lower exposures when the exposure is chronic or repeated.
- There is an abundant literature of case reports and cell culture studies that point to neurotoxicity as a consequence of MeI exposure, typified by onset of symptoms and cell damage after a post-exposure period of apparent normality. Unfortunately, there was inadequate attention given to the very striking case study reports. The reports illustrate the extreme toxicity of MeI. There was insufficient attention to the severity of the case studies and a lack of coverage of all the studies. The studies indicate severe neurotoxicity which appears to persist over long periods of time and which may reflect chronic conditions. The case studies are more important than the limited reported studies because they demonstrate severe long-term effects. These results need to be given greater attention in terms of the implications of the findings even given the lack of exposure data.

There are clearly brain regions and cell types that are especially vulnerable, based on symptomatology and direct assessments of in neuronal cells. Not surprisingly, these are similar to the targets for other neurotoxicants that cause oxidative stress (cerebellum, especially granule cells; Parkinson-like outcomes, likely reflecting targeting of the caudate-putamen, which is highly susceptible to oxidative damage; cognitive problems, likely reflecting hippocampal damage, etc). At least some of this represents oxidative stress, since antioxidants are protective in cell culture models. The brain is especially vulnerable to oxidative stress because of its high metabolic rate, low antioxidant reserves, and unique membrane lipid composition that makes damaging degrees of lipid peroxidation more likely than in other tissues; numerous neurotoxicants act through oxidative stress and it is widely accepted that oxidative stress leads to neurotoxicity, and as noted above, there are regions that are especially sensitive and that happens to correspond to those that would trigger the symptoms reported in the case studies. However, other mechanisms must be operating because the time course for GSH depletion of cultured neurons is not consonant with the much later loss of cell viability. The fact that MeI damages neurons in culture effectively rules out antithyroid actions as the sole mechanism (important for the consideration of *developmental* neurotoxicity, below). In presentations to the SRC, findings from cell culture studies were dismissed specifically because they were *in vitro*; this runs counter to recommendations made in the EPA Inspector General's 2006 report on improving the quality of pesticide safety data under FQPA and other recent endorsements of the use of cell culture models and other modern techniques in risk assessment by both the EPA and independent Panels reviewing pesticide safety.

- Conclusion: There is a strong expectation that MeI is neurotoxic, based on numerous case studies and laboratory findings. The mechanism for this is unclear, and therefore uncertainty factors will need to be applied in considering this endpoint.

Developmental Neurotoxicity (DNT)

- Arysta proposes fetal hypothyroidism as the mechanism that produces developmental neurotoxicity; as a consequence, they argue that the fetotoxicity in rabbits can be ignored because of higher fetal/maternal iodide than humans. The SRC believes that this is clearly wrong: NaI itself does not produce mortality but does elicit the same thyroid abnormalities, both structurally (Inhal Toxicol 21:476, Table 10) and functionally as MeI (Volume I Table 57, shown in Lim & Reed presentation, slide 33 - same exact fetal TSH, T3, T4, T-c depletion, T hypertrophy for MeI and NaI).
- The use of altricial species (i.e. more immature at birth than humans) means that the available data do not take into consideration the equivalent of third trimester exposures in human fetuses, the period of maximal growth; the fact that the original investigators stopped the study in gestation thus ignores what is likely to be a much more sensitive period of vulnerability.
- Recommendation:
    - Absent a convincing mechanistic link, modeling based on serum iodide is inappropriate. Therefore under the Food Quality Protection Act, DPR should

apply an uncertainty factor to the benchmark for the most sensitive developmental endpoint.
    - A factor of at least 10X is needed due to database deficiency due to lack of an adequate study on developmental neurotoxicity (DPR included a 10X factor for this deficiency). Based on the known effects of MeI and the limited data for developmental exposures, DPR should comment on whether or not a 10X UF is sufficient.
- Arysta argues that there is no need for DNT evaluations because their proposed mechanism of fetal toxicity (iodide) effectively rules out the possibility that MeI is a developmental neurotoxicant. This argument is incorrect, even from their own data (see above). It is universally accepted that neurotoxicants target the developing brain more than the adult brain because of the complex processes involved in brain assembly (which lend themselves to temporal disruption of carefully-coordinated events in cell replication, migration and 'wiring'). Also, the developing brain is deficient in antioxidant reserves, both at the level of the neuronal cells themselves, and also because of the relative deficiency of glia, the cells that provide metabolic support that in the adult, protects neurons from oxidative stress. The argument that the effects are all attributable to high iodide in rabbit fetuses can be dismissed based on Arysta's published findings (above).
- Both of Arysta's developmental toxicity studies are deficient in design in terms of these issues: the rabbit studies stop during gestation; the rat studies have a treatment "hole" in late gestation and the early neonatal period that corresponds to one of the most vulnerable periods to neurotoxicants, and further involve daily postnatal maternal separation that adds variability to the test populations, thus potentially obscuring any effects of toxicant exposure (a proper design would have included an additional, unmanipulated control group).
- MeI concentrates in the fetal brain to levels well above those in the mother (see DPR draft, Table 49). Direct neurotoxicant actions are thus likely to occur.
- There is a vast literature on thyroid disruptors and brain development, the main thrust of which is that even nonsymptomatic hypothyroidism during critical periods of brain assembly will lead to adverse neurodevelopmental sequelae. There is no question that MeI causes thyroid disruption in pregnant animals.
- Conclusion: There is a high likelihood that MeI is a developmental neurotoxicant and that there are multiple mechanisms contributing to that endpoint, rather than a single mechanism. Thus, a model based on a single metric such as serum iodide, cannot provide any assurance of human safety. Current legislation (the Food Quality Protection Act) directs EPA and DPR to apply an additional uncertainty factor for compounds for which developmental neurotoxicity is likely, where data are not sufficient to determine that expected exposures are safe. The Panel believes that the data on neurotoxicity available for MeI are far from sufficient to preclude the necessity for the mandated extra uncertainty factor in this case.

Risk Appraisal Comments, including Uncertainty Factors:

- Inadequacy of the database: Based on the fact that MeI induces thyroid perturbations/toxicity, fetal deaths, reduced fetal and pup growth, and neurotoxicity, the

lack of information on long-term effects that may arise after fetal and perinatal exposures is a major deficiency in the MeI database. Long-term effects after early-life exposure that are of particular concern with MeI include neurodevelopmental toxicity and cancer risk. While a 10X uncertainty factor applied to the NOEL or benchmark dose for neurotoxicity and an early life adjustment factor for the cancer potency are certainly justified, it should be recognized that even these adjustments might not be sufficient to account for all the shortcomings of the database.

- The age-dependent adjustment factor should be applied to the MeI cancer potency estimate because this chemical is mutagenic and early life exposures are likely.
- Key points made above consider "development" to occupy a framework of fetal exposure, with the further limit that the animal models do not account for late gestational human exposures. It is abundantly clear that human brain development continues into the neonatal period, childhood, and adolescence. Human bystander exposures, as well as worker exposures are going to involve these developing populations as well. Arysta has provided no data on the effects of MeI for these later developmental stages, and consequently, there is uncertainty about all aspects of MeI toxicity for these vulnerable subpopulations: (1) California allows children as young as 12 years of age to work in the fields, (2) the workers reported that their children were sometimes with them during work, and (3) pregnant women are not excluded from working with fumigants. Clearly these additional exposure periods are highly problematic for endocrine disruption and nervous system development and may produce vulnerability to other endpoints.
- An additional 10-fold safety factor to protect against developmental neurotoxicity is warranted.

Determination of the Mechanisms of Oncogenicity

Genotoxicity

There is strong evidence that MeI is a genotoxic and an alkylating agent. DPR's RCD should place greater emphasis on a genotoxic mechanism. See Appendix E for further discussion on this mechanism:

- The SRC heard from the holder of the MeI pesticide patents that the likely mechanism of pesticidal action is via alkylation.
- Even though MeI was "nonmutagenic" in all but one of the studies that were submitted through the FIFRA process, importantly, it was tumorigenic, mutagenic, clastogenic, or an alkylating agent in 22 of 24 published, peer-reviewed studies (discussed in Appendix E). In one negative study, the authors raised concerns about the inability of a mutagenic test system (the hypoxanthine-guanine phosphoribosyltransferase gene in mouse lymphocytes) to detect a number of well-documented genotoxins. Moreover, this "negative" study did not question whether or not MeI is a genotoxin, given that, in the same paper, MeI was shown to cause mutations at the *thymidine kinase* locus. In the second negative study, the goal was to try to improve a test system (transformation in human C3H 10T1/2 cells) that is notoriously poor at detecting direct-acting alkylating agents. It is also important to note that some of the key FIFRA studies that yielded negative results used non-closed systems in which MeI vapors were not

contained (thus raising serious questions as to whether substantive exposure actually took place).

- Some positive genotoxicity results for MeI (includes studies covered in the DPR RCD): MeI tested positive in Ames strains TA1535 (base-pair), TA1536 (frameshift), TA1537 (frameshift), TA1538 (frameshift), TA98 (frameshift), and TA100 (base-pair) in a closed system with and without S9 activation. MeI was mutagenic in the *E. coli* WP2 uvrA assay and is a chemoselective alkylating agent. MeI also increases the mutation rate in *Saccharomyces cerevisiae* D3, CHO cells at the HGPRT locus (point gene mutation). It also increased mutant colonies at the $TK^{+/-}$ locus and ouabain-resistant locus in mouse lymphoma cell mutation assays (chromosomal aberrations). MeI was positive for morphological transformation in Golden Syrian hamster embryo cells (carcinogen screening assay) and was an alkylating agent of guanine in human lymphoblasts and DNA from lymphocytes and of guanine and adenine in tissue DNA from rats dosed with MeI via gavage or inhalation (Amacher, 1984; Clive, 1979; Gansewendt, 1991; Moore, 1982; Oshiro, 1981; Rosenkranz, 1979; Simmon, 1979; Takahashi, 1987; Xu, 1993).
- MeI alkylated the substrates 4-(p-nitrobenzyl)-pyridine (a synthetic electrophile) and deoxyguanosine at 27% the rate of epichlorohydrin, a potent alkylating agent.
- MeI treatment of *E. coli* induces *alkA* and *umuC*, which indicates that *E. coli* has turned on its SOS response, which is expressed to cope with lethal and mutagenic DNA damage, such as would be caused by methylation following MeI treatment (Takahashi, 1987).
- MeI caused the formation of two hyper-reactive sites at two guanine positions at the promoter and exon 1 of the Fragile-X mental retardation gene in human lymphoblast cells or DNA extracted from lymphocytes from male donors (Cloutier, 2001).

Amacher DE, and Zelljadt I. Mutagenic activity of some clastogenic chemicals at the hypoxanthine guanine phosphoribosyl transferase locus of Chinese hamster ovary cells. *Mutat Res* 136: 137-145, 1984.

Clive D, Johnson KO, Spector JF, Batson AG, and Brown MM. Validation and characterization of the L5178Y/TK+/- mouse lymphoma mutagen assay system. *Mutat Res* 59: 61-108, 1979.

Cloutier JF, Castonguay A, O'Connor TR, and Drouin R. Alkylating agent and chromatin structure determine sequence context-dependent formation of alkylpurines. *J Mol Biol* 306: 169-188, 2001.

Gansewendt B, Xu D, Foest U, Hallier E, Bolt HM, and Peter H. DNA binding of methyl iodide in male and female F344 rats. *Carcinogenesis* 12: 463-467, 1991.

Moore MM, and Clive D. The quantitation of TK-/- and HGPRT- mutants of L5178Y/TK+/- mouse lymphoma cells at varying times post-treatment. *Environ Mutagen* 4: 499-519, 1982.

Oshiro Y, Balwierz PS, and Molinary SV. Morphological transformation of C3H/10T1/2 CL8 cells by alkylating agents. *Toxicol Lett* 9: 301-306, 1981.

Rosenkranz HS, and Poirier LA. Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and noncarcinogens in microbial systems. *J Natl Cancer Inst* 62: 873-892, 1979.

Simmon VF, Rosenkranz HS, Zeiger E, and Poirier LA. Mutagenic activity of chemical carcinogens and related compounds in the intraperitoneal host-mediated assay. *J Natl Cancer Inst* 62: 911-918, 1979.

Takahashi K, and Kawazoe Y. Potent induction of the adaptive response by a weak mutagen, methyl iodide, in Escherichia coli. *Mutat Res* 180: 163-169, 1987.

Xu DG, He HZ, Zhang GG, Gansewendt B, Peter H, and Bolt HM. [DNA methylation of monohalogenated methanes of F344 rats]. *J Tongji Med Univ* 13: 100-104, 1993.

Tumorigenicity

- In the published literature, MeI has been shown to induce tumors in experimental animals. IP injection of MeI induces lung tumors in A/He mice (Poirier et al., 1975). This same study also showed that a variety of other simple alkyl iodides were also tumorigenic (e.g., Me, nPr, iPr, nBu, and sBu were all positive, although Et and tBu were negative). SC injection of MeI induces local sarcomas (fibro-/spindle-cell/round-cell) in BD rats (Druckrey et al., 1970). Though the details of the latter study are lacking (and it is in German), there is no reason to doubt the fundamental conclusion that the tumors were real and MeI-induced, and so this study adds to the weight of the evidence that MeI is tumorigenic.
- In the Arysta study (Harriman 2005), MeI produced a variety of tumors. MeI inhalation gave thyroid follicular cell tumors in male rats (Table 22, p59) and astrocytomas in male rats (p57). [Thyroid hyperplasia was also observed in male and female rats.] Oral MeI gave thyroid follicular cell tumors in male mice (Table 26, p65). Male mice got cervical adenomas/carcinomas ($P<0.05$ at 84ppm, p13 USEPA Cancer Report). Benign tumors were also reported (see below).

Thyroid Tumors—mechanisms of action

- Based on an analysis of both the extensive thyroid tumorigenesis literature and the MeI data, the most likely MOA for MeI-induced thyroid tumors is genotoxic MeI-initiation accompanied by MeI-enhanced TSH-promotion. There can be no threshold for TSH-promotion, because--even if MeI levels are lowered such that TSH levels drop to background--endogenous normal TSH levels have been shown to promote genotoxin-initiated thyroid tumorigenesis. The weight of the evidence is strong that MeI is a genotoxic mutagen/carcinogen, and arguments to the contrary are flawed and not convincing. Arguments favoring a pure "thyroid hormone perturbation" MOA (more properly formulated as: [spontaneous mutagenesis-initiation/TSH-promotion]) are not compelling; e.g. the notion that male rats get more thyroid tumors because they have higher TSH levels does not stand up to careful scrutiny, and, furthermore, a pure genotoxic initiation mechanism involving another thyroid mutagen/carcinogen has been shown to give more thyroid tumors in males than females.
- With two competing MOAs, public health risk assessment should be based on the more worrisome mechanism (in this case: genotoxic MeI-initiation), unless the arguments favoring it are weak, while arguments favoring the less worrisome mechanism (in this case: thyroid hormone imbalance) are strong and compelling. This is not the case, as genotoxic MeI-initiation for thyroid tumors is supported by the weight of the evidence. Thus, public health concerns require the assumption of the genotoxic MOA for risk assessment, and a linear

dose-response curve must be applied. If a correction for MeI-enhanced TSH-promotion is to be included for extrapolation to lower MeI doses, then the linearly extrapolated risk might be raised ~3-10-fold, though this value cannot be estimated with any degree of confidence.

- Support for a genotoxic mechanism include: MeI is mutagenic in several test systems, induces cancer at other tissue sites (eg. astrocytomas and cervical/uterine fibromas), and radiolabeled methyl groups target the thyroid, direct genetic damage by the methyl moiety could induce oncogenic changes in thyroid cells.
- The preferential formation of thyroid tumors in rats could be the consequence of higher adduct levels in this tissues, consistent with the thyroid showing the highest concentrations of ^{14}C-MeI after inhalation exposure (Volume I, Table 1 and 2, p18 and 19).
- One of the key arguments offered in favor of the thyroid hormone imbalance MOA was that male rats get more thyroid tumors from MeI, because they show greater perturbation in their thyroid hormone levels following MeI exposure. The SRC does not consider this mechanism likely. (Please see the reasoning in Appendix E)
- The MeI inhalation concentration that gave significant thyroid tumors was 60 ppm in rats (DPR Volume I, Table 22, p59). The time course of iodide level following inhalation of 25 ppm and 100 ppm MeI by rats is given in the USEPA Cancer Report (Table 9a, p25). Iodide levels fluctuate dramatically (in some cases >40-fold), because MeI dosing is for only 6 of 24 hours and iodide is rapidly excreted. (Rats excrete ~95% of a daily iodide-sufficient intake, which is normally ~20 ug/day). Interpolation to 60 ppm MeI (from 25 ppm and 100 ppm data) gives an estimated peak iodide level of ~70ug/mL, and an average iodide level of ~28 ug/mL. In Takegawa, et al. (2000), rats given 1000 ppm KI in drinking water had a daily iodide intake of ~60 mg/kg. While this value is not equivalent to a measurement of internal iodide level, by making a few assumptions, a value of ~60 ug/mL for internal iodide is probably reasonable, and this level of iodide is in the range of iodide levels following inhalation of 60 ppm MeI.
- The DPR RCD should give more consideration to DNA adducts and the potential role of protein or DNA alkylation in the toxicity and carcinogenicity of MeI. For example, to what extent does residual ^{14}C in tissues at 168 hrs after exposure to ^{14}C-MeI represent macromolecular (protein and DNA) binding? Because specific methylated adducts were identified immediately after 6 hr inhalation exposure to MeI, the comment that DNA adducts may be due to de novo synthesis should be deleted or modified to reflect the experimental findings.
- In the study described in Takegawa, et al. (2000), rats given 1000 ppm KI in drinking water did not develop thyroid tumors, which argues against iodide from MeI hydrolysis being causative in thyroid tumors, if the iodide levels were comparable in the Takegawa, et al. (2000) study vs. the MeI study.
- Takegawa et al (2000) showed that iodide enhances nitrosamine-induced thyroid tumorigenesis, which suggests that iodide can be active as a tumor promoter following administration of a thyroid genotoxin [DHPN, N-bis(2-hrdroxypropyl)nitrosamine]. Iodide promotion has been noted in other studies as well. By analogy, in the MeI case, rat thyroid tumors might require MeI as the genotoxic initiator with breakdown-iodide acting as a tumor promoter. There are several arguments against this MOA.

- DHPN alone induced an equal number of adenomas vs. carcinomas, but iodide-promotion preferentially enhanced carcinomas (~3.5-fold). If iodide-promotion were an important factor in the MeI MOA, then a similar preference for carcinomas might be expected, when in fact it is just the opposite: more adenomas are observed in the MeI study (~2.5-fold).

-The authors point out that rats excrete thyroid hormones rapidly in comparison to humans, because of species differences in thyroid hormone binding proteins, from which they suggest, "Rat thyroids are accordingly prominently sensitive to the promoting effects (of iodide) compared to humans, and such effects are difficult to induce in humans."

-Finally, the authors do not note that iodide promotion is greater in male vs. female rats.

Astrocytomas

- Questions were raised about whether the astrocytomas observed in male rats are due to MeI treatment or were spontaneous. Arguments suggesting that astrocytomas in rats are MeI-induced are more compelling than arguments suggesting they are spontaneous. Not all genotoxic alkylating agents induce brain tumors and this is usually thought to be determined by how well the genotoxin can pass the blood-brain barrier. MNU, however, does cross the blood-brain barrier and is a well-established brain carcinogen in rats. MNU is a simple methylating agent that induces the same spectrum of DNA adducts as MeI, and MNU causes mutations via m6G, which is an adduct also formed by MeI and is likely to be the MeI pre-mutagenic lesion. Furthermore, MeI passes the blood-brain barrier (DPR Volume I, Tables 1 and 2, p18 and 19), so it would be expected to be mutagenic in brain by analogy to MNU. The probability is low that the four astrocytomas observed in male rats happened by chance to be in the MeI treated rats.
- Arysta (in its response to the initial DPR assessment) pointed to historical controls that manifested astrocytomas at a background level (PowerPoint slide #40), arguing that artrocytomas were thus more likely to be spontaneous than exposure-related. Their interpretation of these data, however, is misleading for the following reason: Charles River Laboratories reports in the document "Compilation of Spontaneous Neoplastic Lesions and Survival in Crl:CD (SD) BR Rats From Control Groups" that although astrocytomas were observed in 8 studies (ranging from 0.87% to 3.33%, as noted by Arysta, in another 15 studies (the majority of those reported) no background astrocytomas were observed whatsoever.
- It has been noted that the prevalence data for astrocytomas is uncertain because ~50% of the animals in the 5ppm and 20ppm groups were not analyzed for tumors at terminal sacrifice. On balance, the arguments against the astrocytomas being MeI-induced are not compelling, and it is prudent to consider them to be MeI-

induced. This adds to the weight of the evidence that Me-I is a genotoxic tumorigen and must be evaluated for risk accordingly.

Berleur MP, Cordier S. The role of chemical, physical, or viral exposures and health factors in neurocarcinogenesis: implications for epidemiologic studies of brain tumors. Cancer Causes Control. 1995 6:240-56.

Benign and Other Tumors

Female mice exposed to MeI developed cervix and uterine fibromas (P<0.0.1, Table 7, p14, USEPA Cancer Report), which were characterized as "incidental" by the USEPA. Although these fibromas were not malignant, they indicate a potentially significant problem for women, in that they can cause severe pain, heavy bleeding, incontinence and infertility. The only effective treatment is surgery and cervical cancers are difficult to remove without damaging surrounding tissue. Thus, the health effects of such tumors cannot be dismissed.

Additional Cancer Risk Comments

- Because of potential early life exposure to this mutagenic compound, the SRC recommends that age-dependent adjustment factors be applied to the MeI cancer potency estimate. The recent guidelines developed by OEHHA, DPR's sister agency, should be used. The Panel is concerned about child bystanders, and teenagers who, in addition to receiving exposure as bystanders or nearby residents, may be exposed during work in the fields.
- The now-standard interspecies projection for carcinogenesis is based on differences in pharmacokinetics, which tend to go with metabolic rates. So if the dose is stated in mg/kg-day, a factor is applied that is defined by (Human body weight/animal body weight)^1/4 to make up for the fact that humans typically metabolize chemicals more slowly than the animals. The explanation for why there is no factor for human inter-individual variability applied to cancer potency estimates is somewhat obscure. Basically it goes to the tradition that the toxicologists in charge of EPA assessment policies considered the linear projection to be "conservative" enough. The SRC disagrees, as did a recent NRC committee.
- The mouse chronic study was not adequate to evaluate the full carcinogenic potential of MeI in mice. Most important, the study duration was only 18 months. A carcinogenic study of short duration has markedly reduced sensitivity, especially for late appearing tumors (Haseman et al., 2001). In the MeI study, thyroid follicular cell hyperplasias were observed at increased incidences in male and female mice, while thyroid follicular cell neoplasms were increased only in the highest dose group of male mice. If the mouse study had been conducted for 2 years duration, which is common for most cancer bioassays, it is possible that several of the thyroid lesions would have progressed to neoplasms. Also, the elevated liver tumor response in male mice might have reached statistical significance in a 2-year study. Interestingly, the historical database for studies conducted at the contract laboratory were of 2 years duration.

- Another concern with the mouse study is that the composition of the microcapsules that were used to stabilize MeI in the diet was never specified. And, although the diets were changed out every 2 days, no analyses were provided on the concentration of MeI in test diets from cage samples. If microcapsules blended in the diets deteriorate while in the cage (e.g., due to animals nesting in the feed), MeI would be released and we would not be able to adequately determine the true dose in mice.

National Research Council Committee on Improving Risk Analysis Approaches Used by the U.S. EPA, 2008. Science and Decisions: Improving Risk Analysis National Academy of Sciences Press, Washington, DC, 2008.

Haseman, J., Melnick R., Tomatis, L., and Huff, J. Carcinogenesis bioassays: study duration and biological relevance. Food Chem. Toxicol 39: 739-744, 2001.

Hattis, D. and Barlow, K. "Human Interindividual Variability In Cancer Risks--Technical And Management Challenges" Human and Ecological Risk Assessment, Vol. 2, pp. 194-220, 1996.

APPENDIX A:

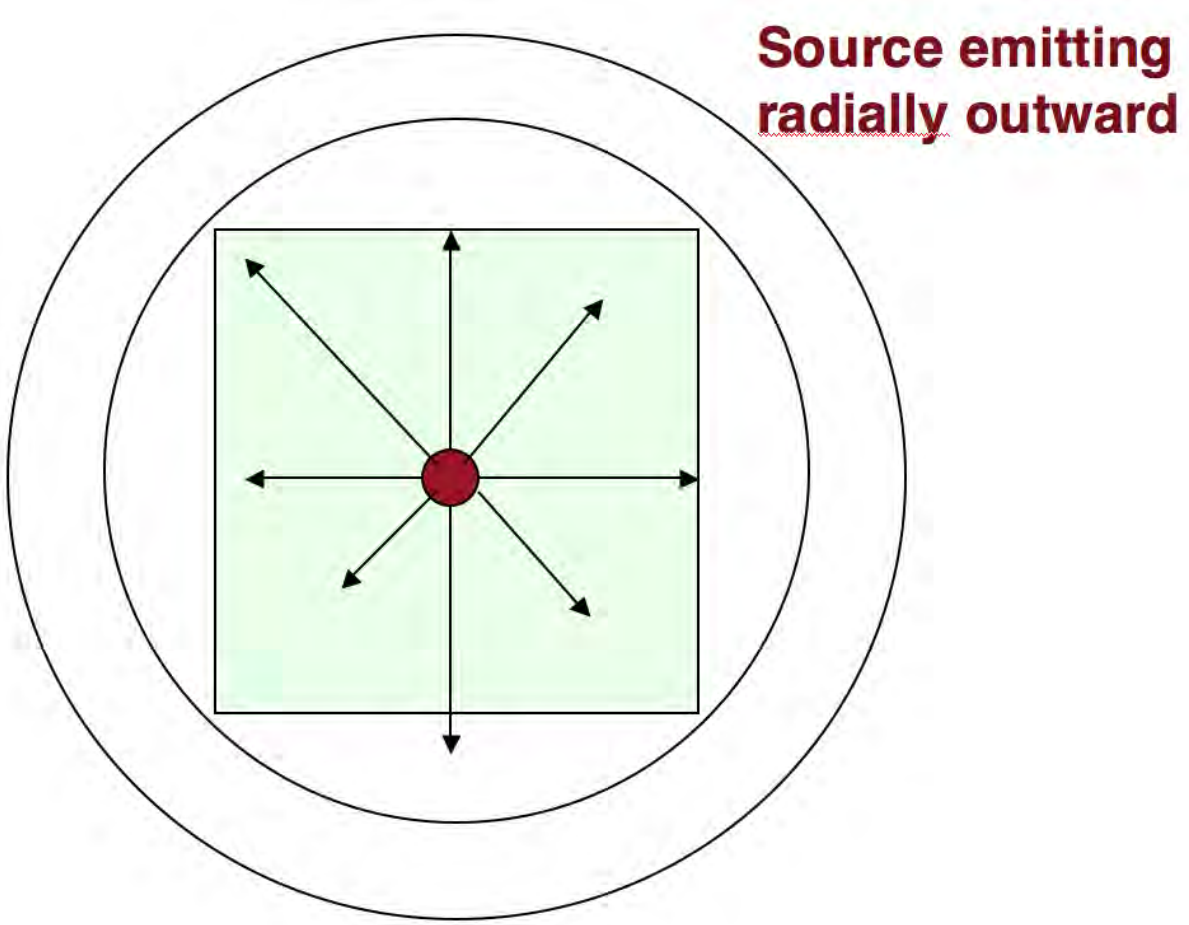
Some ground-truth calculations on air emissions and exposure for MeI
By Dr. Tom McKone

In order to make sense of the bystander exposure calculation, I set up my own method for calculating concentration at distances between 10 and 200 m from a field.

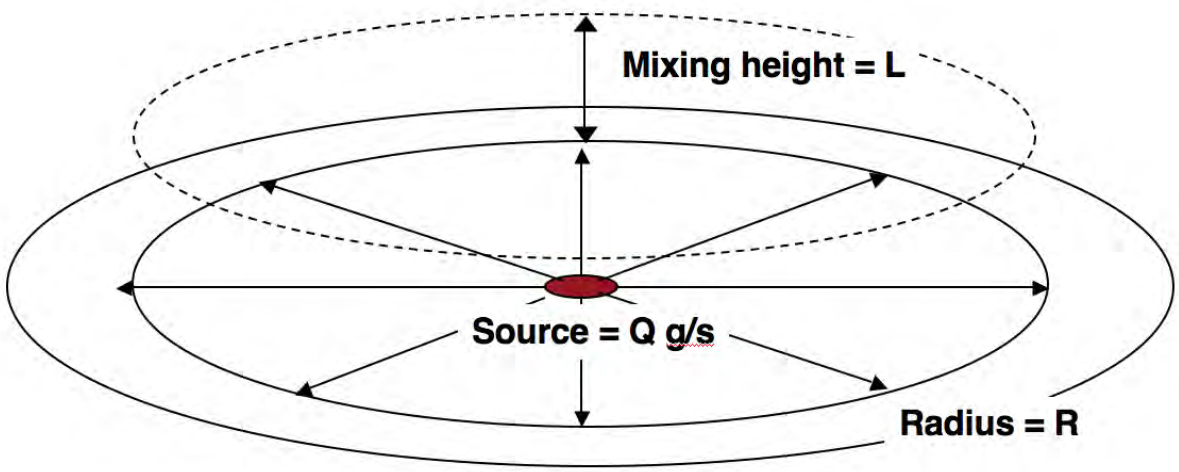
Some of the questions I wanted to address with this approach are:

1. What happens when the field is larger than the assumed 40 acre?
2. What happens when multiple fields are impacting the same bystander?
3. Is the approach appropriate for different seasons?
4. Is the approach for making the inverse calculation to determine emission flux appropriate does it provide plausible numbers?

I developed a "ground-truthing" model that works as follows. The field is placed within a circle of radius R where R is the distance from the center of the field plus the distance between the bystander and the edge of the filed.



All mass emitted from the field passes through a 45° segment of a circle that is at a distance R from the field center. At this distance R the air concentration is diluted by a wind velocity between 1 m/s (assumed short term [24] minimum) or 3 m/s (assumed long term [2-week] minimum) and is confined within a mixing layer of between 10 m (short term minimum) to 100 m (long term minimum). The time of interest is 8 h, 24 h or two weeks.



$$Gains = Losses$$

$$Q = \mathrm{C(R)} \times v_w \times 2\pi R \times L \qquad \mathrm{C(R)} = \frac{Q}{2\pi R v_w L}$$

I considered the case of a Shank, Raised-Bed/Tarp application of 162 pounds/treated-acre (assumed to be 50 pound/total-acre), which in the DPR report results in the highest off-site concentration. With this application rate, and an assumed loss of 30% applied during 24 hours (based on the field and laboratory studies), I estimate a flux of 93 ug/m2/s compared to DPR's estimate of 87.6. Moreover my simple model gives a 24-h average concentration at the field boundary of 4.7 ug/L compared to DPR's estimate of 4.2 ug/L based on the more detailed ISCLT model. It should be noted that my simple model is based on conditions that give rise to plausible upper bounds for off-site concentrations. So it appears that their model is very unlikely to underestimate the 24 hour exposure. I used my simple model to explore how increasing field size changes the result. If the treated field is increased to 80 acres instead of 40 (thus increasing the amount of mass emitted but also increasing the dilution volume), the concentration at 3 m away from the field is 6.7 ug/l instead of 4.7.

Two week long-term average concentration assuming 90% of the applied MeI volatilizes within two weeks is 0.03 ug/l at 10 m from the edge of the field. This gives me confidence that the 0.07 ug/l number in the DPR report is reasonable.

APPENDIX B

Suggested Uncertainty Factor	Value
Utility of PBPK model	**Raise from 3 (DPR) to 10**
Interspecies	**Raise from 3 (DPR) to 10**
FQPA for most sensitive developmental endpoint (neurotoxicity)	**To be determined**
Deficiency in neurodevelopmental studies	**An extra 10x factor on top of DPR's 10x**
Neurotoxicity and lack of MOA	**To be determined**

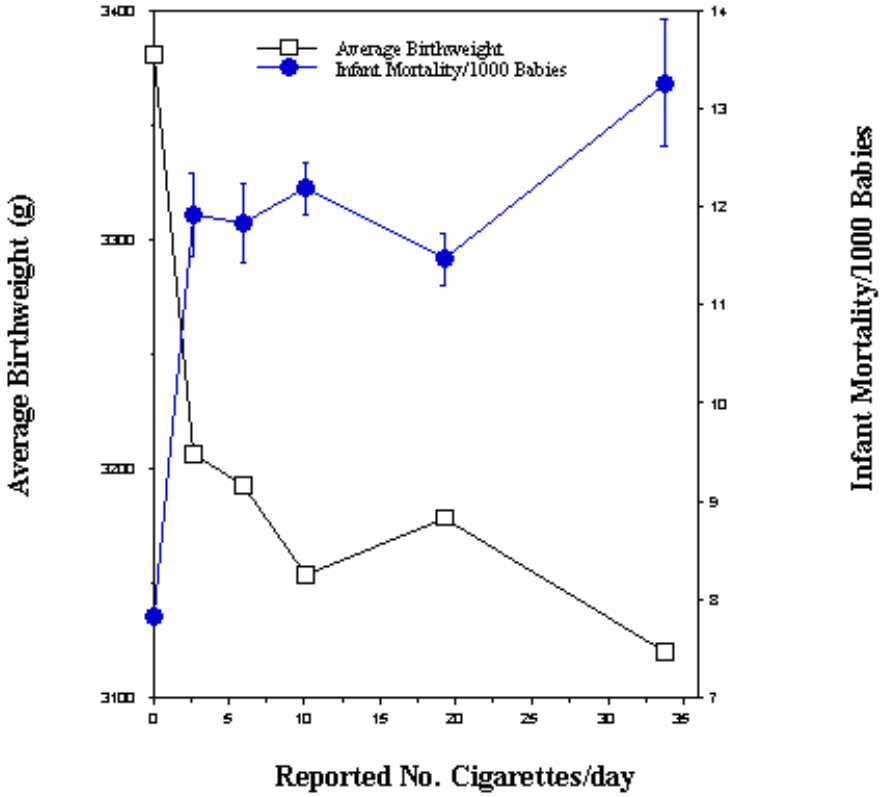
APPENDIX C:

Dr. Dale Hattis

A recent paper summarizes some past and current work on the implications of various amounts of fetal growth restriction in the human context (see in particular Section 4, pp. 50-55). As it happens, a relatively linear dose-response is not uncommon for this response in animal studies; and recent studies in the air pollution context and with cigarette smoking indicate that it is a quite sensitive parameter in humans with potentially serious implications for infant mortality and a variety of developmental endpoints. The best guidance on an appropriate benchmark response level for fetal growth restriction is to look to the analogous degree of fetal growth restriction and infant mortality associated with cigarette smoking, and the fetal growth restriction seen with criteria air pollutants such as particulates. Some calclulations of this approach follow. (Please refer to Hattis, D. and Keaney Lynch, M. "Alternatives to Pollutant-by-Pollutant Dose-Reponse Estimations for Air Toxics" EP-W-05-022 WA 3-80 Task 2 White Paper, 2009.)

> Begin with Figure 1 for cigarette smoking. A pack per day of personal smoking makes a difference of about 200 g in average birth weight. With a standard deviation of about 500 g, this represents about 0.4 standard deviations. You can see from the figure that this is associated with about a 4/1000 increase in infant mortality, from 8/1000 to the neighborhood of 12/1000. These calculations were based on older data (probably from the 1980s) when infant mortality rates were larger than they are now. However if there is an appreciable possibility that these responses are connected, I think it is unreasonable to consider as much as a 1 standard deviation change in fetal weight to be the practical equivalent of a NOAEL for regulatory purposes. I think public policy must aim to keep the magnitude of effect much lower than that. On this scale, a 1% change in birth weight--about 35 g in people, or 0.07 standard deviations, is I think the largest amount that should potentially be considered as representing a LOAEL, and even that I can only recommend with some reservations. A 35 g difference in people is a magnitude of change that can be detected epidemiologically. See for example the estimates of the magnitude of effect seen for several of the criteria air pollutants (Tables 1 and 2 showing work of Bell et al (2007). As you can see the population average birth weight effect of PM2.5 is estimated to be about 80 g, and the effects associated with NO_2 and CO come out at about 32-35 g.

Figure 1: Relationships Between Reported Cigarettes Smoked per Day, Average Birth Weight, and Infant Mortality



Data Source: National Center for Health Statistics. 1996. 1990 Birth Cohort Linked Birth/Infant Death Data Set, NCHS CD-ROM Series 20 No. 6, SETS Version 1.22a, issued May 1996.

Figure previously appeared in Hattis, D. "Role Of Dosimetric Scaling And Species Extrapolation In Evaluating Risks Across Life Stages. IV. Pharmacodynamic Dosimetric Considerations." Draft Report to the U.S. Environmental Protection Agency Under RFQ No. DC-03-0000, January 2004.

Table 1: Basic Birth Weight Reduction Results Based on County-Average Air Pollutant Exposures During Gestation for 358,504 Babies in Massachusetts and Connecticut, Evaluated with Single-Pollutant Models, Controlling for Confounders

Air Pollutant	Grams Reduction Birth Weight per Interquartile Exposure Range	Lower 95% Confidence Limit (g)	Upper 95% Confidence Limit (g)	Mean and Std Dev Exposure	Interquartile Exposure Range	Exposure units
NO_2	8.9	7	10.8	17.4 ± 5.0	4.8	ppb
CO	16.2	12.6	19.7	656 ± 180	303	ppb
SO_2	0.9	-2.6	4.4	4.7 ± 1.2	1.6	ppb
PM_{10}	8.2	5.3	11.1	22.3 ± 5.3	7.4	µg/m3
$PM_{2.5}$	14.7	12.3	17.1	11.9± 1.6	2.2	µg/m3

Source: Bell et al. (2007).

Table 2: Implications for Population Aggregate Birth Weight Changes of the Bell et al. (2007) Results for Pollutant Potencies (gram Reduction in Mean Baby Weights Per Unit Exposure During Gestation) and Suggested Population Aggregate Impacts on Birth Weights

Air Pollutant	Indicated Potency in g Birth Weight Reduction per ppb Gas or (for Particles) µg/m3	Lower 95% Confidence Limit on Potency	Upper 95% Confidence Limit on Potency	Suggested Population Aggregate Effect (g/baby) (Potency x Mean Exposure)
NO_2	1.85	1.46	2.25	32
CO	0.053	0.042	0.065	35
SO_2	Non-significant			
PM_{10}	1.1	0.7	1.5	25
$PM_{2.5}$	6.7	5.6	7.8	80

APPENDIX D:

Dr. Ted Slotkin

Examination of WIL laboratories Neurotoxicity Studies

Neuropathology was done 15 days after exposure and was qualitative only, with 6 animals per sex per group. The findings for CNS are designated "unremarkable." There is no description of whether the observer was blinded to the treatment condition, the number of sections examined, the thickness and location of the sections, or the criteria used. It is evident from the description of the technique as "qualitative" that they were looking only for the grossest kinds of damage and no quantitative or semi-quantitative approaches were taken. There was no evaluation of layer thicknesses in key regions, comparisons of neurons vs. glia to look for glial proliferation typical of damage, or any of the hallmarks generally accepted in standard examinations of neuropathology that would be expected in the scientific literature or in, for example, EPA's standard developmental neurotoxicity screen. Using their criteria, the organophosphate pesticides, lead and even mercury could easily have been found to be negative for neurotoxicity.

In the Appendix, the investigators state that they validate their ability to identify neuropathology by citing an earlier study they performed with trimethyltin. As shown in a 1986 paper they cite for their dosing and technique, this neurotoxicant causes clear-cut damage in specific areas of the brain. Using the protocol from the 1986 paper, they observed a number of neuropathological changes, which they then cite as evidence that they are sufficiently skilled in neuropathology to perform and interpret the studies on MeI. However, examination of their actual outcomes shows that they detected neuropathology in only 1 out of 5 animals exposed to trimethyltin in one of their studies, and 2 out 5 in another; this is not at all in line with the consistent neuropathology caused by trimethyltin exposure, and demonstrates that their laboratory has a high rate of false negatives. Further, they apparently did not find neuropathology in some of the major sites that trimethyltin targets. They provide no evidence that they are capable of conducting the semi-quantitative or quantitative approaches or techniques that have become essential to neurotoxicologic examinations since 1986.

Taken together with their outdated, qualitative approach, this demonstrates a lack of scientific rigor that obviates their conclusion that MeI does not produce neurotoxicity and raises the question of whether studies conducted with the techniques now widely recognized in the field would have come to the same conclusion.

APPENDIX E

Dr. Ed Loechler

1. A CONTRADICTION: THE THYROID RESPONDS TO MeI AS IF IT IS EXPERIENCING LOW IODIDE

In terms of oncogenic MOA, it matters whether MeI treatment leads to a thyroid response that is more like low iodide (I-lo) or high iodide (I-hi), because I-lo alone is oncogenic in rats, while I-hi alone is not oncogenic. Both I-lo and I-hi promote genotoxic-initiated thyroid tumorigenesis.

Rats exposed to I-hi levels initially show a decrease in T3/T4, which results in an increase in TSH in rodents (and humans) via the "Wolff-Chaikoff effect" (reviewed in Ward 1986, and Kanno 1996). Morphological changes in the thyroid are not observed in this initial acute phase. During chronic I-hi administration, rats restore the euthyroid state (after ~50 hours) with normalization of serum TSH levels, which is called "escape from the Wolff-Chaikoff effect" and occurs via down-regulation of the iodide transporter (NIS: sodium-iodide symporter, Spritzweg 1999, Eng 1999, Eng 2001). The thyroid enlarges somewhat from chronic high iodide treatment, most notably through the formation of colloidal-rich follicles, which fuse.

Chronic I-lo in rats causes a persistent decrease in T3/T4, which stimulates TSH release from the pituitary, which in turn triggers goiter (hyperplasia) and the thyroid can enlarge dramically (>10-fold) presumably trying to compensate. (There are other effects)

Chronic MeI treatment causes rat thyroids to enlarge (males only, Table 21, p58), experience hyperplasia (males and females, Table 22, p59), and to undergo vacuolation (males only, Table 22, p59), while TSH levels are elevated (males and females, Table 21, p58 and Inh. Tox. (2009) 21: p484, Table 2). These observations are more consistent with what is observed in rats experiencing I-lo, with the possible exception of vacuolation, which might be the equivalent of the formation of colloidal-rich follicles.

MeI treatment leads to elevated iodide via hydrolysis in rats (USEPA Cancer Report, Table 9a, p25, and Inh. Tox. (2009) 21: p485, Figure 2), so how could the thyroid respond as if it is experiencing I-lo? Three mechanisms seem plausible.

<u>Mechanism 1</u>: The MeI treatment regimen (6hr/day and 5d/week) results in wild fluctuations in iodide ion levels of as much as 40-fold over 24hr (USEPA Cancer Report, Table 9a, p25, and Inh. Tox. (2009) 21: p485, Fig 2). I-hi might trigger down-regulation of the iodide transporter, after which--when MeI treatment is stopped and iodide ion concentration returns to normal--the thyroid might not transport sufficient iodide because

its iodide ion transporter is down-regulated. The next effect would be a low iodide thyroid response to the on-again/off-again MeI treatment regimen.

Mechanism 2: DHPN-initiated thyroid tumorigenesis involves a single or small number of DHPN treatments, which causes a transient elevation of TSH (Hiasa 1991). Thyroid tumorigens might generally elevate TSH, and, since MeI is a probable thyroid tumorigen and MeI is administered throughout the treatment period, then MeI might be causing the TSH increase independently of iodide via an unknown mechanism.

Mechanism 3: It is possible that MeI binds to the iodide symporter and, while bound, alkylates some key amino acid residue, thus inactivating the symporter protein. This would lead to lower symporter activity and the thyroid would react as if iodide levels were low, even though iodide is actually elevated. The fact that MeI levels are higher in the thyroid than other tissues suggests active transport via the iodide symporter, though there is no observational support for this hypothesized mechanism.

2. I-lo ALONE CAUSES THYROID TUMORIGENESIS, WHILE I-hi ALONE DOES NOT.

A higher incidence of thyroid tumors have been reported in various regions of the world with I-lo (reviewed in: Dal Maso 2009, Feldt-Rasmussen 2001, Shi 1991), and there are various reports of thyroid tumorigenesis being associated with goiter. These reports usually acknowledge that goitrogens (e.g., as found in cabbage) might be causative rather than I-lo, which prompted experimental studies in model systems to investigate mechanism. Most studies have been in rats.

Many studies have shown that I-lo increases the frequency of thyroid tumors in rats (Axelrod 1955, Isler 1958, Isler 1959, Kimura 1976, Tsuda 1978, Oshima 1986), with adenomas appearing after ~12mos and carcinomas after ~18mos (though the timing is complex). Several lines of evidence suggest that the mechanism involves I-lo causing low T3/T4, which stimulates the pituitary to release TSH, which in turn stimulates thyroid hyperplasia. For example, hypophysectomy eliminates I-lo enhanced thyroid tumorigenesis (Nadler 1970), while implantation of a TSH-releasing tumor (with normal iodide) enhances thyroid tumorigenesis (Haran-Ghera 1960). More evidence for this mechanism is discussed in the next section.

I-lo enhanced thyroid tumorigenesis is thought to be due to “promotion,” which must arise following initiation via spontaneous mutagenesis. Though the term “co-carcinogen” is more proper to describe the effects of TSH, I will use the term “promoter” as adopted in the literature.

A number of studies have shown that I-hi alone does not enhance thyroid tumorigenesis, though it does promote mutagen initiated thyroid tumorigenesis (Kanno 1992, Zhu 1995, Takegawa 2000).

3. GENOTOXIC MUTAGEN/CARCINOGEN INDUCED THYROID TUMORIGENESIS AND TSH PROMOTION.

N-bis-(2-hydroxypropyl)nitrosamine (DHPN) is the most extensively studied genotoxic thyroid tumorigen, and a variety of findings suggest that it requires TSH promotion. For example, DHPN-initiated thyroid tumorigenesis is enhanced by I-lo (Kanno 1992, Zhu 1995, Takegawa 2000), which is thought to result from TSH elevation because the tumors are not observed following hypophysectomy (Kanno 1996). In addition, goitrogens, which elevate TSH levels (e.g.,. phenobarbital, brabital, 3-amino-1,2,4-triazole, 4,4'-diaminophenylmethane and propylthiouracil) promote DHPN-initiated thyroid tumorigenesis (Hiasa 1982a, Hiasa 1982b, Hiasa, 1983, Hiasa 1984, Kitahori 1984), and in several instances, TSH involvement is implicated by the decline or disappearance of tumors following hypophysectomy or co-treatment with T3/T4 (Nadler 1970, Doniach 1974, Janec 1980).

DHPN treatment alone induces thyroid tumors (Hiasa 1991, Kanno 1992, Zhu 1995, Takegawa 2000), which is not dependent on elevated TSH (Hiasa 1991). Tumorigenesis is lost following hypophysectomy (Kanno 1996).

The findings in the previous paragraph are particularly important, because they show that even a normal level of TSH is sufficient to promote genotoxin-initiated thyroid tumors. Thus, there can never be a threshold for TSH promotion, because even if MeI levels were lowered to a point where putative MeI-enhanced TSH promotion is lost, there will still be TSH promotion via normal, endogenous levels of TSH.

A single dose of MNU alone also induces thyroid tumors (Oshima 1984). Though TSH levels were not measured in this study, MNU treatment alone did not affect thyroid size compared to control, which is predictive of normal TSH levels. This finding is reminiscent of the finding with DHPN and suggests that MNU alone can initiate thyroid tumorigenesis, while relying on normal TSH levels for promotion.

Two additional studies also showed that a single dose of MNU alone causes thyroid tumors (Kaufman 1981, Tsuda 1983), while other studies showed that MNU initiated turmorigenesis can be promoted by the goitrogens propylthiouracil and phenobarbital (Tsuda 1983, Milmore 1982)).

These findings are particularly germane, because MNU is a methylating agent and forms the same kinds of adducts in DNA as does MeI.

I^{131} and X-rays also initiate thyroid tumors when followed by a promotion regimen that leads to elevate TSH, such as I-lo (Nadler 1969, Nadler 1970). The I^{131} study was particularly revealing, as I^{131}-initiation/I-lo-promotion gave significantly more tumors (5.5/animal) compared to I-lo treatment alone (0.75/animal), and both inductions were eliminated by hypophysectomy.

As noted throughout this report, elevated TSH causes thyroid hyperplasia, and TSH has been shown to stimulate growth of thyroid follicular cells in culture for humans (Williams 1987, Williams 1988, Roger 1988), dog (Roger, 1984) and rats (Smith 1984), and DNA synthesis is induced (Roger 1988).

4. MeI IS A GENOTOXIN

[Only references in this section that are not in the DPR report are included in the reference list at the end of this document.]

By many tests and criteria, MeI is (and must be) a genotoxin as outlined below. Furthermore, MeI's genotoxic mechanism is simple and straightforward, which removes concerns about (e.g.) differences in metabolic activation that complicate extrapolations involving other carcinogens that must be metabolically activated to a proximal carcinogen.

MeI is a direct-acting methylating electrophile, which reacts with nucleophiles by a simple SN2 displacement. This reaction is analogous to the reaction of a variety of other direct-acting methylating electrophiles that have been established as mutagens and carcinogens, notably methyl methane sulfonate (MMS) and N-methyl-N-nitrosourea (MNU), both of which IARC ranks in Group 2A (probably carcinogenic to humans). [There are a variety of more sophisticated chemical analogies between MeI, MMS and MNU, such as similar Swain-Scott "s" factors.] MeI reacts with DNA to give DNA adducts, which were characterized as m3A, m7G and m6G in one study (Gansewendt 1991) and had properties of m7G in another (Cloutier 2001). Importantly, m6G is a well-established premutagenic lesion, which is responsible for the mutations induced by the methylating class of carcinogens, such as MMS and MNU. Thus, chemically, MeI behaves analogously to other methylating agents that are well-established mutagens and carcinogens.

A variety of indirect cellular assays provide evidence that MeI reacts with DNA, including the induction of the SOS response in *E.coli*, (Takehashi 1987) the induction of homologous recombination in yeast (Simmon, 1979b), the induction of DNA repair synthesis in human lymphocyte (Andrae 1980), and the induction of chromosome aberrations in CHO cells (Gudi 2001). Each of these tests involves a cellular endpoint that is indicating a genotoxin has reacted with DNA, such that the cell must cope with potentially lethal and mutagenic lesions.

Various studies have shown that MeI can be mutagenic in bacteria [principally using the Ames test in Salmonella (McCann, 1975, Rosenkranz 1979, Simmon, 1977, Simmon 1979a) and the WP2 test in *E. coli* (Hemminki 1980, Takahashi 1987)]. Various studies have shown that MeI can be mutagenic in a variety of eukaryotic cells in culture, including in TK in mouse lymphocytes (Clive 1979, Moore 1982, Moore 1985a/b), in HGPRT in CHO cells (Amacher, 1984) and via acquired Ouabain resistance in mouse lymphocytes (Amacher 1985).

MeI can transform eukaryotic cells in culture, in particular Syrian Hamster Embryo cells (Peinta 1977).

In the published literature, MeI has been shown to induce tumors in experimental animals. IP injection of MeI induces lung tumors in A/He mice (Poirier 1975). This same study also showed that a variety of other simple alkyl iodides were also tumorigenic (e.g., methyl, n-propyl, iso-propyl, n-butyl, and sec-butyl were all positive, although ethyl and t-butyl were negative). SC injection of MeI induces local sarcomas (fibro-/spindle-cell/round-cell) in BD rats (Druckrey 1970). Though the details of the latter study are lacking (and it is in German), there is no reason to doubt the fundamental conclusion that the tumors were real and MeI-induced, and so this study adds to the weight of the evidence that MeI is tumorigenic.

In the Arysta study (Harriman 2005, Mileson, et al., Inhalation Toxicology 21: 583-605 (2009)) MeI gave a variety of tumors. MeI inhalation gave thyroid follicular cell tumors in male rats (DPR Report, Table 22, p59) and astrocytomas in male rats (DPR Report, p57). [Thyroid hyperplasia was also observed in male and female rats.] Oral MeI gave thyroid follicular cell tumors in male mice (DPR Report, Table 26, p65). Male mice got cervical adenomas/carcinomas (USEPA Cancer Report, $P<0.05$ at 84ppm, p13). Other tumors were also noted (see below).

There are two studies in the literature in which MeI was negative for genotoxicity.

In the first negative study, MeI was not detectable as a mutagen in HGPRT in mouse lymphocytes, though the authors do not conclude that MeI is not mutagenic, because in the same study MeI was positive at TK (Clive 1979). It was noted that HGPRT in mouse lymphocytes was also negative for other well-known mutagens/carcinogens (AF-2, 2-AAF and hycanthone) and was only weakly positive for B[a]P. The authors conclude, "On these grounds of greater sensitivity...and the ability to detect the mutagenicity of 2-AAF, hycanthone, AF-2, methyl iodide and B[a]P, we feel the TK locus to have distinct advantages over the HGPRT locus, at least in lymphoma cells." Furthermore, MeI was positive for mutagenesis at HGPRT in CHO cells (Amacher, 1984).

In the second negative study, MeI did not transform human C3H 10T1/2 cells (Oshiro 1981). However Oshira (1981) was seeking to evaluate why many direct acting alkylating agents were negative in this test system. Using large numbers of cells and at high toxicities, MMS, MNU, MNNG, beta-propriolactose and 1,3-propane sultone gave some transformants, while MeI did not. However, steep killing curves were obtained and transformants were only observed in a narrow window of concentration. In the range where transformants might have been detected with MeI, the number of transformed colonies (in parenthesis) was often small: MNNG (16), beta-propriolactose (5), MMS (5), MNU (1) and 1,3-propane sultone (1), implying that the difference between a positive and a negative result was marginal; note: MNU, a well-established and often potent mutagen and carcinogen gave one transformant in this test system in the killing range where MeI gave zero transformants.

I note that in some bacterial Ames test studies, MeI was negative in certain Ames strains, but this is typical as each strain detects a single kind of mutation at a specific site (i.e., a reversion assay), and it is not unusual for a mutagen to be capable of inducing (e.g.) a base substitution at a G:C base pair but not an indel at an A:T base pair.

Thus, 22/24 studies in the published literature show that MeI has properties of a genotoxin and/or a mutagen or carcinogen. Furthermore, in the two negative studies, the authors raise concerns about the inability of the test system to detect all genotoxins, and they do not question whether MeI is a genotoxin.

Three unpublished studies by Arysta indicate that MeI is not a genotoxin. MeI is not mutagenic in bacteria in a variety of test systems (Wagner 2001) or in CHO cells at HGPRT (San 2001), and MeI does not cause chromosomal aberrations in CHO cells (Gudi 2001).

In their Cancer Report, the USEPA offered a series of arguments against a genotoxic mechanism for MeI, which are considered next.

One argument is that tumors formed at terminal sacrifice do not suggest a mutagenic pathway. As far as I am aware, this is not a generally accepted principle, and I have asked for clarification of this point by the USEPA.

A second argument is that evidence in the literature (Gansewendt 1991) shows that MeI adducts form in multiple organs, but tumors are only found in the thyroid. In the Gansewendt (1991) adduct study, though, adduct levels were not measured in the thyroid, so it is unknown whether adduct levels were higher in the thyroid than in the tissues that were tested. Furthermore, tumor rates per unit adduct formed can vary because of differential DNA repair, as well as subsequent downstream responses to those adducts.

A third argument is that the absence of MeI-induced tumors at "the port-of-entry…also demonstrates that mutagenicity is not contributing to carcinogenic profile…" This is an intuitive argument. In fact, carcinogens do not always act at their port-of-entry. There is also an intuitive argument on the other side: if a compound does not react quickly it may reach something approaching tissue equilibrium. MeI seems to fit in this category (DPR Volume I, Tables 1 and 2, p18 and 19).

This question can also be addressed more definitively based on the adduct distribution data from Gansewendt (1991), using F344 rats.

TABLE

m6G adducts	stomach	lung	liver
Oral	1150	220	150
Inhalation	1300	555	350

m7G adducts	stomach	lung	liver
Oral	2850	1430	1350
Inhalation	1550	870	650

M6G and M7G adducts for oral exposure is stomach > lung > liver, which is consistent with a preference for port-of-entry adduction. But for inhalation exposure the order is also stomach > lung > liver, which is not consistent with port-of-entry. This is particularly noteworthy for m6G adducts, which is the likely pre-mutagenic lesion from MeI.

In fact these data are consistent with MeI distributing throughout the body and reaching something approaching tissue equilibrium followed by reaction with DNA. This suggests that route of exposure is not so important and that tumors at the port-of-entry are not necessarily expected. In fact, the preferential formation of thyroid tumors in rats could be the consequence of higher adduct levels due to higher levels of MeI in the thyroid (DPR Volume I, Table 1 and 2, p18 and 19).

A fourth argument involves iodinated glycerol, which is called a "close structural analog" of MeI. The argument is as follows. Iodoglycerol gives tumors at sites other than thyroid; iodoglycerol is a genotoxic carcinogen; if MeI were also a genotoxic carcinogen then it

would also gives tumors in places other than the thyroid, because it is a close structural analog of iodinated glycerol.

Iodoglycerol is not a close structural analog of MeI, because it is larger and has two hydroxyl groups that could very well affect its tissue distribution. In the absence of tissue distribution information for iodoglycerol, this argument must be considered weak.

It is worthy of note that the USEPA Cancer Report states (p24): "…the fact that iodomethane has been shown to have mutagenic properties precludes the exclusion, at this time, of mutagenicity as a contributing factor in thyroid tumorigenesis." In their June 2, 2009 letter to DPR (p2), the USEPA states: "Modest changes in thyroid hormone homeostasis may promote tumor formation in rats." The verb "may" in this statement is telling, in that it does not imply high confidence. (I am assuming that the word "promote" was used to mean "enhance" and was not meant in the sense of a tumor promoter.)

The Arysta PowerPoint slide #45 contained the statement: "One study reported as positive for DNA adduct formation; however, interference from de novo synthesis appears to have occurred," which is their analysis of the Ganeswendt (1991) MeI adduct study, and is argued more extensively in Inh. Tox. (2009) 21: p600-601.

The notion is that the ^{14}C-Me group in MeI has time to enter the one-carbon pool and be incorporated into nucleic acids, which is the reason that ^{14}C-label was found in the adducts m3A, m7G and m6G. While this might explain how ^{14}C-labeled dA and dG might arise, it cannot explain the presence of m3A, m7G and m^{6}G, because normal nucleic acid metabolism does not lead to methylation at the N3A, N7G or O^{6}G positions. Put another way: the methyl groups present at these atoms in m3A, m7G and m^{6}G are only found in DNA following exposure to a methylating mutagen/carcinogen, and in this study the only methylating agent included in the experiment was MeI. The Arysta argument is spurious, and it should be noted that the authors of Gansewendt (1991) conclude about their own work, "These results demonstrate a systemic genotoxic effect of methyl iodide."

Thirteen of the published studies showing that MeI was genotoxic were reviewed by Arysta scientists (Mileson, et al., Inhalation Toxicology 21: 583-605 (2009), who discounted the veracity of all thirteen. It is hard accept that so many mutually corroborative published studies, accepted after peer review, reported over many decades, by so many independent laboratories, and using such a wide variety of test systems could be in error.

Thus, a large body of evidence, using a broad spectrum of assays, support the notion that MeI is a genotoxin that causes both mutations and cancer, while arguments against this conclusion are weak and not convincing.

5. "THYROID HORMONE IMBALANCE" MOA FOR THYROID TUMORS

The fact that I-lo alone causes thyroid tumors in rats, suggests that elevated MeI-induced elevation of TSH alone ("thyroid hormone imbalance" MOA) might be causing thyroid tumors via TSH-promotion following spontaneously mutagenesis initiation. One of the key arguments offered in favor of the thyroid hormone imbalance MOA was that male rats get more thyroid tumors from MeI, because they show greater perturbation in their thyroid hormone levels following MeI exposure.

Genotoxic DHPN treatment alone also gives more thyroid tumors in male than female rats by between 2-10-fold (Hiasa 1985, Hiasa 1991), even though TSH levels are similar in both males and females either treated with DHPN or untreated (Hiasa 1991). This suggests an alternative interpretation of the MeI results: male rats may simply be more prone to genotoxin-induced thyroid tumorigenesis. This notion is reinforced by the observation that castration decreases DHPN-initiated thyroid tumors (Kitahori 1989, Hiasa 1989).

Furthermore, the claim that TSH levels in males are higher than females following MeI exposure is subject to question.

During chronic MeI inhalation exposure, T3 levels are lower at 26, 52 and 104 weeks in males (66%, 89% and 89%, respectively) and in females (73%, 89% and 89%, respectively), but the effects are subtle and not male specific (Table 9b, p24 in the USEPA Cancer Report). T4 levels are variable and can be higher or lower. In contrast, TSH levels increase dramatically at 26 weeks in males (~12-fold) and in females (~7-fold), with a similar trend (though a smaller fold-change) at 52 weeks and 104 weeks.

However, if one considers the TSH data closely, concerns arise, in that the standard deviations are huge and problematic. For example, the value for TSH in females at 60ppm and 26 weeks is 12.92 +/-13.36 ng/mL. This standard deviation implies that a negative value for TSH is possible (i.e., 12.92 - 13.36), which is of course impossible. [This problem arises because in this case log-averaging should have been applied, which is required when there is great variation and values approach zero. Non-log averaging in such cases always exaggerates fold-increases and makes it hard to compare data from different sources; e.g., to be certain that the fold-increase for males is really larger than for females. The appropriate statistical test should be a t-test on the log-transformed TSH levels.]

The dramatic variation implied by the large standard deviations is reinforced in comments made by DPR (Volume I, p56); e.g., TSH levels for animals with carcinomas ranged from 2.27 to 36.86ng/mL. But of course if differences in TSH are to be considered as a possible contributor to the generation of tumors only measurements done before the ultimate manifestation of tumors should be considered. By the "thyroid hormone imbalance" MOA,

the notion is that TSH levels are responding indirectly to breakdown-iodide levels. If true, then these substantial TSH differences probably reflect: (1) the fact that iodide levels fluctuate dramatically throughout the MeI dosing regimen (>40-fold in one case in Table 9a, p25, USEPA Cancer Report), which reflects that MeI exposure regimen (6hr/day, 5d/week), and (2) the fact that the serum half-life for TSH is short (~20min, Lemarchand-Béraud 1981). The most likely inference is that TSH levels were not collected at a fixed time with respect to MeI dosing, such that TSH levels probably reflect test-time vagaries rather than key differences between (e.g.) TSH levels in males vs. females. For example, in week 26 for the 60ppm MeI exposure, the levels in males (30.5 +/-13.7) vs. females (12.9 +/-13.4) show overlapping standard deviations, suggesting that the differences may not be statistically significant.

Furthermore, DPR report notes a lack of correlation between TSH levels and tumor formation (Volume I, p56); e.g., the male with the highest TSH level (50.4ng/mL) had no tumors, while one male with a tumor had a background value (2.27 ng/mL). DPR pointed out that such an observation is inconsistent with the notion that TSH levels were causative for thyroid tumors. However, DPR cautioned that perhaps "changes in hormone level over the course of treatment might be a better predictor of tumor outcome." While this may be true, it is illogical to consider data in support of a particular MOA (i.e., increased TSH levels in males is associated with thyroid tumors), when the data has a severe contradictory element (i.e., the lack of a correlation between TSH levels in specific animals and cancer).

While there is probably no reason to doubt that TSH is also high, and that the wild fluctuations in TSH levels are probably due to the fluctuations in iodide concentration due to the on-again/off-again MeI dosing regimen, these large variations in TSH levels prevent firm conclusions about whether TSH levels are actually higher in male vs. female rats.

In summary one must seriously question the conclusion that a correlation exists between male rats having higher TSH levels and male rats having more thyroid tumors, which was one of the key arguments used to justify the sensibleness of the thyroid hormone imbalance MOA.

8. THE MeI DOSE-RESPONSE RELATIONSHIP

Two MOAs must be considered: (1) the "thyroid hormone imbalance" MOA, which is really more likely to be [spontaneous initiation/TSH-promotion (via MeI exposure)], and (2) [MeI-initiation/TSH-promotion (via MeI exposure)]. MOA (1) might have a threshold in individuals or animals; although different individuals can be expected to differ in their thresholds. MOA (2) will probably be biphasic and will have no threshold.

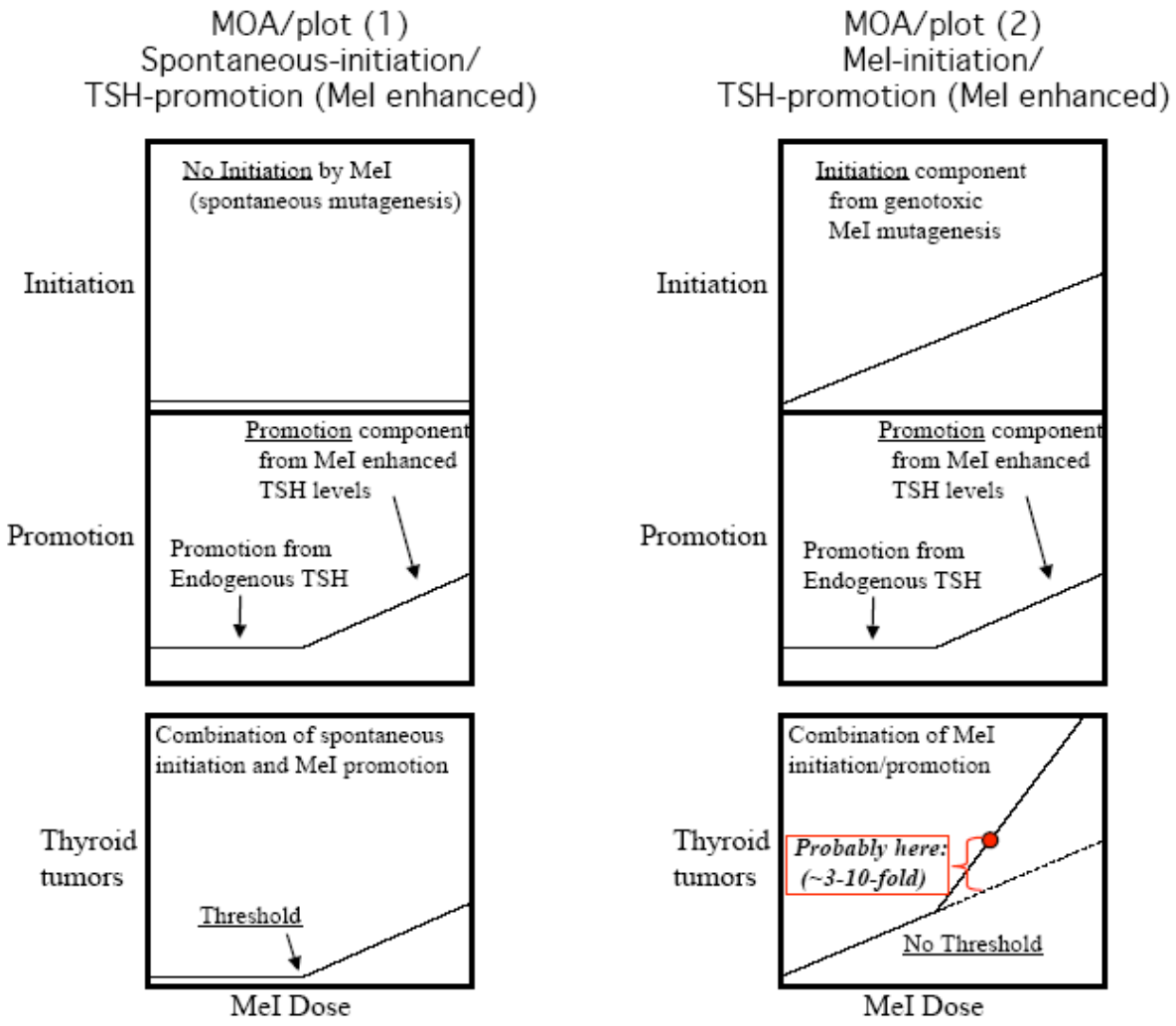
The precise shapes of the dose-response curves for these two MOAs are unknown, but the attached figure shows simplistic versions to demonstrate the principles. Based on evidence

in the literature and the MeI data, arguments favoring MOA/plot (1) are not compelling, and arguments against MOA/plot (2) are weak. On balance, the weight of the evidence favors MOA/plot (2), which must be assumed and a linear extrapolation is prudent. As indicated in a recent national Academy of Sciences report (NRC) interactions with background pathological processes can usually be expected to linearize dose response relationships.

Based on Dr. Ruby Reed's estimates for a linear extrapolation of MeI risk:

Occupational exposure to give a risk of 10^{-5}	1.7ppb
Bystander exposure to give a risk of 10^{-6}	0.04ppb

However, MOA (2) does include TSH-promotion, and 60ppm MeI probably involves some MeI-induced TSH promotion. Although it is impossible to know with certainty where on the MOA/plot (2) the data lie, in large part because the TSH levels fluctuate so wildly, a reasonable extrapolation can be made. At 60ppm MeI, the thyroid gland increases in size by ~3-fold in males (DSP report, Table 21, p58), which is large, although not as large as has been observed with I-lo alone, which can be >20-fold (e.g., Oshima 1984). Based on thyroid gland enlargements from I-lo in Kanno (1992), the TSH-promotion enhancement for DHPN-initiation, based on this,would be in the range of 3-fold-to-10-fold. If this factor held for MeI, then correction for putative MeI-stimulated TSH-promotion would increase these values by ~3-10-fold, although this estimated value (for tumor endpoints) carries considerable uncertainty.


MOA/plot (1)
Spontaneous-initiation/
TSH-promotion (MeI enhanced)
MOA/plot (2)
MeI-initiation/
TSH-promotion (MeI enhanced)
Initiation
No Initiation by MeI
(spontaneous mutagenesis)
Promotion
Promotion component
from MeI enhanced
TSH levels
Promotion from
Endogenous TSH
Thyroid
tumors
Combination of spontaneous
initiation and MeI promotion
Threshold
MeI Dose
Initiation
Initiation component
from genotoxic
MeI mutagenesis
Promotion
Promotion component
from MeI enhanced
TSH levels
Promotion from
Endogenous TSH
Thyroid
tumors
Combination of MeI
initiation/promotion
Probably here:
(~3-10-fold)
No Threshold
MeI Dose

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DATE: January 12, 2010

SUBJECT: Responses to Scientific Review Committee Comments on Methyl Iodide Risk Characterization Document for Inhalation Exposure (Volume I) for August 2009

The following are our responses to comments from the Scientific Review Committee (SRC) compiled report (November 18, 2009) on the August 2009 Methyl Iodide Risk Characterization Document for Inhalation Exposure (Volume I). The SRC comments are italicized with page and section noted. They are assigned numbers for ease of reference. These comments were considered by DPR in the writing of the December 2009 document for SRC review.

Comment 1. Chloropicrin

Page 2: Exposure Assessment

No measurements of concurrent exposures to chloropicrin (added to MeI) or to other pesticides (that may be used in ways leading to co-exposure with MeI) have been done. The Panel is concerned about the potential additive or multiplicative (synergistic) adverse health effects of such co-exposures; without exposure estimates such effects cannot be assessed appropriately.

DPR Response: We recognize that MeI formulations contain various amounts of chloropicrin, ranging from 25% to 75%. There is a potential for cumulative risk issues as both chemicals have similar effects such as GSH depletion and developmental toxicity. While a discussion about chloropicrin toxicity has been added to the revised document, there is no information on the exposure levels of chloropicrin when MeI formulations are used. Since the concentrations of MeI and chloropicrin are relative in the formulations, there should be greater concern about chloropicrin toxicity when the MeI formulation contains 75% chloropicrin, compared to the one containing 25% chloropicrin. The DPR chloropicrin risk assessment, with 100% chloropicrin as the active ingredient and 2% as a warning agent, is being reviewed by the California Scientific Review Panel. When finalized, information in that risk assessment could be used to determine the potential for cumulative toxicity when both chloropicrin and MeI are used.

Comment 2. MeI metabolic pathway
Page 6: Metabolism
Figure 1 in Volume I is inadequate. A more comprehensive figure is required delineating the metabolic pathways for MeI, including identification of intermediates formed prior to the formation of CO_2.

DPR Response: Figure 1 (**page 26**) has been modified with additional details.

Comment 3. MeI as an alkylating agent and toxicity related to alkylation
Page 6: Metabolism:
There does not appear to be a discussion of the relative bond strength between Cl-C, Br-C, and I-C. It is apparent that the I-C bond is weakest and most vulnerable for SN2 reactions that would result in alkylation. The alkylation potential should be addressed at length since it demonstrates an enhanced capability for covalent bond breakage and binding with macromolecules. There is extensive discussion of bond strengths, SN2 reactions, and alkylation in the literature even modest organic chemistry texts. This is a matter of great simplicity with the implication that alkylation will be the key pathway for binding with nucleophiles. This basic organic chemistry should be taken very seriously. This suggests that alkylation is very likely to be a primary mode of chemical binding.

Page 8: Selection of Critical Toxicological Endpoints for Risk Assessment
As an overall comment on DPR's approach to the toxicology, there is a greater emphasis on potential effects of excess iodide than on MeI as an alkylating agent, with potential genotoxic and cellular regulation effects that might result from covalent modification of cellular macromolecules.

DPR Response: A new section (**III.K. MeI as an Alkylating Agent**, **page 128**) has been added to discuss the reactivity of the carbon-iodide bond strength and the implications. MeI has lowest bond strength compared to other haloalkanes; and thus is expected to be more reactive. Specific discussion for alkylating properties for fetal death and oncogenicity is in the **IV.A.** Hazard Identification section. The section on genotoxic MOA (**IV.A.5.b.(2), page 149-151**) has been substantially expanded to emphasize the role of alkylation reaction of MeI in carcinogenesis. As a result, these discussions significantly strengthen the MOA of oncogenicity and support for the evaluation of the oncogenic risk of MeI based on the nonthreshold approach for regulatory purposes.

Comment 4. Use of PBPK Model for interspecies pharmacokinetic extrapolation
Page 6: PBPK Model—Application to Computing HECs

- *The pharmacokinetic model that was used for MeI is very detailed and complicated. Saliently, it introduces both model uncertainty and parameter uncertainty to the overall risk assessment process. Some of the key model parameters included in the PBPK modeling are based on very sparse data; this further increases the uncertainty and limits the reliability of results from the PBPK model. Overall, the data do not appear to be sufficient to allow reliance on PBPK modeling to estimate human equivalent concentrations given the multiple uncertainties present in the model itself, and the questionable assumption made by the modelers that inorganic iodide is the species responsible for causing the adverse effects.*

- *The only mode of action that seems to have been considered in the pharmacokinetic model is excess serum iodide, but the inter-species differences in the distribution of and responses of humans and rodents to serum iodide have not been fully accounted.*

- *It is not appropriate to rely on a model-based dose metric for which the mode-of action has not been clearly established. Both MeI and NaI cause increases in TSH and cause thyroid hypertrophy and colloid depletion in the rabbit fetus, but only MeI reduced the percent of viable fetuses/litter, caused post-implantation loss, and increased late resorptions. The DPR document notes (page 139) "lack of concordance between fetal death from MeI and NaI on equal iodide bases suggests a different MOA than excess fetal iodide." In addition, there was insufficient data on serum iodide from MeI exposures (exposures below 20 ppm and time-course measurements) to establish exposure-response relationships and the PBPK model-based simulations did not provide adequate fits to the serum iodide data.*

- *DPR reduced the 10X factor for interspecies differences to 3 because they relied on the PBPK model... However, since the Panel rejects the utility of this model...*

DPR Response: In **IV.A. Hazard Identification (page 129)**, DPR discusses that PBPK model has sufficient merit over the non-chemical-specific default approach. In the August draft, DPR used PBPK model to characterize marker for exposure and the model can be used to account for additional 16 hours of ambient MeI air exposures by workers. Since the SRC rejected the PBPK model, acute HECs are calculated using the DPR default methodology in the December 2009 final document.

Comment 5. Interspecies pharmacokinetic extrapolation factor in the calculation of HECs
Page 6: PBPK Model-Application to Computing HECs
DPR reduced the 10X factor for interspecies differences to 3 because they relied on the PBPK model to address species differences in pharmacokinetics. However, since the Panel rejects the utility of this model, the 10X factor should be restored.

Page 7: Dose Metrics and HECs
In addition, an interspecies uncertainty factor of 10 instead of 3 should be used to judge the adequacy of margins of exposure.

DPR Response: DPR uses the 10-fold interspecies uncertainty factor when default method is used. When PBPK model is used to address the interspecies PK difference, half of the 10-fold factor, e.g., factor of 3, is used instead. A discussion on the application of the 10-fold uncertainty factor has been added to **V.A. Risk Appraisal- Hazard Identification (page 164)** for further clarification of this conventional practice.

Comment 6: Extrapolation of animal dose to human dose by body weight
Page 7: Dose Metrics and HECs
Because of uncertainties in events subsequent to exposure and the selection of a proper dose metric, and uncertainties in model-based estimates of HECs, extrapolations of animal dose to human dose based on scaling external exposures by body weight$^{3/4}$ should be presented

DPR Response: A discussion has been added to compare adjustment using the body weight factor versus DPR's default breathing rate ratio for non-cancer endpoint HECs in **IV.A. Hazard Identification (page 130)**.

Comment 7. Benchmark dose for point of departure
Page 8: Dose-Response Analysis for non-cancer endpoints

- *Most if not all of the dose points treated as "NOELs" are in fact more conservatively LOAELs.*

- *Because a large number of effects showed elevated responses at the DPR specified NOELs, benchmark dose analyses would be a more appropriate approach to dose response for characterizing health risks of MeI. This approach uses all of the dose-response data and does not simply rely on statistical significance by pair-wise comparisons between small groups of control and exposed animals.*

- *The point-of-departure for estimating a margin of exposure should be an exposure value slightly below the range of the experimental data. The deviation from no effect should take into account the severity of the endpoint in question: e.g. for fetal death a point of departure based on 1% or less rather than a 5% incidence.*

DPR Response:
As much as the benefit of benchmark dose approach is recognized, it is also well understood that some key determinants for its proper application can be subject to scientific debates of diverse opinion and are database- and endpoint-specific. The reason for DPR's hesitation in applying the BMD approach to fetal death endpoint was stated. However, at the recommendation of the SRC, benchmark dose analysis has been incorporated into the revised document. A discussion on the uncertainty associated with the benchmark dose at the level of benchmark response recommended by the SRC is also included in the revised document.

1. **III. Toxicology Profile (page 16, 30, 35, 46, 63, 84, 86)**: The revised document has added benchmark dose analysis and discussion regarding the use of BMD approach to specific datasets and for key toxicity endpoints to study summaries.
2. **IV.A. Hazard Identification (page 136-145)**: In the revised document, benchmark doses are used to calculate the HECs, except for nasal effect which data can not be modeled.

Comment 8. Cholesterol changes
<u>Page 8: Selection of Critical Toxicological Endpoints for Risk Assessment</u>
Effects on cholesterol levels might also be given more attention since they bear relevance for human health. Linear increases in serum cholesterol, including LDLs, are common among rats, dogs, and rabbit fetuses. DPR's MeI document should consider a dose-response analysis and characterization of human risk for cholesterol changes.

DPR Response:
1. **IV.A.3.b.(1) Critical NOEL (page 142)** BMD analysis is conducted for this endpoint.
2. **IV.A.3.b.(2) HEC (page 142)** Since the benchmark doses for cholesterol change are higher than for neurotoxicity, HECs are not calculated. The lower HECs from neurotoxicity will protect against the cholesterol increase with MeI exposure.

Comment 9. Fetal growth restriction as a toxicity endpoint
<u>Page 8: Selection of Critical Toxicological Endpoints for Risk Assessment</u>
The Panel agrees with DPR's selection of critical endpoints for the assessment of MeI, but recommends that fetal growth restriction...be considered as an additional critical endpoint.

Page 8: Dose-Response Analysis for non-cancer endpoints
Dr. Hattis has prepared an analysis that may be helpful for dose-response assessment of fetal growth inhibition, should this endpoint be added to the risk assessment.

DPR Response: As discussed under **III.G.2. Rabbit- Inhalation (page 93)**, the SRC benchmark response criterion of 0.07σ from human data to determine the point of departure for rabbit fetal growth restriction would result in high % of the litters in the control group fitting within the category of "showing adverse effect" definition. Thus this criterion is not used with the MeI dataset. Fetal death remains to be the most sensitive endpoint as the effective doses for fetal body weight are higher than those for fetal death.

Comment 10. Benchmark response for fetal death
Page 8: Dose-Response Analysis for non-cancer endpoints
The point-of-departure for estimating a margin of exposure should be an exposure value slightly below the range of the experimental data. The deviation from no effect should take into account the severity of the endpoint in question: e.g. for fetal death a point of departure based on 1% or less rather than a 5% incidence.

DPR Response:

1. **III.G.2. Rabbit-Inhalation (page 92)** A discussion on the appropriate benchmark response for fetal death and body weight is added.
2. **IV.A.2.a.(3) (page 137)** The HEC is calculated based on LED_{01} of 0.5 ppm using the nested logistic model.
3. **V. Risk Appraisal-Hazard Identification (page 164)** The uncertainty associated with the use of 1% excess risk for fetal death is added. With the wide data variability for this endpoint, the application of 1% BMR to this dataset carries some uncertainty of being overly conservative than the data would allow for the defining the adverse effect level.

Comment 11. Nasal Lesions
Page 8: Dose-Response Analysis for non-cancer endpoints
The total incidence of nasal lesions in rats (pages 42, 43, and 59) and thyroid lesions in female mice (page 51) needs to be provided and analyzed. The current limitation of the analysis to the subtypes and tissue layers is incomplete and potentially misleading.

DPR Response: Total incidences are added to Tables 11, 12, 22, 31, and 32 **(pages 47, 48, 65, 87, and 88).**

Comment 12. Uncertainty factor for Developmental neurotoxicity

Page 9: Developmental Neurotoxicity

- *Absent a convincing mechanistic link, modeling based on serum iodide is inappropriate. Therefore under the Food Quality Protection Act, DPR should apply an uncertainty factor to the benchmark for the most sensitive developmental endpoint.*

- *A factor of at least 10X is needed due to data base deficiency due to lack of an adequate study on developmental neurotoxicity (DPR included a 10X factor for this deficiency). Based on the known effects of MeI and the limited data for developmental exposures, DPR should comment on whether or not a 10X UF is sufficient.*

Page 11: Developmental Neurotoxicity (DNT)

Current legislation (the Food Quality Protection Act) directs EPA and DPR to apply an additional uncertainty factor for compounds for which developmental neurotoxicity is likely, where data are not sufficient to determine that expected exposures are safe. The Panel believes that the data on neurotoxicity available for MeI are far from sufficient to preclude the necessity for the mandated extra uncertainty factor in this case.

Page 12: Risk Appraisal Comments, including Uncertainty Factors

- *It is abundantly clear that human brain development continues into the neonatal period, childhood, and adolescence. Human bystander exposures, as well as worker exposures are going to involve these developing populations as well. Arysta has provided no data on the effects of MeI for these later developmental stages, and consequently, there is uncertainty about all aspects of MeI toxicity for these vulnerable subpopulations: (1) California allows children as young as 12 years of age. to work in the fields, (2) the workers reported that their children were sometimes with them during work, and (3) pregnant women are not excluded from working with fumigants. Clearly these additional exposure periods are highly problematic for endocrine disruption and nervous system development and may produce vulnerability to other endpoints.*

- *An additional 10-fold safety factor to protect against developmental neurotoxicity is warranted.*

DPR Response: DPR has already applied a 10-fold additional uncertainty factor to address potential developmental neurotoxicity. There is no evidence to support deviating from this default factor. As discussed under **V.C.1.c. Additional Uncertainty Factor (page 173)**, DPR considers 10-fold sufficient because the RfCs are 300-fold below the point of departure with a conservative benchmark response for defining the no effect level. RfCs for repeated exposures for neurotoxicity (based on the acute ENEL) are more 10-fold higher than for fetal death. The fetal death RfCs are 6,000-fold and 2,500-fold lower than the PEL and TLV for workers.

Comment 13. Uncertainty factor for Neurotoxicity

Page 10: Neurotoxicity

- *There is an abundant literature of case reports and cell culture studies that point to neurotoxicity as a consequence of MeI exposure, typified by onset of symptoms and cell damage after a post-exposure period of apparent normality. Unfortunately, there was inadequate attention given to the very very striking case study reports. The reports illustrate the extreme toxicity of methyl iodide. There was insufficient attention to the severity of the case studies and a lack of coverage of all the studies. The studies indicate severe neurotoxicity which appears to persist over long periods of time and which may reflect chronic conditions. The case studies are more important than the limited reported studies because they demonstrate severe long term effects. These results need to be given greater attention in terms of the implications of the findings even given the lack of exposure data...*

- *Conclusion: There is a strong expectation that MeI is neurotoxic, based on numerous case studies and laboratory findings. The mechanism for this is unclear, and therefore uncertainty factors will need to be applied in considering this endpoint.*

DPR Response:

1. **Section III.I. (page 116):** A discussion about human case reports and neurotoxicity is added.
2. **Section IV.A.3.b.(1) (page 142)**: A discussion about human case reports and neurotoxicity is added
3. **Section IV.A.3.b.(2) (page 143)**: HECs for subchronic exposure, extrapolated from acute LED using a 3-fold modifying factor are added.
4. **Section IV.A.4.b. (page 145)**: HECs for chronic exposure, extrapolated from acute LED and 10-fold modifying factor are added.

Comment 14. Early life adjustment factor for the cancer potency

Page 11-12: Risk Appraisal Comments, including Uncertainty Factors

- *Inadequacy of the database: Based on the fact that MeI induces thyroid perturbations/toxicity, fetal deaths, reduced fetal and pup growth, and neurotoxicity, the lack of information on long-term effects that may arise after fetal and perinatal exposures is a major deficiency in the MeI database. Long-term effects after early-life exposure that are of particular concern with MeI include neurodevelopmental toxicity and cancer risk. While a 10X uncertainty factor applied to the NOEL or benchmark dose for neurotoxicity and an early life adjustment factor for the cancer potency are certainly justified, it should be recognized that even these adjustments might not be sufficient to account for all the shortcomings of the database.*

- *The age-dependent adjustment factor should be applied to the MeI cancer potency estimate because this chemical is mutagenic and early life exposures are likely.*

Page 17: Additional Cancer Risk Comments
Because of potential early life exposure to this mutagenic compound, the Panel recommends that age-dependent adjustment factors be applied to the MeI cancer potency estimate. The recent guidelines developed by OEHHA, DPR's sister agency, should be used. The Panel is concerned about child bystanders, and teenagers who, in addition to receiving exposure as bystanders or nearby residents, may be exposed during work in the fields.

DPR Response:

1. **IV.C. Risk Characterization (page 161)** Age-dependent adjustment factors (ADAF) is discussed. Current risk assessment practice is to add ADAF to the calculation of risk, not to the potency as the SRC comment suggested.
2. **IV.C.2. Bystanders and Residents (page 162)** ADAF is applied in the cancer risk calculation. The risk is 1.8-fold higher than based on adult lifetime exposure alone.

Comment 15. Mechanisms of Oncogenicity- Thyroid Tumors
Page 12-16: Determination of the Mechanisms of Oncogenicity

- *There is strong evidence that MeI is a genotoxic and an alkylating agent. DPR's RCD should place greater emphasis on a genotoxic mechanism.*

- *Based on an analysis of both the extensive thyroid tumorigenesis literature and the MeI data, the most likely MOA for MeI-induced thyroid tumors is genotoxic MeI-initiation accompanied by MeI-enhanced TSH-promotion. There can be no threshold for TSH-promotion, because--even if MeI levels are lowered such that TSH levels drop to background--endogenous normal TSH levels have been shown to promote genotoxin-initiated thyroid tumorigenesis. The weight of the evidence is strong that MeI is a genotoxic mutagen/carcinogen, and arguments to the contrary are flawed and not convincing. Arguments favoring a pure "thyroid hormone perturbation" MOA (more properly formulated as: [spontaneous mutagenesis-initiation/TSH-promotion]) are not compelling; e.g. the notion that male rats get more thyroid tumors because they have higher TSH levels does not stand up to careful scrutiny, and, furthermore, a pure genotoxic initiation mechanism involving another thyroid mutagen/carcinogen has been shown to give more thyroid tumors in males than females.*

- *With two competing MOAs, public health risk assessment should be based on the more worrisome mechanism (in this case: genotoxic MeI-initiation)...Thus, public health concerns require the assumption of the genotoxic MOA for risk assessment, and a linear dose-response*

curve must be applied. If a correction for MeI-enhanced TSH-promotion is to be included for extrapolation to lower MeI doses, then the linearly extrapolated risk might be raised ~3-10-fold, though this value cannot be estimated with any degree of confidence.

- *The DPR RCD should give more consideration to DNA adducts and the potential role of protein or DNA alkylation in the toxicity and carcinogenicity of MeI. For example, to what extent does residual ^{14}C in tissues at 168 hrs after exposure to ^{14}C-MeI represent macromolecular (protein and DNA) binding? Because specific methylated adducts were identified immediately after 6 hr inhalation exposure to MeI, the comment that DNA adducts may be due to de novo synthesis should be deleted or modified to reflect the experimental findings.*

- *In the study described in Takegawa, et al. (2000), rats given 1000 ppm KI in drinking water did not develop thyroid tumors, which argues against iodide from MeI hydrolysis being causative in thyroid tumors, if the iodide levels were comparable in the Takegawa, et al. (2000) study vs. the MeI study.*

DPR Response:

1. **Section IV.A.5.b.(1)**
   a. **(page 146)** Statistical analysis on TSH levels using the Mann Whitney U-test is added. The difference in TSH levels between males and females are significant at 26 weeks, but not at 52 weeks or 104 weeks.
   b. **(page 148 and 149)** Reports on erythrosine treatment in rats, and iodide supplementation in humans resulting in follicular cell tumors are added. They add to the weight of evidence for the role of excess iodide in thyroid tumor formation. Uncertainty in using total dose to compare results from MeI inhalation and KI drinking water study is also added.
2. **Section IV.A.5.b.(2) (page 149)** The discussion on MeI and genotoxicity is expanded. The discussion on the adduct finding from Gansewendt et al. 1991 is modified.
3. **Section IV.A.5.b.(3) (page 151)** A new section to describe the SRC's TSH Promotion MOAs is added. DPR dose-response assessment of cancer risk for MeI is based on overall tumor incidences that the end of the bioassay period, and includes both threshold and non-threshold possibilities, as well as any promotional effects within the biological system.
4. **III.E.2.b. (page 81)** The description about adducts from de novo synthesis has been modified.

Comment 16: Fibromas
Page 17: Benign and Other Tumors
Female mice exposed to MeI developed cervix and uterine fibromas (P<0.0.1, Table 7, p14, USEPA Cancer Report), which were characterized as "incidental" by the USEPA. Although these fibromas were not malignant, they indicate a potentially significant problem for women, in that they can cause severe pain, heavy bleeding, incontinence and infertility. The only effective treatment is surgery and cervical cancers are difficult to remove without damaging surrounding tissue. Thus, the health effects of such tumors cannot be dismissed.

DPR Response: The cervix and uterine fibroma finding was not dismissed in the DPR risk assessment. It has been included in the weight of evidence (**IV.A.5. p. 145**).

Comment 17. Interspecies Extrapolation factor for cancer potency
Page 17: Additional Cancer Risk Comments
While there is a factor applied (Human body weight/animal body weight)^1/4 to make up for the fact that humans typically metabolize chemicals more slowly than the animals, there is no factor for human inter-individual variability applied to cancer potency estimates. This approach of linear projection without such a factor is considered "conservative" enough by USEPA. The Panel disagrees, as did a recent NRC committee.

DPR Response: As discussed under **V.C.2.a. Evaluation of Benchmarks (page 176)**, the NRC recommends a phase-in when a frame work is developed and then a factor is determined. Therefore, no such factor is added to the DPR calculation at this time. DPR has included age-dependent adjustment to the cancer risk calculation (**IV.C.2. Bystanders and Residents, page 162).**

Comment 18: Mouse Oncogenicity Study
Page 17-18: Additional Cancer Risk Comments

- *The mouse chronic study was not adequate to evaluate the full carcinogenic potential of MeI in mice. Most important, the study duration was only 18 months. A carcinogenic study of short duration has markedly reduced sensitivity, especially for late appearing tumors (Haseman et al., 2001). In the MeI study, thyroid follicular cell hyperplasias were observed at increased incidences in male and female mice, while thyroid follicular cell neoplasms were increased only in the highest dose group of male mice. If the mouse study had been conducted for 2 years duration, which is common for most cancer bioassays, it is possible that several of the thyroid lesions would have progressed to neoplasms. Also, the elevated liver tumor response in male mice might have reached statistical significance in a 2-year*

study. Interestingly, the historical data base for studies conducted at the contract laboratory were of 2 years duration.

- *Another concern with the mouse study is that the composition of the microcapsules that were used to stabilize MeI in the diet was never specified. And, although the diets were changed out every 2 days, no analyses were provided on the concentration of MeI in test diets from cage samples. If microcapsules blended in the diets deteriorate while in the cage (e.g., due to animals nesting in the feed), MeI would be released and we would not be able to adequately determine the true dose in mice.*

DPR Response: The cited mouse study (Harriman, 2005a) is an oral study where the mice were given microencapsulated MeI mixed in the diet for 18 months. Various non-oncogenic effects were reported for the lowest dose tested (60 ppm). The duration of the study met FIFRA guideline requirement of at least 18 months in duration for mice (USEPA Health Effects Test Guidelines OPPTS 870.4300).

DPR has based the evaluation of the oncogenic potential of MeI on the rat inhalation study (Kirkpatrick, 2005). This study was conducted using inhalation exposure, a relevant route for human exposure, and showed significant non-oncogenic and oncogenic effects in the thyroids, a major target organ of MeI.

Comment 19: Appendix B Recommendation of Uncertainty Factors
Page 22:

Suggested Uncertainty Factor	Value
Utility of PBPK model	**Raise from 3 (DPR) to 10**
Interspecies	**Raise from 3 (DPR) to 10**
FQPA for most sensitive developmental endpoint (neurotoxicity)	**To be determined**
Deficiency in neurodevelopmental studies	**An extra 10x factor on top of DPR's 10x**
Neurotoxicity and lack of MOA	**To be determined**

DPR Response: These recommendations have been considered in the appropriate sections of the revised document.

Comment 20: Appendix C Recommendation on Benchmark Response for Fetal Growth restriction
Page 23:
A recent paper summarizes some past and current work on the implications of various amounts of fetal growth restriction in the human context... As it happens, a relatively linear dose-response is not uncommon for this response in animal studies; and recent studies in the air pollution context and with cigarette smoking indicate that it is a quite sensitive parameter in humans with potentially serious implications for infant mortality and a variety of developmental endpoints. The best guidance on an appropriate benchmark response level for fetal growth restriction is to look to the analogous degree of fetal growth restriction and infant mortality associated with cigarette smoking, and the fetal growth restriction seen with criteria air pollutants such as particulates...(see Volume IV for full text).

DPR Response: (see Response to Comment 9)

Comment 21: Appendix D Examination of WIL Laboratories Neurotoxicity Studies
Page 26 *(see Volume IV for full text)*

DPR Response: The SRC comment on the inadequacy of the testing laboratory was noted on page 31 of Volume I. DPR is aware of the contract laboratory's use of trimethyltin to provide the evidence that their procedure would detect histopathological changes. However, without the context of the dose response data, we are unable to respond to the comment citing the incidences as showing that "their laboratory has a high rate of false negatives...they provide no evidence that they are capable of conducting the semiquantitative or quantitative approaches or techniques..." Please note that the 1/5 and 2/5 cited are for different endpoints apparently from a single study, not from separate studies as indicated in the comment.

Comment 22: Appendix E Oncogenic Mode of Action
Page 27 *(see Volume IV for full text)*

DPR Response: The SRC considered the MeI-induced thyroid effects (enlargement, hyperplasia, vacuolation) and elevated TSH reflected a low-iodide, and not high-iodide, response. The most likely MOA involved TSH promotion with no threshold level. Thus, the oncogenicity of MeI should be evaluated only by linear extrapolation (non-threshold) approach.

DPR reviewed the references cited in this Appendix and additional references and determined that the role of excess iodide in the oncogenicity of MeI can not be excluded. Of particular

usefulness was the review by Kanno et al (1996), the study using red dye #3 (described on page 148, Borzelleca, *et al.*, 1987), and the review by Felt-Rasmussen (2001).

As indicated on **page 152** of **Volume I**, the DPR dose-response assessment of oncogenic risk for MeI is based on the overall tumor incidences at the end of the bioassay period, and includes both threshold and non-threshold approaches. These approaches encompass MOAs involving iodide, genotoxicity, and any promotional effect by TSH. For regulatory decision, the RCD favors the linear approach which presents the higher risk; thus, lower allowable exposure level.

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SUBJECT: RESPONSE TO METHYL IODIDE EXTERNAL REVIEW PANEL COMMENTS

During the public presentation on September 24, 2009, a number of the external review panel members raised points of concern with regards to the methyl iodide exposure assessment document (EAD). Subsequently, we received additional written comments from the panel members. The issues that were raised were as follows:

Drs. Blanc, Hammond, and McKone expressed concern about using a respiratory protection factor of 90%. References have been added to the text of the EAD to show from whence this number was derived. An addition, and a discussion of the uncertainties associated with wearing respiratory protection has been added to the Appraisal section of the EAD.

Dr. Blanc, in his written comments, suggested that some component of skin direct contact to liquid MeI with 100% absorption should be factored in to the exposure assessment. Liquid spills are a theoretical concern. However, in my opinion, the manner in which the chemical is normally used makes it highly unlikely that there will be direct dermal contact with the liquid. Nonetheless, the appraisal section of the EAD has been modified to address this issue.

Dr. Blanc, in his written comments, expressed the concern that a protection factor was used for hole-punchers, tarp-removers, and planters even though they are not required by the label to use respiratory protection. This was not the case, and the text of the EAD has been modified to obviate this concern.

Dr. McKone was concerned that puffs of methyl iodide gas, encountered by hole punchers, were not addressed. The air samplers worn by these individuals were drawing air from the breathing zone, so those puffs were sampled.

Dr. McKone was concerned about whether occupied homes should be considered sensitive sites- and thus be ¼ mile away from a treated field. The labels defines sensitive sites as: "... schools, day care facilities, nursing homes, hospitals, prisons, and playgrounds." Thus, occupied homes are not "sensitive sites" according to the label, and may be as close as the edge of the buffer

zone. The Department of Pesticide Regulation estimated resident bystander exposures taking this definition on the label into account.

A number of panel members expressed concern that the default breathing rates utilized in the EAD might underestimate exposures. However, as noted on page 11, the percent retention/absorption of methyl iodide (MI) specifically (and for retention/absorption of all other vapors studied in humans- Frank, 2008) is inversely related to breathing rate. It is not at all clear that increasing the rate of respiration due to exertion or other factors results in an increase in absorbed dose. A discussion of the uncertainties has been added to the Appraisal section of the EAD.

Dr. Blanc was concerned that in the Tables of the EAD there was a single average value for repetitive dosing over a season, even though there are different air concentrations of MI associated with each method. It is unlikely that a bystander would be exposed repetitively, throughout a season to MI levels associated with only one type of application. The tables were clearly misleading, so they have been modified. The footnotes for the tables have been modified to indicate that the absorbed dose is from an average air concentration of MI from all application methods.

Dr. McKone was concerned that the possibility of a differential between indoor and outdoor air was not addressed- specifically that MI or breakdown products might accumulate indoors. Examination of the database concerning differentials between indoor and outdoor concentrations of chemical vapors indicates that when the source of a vapor is outdoors, the outdoor concentrations are higher than the indoor concentrations. The chemical/physical properties of MI do not suggest an accumulation of vapor indoors is likely.

Department of Pesticide Regulation

Mary-Ann Warmerdam
Director

M E M O R A N D U M

Arnold Schwarzenegger
Governor

TO: Joseph P. Frank, D.Sc.
Senior Toxicologist
Worker Health and Safety Branch
(916) 324-3517

FROM: Roger C. Cochran, Ph.D., D.A.B.T. [*Original signed by R. Cochran*]
Worker Health and Safety Branch
Staff Toxicologist (Specialist)
(916) 324-3516

DATE: February 4, 2010

SUBJECT: RESPONSE TO METHYL IODIDE EXTERNAL REVIEW PANEL COMMENTS OF NOVEMBER 18, 2009

A comparison of the concerns raised in the draft comments by the review panel, and the comments received on November 18, 2009, does not indicate any new concerns regarding the exposure estimates for methyl iodide.

Department of Pesticide Regulation

Mary-Ann Warmerdam
Director

M E M O R A N D U M

Arnold Schwarzenegger
Governor

TO: John S. Sanders, Ph.D.
Environmental Program Manager II
Environmental Monitoring Branch

FROM: Randy Segawa *Original signed by*
Environmental Program Manager I
Environmental Monitoring Branch
916-324-4137

DATE: December 14, 2009

SUBJECT: SCIENTIFIC REVIEW COMMITTEE COMMENTS ON THE METHYL IODIDE RISK ASSESSMENT, VOLUME III-ENVIRONMENTAL FATE AND THE DEPARTMENT OF PESTICIDE REGULATION RESPONSES

The Department of Pesticide Regulation's (DPR's) scientific review committee for the methyl iodide (MeI) risk assessment provided comments in November 2009. The following are the comments and responses pertaining to the environmental fate section of the risk assessment.

Comment: The most critical issue with Volume III concerns the inadequate evaluation of the potential for groundwater exposures. The half-life of MeI in water is relatively long, and the potential for contamination of this vital resource is of major importance for California.

Response: DPR added an alternative estimate of ground water concentrations, using bromide ion as a surrogate.

Comment: DPR needs to determine if there are data emerging from Florida and other states where use of MeI is ongoing.

Response: DPR added a description of the status of the Florida monitoring. No groundwater data is available from Florida. Groundwater monitoring in Florida would likely provide little information at this time. It will likely take several years for measurable groundwater contamination to occur. Since MeI has only been registered since 2007, any negative findings in the near future likely do not provide an adequate assessment of the potential for groundwater contamination.

Comment: DPR concluded that groundwater contamination by MeI is unlikely and that groundwater contamination by the iodide anion breakdown product is uncertain. But they also observed that the amount of MeI applied in vulnerable areas (those with high potential for transfer to groundwater) is uncertain and that the amount and timing of water applied after fumigation is uncertain. They note that these issues cannot be fully addressed until additional field dissipation data are obtained.

Response: No changes needed.

Comment: Use of a "water cap" or post-fumigation irrigation may result in an increased risk of groundwater contamination.

Response: DPR clarified that staff specifically evaluated the scenario of additional irrigation in the evaluation of groundwater contamination by methyl iodide. The probability of groundwater contamination is nearly zero.

Comment: One key discussion that is missing in all of the exposure discussions is a summary of the overall fate or mass balance of MeI when it is applied to a field–something analogous to a pharmacodynamics flow chart. The current discussions of environmental fate are rather qualitative and should be more quantitative.

Response: There is insufficient data to estimate a complete mass balance, primarily due to lack of valid field dissipation data. However, DPR added a section describing the available data and that 48–100% of the amount applied volatilizes, based on seven field volatility studies.

Comment: It also appears that there are no good data on the rates of MeI degradation in soil and how these vary with climate conditions and soil properties. The degradation estimates are apparently based on a single study in Watsonville. A single study cannot capture the variability in climate conditions and soil properties.

Response: DPR agrees and requires field dissipation studies in regions and conditions known to be vulnerable to groundwater contamination.

Comment: It would be very useful to provide a fate diagram that illustrates the approximate fractions of a unit mass (1 kg) that follow different transport/transformation pathways–the fraction that evaporates, the fraction that is transformed to the iodide anion, the fraction transformed to other products. Also for the fraction that evaporates it would be useful to have this broken down into the fraction that evaporates within one day, three day, one week and two weeks.

Response: DPR added a figure that shows the cumulative mass volatilized over time as measured in seven flux studies. There are no valid field dissipation studies that provide data on methyl iodide transformation in the soil.

Comment: If groundwater contamination has either been documented already or is likely to occur based on application and physical properties of MeI, and taking all of the uncertainties noted above into account, then the risks associated with oral exposure from drinking water,

dermal exposure from bathing, and inhalation exposure from showering should each be incorporated into the MeI risk assessment.

If supplemental air release or dietary exposure is reasonable based on the above uncertainties, additional risk modeling should also take these routes into account.

Response: Groundwater contamination by MeI has not been documented, or is likely to occur. Groundwater contamination by iodide ion has not been documented, and the probability of occurring is uncertain. The risk assessment includes a worst-case estimate of the risk from drinking water. The uncertainty in water concentrations and uncertainties in resulting exposure from bathing and showering makes the assessment of risk from these scenarios unreliable.

B. Scientific Review Committee Comments and Errata on December 2009 Draft RCD (Comments received on January 21, 2010) and List of Tasks for completion of final RCD (From January 25, 2010 meeting)

Appendix 2

The SRC comments regarding the December 2009 DPR revised RCD on methyl iodide

January 2010

The December 2009 DPR responses to the SRC comments of November 2009 reflect a serious attempt to grapple with a complex critique of wide-ranging sets of issues. These issues involve exposure modeling, adverse health outcome evaluation, and risk assessment. Moreover, this dialogue is all the more to be commended given the inherent differences in between reviewers with a primary biomedical scientific approach to the data at hand as opposed to regulators facing the challenges of a highly structured set of reporting criteria.

That being said, the December 2009 revisions are not as successful as they might be in satisfactorily addressing certain of the key points raised in the initial SRC review. In general terms, the remaining gaps that remain to be bridged can be characterized as follows:

Data Base

There appears to be a string consensus within DPR and the SRC both that the data base we are forced to use has very severe deficiencies that leave key questions unanswered. The standard scientific approach (reflected in the tone and substance of the SRC's critique) is to delineate these data shortcomings and *to discuss their potential implications for any presumed findings*. DPR, consistent with precedent in many previous documents, does not invest a great deal of text to such matters. In this particular case, however, such detailed discussion is quite important. This is acutely apparent in the neurotoxicity endpoint risk assessment and connects back to the issue of a "special" additional adjustment factor.

Exposure Assessment

General Comments

1. The draft Exposure Assessment document, as opposed to Volume 1, seems to have taken a very different approach in responding to the SRC critiques. Unfortunately, this approach, by choosing a point-counter-point balloon-comment format, is counter-productive. Points that may reflect the SRC's confusion from textual omissions are likely to raise similar concerns in other readers. Thus the text itself should be expanded to make clear those points of added information/clarification only.
2. The SRC is concerned that DPR has for the most part rejected our request to address uncertainty, variability, and key sensitivities in the exposure assessment. It is important that DPR avoid single-value assumptions for exposure parameters. If they insist on using single values, then they should consider impacts on the risk assessment results of alternative assumptions. DPR seemed more interested in rejecting and refuting our suggestions about modifications to the exposure assessment rather than trying to address the technical validity of our comments.

3. The unwillingness of the Exposure Assessment document to present, as sensitivity analyses at least, the effect of more real-world realistic exposure calculations (even if not used as the final basis of a risk calculation) is not acceptable. This certainly applies to the overly optimistic 90% attenuation of inhalation exposure with respiratory protection, the failure to consider skin contact because it would be "uncommon," and the unwillingness to take into account 10-hour work days because county agricultural officers could restrict exposure to 8 hours (for applicators alone it seems). [Volume 1 also seems to stick to an 8-hour day cut-off.]
4. The SRC suggests that DPR provide a graph of all of the HEC/PODs and derived RfCs along with estimates of various human exposures scenarios. Such a graph would illustrate the multiple effects of MeI, the magnitude of difference between the most sensitive endpoint and other induced effects, MOEs, and the relationships between estimates of human exposure and derived values of daily exposures that are considered to be without appreciable risk of non-cancer effects during a lifetime (RfC).
5. The inhalations rates were not changed, despite a lengthy discussion at the September meeting. The inhalation rate will make a major difference in the absorbed dose. While a 24-hour average rate may be appropriate for lifetime chronic exposure to a chemical which has stable concentration, evaluation of acute effects that come from brief exposures (a few hours) should incorporate the breathing rates during work or (for children) while at play.

Specific Critiques

1. *p. 10, line 23, Vol. II.* There should be a brief explanation of why the calculation of risks for community residents yields a greater number than the calculation for workers, as this result is counterintuitive. Is it that DPR has maintained the assumption of a 90% protection factor for personal equipment? Is it that the workers are assumed to have only a brief exposure during a single season, where as the community residents are assumed to be repeatedly exposed for many years or a lifetime? Both? Additional factors?
6. *p. 44, r16, Vol. II.* One overall problem is DPR's basic assumption that accidents do not happen. For example in the Exposure Assessment document [this was in response to our comment that the exposure assessment should include a scenario with direct skin contact with MeI liquid]:

   > "DPR conducts risk characterization (and thus exposure assessment) only of uses that are legal and follow all label restrictions and state and federal laws and regulations. Under accidental spill conditions, the directions listed on the MSDS are followed."

   The following was added to the text: "Based upon the techniques involved in handling MI during application, exposure to liquid MI via skin absorption was considered highly unlikely." A similar rationale is used in retaining the 90% protection factor (PF) for respirators (see comment r2 on p. 4). In this case the assumption is also that all respirator rules will be followed. The SRC's request for changeout schedules was ignored. The SRC also requested exposure estimates with and without these protection factors; Table

11 of the main text has estimates only with the PF, as noted in small print in a footnote, but Tables 1 and 2 in Appendix II do present both. Both of these are highly problematic.

7. *Comment r4, Vol. II.* The SRC mentioned the fact that the measurements were made at relatively low temperatures, and requested this be addressed ["The literature on chemical properties indicates that for volatile compounds such as MeI the vapor pressure (the driving force for volatilization) can often double between 20 and 30 °C"]. In comment r4 they basically say it is too difficult to adjust the model for temperature. However, the SRC thinks the text should at least note that concentrations might be higher at higher temperatures.
8. *p. 48, r18, Vol. II.* The response to the SRC's comment about the assumptions of 8-hour workdays and 3-month exposures addressed the effect of repetitive exposures, but not of the longer work day. A 10-hour workday is important for both acute and chronic exposure estimates.
9. *Page 130, line 29, Vol. II.* An explanation of the 16-hour non-working exposure period needs to be included.

PBPK Model—Application to Computing HECs

Specific Critiques

1. *p. 1, lines 29-36, Vol. I.* "endpoint of concern (critical endpoint) for that particular exposure duration." When the data can be modeled by benchmark dose software, the point of departure (POD) is based on benchmark levels based on a fixed response or multiplier of the standard deviation, instead of the critical NOELs. The PODs are converted to HECs, which account for factors such as intake, exposure duration, and pharmacokinetic (PK) differences between laboratory animals and humans using default methodology or PBPK modeling. With PBPK modeling, the HEC is linked to a dose metric for markers of exposure or toxicity, rather than to the external exposure concentration such as the NOEL." It is important for development of further policy that the specific rules for deciding on the point of departure in percentage terms (for quantal effects) or in standard deviation terms (for continuous effects) be defined and grounded in some specific risk management reasoning. This should not be an arbitrary choice to be made up on the fly for each chemical.
2. *Page 2, line 6, Vol. I.* The rationale for rejecting the PBPK model should be based on its validity, not because this was specified by the SRC.
3. *Page 3, Table 1, Vol. I.* The basis for estimating HECs needs to be described in the footnotes; also, many of these are substantially larger than that reported in the previous draft (e.g., for acute olfactory degeneration, HEC for workers changed from 2.8 to 17.1 ppm).
4. *Page 16-17, Vol. I.* The rationale for performing a BMD analysis should be based on the value of this approach, not because this was specified by the SRC. All models that were used should be specified. Explain why a multiplier of standard deviation was sometimes used.
5. *Page 130, lines 5-20, Vol. I.* The selection of maternal iodide as the dose metric may reasonably capture maternal exposure to MeI, but so does direct measurement of the

exposure concentration. However, such a dose metric is not associated mechanistically with the endpoint of greatest concern, i.e. fetal death. This is reflected by the fact that MeI but not NaI caused reductions in viable fetuses/litter and increased late resorptions. Experimental studies could have been conducted to estimate blood time courses of MeI in maternal and fetal blood, e.g., blood sampling during nose-only exposures or measurement of surrogate biomarkers. The lack of such data does not provide a reasonable excuse for the selection of an inappropriate dose metric.

6. *p. 130, lines 5-30,* Vol. I. Use of default interspecies scaling rather than the PBPK model and maternal iodide dose metric for fetal death and other acute effects: the SRC supports DPR's newer approach, corresponding to the committee's previous recommendation. However, considerable skepticism is in order on Arysta's contention that analysts should have greater confidence in the maternal iodide dose metric estimated via the PBPK model than the area under the curve for the parent MeI favored by the SRC. The factors governing elimination of the putative causal form for the acute effects—directly alkylating MeI—are very different from the factors governing elimination of iodide. Iodide is presumably eliminated largely through urinary excretion whereas the parent MeI is likely to be dealt with by some combination of metabolism/GSH conjugation and passive reaction. The SRC agrees that maternal iodide is likely better estimated by the existing model than other possible dose metric but superior measurement does not make this dose metric a superior predictor of the adverse effects in question, and the source of DPR's confidence in the use of this dose metric therefore is elusive. The sponsor did have at its disposal an ability to calibrate the model for integrated MeI dose—hemoglobin adducts as mentioned briefly in the PBPK modeling paper. Such calibration has been done, for example, for acrylamide and its active metabolite based on hemoglobin adduct data in both rodents and people. In the absence of a credible calibration of a PBPK model for the most likely causally relevant dose metric (the integrated Area Under the Curve for the parent MeI in the blood), the SRC thinks the choice must be to revert to default dosimetry assumptions.
7. *Page 130, lines 34 to Page 131, line 30, Vol. I.* The SRC agrees with deriving the HEC based on body weight scaling for PK differences and accounting for PD differences using $10^{0.5}$.
8. *Page 131, line 42 to Page 132, line7, Vol. I.* The 24-hour HECs based on maternal and fetal MeI AUCs are based on simulations and a lack of measurement data for model validation. Thus, the estimated values are not reliable. DPR's support of the PBPK modeling approach lacks scientific merit.
9. *p. 131, lines 32-46, and p. 132, lines 1-2, Vol. I.* These comments appear to flow from PBPK model estimates of the AUC for the parent MeI. As previously noted, the model has not been calibrated against data relevant to assess this dose metric, and therefore, although the SRC believes this is the most likely relevant causal dose metric for risk projection, it is not believed that the model predictions for it can be considered reasonably reliable for use in assessing risks.
10. *Pages 136-145, Vol. I.* The derivation of each of the HECs needs to be fully described.

11. *Page 173, Vol. I.* The HECs are based on the lower confidence limit of a benchmark response, not on no-effect levels (line 11). DPR's "assessment of neurotoxicity" does not address the fact that there is lack of information on developmental neurotoxicity. The fact that the acute RfC is much lower than the current PEL or TLV does not support the adequacy of the 10X UF for database insufficiency. The PEL and TLV cause fetal deaths in rabbits – making them not health protective.

Neurotoxicity

General Comments

1. It is confusing that there are calculations based on supposed measures of "neurotoxicity" when there were in fact no studies of neurotoxicity actually conducted. The studies labeled as "neurotoxicity" were nothing of the sort. The extrapolations described on page 143 of Volume I are based on studies that did not assess neurotoxicity in any sense that would be accepted in the scientific community. The contract lab conducting the studies was demonstrably incapable of detecting neurotoxicity from positive control test compounds and the study design (an acute exposure and a single time point qualitative assessment by apparently non-blinded observers) is not an evaluation of neurotoxicity. As such, there is a disconnect between this revised DPR document and the first set of comments on neurotoxicity sent in by the SRC, which indicated that, in fact, there was no reliable assessment of neurotoxicity.
2. It is still the opinion of the SRC that developmental neurotoxicity cannot be explained by hypothyroidism.
3. Greater uncertainty factors should be applied based on the poor quality of the neurotoxicity studies submitted by the registrant.

Risk Appraisal including Uncertainty Factors

General Comments

1. Residual questions remain regarding intra and interspecies adjustment factors as applied. Overall there was quite a bit of progress made in this area, fundamentally because DPR was very responsive to adopting more public health protective NOEL or benchmarking approaches in almost every instance where such questions arose and where technical exploration of the data indicated this was feasible. In places where changes were not adopted, DRP provided detailed text within the body of Health Risk Assessment (Vol. I). The revision, in attempting to respond to the SRC, may have introduced additional unintended confusion in its attempts to clarify the rationale for the adjustment factors as used. It is likely that this can be clarified through additional dialogue at the upcoming meeting. This discussion should seek to gap any remaining differences viewed as to the appropriate intraspecies adjustments still needed following benchmark calculations where applied (given that the PBPK model was rejected, in agreement with a central SRC

recommendation). There also seems to be confusion arising as to the potential application of a supplemental multiplicative factor of 10 in regard to neuro-developmental and/or neutotoxicity endpoints.

2. The report defends the 10X uncertainty factor as being adequate for database insufficiencies, which include the lack of data on developmental neurotoxicy. Of additional concern is that this UF was applied only to the fetal death endpoint.
3. The claim that an acute toxicity study can be adjusted by 3X and 10X modifying factors to be protective against subchronic and chronic neurotoxicity, respectively, lacks scientific merit.
4. The mentioning of NOELs for endpoints in which BMD modeling was performed seems to be distracting and misleading.

Specific Critiques

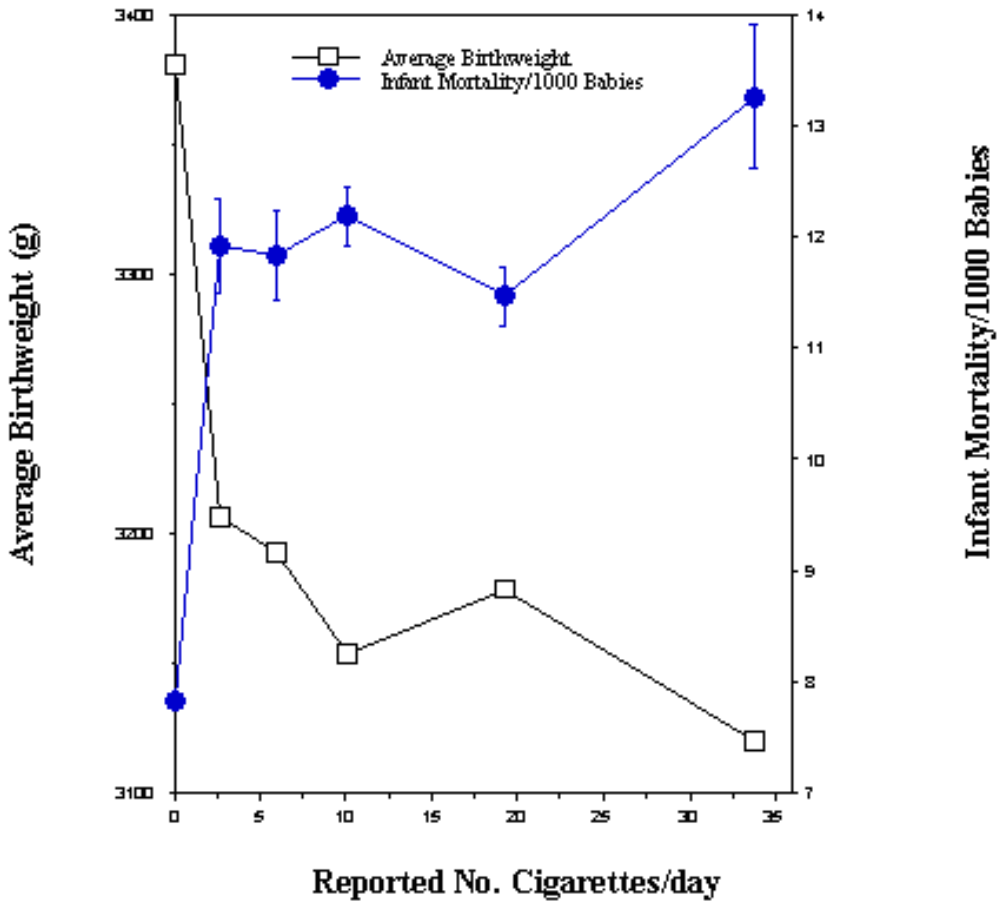
1. *Page 1, line 31, Vol. I.* DPR should provide rationale for selection of a specified level of extra risk for the benchmark response (1%, 2%, 5%, 10%, or a multiplier of the standard deviation) as it pertains to the MeI database. Explain why a multiplier of standard deviation was sometimes used.
2. *Page 2, lines 17, 44, Vol. I.* When LED values are determined, NOELs should not be specified. The NOEL values are distracting and misleading.
3. *p. 2, lines 14-36, Vol. I.* "For acute exposure toxicity, relevant studies are selected from studies described in the Acute Toxicity, Subchronic Toxicity, and Developmental Toxicity sections. The critical endpoints are: fetal death in rabbits, olfactory epithelial degeneration in rats, and neurotoxicity in rats. The NOEL is 2 ppm and the benchmark LED_{01} is 0.5 ppm for the fetal death endpoint from a rabbit teratology study. Since it is the result of maternal exposure, it is applicable only for women of child-bearing age in the workplace or in the general population. Four possible MOAs are explored: thyroid perturbation from excess iodide, glutathione (GSH) depletion, direct alkylation, and altered cholesterol homeostasis. The conclusion is that the data do not support a single predominant MOA for fetal death. The nasal effect and neurotoxicity endpoints with NOELs of 21 ppm and 27 ppm, respectively, are appropriate for all other age groups. Olfactory epithelial degeneration as a local effect was observed in rats from a 13-week study with GSH depletion as a marker for plausible MOA. Since the data cannot be adequately described by current benchmark dose models due to the large difference in response between the NOEL and the LOEL, the HECs are based on the NOEL. Neurotoxicity was indicated by decreased body temperature and motor activity in rats after a 6-hour inhalation exposure to MeI. The $LED_{0.36\sigma}$ for reduced ambulatory motor activity in female rats is 12.8 ppm". The .36 sigma standard is new. There should be some specific reasoning associated with this. Tables 1, 4, and 5: It is hard for readers to interpret and grasp the implications of the complex and detailed results in these tables. There are several places where margins of exposure are calculated as less than 1, and it is unclear what the basis is for the margin of exposure calculations for chronic carcinogenesis. Presumably the usual guideline for noncancer effects is that margins of

exposure should exceed 100 (or perhaps 30, on the basis of the statement at the top of page 8). In order to be reasonably acceptable. For cancer there should be a comparable figure based on a targeted upper confidence limit risk figure.

4. *Page 2, line 39, Vol. I.* Something is wrong with the determination of an LED of 19 ppm for reduced pup weight – this value is essentially the same as an exposure (20 ppm) that caused a significant decrease in mean pub body weight (Table 33). The SRC suspects an LED_{02} for female pups would be about 2 ppm (shown as 3 ppm in Table 1) – should always specify the group, i.e., male or female rats.
5. *Page 2, line 45, Vol. I.* The LED should be 1.3, not 4.3 (as shown in Table 1).
6. *Page 6, Table 4, Vol. I.* Several MOEs increased and the cancer risk values decreased from the previous draft. How can this be if the HECs are lower than before and the cancer slope factor did not change? Did exposures decrease?
7. *Page 9, Table 6, Vol. I.* The additional UF of at least 10X for database insufficiency must be applied to all child- and infant-based HECs used for the determination of the RfC, i.e., UF=300. There are no studies on developmental neurotoxicity or other long-term effects associated with fetal or perinatal exposures.
8. *p. 18, lines 5-9, Vol. I.* "The analysis involves fitting a mathematical model to the entire dose-response dataset for an endpoint, and allowing the model to estimate the threshold dose (benchmark dose, BMD) corresponding to a level of benchmark response (BMR). The words "threshold dose" are confusing and incorrect in this context. These two words should be deleted. The BMD analysis does not involve estimating a dose at which there is zero incremental effect over background—the usual definition of a "threshold". Instead it involves estimating the dose (BMD) and its lower confidence limit (BMDL) at which the data and models used indicate that the chemical will cause an incidence of harm equal to a finite benchmark response level.
9. *p. 18, lines 15-16*, Vol. I. The MeI data are analyzed for 1 to 10% response (for quantal outcome parameters such as the incidence of fetal death) as well as at 0.61σ or 0.36σ (for continuous outcome parameters like fetal weight). The LED is selected as equivalent to a no-effect level in this document. The SRC appreciates the fact that some standards for analysis of changes in continuous parameters have been specified. However for both the quantal and the continuous criteria, the reader should be informed of the general basis for setting the analysis parameters as they were.
10. *Page 31, lines 7-12, Vol. I.* It is not clear why LED_{05} or LED_{10} were not selected for the POD.
11. *Page 35, Vol. I.* The ED_{10} for GSH depletion in blood at 3 hrs was estimated to be 35 ppm; however, the decrease at 25 ppm is more than 10%. DPR should explain this discrepancy.
12. *Page 46, lines 13-16, Vol. I.* An LED rather than a NOAEL should be determined for increased liver weight.
13. *Page 46, Vol. I.* Why was only the week-6 body weight data for males used in the BMD analysis? Why were the endpoints and the LED_{05} or LED_{10} values in this table not considered critical and included in Summary Table 1 and in the MOE analysis?

14. *Page 49, line 42, Vol. I.* An LED rather than a NOAEL should be determined for the forestomach lesions. There are several instances where NOAELs are specified when BMD modeling should have been performed.
15. *Page 62, lines 31-35, Vol. I.* The paragraph on identifying NOELs for salivary gland atrophy and metaplasia should be deleted.
16. *Page 63, Vol. I.* LEDs for salivary gland atrophy should be included in this table.
17. *Page 73, line 40, Vol. I.* An LED rather than a NOAEL should be determined for effects in dogs, including increased cholesterol, increased liver weight, etc.
18. *Page 83, line 44; page 84, line 13, Vol. I.* The mention of NOAELs in the range finding study is inappropriate and unnecessary.
19. *Page 84, Table, Vol. I.* Where are the data that were used in the BMD analysis? Why weren't the other endpoints mentioned on pages 83 and 84 also included in this analysis?
20. *Page 86, line 17-18, Vol. I.* This sentence should be deleted since an LED value will be used.
21. *Page 86, table, Vol. I.* Where is the 22-week body weight data used to derive LEDs for the first two rows? The ED_{05} for vaginal patency (22 ppm) is above an exposure that caused a significant increase (20 ppm). Therefore, an LED_{02} should have been derived.
22. *Page 91, lines 34-36.* Based on the subsequent discussion, the sentences on specified NOAELs should be deleted.
23. *Page 93, Vol. I.* This may not matter since the lowest POD will still be based on the LED_{01} (0.5 ppm) for fetal death. The SRC was trying to lay the groundwork for more systematic discussion of how one should choose a benchmark response level for a continuous variable that is strongly related to a very bad outcome in people—infant mortality. So although this may not provide the lowest POD in this particular case, it still may contribute to DPR's thinking in general about how to arrive at benchmark response levels for continuous biomarkers if they don't simply adopt EPA's standard procedure to just set the level at a full standard deviation of variable in the control population. The SRC's specific advice in this case is to use a 35 gram change in population birth weights—corresponding to about 1% of a baseline mean birth weight of about 3500 gram. In comparison, direct cigarette smoking makes a difference of a couple of hundred grams (about 6%). As per the plots supplied (see below) this degree of change in birth (6%) weights is associated with about a 3/1000 change in infant mortality rate (from a baseline of about 8/1000 to about 12/1000), so the SRC believes an appropriate number for consideration as the analog for a NOAEL should be much less than this.

Relationships Between Reported Cigarettes Smoked per Day, Average Birth Weight, and Infant Mortality



24. *p. 93, line 6 and again on line 20, Vol. I.* Use of the word "threshold" here is ill-advised as there is no showing of no effect below the chosen level. The SRC would suggest substituting "point of departure".
25. *Page 133-144, Vol. I.* While PODs have been determined based on BMD modeling and paragraphs reflecting that effort have been inserted, the repeated specification of NOAELs is distracting and misleading. The risk assessment section should be rewritten with strict emphasis on PODs and elimination of NOELs.
26. *Page 143, lines 38-40, Vol. I.* How well a 3X-modifying factor of an acute response reflects potential subchronic toxicity is very uncertain. Thus, the comment that the use of 4.3 ppm as a POD would be protective against neurotoxicity is highly speculative.
27. *Pages 161 and 162, Vol. I.* A MOE is not a risk value (page 161, line 6; page 162, line 23).
28. *Page 174, Table 66, Vol. I.* An additional UF of at least 10X for database insufficiency must be applied to all child- and infant-based HECs used for the determination of the RfC, i.e., UF=300. There are no studies on developmental neurotoxicity or other long-

term effects associated with fetal or perinatal exposures. Based on DPR's belief that GSH depletion represents the MOA for olfactory degeneration, an additional UF (3-10X) should be applied to this endpoint because of the much lower levels of glutathione in the olfactory epithelium of rats compared to humans.

29. *p. 130, lines , and p. 131 lines 1-30, Vol. I.* DPR's response to the SRC seems sound here. The Body Weight$^{3/4}$ projection does mainly adjust for adult differences in pharmacokinetics. The additional intake rate adjustment will cover cases where children have increased exposure relative to adults. After those adjustments are made, retaining an additional 3-fold to account for pharmacokinetic differences should generally be in line with prior practice. A separate consideration, however is the up to 10-fold Food Quality Protection Act factor called for to compensate for the absence of suitable neurodevelopmental studies to allay concerns for this distinctive type of effect in a chemical with established neurotoxicity. It is possible that the SRC's earlier discussion inadvertently conflated these distinct considerations (for interspecies projection and neurodevelopmental effects). However it is not clear from the regulatory history that the FQPA factor is appropriately applied to acute effects. The SRC and DPR may wish to consider this issue in its own right at the next meeting in late January. If the application of the FQPA factor and/or a database deficiency factor is considered appropriate for longer term effects, then this should be explicitly treated in the subchronic or chronic effects section of the discussion (p. 142, lines 25-34 and p. 143, lines 19-40).
30. *p. 149, Vol. I.* "In summary, MeI-induced thyroid tumors in rodents can be due to thyroid perturbation as the MOA because the increase in serum iodide levels and the pattern of changes in the thyroid function and pathology in rats after MeI exposure are consistent with known effects of iodide on the thyroid. The studies with erythrosine in rats (Borzelleca, *et al.*, 1987)[*] and iodine prophylaxis in human (Felt-Rasmussen, 2001)

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[*] Lifetime toxicity/carcinogenicity study of FD & C Red No. 3 (erythrosine) in rats. Borzelleca JF, Capen CC, Hallagan JB. Food Chem Toxicol. 1987 Oct;25(10):723-33.

Medical College of Virginia, Department of Pharmacology and Toxicology, Richmond 23298.

FD & C Red No. 3 was fed to Charles River CD rats as a dietary admixture in two long-term toxicity/carcinogenicity studies. The studies consisted of an in utero and an F1 phase. In the former, the compound was administered to five groups of the F0 generation rats (60 of each sex/group) at levels of 0.0, 0.0, 0.1, 0.5 or 1.0% ('original study') and 0.0 or 4.0% ('high-dose study'). The concurrent control groups received the basal diet. After random selection of the F1 animals, the long-term phase was initiated using the same dietary levels and 70 rats of each sex/group, including the three control groups. Rats were exposed for a maximum of 30 months. No compound-related effects were noted in the in utero phase. Mean body weights of the female F1 rats on 4.0% FD & C Red No. 3 (3029 mg/kg/body weight/day) were significantly lower than those of controls (P less than 0.01) throughout the study. Food consumption increased in all treated groups in a dose-related manner. There were no significant effects on the haematology, serum chemistry and urinalysis and no compound-related effects on survival. In male rats receiving 4.0% FD & C Red No. 3 (2464 mg/kg/day) thyroid weights were increased, with a mean weight of 92 mg compared to 44 mg for controls, and statistically significant increases in the incidence of thyroid follicular cell hypertrophy, hyperplasia and adenomas were recorded. A numerically increased incidence of thyroid follicular adenomas in female rats given 0.5, 1.0 or 4.0% FD & C Red No. 3 was not statistically significant. The no-observed-adverse-effect levels established in these studies were 0.5% (251 mg/kg/day) for male rats and 1.0% (641 mg/kg/day) for females.

provide additional support for this MOA. "[**] This is contrary to the conclusion reached by the SRC. The SRC noted in particular that experiments where iodide salts were administered by themselves did not result in tumors, although there is some change in thyroid parameters at high doses. Research on this issue shows that there is at least some evidence of tumor promotion by iodide at very high doses after administration of a mutagenic initiator.[*] No excess tumors were seen at any dose of iodide in the absence of the initiator treatment. Some promotion of tumors was evident in animals given the

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[**] **Iodine and Cancer**

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Ulla Feldt-Rasmussen. Thyroid. May 2001, 11(5): 483-486. doi:10.1089/105072501300176435.

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Thyroid carcinomas are the most frequent endocrine malignancies. Among thyroid carcinomas the most frequent types are the differentiated forms (follicular, papillary or mixed papillary-follicular), whereas anaplastic thyroid carcinoma and medullary thyroid carcinomas are rare. Animal experiments have demonstrated a clear increase in incidence of thyroid epithelial cell carcinomas after prolonged iodine deficiency leading to a situation of the thyroid gland by thyrotropin and possibly other growth factors. However, the overall incidence of differentiated thyroid carcinoma is generally not considered to be influenced by the iodine intake of a population, whereas the distribution of the types of thyroid carcinoma seems to be related to the intake of iodine, with fewer of the more aggressive follicular and anaplastic carcinomas and more papillary carcinomas in iodine rich areas. Populations starting iodine prophylaxis demonstrate an increase in the ratio of papillary to follicular carcinoma. Because a population with higher iodine intake usually has fewer benign nodules in the thyroid gland and the incidence of thyroid carcinomas is similar to an iodine-deficient region, the diagnostic work-up of nodules in the thyroid gland may become affected. The incidence of other cancers, such as breast cancer, may be influenced by the iodine intake, but too few studies are available at present. The present article summarizes available data from both epidemiological studies, animal experiments, and basic gene transfection studies. The overall incidence for a relationship between iodine and cancer is poor and future studies are warranted.

- The full abstract of this paper is:

Kanno J, Onodera H, Furuta K, Maekawa A, Kasuga T, Hayashi Y. Toxicol Pathol. 1992;20(2):226-35.
Department of Pathology, Faculty of Medicine, Tokyo Medical and Dental University, Japan.

Thyroid tumor-promoting effects of iodine deficiency and iodine excess were investigated in a rodent 2-stage model to estimate an optimal iodine intake range that would not effectively promote development of thyroid neoplasia. Six-week-old male F344 rats were given a single subcutaneous injection of 2,800 mg/kg body weight N-bis(2-hydroxypropyl)-nitrosamine (DHPN) or saline vehicle, maintained on Remington's iodine-deficient diet (21 +/- 2 ng/g iodide), and supplemented with various amounts of potassium iodide up to 260 mg/liter in drinking water to generate conditions ranging from severe iodine deficiency to severe iodine excess. In DHPN-treated rats, both conditions significantly increased thyroid follicular tumorigenesis. In DHPN-untreated rats, iodine deficiency produced diffuse thyroid hyperplasia, characterized by small follicles with tall epithelium and reduced colloid, together with a decrease in thyroxine (T4) and an increase in thyroid-stimulating hormone (TSH). On the other hand, iodine excess produced colloid goiter, characterized by large follicles with flat epithelium and abundant colloid admixed with normal or small-sized follicles lined by epithelium of normal height, together with normal serum T4 and slightly decreased TSH. These effects were directly proportional to the severity of iodine deficiency or extent of iodine excess and suggest that each condition has a different thyroid tumor promotion mechanism. Iodine intakes that showed the least tumor promotion were 2.6 and 9.7 micrograms/rat/day in this study. Promoting mechanisms and the problem of statistically estimating recommended daily iodine intake range are briefly discussed.

initiator and iodide in the region of 0.760 and 3 mg/day. No detectable excess was found in initiated animals given iodide at 2.6 micrograms per day. For comparison, the animals given the highest inhalation exposures of MeI (60 ppm) are likely to have inhaled about 17 mg of iodide per day on the 5-7 days/week when they were exposed given a 8.3 liter/hour breathing rate for mature .625 kg rats. Therefore the iodide amounts taken in are in a range that has previously been associated with promotion, although iodide has not been observed to be a complete carcinogen even at these high levels.

31. *p. 176*, Vol. I. ADAF discussion: actually the result of application of EPA's ADAF is a factor of 1.6, slightly less than the 2 reported here, although perhaps the difference is due to rounding. Rounding is not helpful here.

Mechanisms of Oncogenicity

General Comments

1. The extensive input from the SRC regarding oncogenicity has not been put to as good use as it might have been in the revision. Although the SRC comments have been preserved as an appendix, it would be good to see more of this content incorporated in the body of Vol. I. From a practical point of view, a reordering of the existing text regarding mechanism of action would be preferred. By retaining the format of presentation beginning with non-alkylating mechanisms first, the document may unintentionally seem to promote this pathway above the far more likely alkylating mechanism of action endorsed strongly by the SRC as being more consistent with the literature.
2. The revised document claims that the MOA for MeI-induced thyroid tumors "involves the perturbation of thyroid function," while a genotoxic MOA is "plausible". This view understates the role of DNA alkylation and mutagenicity in the carcinogenicity of MeI.
3. DPR's report did not comment on the inadequacy of the mouse oncogenicity study.
4. Please see the appendix for thorough comments from Dr. Ed Loechler on oncogenicity.

Specific Critiques

1. Regarding conventions: Endocyclic ring atoms have their numbering on-line (e.g., N7G), while exocyclic ring atoms have their numbering superscripted (e.g., O^6G). For S_N2 reactions, the "N" is subscripted and the "2" is on-line.
2. *p. 10, Vol. I.* What does the following sentence mean: "These are below the 1 ppb level that protects against excess iodide from MeI beyond iodide exposure standards."
3. *p. 10, Vol. I.* "The effects of greatest concern are death of fetuses as a result of maternal exposure, nasal effects and neurotoxicity observed in adult animals, and perturbation of thyroid function in adult and fetal animals." Carcinogenesis should at least be among the list of "effects of greatest concern." Additionally the SRC believes the implications of the fetal growth restriction observations, including a possibility of infant mortality changes, deserve mention.
4. *Page 24, line 21, Vol. I.* Should note that formaldehyde is a known human carcinogen.

5. *p. 68,* Vol. I. What does the following sentence mean: "the investigators considered the non-significant increase in follicular cell tumors in 600-ppm males...to be treatment-related." This seeming contradiction continues on p. 69 with the sentences: "...fibroma in the cervix in high dose females was treatment related cannot be dismissed at this time." Vs. "However, uterine and cervical fibromas were no considered treatment-related for reasons similar to those put forth by the pathology Working Group." Could DPR state its own conclusion at this point in the text?
6. *p. 69, Vol. I.* Could DPR weigh in about whether they agree with the following statement by The Pathology Working Group: "5. No known clinical or biological significance for these fibromas in animals or humans." It is the understanding of the SRC that, while not malignant, these types of fibromas in humans can be extraordinarily painful and unpleasant, and require surgery and recovery to rectify.
7. *Page 69, Vol. I.* Comments are needed on the inadequacy of the mouse oncogenicity study.
8. p. 77, Vol. I. Regarding Arysta's Ames test, DPR states, "This study was considered acceptable to DPR", and yet subsequent sentences seem to indicate otherwise.
9. *Page 128, Vol. I.* The added paragraph is not particularly informative. The systemic distribution and alkylating potential of MeI is demonstrated by the finding of MeI-derived DNA adducts in multiple organs in exposed rats and the depletion of GSH in the kidney and liver, while GSH depletion and formation of S-methylcysteine in the fetus of exposed does demonstrates the placental transfer of MeI.
10. *Page 146-149, Vol. I.* This declaration that the MOA for MeI-induced thyroid tumors "involves the perturbation of thyroid function," while a genotoxic MOA is "plausible" minimizes the likely role of DNA alkylation in the carcinogenicity of MeI. This section needs to be rewritten with greater emphasis on the genotoxic MOA, especially since KI and other iodide salts produced similar thyroid effects (e.g., increased TSH by NaI) but did not induce thyroid tumors. The implication that the difference in thyroid tumor response between MeI and KI might be due to different routes of exposure is not reasonable if the MOA involves iodide-induced perturbation of thyroid function.

Miscellaneous

1. DPR still defends the use of serum iodide as the dose metric for fetal death.
2. A graphical presentation of all of the HEC/PODs and derived RfCs along with estimates of various human exposures scenarios would be very informative. Such a graph would illustrate the multiple effects of MeI, the magnitude of difference between the most sensitive endpoint and other induced effects, MOEs, and the relationships between estimates of human exposure and derived values of daily exposures that are considered to be without appreciable risk of non-cancer effects during a lifetime (RfC).
3. *p. 24, line 22, Vol. I.* The SRC believes the reader needs to be given some implication for this finding. A suggestion would be to say, "therefore sulfhydryl conjugation may not be completely detoxifying. It results in the formation of at least one reactive metabolite (formaldehyde) that might later be shown to be a mediator of damage to some tissues.
4. *p. 28, Vol. I.* The SRC does not see the value of this paragraph as a summary of the previous material.
5. *p. 162, line 24, Vol. I.* Change 4×10^{-4} to 1.4×10^{-4}.

6. *p. 166, lines 10-12, Vol. I.* This sentence needs clarification.
7. *p. 129, Vol. I.* DPR refers to *Inhalation Toxicology*, in which Arysta has published their experimental results, as a "peer-review journal". It may be "peer-review" in form but it is not peer-reviewed in substance. Characterizing *Inhalation Toxicology* as "peer-review" is a travesty and an insult to journals that truly are peer-reviewed in the true sense of the phrase. An article the SRC read carefully (Mileson et al., *Inhalation Toxicology* :583) was received on October 15, 2008 and accepted on October 31, 2008. It is unprecedented for true peer-review, which is independent, anonymous and objective, to be completed in sixteen days. Furthermore, in reading Mileson et. al., it was clear that the review could not possibly have been objective, both because of the lack of honest reflection on the data and because of the lack of scholarship as demonstrated by missing references and bias in the evaluation of the findings in references. The SRC notes that most of the other Arysta-sponsored articles in *Inhalation Toxicology* were submitted on-or-about October 15, 2009 and were accepted on-or-about November 4, 2009. DPR should not acknowledge that articles published in *Inhalation Toxicology* are truly "peer-reviewed."

Appendix
Comments by Dr. Ed Loechler of the SRC

1. SUGGESTIONS REGARDING DPR'S PRESENTATION:

1. DPR should abandon the term "antithyroid MOA" to describe the class of compounds that increase TSH, which acts as a thyroid tumor promoter on thyroid cells that have initiated (i.e., "transformed"). The term "thyroid tumor promoter" should be used.

While the term "antithyroid" is favored by the USEPA, it is vague and even arguably deceptive. "Antithyroid" is phenomenological, and it gives the impression of a nebulous mechanism of action involving general disruption of thyroid function, and it does not include a key word like "tumor" or "cancer." In most cases these "antithyroid" compounds have been shown to elevate TSH, thus promoting thyroid proliferation, which then promotes mutagen-initiated thyroid tumors. In some cases, "antithyroid" compounds can lead to thyroid tumors without the inclusion of a thyroid mutagen, which probably involves tumor initiation via spontaneous mutagenesis and then promotion by the "antithyroid" compound.

The term "thyroid tumor promoter" MOA is more mechanistic and is consistent with how the majority of scientists describe the functioning of this class of thyroid tumorigens.

In this document, however, I will use the term "antithyroid" for the purposes of communication.

2. If DPR agrees with my arguments in Section 2 (below) that the antithyroid MOA is unlikely, then I make some recommendations at the end of Section 2. If DPR rejects the arguments in Section 2 (below), then I would suggest the following.

The presentation of thyroid cancer risk in the first ten pages of Volume 1 is obtuse in several ways.

(1) While it is implied, nowhere in the first ten pages is it clearly stated that two MOAs were judged to be plausible for thyroid cancer, and that a risk assessment was made for each.

<u>Recommendation</u>: At several key junctures when thyroid cancer risk is discussed in the first ten pages, this should be addressed. For example the sentence on p3, beginning "The highest cancer incidence..." should be changed as follows. "The highest cancer incidence is associated with thyroid tumors, and two MOAs were considered plausible: "genotoxic MeI initiation" and "MeI thyroid tumor promotion."

(2) Nowhere in the first ten pages is it clearly stated that the final cancer risk assessment was based on the genotoxic MOA, because of its greater risk; i.e., nowhere in the introduction is there language like on p152 "...the need to reduce exposure will be based on the approach that showed the higher risk value..."

Recommendation: On p3, after the sentence mentioned in (1) above, add a sentence: "Though two MOAs were plausible, the genotoxic MOA is appropriate for exposure reductions since it showed the higher risk value."

Recommendation: Amend the sentence on p10, which begins: "Lifetime exposure of workers..." To: "Concerning thyroid cancer risk, the need to reduce exposure is based on the approach that showed the higher risk value, which was the genotoxic MOA, and thus, lifetime exposure of workers..."

(3) I am concerned with potential perceptions given DPR's presentation of two MOAs for thyroid tumors. One can imagine a reader misrepresenting this document by stating: "The genotoxic MOA is probably wrong--after all the USEPA concluded that it is unlikely, and, thus, the antithyroid MOA is more likely to be correct, so there is no reason to be concerned about a cancer risk from MeI since the antithyroid MOA has a threshold that can be met in the field.

Recommendation: Add two sentences, probably at the end of p10. "It is important to note that, even if the non-genotoxic mechanism were responsible for MeI induced thyroid tumors, MeI's genotoxic potential cannot be dismissed, because a genotoxic MOA is likely for the induction of astrocytomas and cervical/uterine fibromas by MeI. Furthermore the genotoxic potential of MeI is well-established in the scientific literature, where MeI has been shown to react with DNA to form a well-established premutagenic DNA adduct, MeI has consistently tested positive in many mutagenesis assays (as conducted by many investigators over many years), MeI can transform eukaryotic cells in culture, and MeI has been shown to be tumorigenic in other animal test systems."

3. DPR should consider reorganizing its section on genotoxicity to follow a more logical progression: chemistry -> adducts -> mutations -> transformation -> cancer. The following sections (in this order) would make more sense.

1. Alkylation chemistry, and why MeI should behave like other known methylating mutagens/carcinogens based upon chemical analogy.

2. Evidence that MeI reacts with DNA to form DNA adducts.

3. Indirect evidence for MeI adduct formation based upon tests showing that MeI induces cellular responses that are known to be induced by DNA damage, including: induction of the SOS response in *E.coli*, the induction of homologous recombination in yeast, the induction of DNA repair synthesis in human lymphocytes and the induction of chromosome aberrations in CHO cells and fragile X.

4. Studies showing MeI is mutagenic in (i) bacteria and (ii) eukaryotic cells.

5. Studies showing that MeI can transform cells in culture.

6. Studies showing that MeI is tumorigenic.

2. ANTITHYROID MOA FOR MeI IS LESS LIKELY THAN GENOTOXIC MOA

2.1 INTRODUCTION

Compounds in the antithyroid category of thyroid tumorigens have diverse mechanisms of action, but they all converge on the same effect: they perturb some aspect of the Hypothalamic-Pituitary-Thyroid axis and elevate TSH levels, which stimulates thyroid proliferation. In the literature there is agreement that TSH is acting as a thyroid tumor promoter in the classic "initiation/promotion" model of tumorigenesis as originally proposed by Rous and Kidd, and elaborated by Berenblum. Elevated TSH can promote both genotoxin-initiated thyroid cells (e.g., following treatment with a thyroid mutagen like DHPN or MNU) or can promote spontaneously initiated thyroid cells (i.e., those initiated via spontaneous mutagenesis). For example, iodide deficiency leads to low T3 levels, which stimulates elevation of TSH, while deiodinase inhibitors, like erythrosine, inhibit the T4-to-T3 conversion, which also lowers T3 and leads to elevated TSH.

In such studies, antithyroid compounds, like erythrosine or a low iodide diet, lead to a relatively rapid increase in TSH level in all treated animals, and these levels remain elevated for as long as the animals are treated. If MeI is acting by an antithyroid model, then all rats treated with (e.g.) 60ppm MeI should have elevated TSH levels, which should cluster around a mean that is higher than the TSH level in untreated animals.

There is a second model. MeI could be inducing a genetic change that results in high TSH levels. This model leads to different predictions. (1) Some MeI treated animals will still show relatively normal TSH levels, because they have not yet undergone the genetic change that leads to high-TSH. (2) If elevated TSH is a genetic change along the path to disease, then high-TSH levels should be more prevalent in diseased animals.

The data discussed below seem more consistent with the second model involving a MeI-induced genetic change leading to high-TSH. By saying this I do not mean to imply that the data prove that the genetic model is correct and the antithyroid model is wrong. A hypothesis driven research program would be required to distinguish between these mechanisms more definitively. The best that one can do with the data in hand is to try to evaluate which of the two models seems best able to rationalize the available data, and, thus, to try to establish which model is better supported by the weight of the evidence. I think the weight of the evidence is tipped toward the model that MeI induces elevated TSH levels by causing a genetic change and not by an antithyroid effect. If true, then this suggests that MeI is capable of causing thyroid tumors by a gentoxic mechanism.

The data in this analysis come from Table 22 (p65) and Table 23 (p66) in the DPR's December 2009 Report, along with additional data, some of which is given in two tables at the end of this Section.

2.2 ANALYSIS

While rats treated with 60ppm MeI have elevated TSH on average (Table 22, p65), the animals do not respond uniformly to MeI. TSH levels in individual MeI treated rats vary widely. At 52 weeks for animals with thyroid effects (Table 23, p66), TSH levels vary by ~40-fold (1.18, 2.72, 3.04, 3.20, 9.92, 13.52, 13.80, 18.12, 26.02 and 48.30. At 104 weeks for animals with thyroid effects (Table 23, p66), TSH levels vary by ~20-fold (2.27, 2.30, 2.62, 6.23, 10.53, 11.24, 20.41, 32.33, 36.86 and 50.40). Variability of this magnitude does not seem consistent with random fluctuations around a common mean, but rather suggests that an important variable is being overlooked.

Table 1 (next page) shows individual male rats with adenomas in the 60ppm MeI treatment group combined for both 52 and 104 weeks for all animals tested for TSH levels (from Table 23, p66). They seem to fall into two groups: (1) [low-TSH/no hyperplasia] and (2) [high-TSH/hyperplasia].

The [low-TSH/no hyperplasia] animals with adenomas seem to have TSH levels that are about normal when compared to untreated (0ppm MeI) and unaffected animals (Table 1), though establishing this would require more animals and a careful evaluation of what the range is for normal TSH levels in untreated (0ppm MeI) and unaffected animals. Establishing a cut-off for the [low/high] TSH border would require a careful analysis (and more data), but I am going to use ~10ng/mL.

Table 1 also shows TSH levels for individual male rats with no thyroid effects in the 60ppm MeI treatment group combined for both 52 and 104 weeks, and all but one were in the [low-TSH/no hyperplasia] category. The mean TSH-level is about the same as for untreated/unaffected control animals.

These data are not consistent with an antithyroid model, where the expectation is that TSH levels would be low in untreated rats, while all rats treated with 60ppm MeI should have elevated TSH levels that cluster around a single higher mean. The data is also inconsistent with the antithyroid model because there are a higher percentage of animals in the high-TSH category for the affected (adenoma) group than in the unaffected group, and there is nothing in the antithyroid mechanism (that I can think of) that can account for this difference. In contrast, this pattern seems consistent with the model that high-TSH is due to a MeI-induced genetic change that is on a pathway to about half--<u>though not all</u>--of the adenomas (see below). Furthermore, half of the adenomas have both high-TSH and hyperplasia, as if both are required in this pathway of disease (see below).

TABLE 1: Male Rats, Either Treated with 60ppm MeI or Untreated (0ppm MeI)

60ppm/Adenomas		60ppm/No Effects		0ppm/Adenomas		0ppm/No Effects	
Hyperplasia	TSH(ng/mL)	Hyperplasia	TSH(ng/mL)	Hyperplasia	TSH(ng/mL)	Hyperplasia	TSH(ng/mL)
no	3.04	no	4.06	no	1.44	no	2.5 +/- 1.2**
no	2.62	no	8.32	no	??	no	2.2 +/- 0.9**
no	2.30	no	0.88			no	2.4 +/- 1.1**
no	2.27	no	2.18				
mean	2.6+/-0.3	no	1.28				
		no	4.68				
yes	13.80	no	3.78				
yes	50.40	no	1.58				
yes	11.24	no	2.14				
yes	10.53	no	3.38				
yes	48.40	no	1.83				
mean	26.9+/-18	no	1.31				
		no	2.08				
		no	7.95				
		no	0.92				
		no	11.20*				
		mean*	3.1 +/-2.3				

"mean*" indicates that the 11.20 value was excluded from the calculation. Values with ** are means at 26, 52 and 104 weeks and not data for individual animals.

Regarding untreated (0ppm MeI) male rats, two had adenomas, and both had no hyperplasia with one showing low-TSH, while the second was not tested for TSH (Table 1).

Table 2 shows the number of male rats with adenomas at 104 weeks in several categories. Unfortunately, not all individuals were tested for TSH levels at 104 weeks, or some were tested

but at some other time. Animals with adenomas in the [high-TSH/hyperplasia] category are only observed in rats treated with 60ppm MeI, which suggests that the [high-TSH/hyperplasia] category is MeI-induced.

If we assume that low-TSH is coupled to no-hyperplasia, then [low-TSH/no hyperplasia] rats are [2/2/4/6] for [0/5/20/60ppm]. This suggests that the [low-TSH/no hyperplasia] category of adenomas can occur spontaneously, though the slight increase with increasing MeI dose may indicate that MeI can also induce this category of adenoma, but this cannot be settled statistically.

I note that with 60ppm MeI treated animals at 52 weeks, both categories of adenomas were also observed: two animals were in the [high-TSH/hyperplasia] category and one was in the [low-TSH/no hyperplasia] category (see the Table at the end of this Section).

TABLE 2: Number of Rats with adenomas that are at 104 weeks considering hyperplasia and TSH levels.

	Males ppm				Females ppm			
	0	5	20	60	0	5	20	60
[low-TSH/no hyperplasia]	1	1	4	3		0	0	0	1
[????-TSH/no hyperplasia]	1	1	0	3		1	1	0	0
[high-TSH/hyperplasia]	0	0	0	3	0	0	0	1
[????-TSH/hyperplasia]	0	0	0	1	0	0	0	0

Admittedly, there are not enough animals in Table 2 to make definitive statements, but the patterns are suggestive of two pathways to the formation of thyroid adenomas.

The fact that all animals with adenomas are either [low-TSH/no hyperplasia/adenoma] or [high-TSH/hyperplasia/adenoma] and that no animals with adenomas are either [low-TSH/hyperplasia/adenoma] or [high-TSH/no hyperplasia/adenoma] suggest the following two pathways to adenomas.

Pathways to Adenomas

1. Normal → adenoma

2. Normal → [hyperplasia] → [high-TSH/hyperplasia] → [high-TSH/hyperplasia/adenoma]

Normal → [high-TSH] → [high-TSH/hyperplasia/adenoma]

Regarding Pathway 2, intermediates would be expected. Of the 16 animals in the 60ppm MeI treatment group with no thyroid effects (Table 1), one has high-TSH and might be an intermediate in Pathway 2. Of animals with hyperplasia, but without adenomas (or carcinomas) (Table 3), four have low-TSH and four have high-TSH, and each might be an intermediate in Pathway 2. (I note that a TSH value like 6.23 and 9.92 might be elevated and might indicate that I have not chosen a proper [low/high] cutoff.)

The data in Table 3 seem inconsistent with the expectations for the antithyroid MOA, because, once again, TSH levels in the 60ppm MeI treatment group should be higher than control, but clustered around a single mean.

TABLE 3: Numbers of Male Rats treated at 60ppm MeI with Hyperplasia having low-TSH vs. high-TSH

Hyperpasia	TSH(ng/mL)
yes	2.72
yes	3.20
yes	6.23
yes	9.92
yes	13.52
yes	26.02
yes	18.12
yes	32.33

The same kind of analysis can be applied to female rats (Table 2), though, admittedly, the numbers are so low that statistically meaningful statements are impossible. The [low-TSH/no hyperplasia] category for female adenomas appears to be MeI-dose independent (1/1/0/1 for 0/5/20/60ppm, Table 2), while the only [high-TSH/hyperplasia] adenoma is observed in the 60ppm group. Though this line of thinking for females is not statistically significant, the pattern is reminiscent of males, except that females have fewer spontaneous adenomas and fewer induced adenomas.

No data permits an assessment of whether high-TSH levels in some animals depends on a genetic change alone or whether it depends on a genetic change plus sustained MeI treatment, and, if so, whether cessation of MeI treatment would lead to cessation of adenoma growth. Whichever is the case, however, both require MeI to be acting as a genotoxin to induce several critical genetic changes that are important to adenoma formation.

2.3 AN ALTERNATIVE EXPLANATION

The analysis in Section 2.2 is based on pattern association, and without a mechanistic investigation into cause-and-effect, one could argue that, in fact, there is only one pathway to thyroid adenomas, which requires neither hyperplasia nor an increase in TSH levels, and the fact that some thyroid adenomas have hyperplasia and high-TSH is merely fortuitous and not mechanistically important to the disease pathway. However, this line of thinking also argues against an antithyroid mechanism, because it argues that high-TSH levels are not mechanistically important to MeI-induced thyroid adenomas, and, thus, that the genotoxic mechanism is more likely.

It is important to point out that--though the data do not allow a definitive decision about whether hyperplasia and high-TSH contribute mechanistically to some of the MeI-induced thyroid adenomas--hyperplasia is often viewed as an intermediate in the pathway to tumorigenesis, and elevated TSH is known to contribute to thyroid tumorigenesis. Thus, it is not unreasonable to imagine a thyroid adenoma pathway with hyperplasia and high-TSH as mechanistic intermediates.

2.4. RECOMMENDATIONS:

In conclusion, the high variability in TSH levels in rats treated with 60ppm MeI is not consistent with the antithyroid MOA, which should give elevated TSH levels in all animals. Rather for at least some of the animals, the data seem more consistent with MeI treatment inducing a type of adenoma in some animals that requires a series of mutational genetic changes leading to hyperplasia and high-TSH. The latter pathway is consistent with MeI acting as a genotoxin, and, thus, the "genotoxic" MOA seems more likely to be operating for MeI-induced thyroid tumors. Once again, I emphasize that these data and this analysis does not constitute proof that the genotoxic mechanism is operating; it is simply easier to rationalize the data (e.g. the high-TSH levels) with a genetic/mutational mechanism than with an antithyroid mechanism. Of course, numerous unanswered mechanistic questions remain, such as what genes in what tissues are being mutated and can these mutations be shown to be causative. Nevertheless, the weight of the evidence seems more consistent with a genotoxic MOA.

1. Both the genotoxic MOA and the antithyroid MOA should be presented in DPR's report.
2. An analysis like the one above should be presented to argue that the genotoxic MOA is better supported by the weight of the evidence and that the antithyroid MOA is unlikely.
3. Risk assessment should be presented only based on the genotoxic MOA and all discussions (e.g.. in the first ten pages) should clearly state that, while two MOAs were considered, the antithyroid MOA seems unlikely, while the genotoxic MOA is favored by the weight of the evidence.

ADENOMAS in MALES

#	SEX	MeI	Wk*	AD	CA	HYP	TSH	T3	rT3
3361	M	0	104	+	-	no	1.44	56.01	0.05
3356	M	0	104	+	-	no			
3492	M	5	26	+	-	no	5.64	61.62	QNS
3246	M	5	104	+	-	no	4.65	66.49	0.05
3205	M	20	104	+	-	no	4.67	21.35	0.00
3236	M	20	104	+	-	no	3.19	37.70	0.06
3427	M	20	104	+	-	no	1.33	47.12	0.05
3487	M	20	26	+	-	no	2.76	86.26	QNS
repeat			104			no	1.99	32.09	0.05
3222	M	60	52	+	-	no	3.04	40.50	
3371	M	60	104	+	-	no	2.62	24.00	0.04
3374	M	60	104	+	-	no	2.30	32.58	0.05
3234	M	60	104	+	+	no	2.27	41.36	0.05
3442	M	60	52	+	-	yes	48.40	37.96	
3509	M	60	52	+	-	yes	13.80	27.04	
3331	M	60	104	+	-	yes	50.40	54.93	0.13
3412	M	60	104	+	-	yes	11.24	58.02	0.18
3338	M	60	104	+	+	yes	10.53	44.54	0.12
repeat			104				28.37	67.55	0.26
3439	M	60	104**	+	-	yes			
3359	M	60	85**	+	-	no			
3216	M	60	75**	+	-	no			

CARCINOMAS in MALES

3460	M	0	104	-	+	no	4.77	25.58	0.00
3356	M	0		-	+	no			
3282	M	60	104	-	+	no	36.86	47.61	0.14

HYPERPLASIAS in MALES

3235	M	5		-	-	yes			
3322	M	60	52	-	-	yes	2.72	55.62	
3438	M	60	52	-	-	yes	3.20	23.12	
3369	M	60	52	-	-	yes	9.92	40.92	
3269	M	60	52	-	-	yes	13.52	53.70	
3484	M	60	52	-	-	yes	26.02		0.18
3244	M	60	52	-	-	yes	18.12	QNS	QNS
3283	M	60	104	-	-	yes	6.23	31.50	0.11
3286	M	60	104	-	-	yes	32.33	37.48	0.02
3220	M	60	104	-	-	yes			
3310	M	60	104	-	-	yes			
3373	M	60	104	-	-	yes			
3292	M	60	104	-	-	yes			

NO EFFECT in MALES

3219	M	60	52	-	-	no	4.06	43.10	0.12
3230	M	60	52	-	-	no	8.32	48.56	QNS
3268	M	60	52	-	-	no	0.88	32.12	QNS
3271	M	60	52	-	-	no	2.18	35.08	QNS
3330	M	60	52	-	-	no	11.20	43.14	QNS
3351	M	60	52	-	-	no	4.68	QNS	0.21
3402	M	60	52	-	-	no	3.78	12.8	0.24
3405	M	60	52	-	-	no	1.58	46.4	QNS
3453	M	60	52	-	-	no	2.14	QNS	0.14
3479	M	60	52	-	-	no	3.38	28.96	0.24
3221	M	60	104	-	-	no	1.83	35.77	0.05
3324	M	60	104	-	-	no	1.31	91.45	0.07
3388	M	60	104	-	-	no	2.08	36.54	0.03
3398	M	60	104	-	-	no	7.95	48.12	0.02
3401	M	60	104	-	-	no	0.92	43.53	0.04
3456	M	60	104	-	-	no	1.28	44.56	0.05

Wk* indicates weeks at which the hormone levels were assayed. Wk** is when rat died or was sacrificed.

ADENOMAS in FEMALES

#	SEX	MeI	Wk	AD	CA	HYP	TSH	T3	rT3
3549	F	0		+	-	no			
3847	F	5	26	+	-	no	1.38	29.76	0.09
3763	F	60	104	+	-	no	1.11	31.29	0.23
3817	F	60	104	+	-	yes	28.37	67.55	0.23

CARCINOMAS in FEMALES

3613	F	0	104	-	+	no	2.04	73.31	0.05
3704	F	20	104	-	+	no	1.83	88.45	0.51
3871	F	60		-	+	?			
3579	F	60		-	+	?			

HYPERPLASIAS in FEMALES

3560	F	5	104	-	-	yes	4.85	104.27	0.07
3756	F	5		-	-	yes			
3729	F	60	104	-	-	yes	4.17	71.72	0.24
3813	F	60	104	-	-	yes	3.69	63.95	0.13

NO EFFECT in FEMALES

3585	F	60	104	-	-	no	1.43	57.58	0.46
3628	F	60	104	-	-	no	3.56	88.58	0.32
3639	F	60	104	-	-	no	6.85	81.08	0.22
3640	F	60	104	-	-	no	1.07	87.55	0.35
3659	F	60	104	-	-	no	1.53	94.31	0.44
3663	F	60	104	-	-	no	1.31	33.32	0.17
3669	F	60	104	-	-	no	1.73	38.78	0.12
3678	F	60	104	-	-	no	0.85	76.11	0.49
3710	F	60	104	-	-	no	3.94	37.34	0.16
3743	F	60	104	-	-	no	2.23	86.99	0.21
3818	F	60	104	-	-	no	1.67	52.58	0.09
3824	F	60	104	-	-	no	1.61	82.10	0.21
3844	F	60	104	-	-	no	2.56	32.31	0.15
3861	F	60	104	-	-	no	4.01	83.57	0.13

3. OTHER SIGNIFICANT CONCERNS

3.1. On p149, DPR states: "The relative reactivity of MeI to alkylate DNA compared to endogenous agents is unknown since the study (Gansewndt 1991) did not include a control group."

DPR is casting doubt on whether the findings in Gansewendt 1991 actually show that the methyl groups in N7MeG and O^6MeG came from MeI alkylation of the N7G and O^6G position. Gansewendt 1991 showed that macromolecular ^{14}C-methyl groups from MeI must have entered the C1-pool, which were subsequently incorporated into nucleotides and then into macromolecular DNA. It is theoretically possible that endogenous methylating agents reacted at N7G and O^6G in this ^{14}C-DNA, and this is the source of radiolabeled N7MeG and O^6MeG. Unfortunately, the experimental approach taken by Gansewendt 1991 did not allow them to determine the levels of N7MeG and O^6MeG in an untreated control group.

It is well-known that endogenous methylating agents give rise to such low levels of N7MeG and O^6MeG that they are virtually undetectable. One classic set of papers consistently established

that endogenous O^6MeG levels are <0.05fmol/mg DNA in rat livers and leukocytes. In Gansewendt 1991, O^6MeG following MeI treatment was 100-400 fmol/mg DNA in rat livers (and higher in lung, stomach and forestomach), which was, thus, >2000-fold higher than the endogenous O^6MeG levels in rat livers. Thus, it is extremely unlikely that Gansewendt 1991 was detecting O^6MeG caused by endogenous methylation.

Souliotis VL, Valavanis C, Boussiotis VA, Pangalis GA, Kyrtopoulos SA. Comparative dosimetry of O6-methylguanine in humans and rodents treated with procarbazine. Carcinogenesis. 1994 15:1675-80.

Souliotis VL, Chhabra S, Anderson LM, Kyrtopoulos SA. Dosimetry of O6-methylguanine in rat DNA after low-dose, chronic exposure to N-nitrosodimethylamine (NDMA). Implications for the mechanism of NDMA hepatocarcinogenesis. Carcinogenesis. 1995 16:2381-7.

Souliotis VL, Valavanis C, Boussiotis VA, Pangalis GA, Kyrtopoulos SA. Comparative study of the formation and repair of O6-methylguanine in humans and rodents treated with dacarbazine. Carcinogenesis. 1996 17:725-32.

3.2. DPR continues to claim that male rats have higher TSH than females at 52 weeks, which is based on a statistical analysis (Mann Whitney U-test) for data in Table 21 (p64). While statistical analysis may suggest this is true the values being used for averaging vary by over 40-fold; e.g., at 52 weeks for animals with tumors: 1.18, 2.72, 3.04, 3.20, 9.92, 13.52, 13.80, 18.12, 26.02 and 48.30. Variability of this magnitude is indicating more than mere random fluctuation around a common mean; in fact, such variability suggests that an important variable, which is dominant, is being overlooked. While TSH levels may be higher in MeI treated animals than in the controls, it is unlikely that anything can be concluded about whether TSH levels are higher for males than females.

3.3. p146 "However, there were uncertainties in the results of these older studies because the reports did not provide sufficient details on the methods or results, and the use of a mouse strain known to be sensitive to chemical inducers of lung tumors (Maronpot *et al*., 1986)." DPR should strike "...use of a mouse strain known to be sensitive..." The significance of the Poirier study is being underestimated. One key question is whether MeI might only cause thyroid tumors because of a non-genotoxic antithyroid MOA. Other studies show MeI's genotoxic potential, but The Poirier study establishes that MeI can indeed cause tumors in a system that has no potential confusion from a non-genotoxic "anti-organ" mechanism, since there no reason to think that MeI has any kind of "antilung" effect. The fact that the mice used in this study are prone to lung tumors (probably because some lung tumor gene is already mutated in this strain) is of little importance, except if this study were being used for a quantitative assessment. This criticism would be analogous to suggesting that mutagenesis test results with the Ames strains should be considered suspect because the Ames strains have been altered to enhance *Salmonella*'s sensitivity to mutagenic DNA damage. The fact that Ames strains are more sensitive than regular *Salmonella* to mutagens does not mean that a chemical without genotoxic potential suddenly acquires genotoxic potential in the Ames strains; the strains are merely more

sensitive to the dectection of genotoxins. The same statement can be made about the Strain A mice used in the Poirier 1975 study.

3.4. In my previous report (Appendix E, Scientific Review Comments Compiled), I pointed out numerous analogies between MeI and both MMS and MNU, in terms of genotoxic endpoints, such as reaction to give m6G, and more importantly that MNU--a methylating agent like MeI--causes thyroid cancer. DPR should consider including this literature in their report.

3.5. I note that DPR rejected the SRC recommendation in the following cases.

a. (p130) DPR rejects "dose metric" and used maternal rather than fetal serum iodide levels for analysis.

b. (p131) DPR thinks that the $BWt^{3/4}$ treatment adequately compensates for interspecies UF, and thus they apply the 3-fold and not 10-fold safety factor as suggested by the SRC, because of the lack of a "clear rationale."

c. (p131) DPR notes that the SRC recommendation to use the dose-metric for MeI rather than for iodide would lead to a less conservative outcome, which was elaborated on p132.

Errata

[Dr. Ed Loechler has placed the "Errata" section before Section 2.1 of the appendix of the second set of comments from the SRC to DPR, though it could be placed elsewhere.]

2. ANTITHYROID MOA FOR MeI IS LESS LIKELY THAN GENOTOXIC MOA

ERRATA:

I have been conflicted and confused about whether MeI might plausibly be a thyroid tumor promoter, for which DPR is using the term "antithyroid compound". In Section 2 of my comments in the appendix of the SRC' second set of comments to DPR I analyzed data and concluded that the antithyroid MOA for MeI was unlikely.

However, since writing Section 2, I noticed and came to appreciate the implications of data for individual rats exposed to 60ppm MeI for at 26 weeks, which is data not in DPR's December 2009 Report, but which I requested and received shortly before the meeting on January 25, 2010. The 26-week data allow me to be comfortable with labeling MeI as a plausible thyroid tumor promoter (an "antithyroid" compound) and, thus, I must amend my previous analysis in Section 2.

Classic thyroid tumor promoters act by causing sustained, elevated TSH, where all treated animals cluster around an elevated mean for TSH levels.

At 26 weeks, TSH levels are clustered around an elevated mean for all individuals treated with 60 ppm MeI (10.72, 17.96, 20.24, 22.88, 32.04, 33.76, 40.8 and 43.48 ng/mL), when compared to normal TSH in untreated rats (~2.5 ng/mL, Table 21). These data are not in DPR's December 2009 Report, but probably should be, as they are the most convincing data to support the notion that MeI can plausibly be considered to act as thyroid tumor promoter, since other studies in the literature have shown that TSH elevation for 26 weeks is long enough for a compound to be active as a thyroid tumor promoter.

In DPR's report, data for individual rats were presented for 52 weeks and 104 weeks (Table 23), and while TSH levels are elevated on average, the values for individual rats do NOT cluster around a single elevated mean; i.e., the TSH values are spread over a huge range, which is indicative of something more complex. It was the data at 52 weeks and 104 weeks that I analyzed in Section 2 of this appendix.

Rats receiving high iodide in their drinking water modulate thyroid iodide intake via down-regulation of their thyroid sodium-iodide symporter (NIS), which leads to a phenomena called "escape from the Wolff-Chaikoff effect." Rats receiving high iodide dosing via MeI inhalation probably do not down-regulate their NIS, and thus, do not "escape from the Wolff-Chaikoff effect," thus resulting in sustained TSH elevation.

What follows (Sections 2.1. - 2.4.) is my analysis of the data at 52 weeks and 104 weeks, which I submitted before I had noticed and appreciated the implications of the data for individual rats treated with 60 ppm MeI at 26 weeks. The analysis that follows is correct in that the data do suggest that there are two pathways leading to the formation of thyroid adenomas. However, the following analysis is NOT correct in its conclusion that MeI is more likely to be acting via a genotoxic mechanism, because the genetic changes that are inferred in this analysis could also have arisen via MeI acting as a thyroid tumor promoter, which stimulated thyroid cell growth and led to the accumulation of spontaneous transforming mutations.

In conclusion, I continue to believe that the genotoxic MOA is plausible for MeI-induced thyroid tumors. However, I now also agree with DPR that the antihyroid MOA (thyroid tumor promoter MOA) is also plausible; i.e., I believe that no data exists that allows one to decide whether the genotoxic MOA or the antithyroid MOA is more probable, and, thus, both MOAs must be considered in the risk assessment.

2.1 INTRODUCTION

Compounds in the antithyroid category of thyroid tumorigens have diverse mechanisms of action, but they all converge on the same effect: they perturb some aspect of the Hypothalamic-Pituitary-Thyroid axis and elevate TSH levels, which stimulates thyroid proliferation. In the literature there is agreement that TSH is acting as a thyroid tumor promoter in the classic "initiation/promotion" model of tumorigenesis as originally proposed by Rous and Kidd, and elaborated by Berenblum. Elevated TSH can promote both genotoxin-initiated thyroid cells (e.g., following treatment with a thyroid mutagen like DHPN or MNU) or can promote spontaneously initiated thyroid cells (i.e., those initiated via spontaneous mutagenesis). For example, iodide deficiency leads to low T3 levels, which stimulates elevation of TSH, while deiodinase inhibitors, like erythrosine, inhibit the T4-to-T3 conversion, which also lowers T3 and leads to elevated TSH.

[Continues on as before.]

Appendix 3

Afternoon notes from the January 25 methyl iodide meeting of the SRC and DPR

Afternoon Session

Tasks agreed upon by the SRC and DPR based on the morning discussion:

1. Comments in balloons now will be put into text where appropriate.
2. There are four areas of exposure modeling/assessment which the value as used could be systematically underestimated. The four values shouldn't be changed in principle finding but an alternative value will be presented for the point of discussion as to what the impact would be on a different approach (numeric value). These four areas are the 50% respiratory protection factor, some level of skin exposure, an increased breathing rate (the SRC suggests using the OSHA rate), and a 10-hour work day. These aren't necessarily the final values put forward by DPR but are alternative calculations that may yield different numbers and are warranted by scientific argument.
3. DPR will discuss in the text whether or not temperature will be a driving factor in the model.
4. DPR doesn't have the science to quantitate the effect of temperature on emission. Therefore they will mention that temperature has an effect but it cannot be quantified. DPR couldn't detect a reasonable pattern with temperature. Soil temperature is a major factor and there is a smaller temp variation. They will add a narrative to cover these points.
5. Small points to address panel comments on Vol. I to the extent that is appropriate.
6. Add a factor of 10 for database uncertainty since the acute neurotoxicity study did not adequately examine neurotoxicity, and there is no neurotoxicity study for repeated exposures. Human exposure showed neurotoxicity after repeated exposures.
7. Regarding Table 6 in Vol. I, currently it is not clear why there are two rows for "Lifetime"/"Thyroid Tumors in rats" and what each row represents. There was also an agreement that these two rows would be reversed in order and that each would have a titled added (something like): Genotoxic MOA (non-threshold) and Antithyroid MOA (threshold).
8. Include comments about the absence of any data on developmental neurotoxicity (as distinct from chronic exposure neurotoxicity in the adult and the high likelihood that MeI would prove to be a developmental neurotoxicant.
9. Genotoxic MOA will be added before thyroid perturbation MOA. Reverse text in all places including tables.
10. Add a paragraph on the genotoxicity that will be stronger and evoke the correlary among the other methylating agents that act at the same site as MeI. Add electrophilic chemistry as appropriate, specifically mention MMU and MMS. (MNU--a methylating agents like MeI--is an established thyroid mutagen. Furthermore, MeI is an S_N2 electrophile, like

MNU and MMS, and all three form the same kind of mutagenic adduct (m6G), where MNU and MMS are both 2A carcinogens according to IARC. This kind of information, along with references, can be found in the Appendix that Dr. Loechler submitted back in November 2009).

11. Take out "reject the PBPK model" sentence and revise. Include DPR rationale for dropping model and using default model.
12. Include inadequacy of the protocol for 18 mo study.
13. When the point of departure is based on BMDL, take out the statements that indicate the use of NOEL for that endpoint for hazard identification.
14. Take out acceptability statement regarding Wagner and Dakoulas (2001) as to emphasize DPR's concerns about the study in agreement with USEPA which called in a "No-test".
15. Linear projection of low-dose extrapolation deserves first mention because the most likely mode of action is direct reaction of MeI with DNA.

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DATE: January 29, 2010

SUBJECT: Responses to Scientific Review Committee Final Comments on Methyl Iodide Risk Characterization Document for Inhalation Exposure (Volume I) for the December 2009 Draft

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The Department of Pesticide Regulation (DPR) submitted to the Scientific Review Committee (SRC) a revised draft of the risk assessment (dated December 2009). For Volume I, the SRC provided initial comments and compiled final written comments (January 21, 2010). On January 25, 2010, DPR staff met with the SRC to discuss the comments and responses, and the specific points for revision in the final document. This memorandum presents our responses to the specific points discussed at that meeting and changes made for the revised document. In addition, the oncogenicity mode of action discussion will be updated with the SRC current position on the MOA which is reflected in the January 21, 2010 comment and the subsequent Errata.

Point #5: Errors and minor clarifications
Small points to address panel comments on Vol. I to the extent that is appropriate.

DPR Response: All errors will be corrected, and other points as appropriate will be revised.

Point #6: Neurotoxicity
Add a factor of 10 for database uncertainty since the acute neurotoxicity did not adequately examine neurotoxicity, and there is no neurotoxicity study for repeated exposures. Human exposure showed neurotoxicity after repeated exposures. .

DPR Response: A 10-fold UF for database deficiency will be added to the benchmark MOE and the calculation of the reference concentrations. This change will be reflected in the text and the appropriate tables.

Point #7: Table 6
Regarding Table 6, currently it is not clear why there are two rows for "Lifetime"/"Thyroid Tumors in rats" and what each row represents. Here was also an agreement that these two rows would be reversed in order and that each would have a title added (something like): Genotoxic MOA (non-threshold) and Antithyroid MOA (threshold).

DPR Response: The table will be clarified.

Point #8: Neurotoxicity
Include comments about the absence of any data on developmental neurotoxicity (as distinct from chronic exposure neurotoxicity in the adult) and the high likelihood that MeI would prove to be a developmental neurotoxicant.

DPR Response: The absence of this type of data and MeI can potentially cause developmental neurotoxicity have already been discussed under section V.C.1. The risk assessment applied a 10-fold additional uncertainty factor for this concern. This factor is applied to the fetal death endpoint because this effect is the most sensitive endpoint as a result of fetal exposure to MeI. We agree with the SRC that fetal death and the potential for developmental neurotoxicity are most likely not of the same MOA. When an effect that has not been tested (i.e. developmental neurotoxicity) but can realistically be expected to be more sensitive than the most sensitive endpoint detected (i.e., fetal death), an additional UF or database deficiency MF (modification factor) is applied to the latter. This default approach does not necessarily imply the same MOA for the two endpoints.

Point #9: **Thyroid tumor mode of action discussion**
Genotoxic MOA will be added before thyroid perturbation. Reverse text in all places including tables.

DPR Response: The SRC recommended putting the genotoxic MOA first because it is more likely than the antithyroid MOA, and effects from a genotoxic MOA (non-threshold) would occur first, before the antithyroid MOA which requires a threshold dose to be reached. DPR maintains that both MOAs are plausible and the data do not indicate which one is more likely than the other. The paragraphs will be rearranged with the genotoxicity MOA as suggested. This would also reflect the greater risk based on genotoxic MOA using the non-threshold approach to calculate the risk.

Point #10: Mutagenicity of other methylating agents
Add a paragraph on the genotoxicity that will be stronger and evoke the correlary among the other methylating agents that act at the same site as MeI. Add electrophilic chemistry as appropriate, specifically mention MMU and MMS. (MNU-a methylating agents like MeI-is an established thyroid mutagen. Furthermore, MeI is an S_N2 electrophile, like MNU and MMS, and all three form the same kind of mutagenic adducts (m6G), where MNU and MMS are both 2A carcinogens according to IARC. This kind of information, along with references, can be found in the Appendix that I [Loechler] submitted back in November 2009.

DPR Response: Additional text about MeI and MMU and MNS with respect to alkylating ability and DNA adduct formation to the induction of thyroid tumors will be added to the genotoxic MOA discussion.

Point #11: PBPK Model
Take out "reject the PBPK model" sentence and revise. Include DPR rationale for dropping model and using default model.

DPR Response: The cited sentences will be revised to explain why the risk assessment change from the use of PBPK model to default method in the calculation of HECs.

Point #12: Mouse oncogenicity study
Include inadequacy of the protocol for 18 mo study

DPR Response: The study duration and potential increased incidences will be added to the review for this study. While the duration of the study met FIFRA guideline requirement of at

least 18 months for mice (USEPA Health Effects Test Guidelines OPPTS 870.4300), it is shorter than the 2 year in the rat inhalation study (Kirkpatrick, 2005).

Point #13: Clarification on the use of NOEL and BMD.
When the point of departure is based on BMDL, take out statements that indicate the use of NOEL for that endpoint for hazard identification.

DPR Response: When the point of departure is based on BMDL, statements that indicate the use of NOEL for that endpoint in the hazard identification will be deleted. These statements were left over from previous draft where only NOELs were used.

Point #14: Clarification on the genotoxicity study
Take out acceptability statements regarding Wagner and Dakoulas, 2001 as to emphasize DPR's concerns about the study in agreement with USEPA which called in a "No-test".

DPR Response: The acceptability statement for this study will be removed. It is inconsistent with the DPR's stated concern on the conduct of the study.

Point #15: *Linear projection of low-dose extrapolation deserves first mention because the most likely mode of action is direct reaction of MeI with DNA.*

DPR Response: (see response to #9)