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.

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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.

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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:

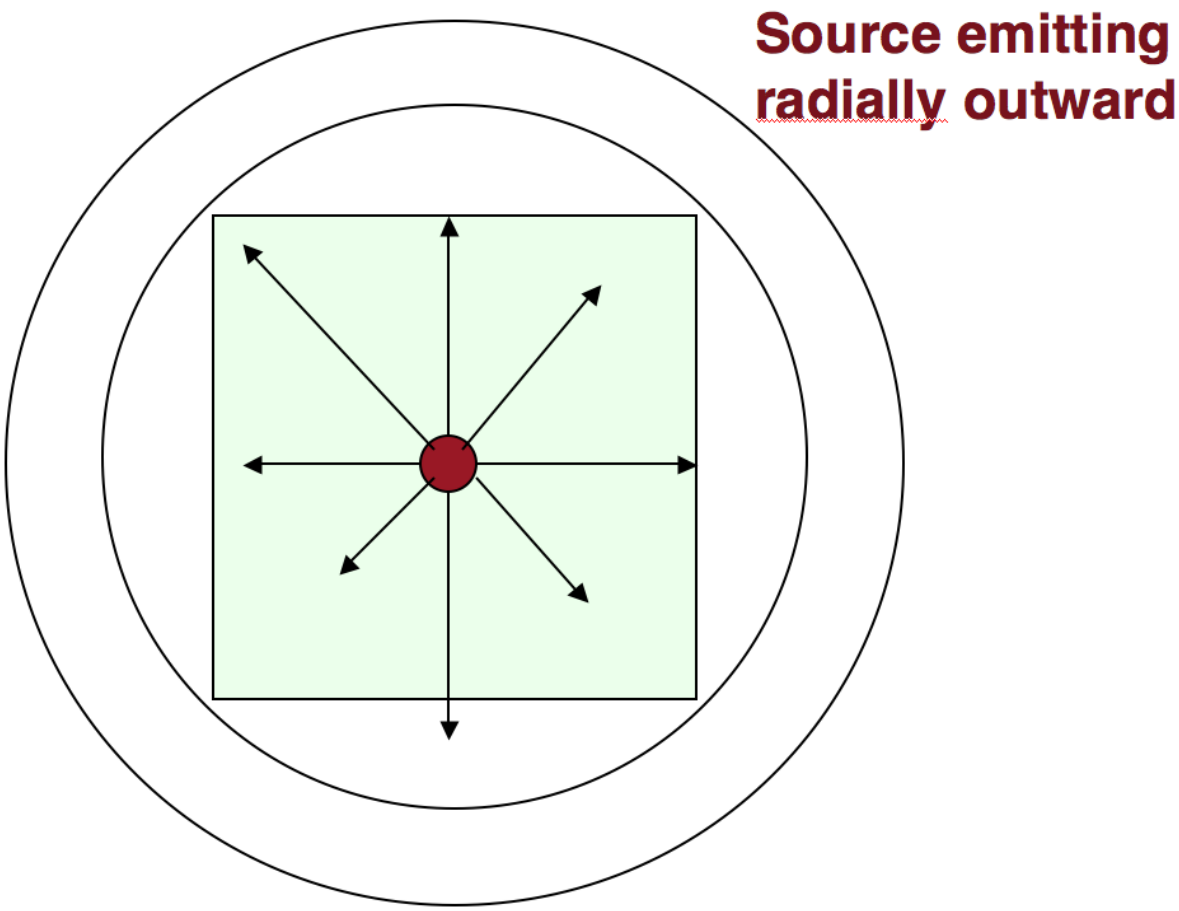
Some ground-truth calculations on air emissions and exposure for MeI
By Dr. Tom McKone

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.

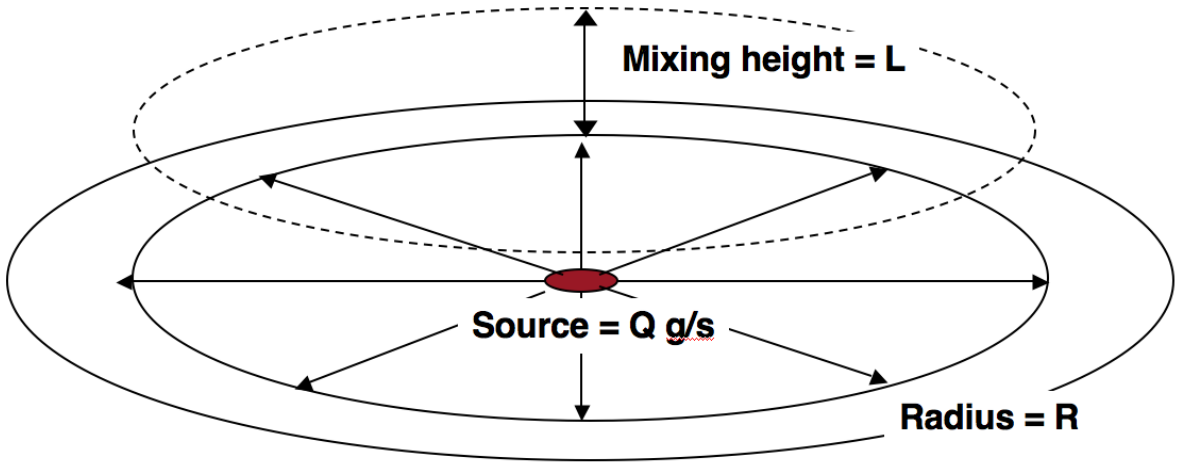
Some of the questions I wanted to address with this approach are:

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**

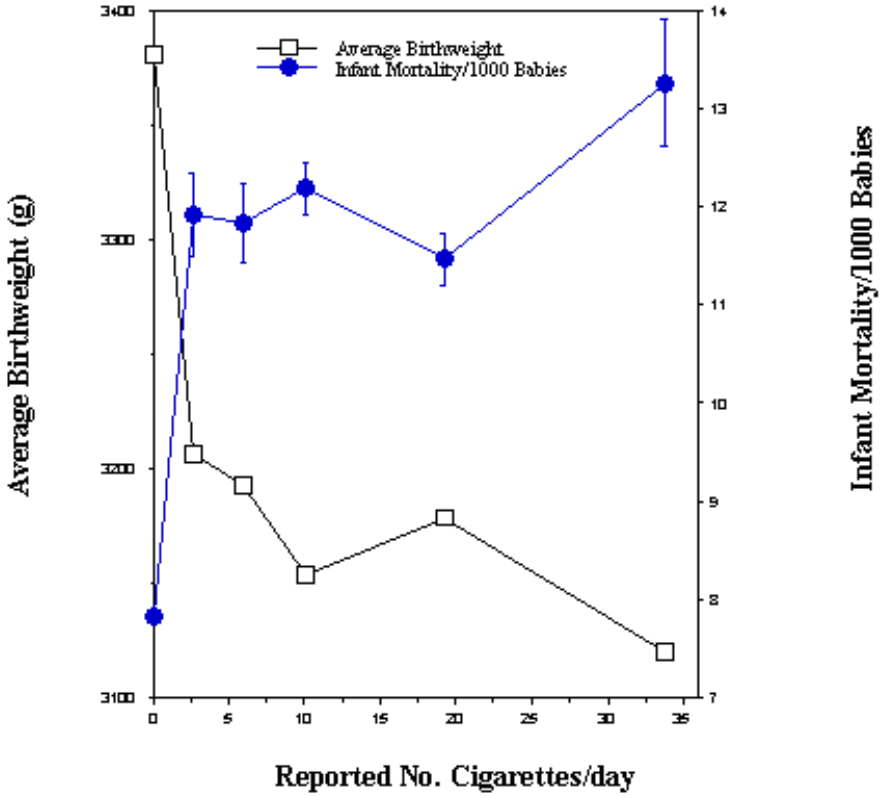
APPENDIX C:

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.

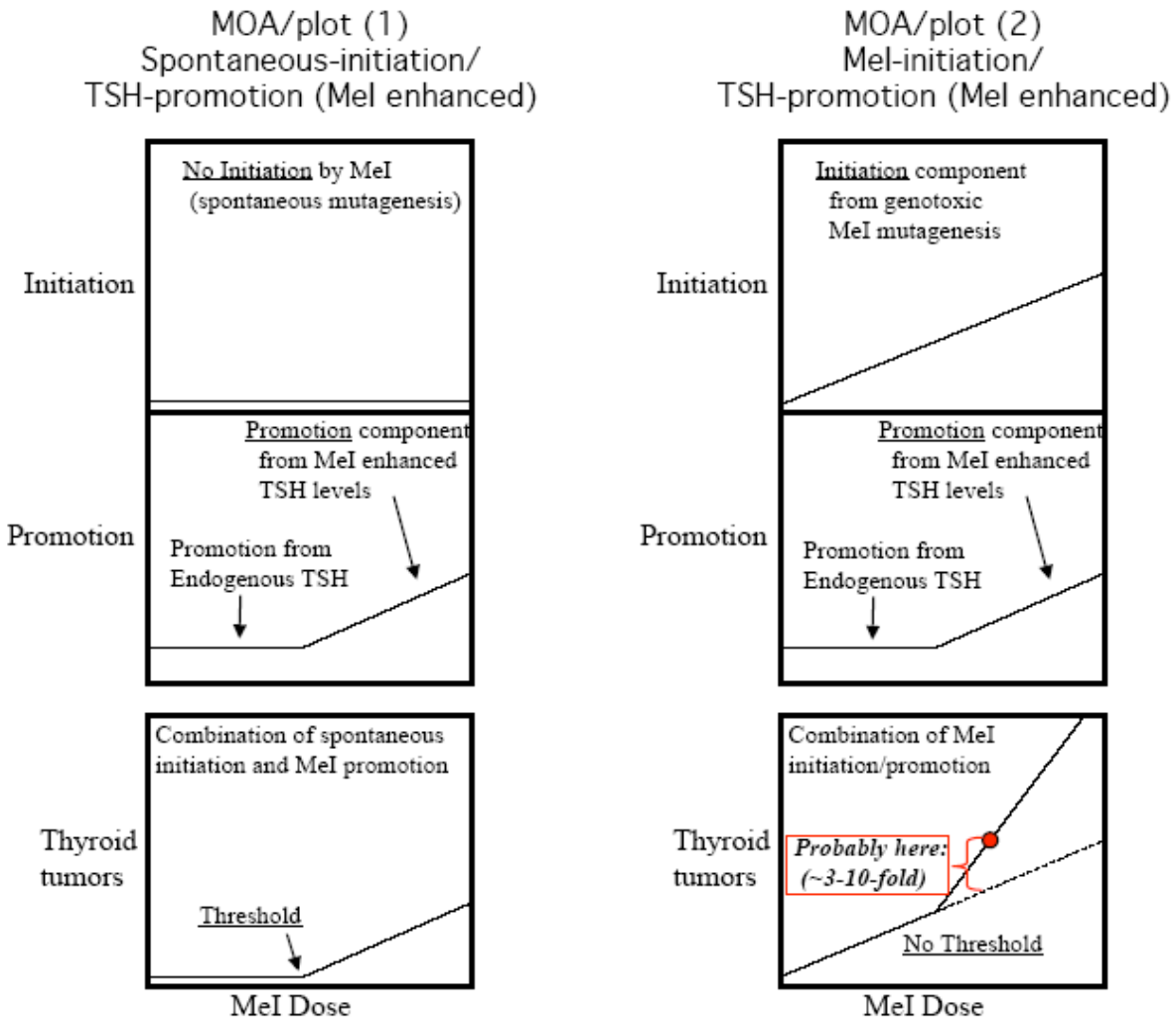
The precise shapes of the dose-response curves for these two MOAs are unknown, but the attached figure shows simplistic versions to demonstrate the principles. Based on evidence

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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