Appendix 2

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

January 2010

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

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

Data Base

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

Exposure Assessment

General Comments

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

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

Specific Critiques

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

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

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

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

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

PBPK Model—Application to Computing HECs

Specific Critiques

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

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

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

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

Neurotoxicity

General Comments

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

Risk Appraisal including Uncertainty Factors

General Comments

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

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

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

Specific Critiques

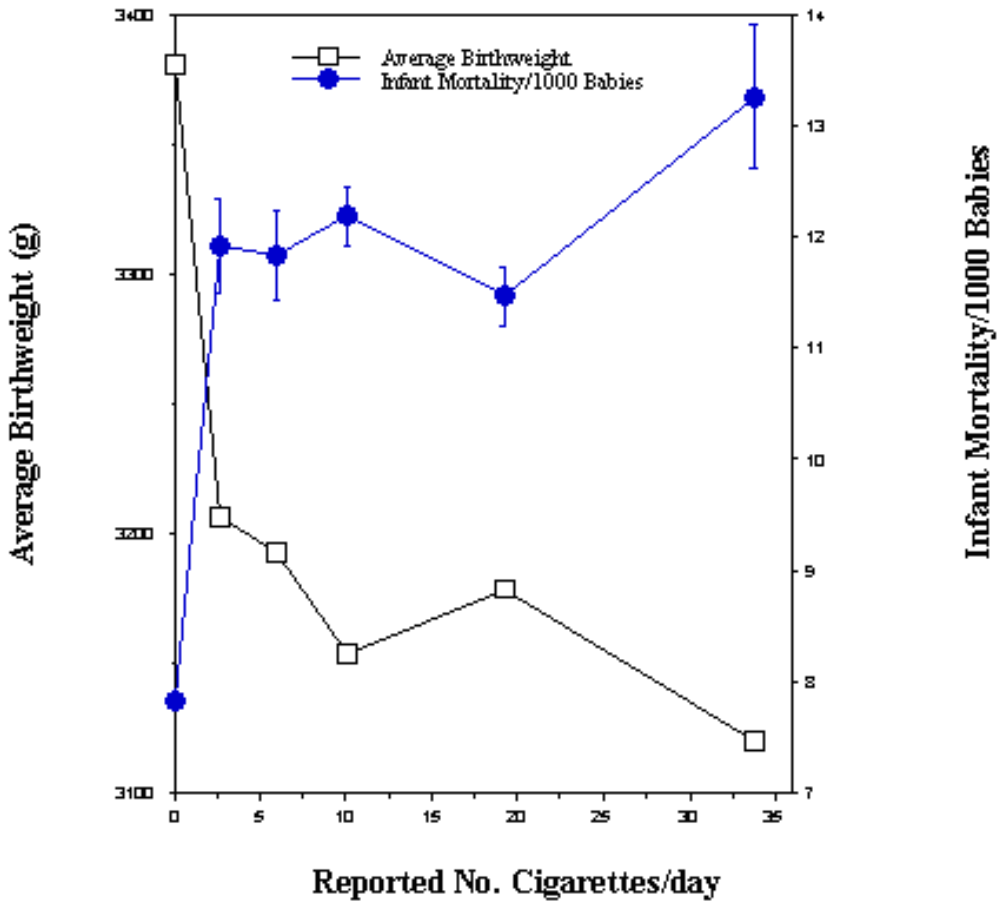
1. *Page 1, line 31, Vol. I.* DPR should provide rationale for selection of a specified level of extra risk for the benchmark response (1%, 2%, 5%, 10%, or a multiplier of the standard deviation) as it pertains to the MeI database. Explain why a multiplier of standard deviation was sometimes used.
2.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

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

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

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

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



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

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

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

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

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

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

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

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[**] **Iodine and Cancer**

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

- The full abstract of this paper is:

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

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

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

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

Mechanisms of Oncogenicity

General Comments

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

Specific Critiques

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

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

Miscellaneous

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

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

Appendix
Comments by Dr. Ed Loechler of the SRC

1. SUGGESTIONS REGARDING DPR'S PRESENTATION:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

6. Studies showing that MeI is tumorigenic.

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

2.1 INTRODUCTION

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

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

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

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

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

2.2 ANALYSIS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Pathways to Adenomas

1. Normal → adenoma

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

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

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

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

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

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

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

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

2.3 AN ALTERNATIVE EXPLANATION

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

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

2.4. RECOMMENDATIONS:

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

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

ADENOMAS in MALES

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

CARCINOMAS in MALES

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

HYPERPLASIAS in MALES

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

NO EFFECT in MALES

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

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

ADENOMAS in FEMALES

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

CARCINOMAS in FEMALES

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

HYPERPLASIAS in FEMALES

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

NO EFFECT in FEMALES

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

3. OTHER SIGNIFICANT CONCERNS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Errata

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

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

ERRATA:

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

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

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

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

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

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

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

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

2.1 INTRODUCTION

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

[Continues on as before.]