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Dear Dr. Hill-Hammond,

I attended the IRIS presentation and evaluation of TBA and ETBE by the SAB in Arlington, VA on August 15-17, 2017, and am providing some follow up comments regarding some of my impressions of the discussion and presentations. I am attaching the previous comments that I submitted on both TBA and ETBE which provide considerably more detail and also the references. I am not repeating the reference list or details in these follow up comments.

My overall impression was the glaring lack of pathology expertise on the panel, for a series of questions that relied heavily on pathology expertise. The need for pathology expertise in the evaluation of the TBA and ETBE IRIS assessment was emphasized at the hearings at the June, 2016 meeting, and for some reason, this has been largely ignored by the IRIS group in their revised write up, as well as a lack of pathology expertise being listed as one of the necessary expertise for the panel and the lack of a pathologist on the panel. Having a pathologist on the panel would have readily addressed many of the questions and issues that arose, especially regarding charge questions 3 and 4. The pathologist(s) could have corrected many of the misstatements being made by the panel members who clearly lacked pathology expertise. Not having a pathologist on the panel was a glaring weakness in the overall evaluation.

For both TBA and ETBE, major issues were the non-cancer findings in the kidney. The IRIS document is not accurate in its assessment, and the confusion related to the interpretation and human relevance of the rat findings was glaring in the panel discussions. It also resulted in a markedly divided panel decision in response to the charge questions. Some of the confusion was based on a lack of basic knowledge of kidney pathology. Let me summarize again the basic information regarding the kidney pathology:

- 1) Chronic progressive nephropathy (CPN) is a disease, not a collection of histopathologic changes, as suggested in the IRIS document. The explicit criteria are published in the INHAND classification which was developed by Societies of Toxicologic Pathology worldwide and used by pathologists for its standardization.
- 2) CPN is not relevant to humans.
- 3) The type of urothelial hyperplasia found in the kidneys of TBA and ETBE-treated rats is actually a hyperplasia of the lining of the renal papilla, not a true urothelial hyperplasia of the kidney pelvis. This type of "urothelial" hyperplasia is part of CPN, not independent of it, and is only seen in high grade CPN cases. The fact that this type of hyperplasia is part of CPN is explicitly stated in the NTP report on TBA on page 56. This is ignored in the IRIS document, and only a few members of the panel appeared to be aware of this,

despite it being stated in my previous comments. Clearly, many of the panel members had not had time to read the submitted outside public comments.

- 4) This type of urothelial hyperplasia is pathognomonic for CPN. This means that it is only seen with CPN.
- 5) The statistical analyses by EPA trying to show the independence of some of the histopathologic findings from CPN is seriously flawed as described in detail in my previous comments, because of difficulties in both the numerator and denominator of the analyses. This is most glaringly obvious in their analysis of the urothelial hyperplasia trying to show that it is an independent marker. Since it is only seen with CPN, trying to statistically show that it is not is an exercise in futility and a demonstration of the flawed nature of the statistical analyses.
- 6) Some of the statistical analyses tried to show that there is a dose response for the urothelial hyperplasia in the male rats and that this is independent of the dose response for CPN incidence. Dose response for the urothelial hyperplasia is actually related to the enhanced grade of CPN that was dose related. Since the hyperplasia occurs only in high grade CPN, the dose response for the enhanced grade of CPN explains the dose response for the urothelial hyperplasia. This also explains why the changes in the male were greater than the female since the grade of CPN in the males was higher than in the females, or was present for a longer period of time.
- 7) Lastly, this type of hyperplasia (of the lining of renal papilla) has never been reported in humans (see citations in my previous comments for documentation). Thus, even if the panelists were to conclude that the urothelial hyperplasia is independent of CPN (which it is not), it is still not relevant to humans.
- 8) All of the kidney effects produced by TBA and ETBE, including the tumors, are due to α_{2u} -globulin nephropathy, CPN and cortico-medullary calcification, and none of these are relevant to humans. The kidney tumors in the TBA-treated rats was due to a synergy between α_{2u} -globulin nephropathy and enhanced CPN, neither of which is relevant to humans.
- 9) The pathology working groups (PWG) for both TBA and ETBE specifically examined tubules not affected by either α_{2u} -globulin or CPN for other types of changes. There were none. Thus, the other effects of TBA and ETBE on the kidney hypothesized by the IRIS report, has no foundation in fact.
- 10) An indication of the lack of expertise and knowledge regarding the pathology was evident in statements (oral and in the written bullet points presented Thursday) by several panel members referring to α_{2u} -globulin (the correct term) as $\alpha_{2\mu}$ -globulin or α_2 -microglobulin. α_2 -Microglobulin is a different protein and unrelated to this nephropathy.

It was my impression in reading the IRIS document and listening to their presentation and defense at the meeting, that the EPA scientists are presenting a highly biased evaluation of TBA and ETBE. This was evident in their lack of pathology expertise in the document and in their presentation, and in their giving credence to several publications for which there is considerable doubt regarding their validity. Some of these were commented on by panel members. For example, the studies that were referred to as the initiation and promotion studies for ETBE are seriously flawed, and the panel generally disregarded these, despite heavy

emphasis in the IRIS document. In the IRIS document, there was no critical evaluation of these studies (see my previous comments for details). Also, there was a lack of awareness of the meaning of many of the pathologic findings, in addition to the kidney. For example, in the IRIS document they indicate that the early appearance of centrilobular hypertrophy in the liver without its presence in the two year bioassay indicated that it could not be related to the liver tumors. This reflects a complete lack of knowledge about centrilobular hypertrophy in liver carcinogenesis for which there is an extensive literature.

This also raises in my mind a difficulty with the systematic review process performed by the EPA (charge question 1). A major part of this review process is an evaluation of the quality of the articles that are being evaluated. However, even more importantly, there needs to be the expertise available in the reader to be able to appropriately evaluate the meaning of the findings in the paper, whether the studies are good or bad. For example, not knowing that centrilobular hypertrophy is only present in the early phase of treatment and is not present at two years, and yet is a clear indicator of nuclear receptor activation makes it impossible for the individual to adequately review the publications. The difficulty I have with systematic review is that it is focused on papers only with the chemical being evaluated. There needs to be some kind of follow up for an evaluation, of at least basic information, on the topics that are being evaluated, such as α_{2u} -globulin, CPN, centrilobular hypertrophy, etc. Systematic review is a worthwhile goal, but attention to quality is essential.

A few members of the panel and outside commenters recommended that EPA convene a panel of experts to develop an evaluation of CPN and its relevance to humans, similar to what has previously been done for α_{2u} -globulin nephropathy. I strongly support convening such a panel, and encourage EPA to do so as quickly as possible. I imagine that financial support for such a panel could be available from industry, even if the meeting is organized in conjunction with the EPA. A mechanism for such a meeting could be through the ILSI Health and Environmental Sciences Institute (HESI) in Washington, DC, which has previously organized meetings in collaboration with EPA on a variety of topics, such as the relevance of rodent liver tumors for humans. Since the issue of CPN pertains to a variety of chemicals, it is timely that such an evaluation be performed. I would be happy to provide a list of possible individuals for such a meeting, including pathologists from EPA, FDA, and NTP.

In the documents on TBA and ETBE, the IRIS reports struggle with the presentation and evaluation of modes of action and human relevance. I strongly encourage them to utilize the EPA/IPCS (International Programme on Chemical Safety) framework, which is widely used by other branches of EPA and is incorporated into the 2005 EPA Cancer Guidelines. This framework was developed under the sponsorship of EPA and Health Canada, involved numerous scientists from EPA and Health Canada, and was eventually extended worldwide by IPCS. This framework is designed to present data in a comprehensive, rigorous, disciplined, and transparent way, with specific processes for evaluation for the hypothesized mode of action, identification of data gaps and whether they are necessary or not for the risk assessment, evaluation of alternative modes of action, and most importantly, how to extrapolate from the animal model to the human. For some reason, IRIS has refused to utilize this framework, which

I believe would be very useful for their evaluations. It is used for the evaluations by other divisions of EPA. For example, in registration documents for OPP, it is actually required that the data be presented in the format of this framework.

Some of the panel members referred to the presentation and publications by Melnick et al. regarding CPN. There are serious flaws in the publications by Melnick as detailed in Hard et al., 2013. The details of the Hard et al. analysis are not presented in the IRIS document for TBA or ETBA. Again, pathology expertise is required for an evaluation of the Melnick et al. publications and those by Hard et al. Also, it was inferred by members of the panel that Dr. Melnick is a pathologist. He is not.

I would like to thank the organizers of the meeting for their assistance. I appreciate the opportunity to present at the meeting, and to send additional comments for the panel and the EPA scientists to consider.

Sincerely yours,

A handwritten signature in black ink, appearing to read 'S.M. Cohen', with a stylized flourish at the end.

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