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Mr. Robert Fensterheim
Executive Director
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I am writing in response to your request for me to comment on specific issues arising from the discussion by the EPA Science Advisory Board of the document titled "Toxicological Review of Acrylamide."

I am a board certified pathologist (ACVP) and toxicologist (ABT) with over 30 years experience in the design, conduct and interpretation of rodent bioassays. Over that period I have participated in more than 200 cancer bioassays and examined the histopathological slides from most of them. I was Chief of the Pathology Branch at the National Toxicology Program which was responsible for assuring the diagnoses from the NTP bioassays were accurate. Subsequently, I was the Director of the Toxicology Research and Testing Program of the NTP where I directed all aspects of their studies. Since leaving the NTP, I have continued to participate in the design and interpretation of numerous bioassays and act as a reviewer of the histopathologic slides from them. This experience has provided me with an insight into what these bioassays really mean and their limitations. It is in light of this experience that I offer my opinion on the following points:

1. Combining of tumor types.

2. The progression of adenomas to carcinomas.

3. What the pathology of acrylamide tells us about its mode of action.

1. Combining of tumor types.

The basis for the below comments and conclusions is derived from the National Toxicology Program's (NTP) procedures for combining tumors for determining a treatment-related effect in rodent bioassays and are described in detail in the paper by McConnell et al. (1986). This paper describes the rationale for why combining certain tumors together for statistical purposes is scientifically based and provides guidance for what should and should not be combined.

McConnell, E.E., Solleveld, H.A. and Boorman, G.A.: Guidelines for Combining Neoplasms as Evidence of Carcinogenicity in Long-term Toxicity and Carcinogenesis Studies. J. Natl. Cancer Institute 76(2):283-289, 1986.

For brevity, the following comments are restricted to the acrylamide (AA) studies of Johnson, *et al.*, 1986 and *Friedman et al.*, 1995.

1.1. Appropriateness of Combining Brain Tumors

The basic question in the IRIS document with regard to the brain tumors reported in the Friedman study is whether tumors referred to as "malignant reticulosis" should or should not be combined with glial cell tumors. The guidelines described by McConnell *et al*. (1986) and current practice by the NTP *clearly* suggest that they should not be combined to determine a treatment-related increase in brain tumors. The reason for this is that they arise from distinctly different stem cells. There are three types of gliomas that typically arise in the brains of rats, i.e. astrocytoma, oligodengrolioma and mixed gliomas. These glial cell tumors all arise from the same stem cell that is derived from the neuroectoderm and, therefore, it is appropriate to combine these tumors. In contrast, the stem cell for malignant reticulosis is entirely different, *i.e*. these tumors arise from the reticuloendothelial system, and are more in harmony with lymphomas and lymphosarcomas. In fact, the term reticulosis is no longer used for these lesions. They are more correctly termed "histiocytic sarcomas." Therefore, because of their fundamental difference in histomorphogenesis, they should not be combined with glial cell tumors.

Solleveld, H.A. and Boorman, G.A. 11. Brain. *In* Pathology of the Fischer Rat. Boorman, G.A. *et al*., Academic Press, 1990.

Solleveld, H.Y. and Zurcher, C. Neoplasms of the Central Nervous System. *In* Pathology of the Aging Rat, Vol 2. ILSI Press, 1994.

1.2. Appropriateness of Combining Mammary Tumors

The basic question in the IRIS document in regard to combining mammary tumors is whether it is appropriate to combine adenomas with fibroadenomas. At the time of the writing of the guidelines (McConnell *et al*., 1986), it was suggested that it was appropriate to combine adenomas with fibroadenomas because they were thought to arise from the same stem cell, *i.e.*, glandular epithelium. However, since that time it has been shown that these tumors are *clearly* different in their histomorphogenesis. The fibroadenomas arise from the ductal epithelium and are comprised primarily of hyperplastic fibrous connective tissue, have only small portions of a tubular (epithelial) component and rarely become malignant. In contrast, the adenomas arise from the acinar portion of the mammary gland, are composed primarily of epithelial cells and have the potential to progress to adenocarcinomas. This is why the current NTP policy is not to combine adenomas and fibroadenomas for determining a treatment-related effect.

2. Do Adenomas Progress to Carcinomas?

The basic premise behind the guidelines for combining tumors (McConnell *et. al*., 1986 and NTP convention) is that most adenomas have the ability to progress to adenocarcinomas. However, because they have the potential to progress does not mean that they will progress. Adenomas certainly have a different biological potential being classified as benign and why they are viewed much differently in the clinical setting with regard to surgical, etc. intervention. That is also why the NTP continues to differentiate benign from malignant tumors. The scientific basis for differentiating benign from malignant tumors is because it is generally recognized that carcinogenesis is a multistep process requiring a series of sequential mutational events. It is also well known that benign tumors can revert to normal tissue if the stimulus (chemical) for their induction is removed. As pointed out in the Guidelines for Combining Neoplasms (McConnell *et. al*., 1986) “Thus differentiating benign and malignant neoplasms may provide information relevant to the mechanistic action of the chemical.”

3. What the pathology of acrylamide tells us about its mode of action.

It is my opinion that the tumor data on acrylamide (AA) are more in line with a hormonal mode of action (MOA) for the induction of the tumors arising in the mammary gland, thyroid gland and tunica vaginalis testes rather than a direct genotoxic event as suggested in the IRIS document. My reasons for this opinion are as follows:

Although noted in the pathology tables reporting the tumors in the Johnson *et al.* (1986) and Friedman *et al*. (1995), the IRIS document does not give the proper weight or emphasis to the biological potential of the tumors of either the thyroid or mammary gland. Insight into the potential MOA can be learned by examining the pattern of the tumor response. As noted in both the studies, the tumor response was only statistically significant with regard to benign neoplasms of the thyroid (adenomas – both sexes) and mammary gland (fibroadenomas – females only). I find this very important data because, for me, it sheds light on the probable MOA. Although I consider all three types of tumors related in terms of a MOA, I will speak to mesothelial tumors of the tunica vaginalis testes (TVM) later in this document.

First, while I'm an advocate of combining benign and malignant tumors of the same tissue type for determining the carcinogenic activity of a material and have published the seminal paper on the subject (McConnell *et. al*., 1986), there is also a great deal more to this exercise than just combining the two morphological types. For example, in my experience most xenobiotics that appear to cause a neoplastic response in rodent models *via* a genotoxic MOA typically result in a malignant tumor response, often at the expense of the benign tumors. In fact, it is more common than not for the primary tumor response with genotoxic chemicals to be *primarily* malignant. In contrast, xenobiotics that cause neoplasia *via* an epigenetic mechanism, *e.g.*, perturbation of a hormonal pathway, irritation, etc., often cause a primary increase in benign tumors, although an increase in malignant tumors is possible depending on the potency of the agent.

Second, the organ distribution of the tumors can be important for understanding a potential MOA. Xenobiotic agents that cause neoplasia *via* a genotoxic MOA typically cause tumors at the site of highest concentration of the parent compound or the site of metabolism to the "active" metabolite, *e.g*., liver, or in an organ with a high affinity for the chemical or its metabolites, *e.g*., bone marrow, kidney, etc., or organs with active cell replication, *e.g*., liver, bone marrow,

intestine, etc. Chemicals that cause tumors *via* a hormonal MOA typically cause tumors in endocrine organs or organs directly influenced by hormones. Therefore, in the case of AA, the organ distribution of tumors in the thyroid and mammary gland would argue more for a hormonal rather than a genotoxic MOA. Also, if genotoxicity were the primary MOA one would expect tumors, especially of a malignant nature, in the liver because this is the site of primary metabolism and possibly other non-endocrine organs.

Finally, if the MOA was based on genotoxicity one would expect to see non-neoplastic "toxic" lesions, *e.g.*, liver necrosis, inflammation, etc. In addition, if AA caused tumors *via* a genotoxic MOA one would also expect to find an increase in tumors of the mammary gland of male rats, which was not the case. At a minimum, I think there would be dose-related pathology in the male mammary gland, which again was not the case.

At first glance, the mesothelial tumors of the tunica vaginalis testes (TVA) would appear not to fit into the hormonal MOA paradigm. However, there are two arguments that suggest that this is not the case. First, in my opinion there is nothing unique about the mesothelium that lines the testes or scrotum and that of the peritoneal cavity. The only unique feature is its location. The mesothelium is morphologically essentially the same as that which lines the rest of the abdominal and thoracic cavities. If the MOA for the TVMs were a genotoxic event, why would the TVMs only start in this location? If however, the MOA was hormonal, one could envision higher levels of the inciting hormone in the tunica vaginalis merely by its being in a microenvironment adjacent to the testes which often contain Leydig (interstitial) cell tumors in this strain (Fischer 344) of rat, *i.e.*, 90+%. It is known that these Leydig cell tumors are hormonally active, so local hormone levels could be higher than in the general circulation. In fact, the analysis by Iatropolis *et al*. (1998) that compared the amount of the testicle occupied by Leydig cell tumors to the development of TVMs shows a fairly strong correlation with the presence of the TVMs. A more in-depth discussion of this hypothesis can be found in the report of a Pathology Working Group (PWG) on the TVMs from the Friedman study, which I understand was earlier submitted to the Agency.

Iatropolis, M.J., *et al*. Microscopic evaluation of proliferative mesothelial lesions diagnosed previously as mesotheliomas of the tunica vaginalis testes. Unpublished report to Cytec Industries, October 14, 1998.

I would like to offer a second possible MOA for the TVMs that also deserves pointing out in the IRIS document. As pointed out by Iatropolis, there is a fairly strong correlation between the size of the Leydig cell tumors and the presence of TVMs. With this in mind I propose a further hypothesis for the restricted location of these mesotheliomas (TVMs). The scrotum of the rat "tightly" encloses the testes; is not pendulous like in higher developed species. Therefore, any enlargement of the testes results in an increase in testicular pressure on adjacent tissues. That being the case, the findings of Iatropolis suggest that it is possible that the testes bearing Leydig cell tumors are overall enlarged, and therefore cause increased pressure on the epididymus and its mesothelial covering. This pressure would in turn cause physical irritation of the adjacent mesothelium resulting in mesothelial hyperplasia and eventually TVMs. This is further supported by the fact that the Fischer 344 rat has both the highest incidence of both Leydig cell tumors and TVMs of any of the commonly used strains of rats. It is my understanding that this potential MOA was also pointed out in the same PWG report.

In summary, I believe the organ distribution and benign nature of the tumors found in the Johnson *et al.* (1986), and Friedman *et al.* (1995) oral acrylamide rat studies do not support a genotoxic MOA. I further propose that a hormonal MOA better fits the data, especially for the mammary gland and thyroid gland. This may also apply to the TVMs, but a further MOA such as physical irritation from an enlarged tumor-bearing testicle is also plausible.

Thank you for the opportunity to provide these opinions.

Sincerely,

Ernest (Gene) McConnell