# Comments on the SAB Review

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My name is Annette Shipp. I'm a Principal with Environ International Corporation and have for about 25 years been involved in the investigation of the potential for chemicals to pose a risk to people exposed to chemicals in the environment, the workplace, or in products used daily life. Acrylamide is an extremely interesting compound and presents a number of challenges to both the EPA and the SAB in that it has a rich and complex body of data that must be considered in its entirety rather than as individual pieces of data. I published a review of acrylamide toxicity in 2006 in Critical Reviews in Toxicology [Shipp et al. (2006). Critical Reviews in Toxicology. 36(6-7): 481-602.] I am respectively submitting these comments at the request of NAPPA. My comments are focused on the use of the Human Relevance Framework.

I respectively submit that while the SAB concurred that the EPA followed the Human Relevance Framework (HRF), the application of that framework did not apply the same standard of evidence to the mutagenic mode of action (MOA) that was applied to the non-genotoxic MOAs. The initial step in the HRF directs an investigator to identify the key events in the animal model, i.e., biological, cellular, or molecular, by which a chemical could cause the tumors noted. The method by which this step is implemented is not explicitly defined but implicit in that step are the following approaches:

- In approach #1: When a tumor arises from a direct mutagenic event events that do not occur under normal physiological conditions in the absence of an exogenous chemical, the initial step in the HRF works well and cause/effect may be discerned from only the chemical-specific data.
- In approach #2, which applies to acrylamide: When the increase in tumors occurs for those types of tumors that arise spontaneously, due to age-related or species/strain-related normal physiological changes, such as Leydig cell tumors in

Fischer 344 (F344) male rats or liver tumors in B6F3C1 male and female mice, a different starting point in the evaluation is necessary. The following must be considered:

- What is the basic biology of that organ system and the physiological controls, such as feed back loops, that explains normal functioning;
- What are the key steps in that biological/physiological flow of normal functioning that are impacted by changes due to aging resulting in changes in that cell or organ system's homeostasis; and,
- What is the key step(s) or biological "trigger(s)" that is the obligatory precursor step that provides the underlying stimulus, even in the absence of exogenous chemicals that "push" a normally functioning cell in an organ to become a neoplastic cell resulting in a tumor-containing organ.
- Stated differently, what are the biological/physiological changes that occur in the development of "spontaneously" occurring tumors in specific organs that can be exacerbated by exogenous chemicals, such as acrylamide?

When an exogenous chemical acts by enhancing age- or species/strain-related tumors, the operative question is how does the chemical of interest add to the influence/effects of naturally occurring, usually age-related, biological "trigger"?

Take for example, the production of TVMs as described by Dr. Maronpot in his submission to the SAB. Dr. Maronpot has concluded that the production of TVMs by acrylamide is by way of a non-genotoxic MOA and linked to the increased production of Leydig Cell tumors (LCT). The MOA for spontaneously occurring (LCT) in F344 rats is the result of an age-related increased response to a dopamine signal at the pituitary leading to decreases in prolactin, down-regulation of LH receptors, and decreases in testosterone, ultimately leading to LCTs because of age-related stimulation of feedback loops.

***It is less of a stretch to say that acrylamide in aging male rats exacerbates the change in dopaminergic tone (there is evidence that acrylamide inhibits the re-uptake of dopamine into nerve ends) that, when occurring in the aging male F344 rats who have become more sensitive to that dopaminergic signal (for which there is experimental evidence), results in an age-related decline in circulating prolactin and testosterone levels (for which there is specific evidence for acrylamide) leading to and enhancing the biological cascade that results in an increase in spontaneous, age-related production LCT (a known and well-accepted MOA for dopamine agonists in***

***the production of LCTs) than to say that acrylamide forms unspecified DNA adducts, which, by vaguely described and essentially unknown steps, results in tumors.***

Invariably, there will be gaps that can then be used to claim that the MOA is undefined and uncertain. This is especially true for chemicals, such as acrylamide, that may be opportunistic rodent carcinogens that are exacerbating age-related changes in a highly susceptible rodent strain. When these "pre-steps", that is, understanding the normal biology/physiology of a system and how that system changes with age, are included in the HRF, then one does not have to "connect all the dots" as the EPA and others are expecting the acrylamide data to do, but rather one can use a wider array of data both for that physiological process and data from other chemicals that affect that process.

While we may not have all of the data for a chemical for all of the "steps" in a biological cascade, we can integrate data for a chemical, in this case acrylamide, to determine at what point in that biological cascade the chemical is likely acting and ask if that interaction influences what would be considered an obligatory precursor event. The EPA indicated that any association with a hormonal MOA was based on limited data and speculative. However, what are the data for acrylamide that identify, based on acrylamide-specific experimental data, the biological cascade from an acrylamide or glycidamide DNA adduct to the increased production of TVMs, or mammary fibroadenomas, or thyroid tumors? What are the acrylamide-specific experimental data that reconciles the lack of tumors in organs, such as the liver and lung, that had more DNA adducts? Further, if the MOA were a genotoxic MOA due to the production of an epoxide-type reactive metabolite, an acrylamide-specific explanation as to why tumors were not found in organs in which this metabolism would be concentration should have been provided.

Hence, application of this HRF framework requires an integration of all relevant data in a weight-of-evidence analysis of those data to: 1) discern patterns of effects that are indicative of a potential MOA; 2) reconcile apparent conflicting data, in particular when studies in animals are not in agreement with epidemiological data; and, 3) determine the relevance of these findings to human health based upon the integration of data of pharmacokinetic and pharmacodynamic differences.

Acrylamide presents a unique challenge to the EPA and the SAB in providing the opportunity for scientists to apply the HRF in such a way as to find the underlying obligatory precursor step(s), e.g., acrylamide's blocking of dopamine uptake into neurons, that may identify in traditional "noncancer" endpoints, such as neurotoxicity, that could be used to understand the biological cascades leading to the cancer effects seen in F344 rats, and possibly harmonize the underlying initial step in some of the non-cancer effects with the cancer effects.