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PATHOLOGY AND MICROBIOLOGY

March 24, 2010

Hon. Lisa Jackson
Administrator
US Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Ave., N.W.
Washington, D.C. 20460

Dear Administrator Jackson:

I am writing regarding NCEA's Science Advisory Board meeting concerning the IRIS toxicological review of inorganic arsenic for cancer that is scheduled for April6-7, 2010. I have a long standing history of research in chemical carcinogenesis with extensive experience regarding the urinary bladder. Because I am also trained as a medical doctor specializing in surgical pathology, I have been involved with research in humans as well as in animal models. I began working in the field of arsenic toxicology and carcinogenesis approximately 15 years ago, and have published extensively in this research area. In 1997, I served on a Science Advisory Panel for EPA regarding arsenic.

Originally, my group's research focused on dimethylarsinic acid (DMA^V), including establishment of the mode of action (MOA) for its effects on the urinary bladder in rats. Our data were used extensively by the EPA for its review on DMA, and formed the basis of many of the conclusions by the previous SAB Panel (2005) that was charged with questions regarding DMA and arsenic. Much of this work has been corroborated and extended by other investigators, including several at EPA's NHERL laboratory. During the past five years, my laboratory has increasingly focused on the issue of inorganic arsenic rather than organic arsenicals.

I have reviewed the draft IRIS document, Toxicological Review of Inorganic Arsenic, which became publically available in February, 2010. I have also reviewed the recently released charge questions to the arsenic panel that is to be convened April 6-7.

I would like to make a few comments regarding some specific issues raised in parts of the IRIS document. There is an enormous amount of material that is covered in the document, with considerable effort made in obtaining the necessary literature through the year of 2007; nevertheless, there are major publications which are not included in the reference list, including studies *in vitro* and *in vivo* as well as interactions with specific cellular molecules. Many of these papers contain information that conflicts with the mode of action and hypotheses conveyed in the IRIS document or provide alternative explanations.

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The charge questions appear to be primarily focused on the interpretation of epidemiology studies, but I strongly believe that to properly interpret these questions the overall knowledge concerning mode of action for inorganic arsenic-induced cancer should be considered. The question of mode of action was addressed by the SAB 2005 Panel, with the conclusion that the specific mode of action for inorganic arsenic was not known, and therefore the agency should default to the linear extrapolation of the dose response data in humans (primarily based on the southwest Taiwan population). However, the Panel concluded that "[t]his issue is an extremely important area for research attention, and it is an issue that should be evaluated in EPA's continuing risk assessment for iAs." (p. 6)

Although the specific mode of action for inorganic arsenic-induced carcinogenesis is not known, the 2005 SAB clearly indicated in its conclusions as well as in its discussion that all of the possible modes of action involve not only a non-linear dose response but most likely involve a threshold.

It has been demonstrated conclusively that arsenicals will not react directly with DNA. Much of this work was performed at the NHERL laboratories of the EPA. Rather than default to a linear extrapolation, I believe that it is appropriate and sufficiently conservative to utilize a non-linear, threshold consideration for the dose response, taking into account what we know from animal studies and from studies in humans.

As indicated in the IRIS document, there are considerable differences between species in the carcinogenic potencies of inorganic arsenic. It would appear that the human is more susceptible than rats or mice. These differences in susceptibility appear to be primarily related to differences in toxicokinetics between species rather than in cellular responses to exposures to the arsenicals. The kinetic basis for the interspecies differences is stated in the IRIS document (page 75).

In all species, as indicated in the IRIS document, it is quite clear that of the various arsenicals it is the trivalent forms of arsenic are the critical molecules causing the toxicity and carcinogenicity. Following exposures to inorganic arsenic there are three trivalent forms present in the body. These are: inorganic arsenic itself, in the form of arsenite, and the monomethyl (MMA^{III}) and dimethyl (DMA^{III}) arsenicals. In humans, trimethyl arsenicals do not occur except at exceedingly high exposure levels, and it does not appear that the trivalent form of trimethyl arsenic is produced in humans.

Although we do not know the specific biological consequences that are critical to arsenic-induced carcinogenesis, the overall mode of action actually is already discernible at the present time. This involves exposure to arsenic, leading to generation of trivalent metabolites. These trivalent metabolites react with critical cellular sulfhydryl groups (most likely specific proteins), leading to consequent biological responses. It is quite likely that multiple biological effects by these trivalent arsenicals are essential for the eventual induction of tumors.

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These three trivalent forms of arsenic that occur in humans are highly reactive chemicals. The reactivity is with thiol groups existing either in small molecules, such as glutathione, or more commonly, in proteins as free sulfhydryl groups. The reactivity of the three trivalent forms of arsenicals with these various thiol groups differs, and there is variation between species. The interspecies differences in reactivity are primarily due to differences in the amino acid sequences of specific proteins, with free sulfhydryl groups sometimes being present in the corresponding protein in one species but not in others. This is demonstrated by hemoglobin. In the rat hemoglobin, there is an additional free sulfhydryl group to which DMA^{III} binds, acting as a "storage sink" for arsenic in the rat. This is the primary reason for the unique toxicokinetics of all arsenicals in rats compared to other species, including humans. In the mouse, the accumulation of arsenic appears to pool in the urothelium of the lower urinary tract, including the urinary bladder, where it is stored in the form of arsenite within the mitochondria. In humans, there does not appear to be a storage or accumulation site for arsenic, and that explains part of the difference in susceptibility between humans and rodents.

The specific mode of action for arsenic in the various tissues of different species is dependent on the toxicokinetics of the trivalent forms of arsenic, and also importantly, is dependent on the reactivity of these trivalent arsenicals with specific proteins in the target tissues. It is not surprising that the primary cancer tissues for arsenic are the skin, lung, and urinary bladder, since these tissues contain significant levels of sulfhydryl groups with which trivalent arsenicals can react.

In addition to hemoglobin, the ability of trivalent arsenicals to react with proteins has been extensively evaluated for several specific proteins, including metalloproteinases, estrogen receptors and related peptides. Much of this research has been performed in the laboratories of Dr. Chris Le at the University of Alberta and Dr. Kirk Kitchin at EPA's NHERL.

Depending on the cellular proteins that are affected by trivalent arsenicals, a potential different mode of action and very different biological consequences could occur. For example, there is considerable evidence that a common consequence of this reaction is cytotoxicity followed by regenerative proliferation. Other possibilities include indirect genotoxic effects, direct mitogenesis, or apoptosis. Several of these biological consequences are listed as key events in the IRIS document.

Although there are several theoretical possibilities for the carcinogenic MOA of arsenic, there is considerable information *in vivo* in animals as well as in humans as to which of these is more relevant. Although important information can be garnered from *in vitro* studies, such results are fraught with considerable uncertainty in extrapolating back to the intact organism. For example, it is essential that appropriate cell types be assessed in *in vitro* studies. Specifically, in animal models as well as in humans, carcinogenicity occurs in epithelial tissues. Therefore, *in vitro* studies should involve epithelial cells from the bladder, skin, or lung, and possibly some other target sites for the arsenicals. Evaluation of arsenicals in various *in vitro* systems involving hematopoietic or mesenchymal cells is not relevant to the critical biological effects..

Oxidative damage is also mentioned in the IRIS document as a possible key event. However, the data supporting this effect is unclear since much of it was derived from *in vitro* studies at cytotoxic concentrations. As the IRIS document points out, *in vitro* studies with trivalent arsenicals at concentrations above 10 μM probably involve cytotoxicity and cell lethality. In fact, trivalent arsenicals are lethal *in vitro* with epithelial cell systems at concentrations greater than 1.0 μM.

The information concerning oxidative damage is unclear regarding cause or effect, as is much of the information concerning indirect genotoxicity. Apoptosis is an unlikely key event *in vivo*, as it is very uncommon in urothelium or lung, and there is no evidence for it in human skin lesions secondary to arsenic exposure. Thus, the most likely modes of action are cytotoxicity and regeneration and/or direct mitogenesis.

In identifying the response in humans, it thus becomes essential to identify the concentrations of the trivalent arsenicals that must be produced for the reaction with critical cellular sulfhydryl groups to occur, leading to a biological consequence, such as cytotoxicity, cell proliferation, or indirect DNA damage. Based on *in vitro* studies with epithelial cell systems, the critical concentrations were shown to be 0.01-1.0 μM. In animal models of the urothelial effects, the concentration appears to be somewhere between 0.1-1.0 μM. As quoted in the IRIS document (page 88), the concentration of trivalent arsenicals present in the tissues of humans and other species exposed to high doses *in vivo* are concentrations that produce biological effects *in vitro*.

The key question is what is the minimal concentration of arsenic required to produce the biological consequences. Or, in other words, what is the level of arsenic to which humans must be exposed to generate these levels of trivalent arsenicals in the target tissues. In populations exposed to high levels of inorganic arsenic (usually greater than 400 ppb) trivalent arsenicals were detected in the urine at levels that correspond with the critical *in vitro* concentrations. Direct measurements of samples of individuals exposed to much lower levels of arsenic (<10 ppb) should be able to ascertain critical levels of trivalent arsenicals necessary to produce toxicological consequences. Unfortunately, levels of the trivalent arsenic compounds in the urine or other body fluids or tissues have not yet been measured in populations with lower exposures. Until such measurements are made, some of the PBPK modeling that has been performed by Dr. Elaina Kenyon at the EPA laboratories is helpful in this regard. Also, animal studies provide indication that exposures to inorganic arsenic by humans need to be in excess of the current standard for inorganic arsenic in the drinking water (i.e., 10 ppb) to generate biologically relevant concentrations of trivalent arsenicals to cause effects. The minimum level of detection for these trivalent species is less than 0.05 μM, which is well below the concentration necessary to produce a biological response in tissue culture or in organisms.

Thus, although the exact details of the mechanism of arsenic-induced carcinogenesis are not known, sufficient information is available to ascertain the overall mode of action for inorganic arsenic in animal models as well as in humans, involving the key events that I described above.

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The currently available information is sufficient for enabling us to quantitatively evaluate a threshold response to environmental inorganic arsenic in humans.

At the bottom of page 99, the mode of action that we have put forth regarding DMA induction of bladder cancer in rats is described. We and others have demonstrated that a similar mode of action is likely to be occurring in the urinary bladder of mice and rats in response to exposure to inorganic arsenic. The question, of course, is whether this mode of action is relevant to humans. There is considerable evidence that cytotoxicity and regeneration occurs in response to high exposures of arsenicals in humans. The preneoplastic lesions for arsenic-induced skin cancer involve a specific type of pigmentation change and a proliferative response referred to as actinic keratosis. This keratosis involves a chronic inflammatory infiltrate and related increased epidermal proliferation and keratin production. Regarding bladder cancer, a recent occupational accident in China resulted in exposures to extremely high levels of inorganic arsenic, with nearly one third of the affected individuals developing hematuria (blood in the urine). Hematuria is a sign of severe toxicity of the urothelium. Urothelial toxicity could also occur without evidence of hematuria if the toxicity does not penetrate the full thickness of the urothelium. This is similar to scraping the skin versus cutting the skin. If the scrape is superficial, there is no bleeding, but there is still cell death with consequent regeneration. If a cut penetrates the full thickness of the epidermis, it leads to bleeding as well as consequent regeneration.

In summary, it is my belief that the current understanding of the mode of action of inorganic arsenic-induced cancer is sufficiently known so that we can perform a risk assessment without defaulting to a linear extrapolation. This should be taken into consideration in evaluating the epidemiology data that are being presented to the SAB as well as responding to the charge questions. I would be happy to provide more detailed information regarding these statements as well as appropriate references. I also plan to present at the meeting on April 6 or 7, 2010.

Sincerely yours,

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