

Comments of

Samuel M. Cohen, MD, PhD

Professor
Department of Pathology and Microbiology
University of Nebraska Medical Center
Omaha, NE

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Thank you for the opportunity to present to you regarding the carcinogenicity of inorganic arsenic. I am mostly addressing your charge question 2. I have been involved with chemical carcinogenesis research for more than 40 years, with an emphasis on the urinary bladder and I have been involved with arsenic research for more than 15 years. I have served on an US EPA Science Advisory Panel on arsenic in 1997. I have also been actively involved for more than 10 years on the WHO IPCS effort on development of the mode of action framework and evaluation of human relevance from animal data.

It is my belief that an understanding of mode of action is essential to put epidemiologic effects into perspective.

There are fundamentally only two ways that chemicals can cause an increased risk of cancer:

1. The chemical can directly damage DNA, increasing the number of mistakes that occur every time DNA replicates; or
2. It can increase the number of times pluripotential cells replicate, increasing the opportunities for spontaneous errors to occur in the DNA.

It has been clearly demonstrated that arsenicals do not interact directly with DNA. An increase in cell proliferation can occur either by cytotoxicity with consequent regenerative proliferation or by direct mitogenesis. As was described in the IRIS report, with specific reference to the research from my laboratory, dimethylarsinic acid acts by producing cytotoxicity and regeneration of the urinary bladder (Fig. 1). During the past five years, increasing evidence has demonstrated that a similar process occurs with inorganic arsenicals in both rats and mice (Fig. 2), and likely also occurs in humans.

Regardless of the target tissue, and regardless of the specific toxicologic response, arsenic produces biologic effects by metabolism to the trivalent forms (Fig. 3). It appears that the different trivalent forms of arsenic can produce similar types of toxicity, although with varying potencies depending on the toxicological effect, specific tissue, and species. As indicated in the IRIS report, differences between species are primarily due to toxicokinetic variations. Trivalents produce effects rather than pentavalent forms because of their ability to interact with sulfhydryl groups, particularly in proteins. This requires a minimal amount of the trivalents to interact with a cell to produce a toxicologic response, that is, a threshold. Recently, sulfur analogs of the arsenicals have been identified as metabolites of inorganic arsenic (Fig. 4), but rapidly are taken up by cells and converted to the trivalent oxygen-containing forms.

Although the specific proteins that serve as the targets for the various arsenicals in the different tissues are not known, the basic mode of action is well delineated (Fig. 5). It involves conversion of arsenic to one or more of the trivalent forms (arsenite, MMA^{III} , or DMA^{III}) leading to an interaction with specific critical cell proteins with a consequent toxicologic response. Regardless of the specific cellular target, this is the basic mechanism and will always involve a

non-linear dose response, and essentially will always involve a threshold. Non-linearities for inorganic arsenic have been demonstrated regarding metabolism, cell transport, and minimum levels necessary to react with critical sulfhydryl groups to produce a biologic effect. There is a convergence of information regarding mode of action and dose response from a wide variety of research approaches, including from *in vitro* models with animal and human cells, from animal models, and from investigations in humans. Pathological, biochemical, molecular and genomic technologies have been utilized.

Based on the 2005 cancer guidelines as well as the evolving mode of action/human relevance framework, at the very least the EPA should be evaluating the dose response relationship based on a non-linear, threshold approach, not resorting to a default of linear, non-threshold. Such a default assumption for arsenic-induced cancer is no longer scientifically tenable.

The evidence from studies in humans strongly supports a non-linear, threshold dose response. These include not only the epidemiology studies that you have been charged to review, but also basic information regarding mode of action. The evidence for the urinary bladder strongly suggests that the process involves cytotoxicity with regenerative proliferation as was observed in a recent occupational accident in China (Fig. 6). A similar process is likely to occur in the skin, since the preneoplastic lesion in the skin in humans, referred to as actinic keratosis, is an inflammatory lesion with proliferation of the epidermis.

In summary, much is already known about the mode of action of arsenic-induced cancer, and involves a non-linear, threshold response. Information is available not only in animal models, but also from *in vitro* systems as well as evidence supporting it from examination of human specimens.

Thank you for your time. I would be happy to address any questions that you have of me.