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Review

# Inorganic arsenic: A non-genotoxic carcinogen

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ABSTRACT

Inorganic arsenic induces a variety of toxicities including cancer. The mode of action for cancer and non-cancer effects involves the metabolic generation of trivalent arsenicals and their reaction with sulfhydryl groups within critical proteins in various cell types which leads to the biological response. In epithelial cells, the response is cell death with consequent regenerative proliferation. If this continues for a long period of time, it can result in an increased risk of cancer. Arsenicals do not react with DNA. There is evidence for indirect genotoxicity in various *in vitro* and *in vivo* systems, but these involve exposures at cytotoxic concentrations and are not the basis for cancer development. The resulting markers of genotoxicity could readily be due to the cytotoxicity rather than an effect on the DNA itself. Evidence for genotoxicity in humans has involved detection of chromosomal aberrations, sister chromatid exchanges in lymphocytes and micronucleus formation in lymphocytes, buccal mucosal cells, and exfoliated urothelial cells in the urine. Numerous difficulties have been identified in the interpretation of such results, including inadequate assessment of exposure to arsenic, measurement of micronuclei, and potential confounding factors such as tobacco exposure, folate deficiency, and others. Overall, the data strongly supports a non-linear dose response for the effects of inorganic arsenic. In various *in vitro* and *in vivo* models and in human epidemiology studies there appears to be a threshold for biological responses, including cancer.



## Introduction

Arsenic has been known as a toxic, poisonous substance for many centuries (Cullen, 2008). Its possible relationship to cancer was first described more than a century ago in individuals being administered various solutions as potential therapeutic agents. Its association with skin changes (arseniasis) and ultimately cancer (basal cell and squamous cell carcinomas) was confirmed by observations in patients given an arsenical for treatment of syphilis that had been developed by Ehrlich, for which he received the Nobel Prize (Cullen, 2008; Neubauer, 1947). Beginning with the seminal publication by Chen et al. (1985) in the early 1980s, an awareness developed of a relationship between high exposure to inorganic arsenic in the drinking water and cancer of the urinary bladder. Exposure to inorganic arsenic in various mining occupations led to the discovery that it also could produce cancer of the lung, which was confirmed later as also arising from oral exposure (NRC, 1999, 2001). Subsequently, other tumors have been identified as being associated with inorganic arsenic such as tumors of the

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kidney and liver (Cohen et al., 2013). However, recently it has been demonstrated that the kidney tumors were actually those arising from the kidney pelvis, not the renal parenchyma, and are urothelial tumors similar to those in the urinary bladder (Ferreccio et al., 2013). The kidney pelvis is lined by the same type of epithelium, the urothelium, as the urinary bladder. Thus, the kidney pelvis tumors are likely due to a similar mechanism that is involved with the urinary bladder. The association with liver cancer has been recently described in various epidemiology studies, although the evidence is not as strong as for the skin, urothelium, and lung (Cohen et al., 2013).

The studies that demonstrated a relationship of exposure to inorganic arsenic with various types of cancer involved exposure to very high levels, either in the drinking water or the air (by inhalation). Inorganic arsenic exposure by inhalation is related to certain mining occupations. This exposure has been significantly reduced due to protective measures that have been implemented (IARC, 2012). However, exposure to inorganic arsenic in the drinking water in some parts of the world remains at extremely high levels, such as in Taiwan, China, Bangladesh, India, Chile, Argentina, and Mexico (IARC, 2012; Cohen et al., 2013). Most of the world, including the United States, Europe, and most of Asia has exposures in the drinking water at substantially lower levels than those that have been described in association with various types of cancer (NRC, 2001; IARC, 2012; Cohen et al., 2013).

The dose response for inorganic arsenic has not been clearly delineated in human populations. The concern is that it extends to low exposures rather than involving only high exposures. Whether exposure to inorganic arsenic at lower levels (less than 10 parts per billion in the drinking water) might be associated with an increased risk of cancer can only be determined by an understanding of the mode of action by which inorganic arsenic induces cancer. Abernathy et al. (1996) nearly two decades ago suggested that inorganic arsenic is a threshold carcinogen. Based on our understanding of the mode of action involved with arsenical induction of various types of cancers, the scientific evidence involving investigations *in vitro*, *in vivo* and in epidemiology, now strongly supports such a conclusion (Cohen et al., 2013). The evidence for this will be presented in this manuscript.

## 1. Effect of cancer mode of action on dose–response relationship

Cancer is due to multiple errors in DNA that can either be inherited or occur during DNA replication (Cohen and Arnold, 2011). The multiple genetic errors must be present in a single cell for cancer to develop, since cancer is a clonal disease. Although known for many decades, it is also now well-accepted that cancers arise from pluripotential cells in tissues, that are commonly referred to as tissue stem cells (Armitage and Doll, 1954; Moolgavkar and Knudson, 1981; Greenfield et al., 1984; Cohen and Ellwein, 1990; Cohen and Arnold, 2011). The errors can occur during DNA replication either by direct damage to the DNA (DNA reactive, genotoxic) or by "spontaneous" errors that occur during DNA replication. If the number of DNA replications is increased by environmental stimuli, the number of these spontaneous errors can be increased. Substances that directly damage DNA are referred to as DNA reactive carcinogens. A broader term for agents that damage DNA is genotoxic carcinogens. Substances that increase the risk of cancer by increasing the number of cell replications without direct damage to DNA are referred to as non-genotoxic or non-DNA reactive carcinogens.

It has been assumed for several decades that DNA reactive carcinogens do not involve a threshold, although there is some evidence that thresholds might also be involved in such instances (Doak et al., 2007). Nevertheless, if genotoxicity is produced indirectly rather than by direct interaction with DNA, or if cancer is induced by a non-genotoxic mechanism, a threshold response is involved.

Genotoxicity can be produced either by direct interaction of the agent with DNA (DNA reactive) or by indirect effects that produce errors in DNA (Cohen and Arnold, 2011). These indirect effects can involve interactions with a number of proteins involved in the mitotic process, such as tubulin, or processes that lead to micronucleus formation or chromosomal aberrations. In addition, inhibition of DNA repair enzymes could also lead to an indirect genotoxic process (Cohen and Arnold, 2011; Cohen et al., 2013).

Indirect effects on the DNA have also been postulated to occur either by oxidative damage or by peroxidation. Although examples have been identified in certain *in vitro* (Gentry et al., 2010; Yager et al., 2013) and *in vivo* animal models (Wei et al., 2005), it remains unclear whether oxidative stress itself can actually lead to an increase in cancer (Snow et al., 2005; Cohen et al., 2013; Gentry et al., 2014a, 2014b; Scudellari, 2015) (For a more detailed discussion, see below).

Increased cell replications in the stem cell population can occur either by increasing cell births or decreasing cell deaths (which increases the number of cells) (Cohen and Ellwein, 1991; Cohen and Arnold, 2011). It is not the rate of cell replication that is critical but the total number of replications. Thus, if the number of cells is increased by decreasing cell death, even if the rate of replication is at normal levels there will be an increase in DNA replications. This appears to be particularly critical in tissues in which there already is a high replication rate, such as colon, skin, or bone marrow. Increased cell births can be produced either be direct mitogenesis, which usually involves certain hormones or growth factors, or by cytotoxicity with consequent regeneration. In epithelia such as the skin, bladder, or lung, which have cell layers, the increase in cell number is evident in the form of hyperplasia. Most commonly hyperplasia involves not only an increase in the cell number but an increase in the replication rate.

## 2. Arsenic metabolism

To better understand the mode of action involved with inorganic arsenic-induced cancer (Fig. 1), a basic understanding of the metabolism of inorganic arsenic is necessary. Inorganic arsenic undergoes a series of reductions of the +5 oxidative state to the +3 oxidative state followed by oxidative methylation (Thomas, 2007; Cullen, 2008; Cohen et al., 2013). The sequence appears to involve inorganic arsenate ($iAs^{+V}$) being reduced to arsenite ($iAs^{+III}$), then methylated to monomethylarsonic acid ($MMA^{V}$), which is reduced to monomethylarsonous acid ($MMA^{III}$) and then methylated to dimethylarsinic

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