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Review

Assessment of the mode of action for hexavalent chromium-induced lung cancer following inhalation exposures

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ABSTRACT

Inhalation of hexavalent chromium [Cr(VI)] is associated with increased lung cancer risk among workers in several industries, most notably chromate production workers exposed to high concentrations of Cr(VI) ($\geq$100 $\mu g/m^3$), for which clear exposure–response relationships and respiratory irritation and tissue damage have been reported. Data from this industry are used to assess lung cancer risk associated with environmental and current occupational exposures, occurring at concentrations that are significantly lower. There is considerable uncertainty in the low dose extrapolation of historical occupational epidemiology data to assess risk at current exposures because no published or well recognized mode of action (MOA) for Cr(VI)-induced lung tumors exists. We conducted a MOA analysis for Cr(VI)-induced lung cancer evaluating toxicokinetic and toxicological data in humans and rodents and mechanistic data to assess plausibility, dose–response, and temporal concordance for potential MOAs. Toxicokinetic data support that extracellular reduction of Cr(VI), which limits intracellular absorption of Cr(VI) and Cr(VI)-induced toxicity, can be overwhelmed at high exposure levels. *In vivo* genotoxicity and mutagenicity data are mostly negative and do not support a mutagenic MOA. Further, both chronic bioassays and the epidemiologic literature support that lung cancer occurs at exposures that cause tissue damage. Based on this MOA analysis, the overall weight of evidence supports a MOA involving deposition and accumulation of particulate chromium in the bifurcations of the lung resulting in exceedance of clearance mechanisms and cellular absorption of Cr(VI). Once inside the cell, reduction of Cr(VI) results in oxidative stress and the formation of Cr ligands. Subsequent protein and DNA damage lead to tissue irritation, inflammation, and cytotoxicity. These effects, concomitant with increased cell proliferation, result in changes to DNA sequences and/or methylation status that can lead to tumorigenesis. This MOA supports the use of non-linear approaches when extrapolating lung cancer risk occurring at high concentration occupational exposures to environmentally-relevant exposures.

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1. Introduction

Inhalation of hexavalent chromium [Cr(VI)] is associated with increased lung cancer risk based on numerous observations in occupational epidemiology studies (IARC, 1990). The increase in lung cancer risk has been observed among workers in chromate production, plating, pigments and ferrochrome production industries where historical exposures to Cr(VI) have been well in excess of 100 $\mu g/m^3$. Among workers with increased lung cancer risk, nasal irritation and perforation have also been reported (Gibb et al., 2000a; IARC, 1990; Luippold et al., 2003). Consistently, based on animal data collected over several decades, it has been argued that the carcinogenicity of Cr(VI) is associated with tissue damage and inflammation induced by high dose exposure to bronchial tissues or microenvironments within the lung (Steinhoff et al., 1986; Levy et al., 1986; Glaser et al., 1990; Beaver et al., 2009b). Although the mode of action (MOA) for Cr(VI)-induced lung tumors has not been established to date, these observations would generally support a non-mutagenic MOA with a threshold for carcinogenicity.

The cancer risk assessment for chemicals that act by a mutagenic MOA are typically quantified assuming no threshold for carcinogenicity (U.S. EPA, 2005), and Cr(VI) has been observed to be genotoxic and mutagenic *in vitro* and *in vivo* by non-natural exposure routes (*e.g.*, intraperitoneal injection) and at highly toxic doses. Although Cr(VI) demonstrates very limited genotoxicity *via* natural routes of exposure and at environmentally-relevant levels (Holmes et al., 2008; Nickens et al., 2010; Thompson et al., 2013), some have concluded that the MOA of Cr(VI)-induced cancers, regardless of the tumor location, is mutagenicity wherein Cr(VI) induces mutations early in the carcinogenic process (McCarroll et al., 2010; Zhitkovich, 2011). Others have suggested that Cr(VI)-induced carcinogenesis arise from epigenetic changes due to increase in genomic instability characterized by chromosomal instability and/or microsatellite instability (MSI) from loss or hindrance of DNA mismatch repair (MMR) genes (Holmes et al., 2008; Nickens et al., 2010).

Risk assessments for Cr(VI)—particularly those conducted for the purposes of setting regulatory guidelines—have mostly relied upon linear low-dose extrapolation based on the conclusion that Cr(VI) may act by a non-thresholded genotoxic mechanism (Seidler et al., 2013; NIOSH, 2013; OSHA, 2006). It should be noted that DNA reactivity, such as the formation of DNA adducts, is not equivalent to mutagenicity. Chemicals that are DNA reactive do not necessarily induce tumors from direct DNA interactions; thus, considering the potential MOAs for Cr(VI)-induced lung tumors, evidence for DNA reactivity should not be taken as specifically supporting a mutagenic MOA.

The United States Environmental Protection Agency (EPA) Cancer Guidelines (U.S. EPA, 2005) as well as the Agency's draft Framework for Determining a Mutagenic Mode of Action for Carcinogenicity (U.S. EPA, 2007) emphasized that a weight of evidence approach should be applied for determining the MOA for carcinogenicity. An observation of mutation in one mutation assay is not sufficient for concluding that a chemical causes specific tumors by a mutagenic MOA or that the mutations is the only key event in the carcinogenic process (U.S. EPA, 2007). Not all carcinogenic chemicals that can interact with DNA will act by a mutagenic MOA, in which mutagenicity must be an obligatory early action of the chemical that precedes other key events such as cytotoxicity and regenerative proliferation in the carcinogenic process (U.S. EPA, 2007).

Risk assessment approaches for inhalation exposure to Cr(VI) have typically relied on epidemiologic data from occupational exposure in the chromate production industry, and linear models have been used to extrapolate from high concentration occupational exposures to much lower levels for the purpose of risk assessment (OSHA, 2006; NIOSH, 2013; U.S. EPA, 1984; Crump et al., 2003; Park et al., 2004; Seidler et al., 2013; Haney et al., 2014). These assessments either did not consider MOA or assumed that Cr(VI) acted by a mutagenic or genotoxic MOA and that no threshold for carcinogenicity exists or can be measured. However recently, a non-linear cancer risk assessment for inhaled Cr(VI) was developed based on evidence supporting that Cr(VI)-induced lung cancers may be thresholded due to kinetic detoxification at low exposures (Haney et al., 2012). In this paper, a kinetic threshold for Cr(VI) was quantified based on the reductive capacity of the lung and used with epidemiologic data for lung cancer to propose a chronic inhalation reference value of 0.24 $\mu g/m^3$. This value was developed to be protective of lung cancer for continuous environmental exposures and was reported to be 300-fold higher than an equivalent value calculated using linear low-dose extrapolation for an increased risk of 1 in 100,000 (Haney et al., 2012).

Given the divergent MOA opinions and approaches to risk assessment in the regulatory and scientific literature, a rigorous examination of a plausible MOA for lung tumors is important for informing the low-dose extrapolation of cancer risk assessment for Cr(VI). Thus, we conducted a MOA analysis for Cr(VI)-induced lung cancer with the available kinetic, human, animal, and mechanistic data. Based on the modified Hill Criteria outlined in