Scientific Workshop

Factors affecting the reduction and absorption of hexavalent chromium in the gastrointestinal (GI) tract: Potential impact on evaluating the carcinogenicity of ingested hexavalent chromium

Hexavalent chromium is a known human carcinogen via inhalation (IARC, 2012; OSHA, 2006; U.S. EPA, 1998a), but less is understood about the risks of hexavalent chromium when ingested. Observational epidemiology studies of a population in China have reported conflicting results on whether an association exists between consumption of drinking water contaminated with hexavalent chromium and stomach cancer (Kerger et al., 2009; Beaumont et al., 2008; Zhang and Li, 1997). In addition, a reanalysis of chromate worker populations did not suggest an increased risk of stomach cancer following inhalation exposures to hexavalent chromium (Gatto et al., 2010). In 2008, the National Toxicology Program (NTP) released its final report on the carcinogenicity of hexavalent chromium following chronic drinking water exposure in rats and mice (NTP, 2008). The NTP report concluded that there was clear evidence of carcinogenicity via ingestion in both male and female rats and mice based on increased incidences of neoplasms in the oral cavity and small intestine, respectively. EPA is currently evaluating information regarding exposure to hexavalent chromium via ingestion for the development of an updated Integrated Risk Information System (IRIS) toxicological assessment.

Chromium exists in multiple oxidation states, but it is the hexavalent and trivalent states that are most prevalent biologically. When ingested, hexavalent chromium can be reduced to trivalent chromium by a number of reducing agents within the gastrointestinal (GI) tract, but the reverse of this process, oxidation of trivalent to hexavalent chromium, will not occur in the human body. Once in the trivalent state, chromium is poorly absorbed by cells and thus poses little or no carcinogenic risk to humans (NTP, 2010; U.S. EPA, 1998b). However, chromium in the hexavalent state can be readily absorbed by cells lining the gastrointestinal (GI) tract via nonspecific anionic transporters, potentially leading to toxic or carcinogenic effects (reviewed in Zhitkovich, 2011; O'Brien et al., 2003). Therefore, an understanding of these two simultaneous and competing processes – absorption of hexavalent chromium by cells lining the GI tract and reduction of hexavalent chromium to trivalent chromium outside of the cell – is important in evaluating the potential carcinogenicity of ingested hexavalent chromium in humans.

As noted above, the NTP rodent bioassay (NTP, 2008) reported tumors in the oral cavity in rats and the small intestine in mice. Interspecies differences in reduction may affect the relative concentrations in the GI tract of ingested hexavalent chromium and subsequent absorption by GI mucosae; these could account for interspecies differences in carcinogenicity, but *in vivo* measurements of the reductive capacities or rates of reduction of hexavalent chromium in the

rodent or human GI tract are not available. As a means of estimating the levels of hexavalent chromium reduction and uptake, several *in vivo* studies have compared total chromium levels in tissues, blood, or urine following exposures to either trivalent or hexavalent chromium in humans (Finley et al., 1997; Kerger et al., 1997; Kerger et al., 1996) and rodents (Collins et al., 2010). Other studies have attempted to estimate the reductive capacity of gastric fluid from humans (Kirman et al., 2013; De Flora et al., 1997) or rodents (Proctor et al., 2012) *ex vivo*. While these data have been used to estimate reductive capacity, the measurements are inherently imprecise given variations in test conditions (e.g., pH, dilution, time elapsed, diet, food present during collection).

Because of the importance in evaluating all existing information on the competing processes of reduction and absorption of ingested hexavalent chromium, the transit of chromium species through the GI tract prior to absorption, and how these processes differ between humans and rodents, EPA has decided to convene a workshop. A panel of scientists will be invited to discuss the available studies of extracellular reduction, absorption, and transit in the GI tract for metals in general and hexavalent chromium in particular. These discussions will help inform a state of the science assessment of the human health risk from ingested hexavalent chromium. This workshop will focus only on the specific scientific issue of extracellular reduction.

The workshop will discuss the following toxicokinetic issues that may inform estimates of the amount of ingested hexavalent chromium absorbed in unreduced form in different portions of the GI tract as a function of species and dose:

- What is known regarding hexavalent chromium reduction, absorption, and transit in the human GI tract?
- What are the factors that could affect the transit of ingested hexavalent chromium in the GI tract, both before and after reduction?
- Do the rates and capacities for hexavalent chromium absorption and reduction vary along the GI tract?
- What are the interspecies differences in structure and function of the GI tract that affect reduction, absorption, and transit of ingested hexavalent chromium?

In addition to the toxicokinetic issues noted above, the workshop will also address what is currently known about how these toxicokinetic processes may vary among human populations and lifestages, thereby affecting susceptibility to toxic effects. The workshop will seek to elucidate the specific factors that may affect the reduction, absorption, and/or transit of hexavalent chromium in the human GI tract of potentially susceptible subpopulations or lifestages, such as:

- Potential conditions or disorders of the GI tract, including infection, chronic disease, or surgical procedures;

- The use of antacids or other medications that lower stomach pH by inhibiting gastric acid production;
- Other factors such as diet, age, genetic variability, or the natural cycling of digestion.

The following table contains studies that have determined reduction or absorption kinetics of hexavalent chromium in humans and animals. These references were identified from literature searches and expert consultation. This table is not meant to be a full systematic review of the literature; a complete literature search for the hexavalent chromium toxicological review will be available at a later date. If any additional references are identified by the public or the expert panelists this table will be revised and made available to the public. For the purposes of this workshop, the term "reduction capacity" is defined as the total mass of hexavalent chromium that can be reduced to trivalent chromium in a unit volume or mass of the defined media.

Selected studies of hexavalent chromium reduction and absorption

Reference	Species/media	Findings
Estimates of bodily fluid reduction capacity (*ex vivo*)[a]		
Proctor et al. (2012)	Stomach contents (rat)	Study estimate: 15.7 µg/mL[b]
	Stomach contents (mouse)	Study estimate: 16.6 µg/mL[b]
Kirman et al. (2013)	Gastric fluid (human)	Study estimate: 20 µg/mL [based on a mean of 7 µg/mL (fasted) from this study and a median of 30 µg/mL (fed) from De Flora et al. (1987)][c]
De Flora et al. (1987)	Gastric fluid (human)	8.3±4.3 µg/mL (fasting), 31.4±6.7 µg/mL (fed)
Petrilli and De Flora (1982)	Saliva (human)	1.4±0.2 µg/mL
Petrilli et al. (1986)	Epithelial lining fluid (human)	23.7±15.9 µg/mL
Estimates of cellular or organ reduction capacity[a]		
De Flora et al. (1997)	Intestinal bacteria (human fecal)	3.8±1.7 µg/10^9 bacteria (elimination via feces)
	Liver (human)	2.2±0.9 µg/g liver homogenate
	Blood (human)	52.1±5.9 µg/mL intact whole blood
	Red blood cells (human)	63.4±8.1 µg/mL RBC lysate soluble fraction
Petrilli et al. (1986)	Pulmonary alveolar macrophages (human)	4.4±3.9 µg/10^6 PAM S9 fraction
De Flora et al. (1987)	Peripheral lung parenchyma (human)	200±70 µg/g lung S12 fraction
Upreti et al. (2005)	Intestinal epithelial cells and gut bacteria (rat)	Most Cr(VI) at 10 ppm completely reduced by bacteria in 6 h. Complete reduction by some cells may take 24 h.
Novel *in vitro* systems		
Skowronski et al. (2001)	Artificial gastric fluid [Cr(III)/(VI) in soil]	Cr(III) and Cr(VI) are both bioaccessible in contaminated soil.
Gammelgaard et al. (1999)	Artificial gastric fluid	1st order reduction kinetics, $t_{1/2}$ = 23 minutes. Also calculated permeability of various Cr compounds in rat jejunum in vitro.
Yu et al. (2012)	In vitro GI method [Cr(VI) processing residue + food]	Concludes that gastric juice alone may not entirely reduce Cr(VI); concurrent food ingestion increases reduction.
Sialelli et al. (2010)	In vitro GI [Cr(VI) in urban soils]	Cr accessibility highest in the intestinal phase (versus stomach).

Reference	Species/media	Findings
Fébel et al. (2001)	Intrajejunal dosing to rats	Absorption of inorganic, trivalent, and hexavalent chromium estimated.
Selected pharmacokinetic studies informing reduction of Cr(VI) and uptake of Cr(III) and Cr(VI)		
Kirman et al. (2012)	Chronic PK study in rats and mice [Cr(VI) in drinking water]	Higher blood and GI tissue concentrations in mice vs rats at comparable exposures.
Witt et al. (2013); Collins et al. (2010); NTP (2010); Stout et al. (2009); NTP (2008)	Male and female rats and mice; dietary and drinking water Cr(III) and Cr(VI) [and gavage Cr(III)]; multiple tissues and excreta	Higher body burdens in rodents exposed to Cr(VI) vs Cr(III). Uncertainties are introduced by 2-day "washout" period prior to tissue collection from chronic studies and differences in exposure media and formulas.
Finley et al. (1997); Kerger et al. (1997); Kerger et al. (1996)	Human volunteer drinking water Cr(VI) and Cr(III) study; urine, RBC and plasma	Elevated Cr levels for all doses. Study authors suggest this is due to Cr(III) absorption.
Sutherland et al. (2000)	Chronic PK study in rats [Cr(VI) in drinking water]	No elevated systemic Cr at 0.5 ppm (~0.06 mg/kg-d); elevated organ levels at 3 ppm (0.3 mg/kg-d) and 10 ppm (0.7 mg/kg-d). Uncertainties are introduced by 4–6 day "washout" period.
Sayato et al. (1980)	IV and oral gavage PK study in rats [Cr(VI) and Cr(III)]	GI absorption of both Cr(VI) and Cr(III) below 1%. Longer half-life for Cr(III).
Thomann et al. (1994)	Chronic PK study in rats [Cr(VI) in drinking water]	Long terminal-phase half-life observed.
Mertz et al. (1965)	IV study in rats [Cr(III)]	Long terminal-phase half-life observed.
Kargacin et al. (1993)	IP injection, and chronic drinking water [Cr(VI)] PK study in rats and mice	Higher blood Cr in rats vs mice; higher liver concentrations in mice vs rats.
Reviews		
Thompson et al. (2013); Witt et al. (2013); Zhitkovich (2011); Stern (2010); De Flora et al. (2008); Sedman et al. (2006); De Flora (2000)		

[a]Reduction capacities represent the mass of Cr(VI) that can be reduced by a tissue or fluid, per unit mass or volume of the media.
[b]An alternative interpretation of rodent data by EPA assumed multiple pathways for Cr(VI) reduction. Reduction capacities ranged from 2-30 µg/mL for the reactions.
[c]Alternative interpretation of human data by EPA assumed 1 pathway for Cr(VI) reduction, with a reduction capacity of 10 µg/mL

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