Received by email on November 12, 2015

Dear Dr. White,

I am writing to provide comment on the Revised Draft: Report on Carcinogens Monograph on Cobalt and Cobalt Compounds That Release Cobalt Ions In Vivo (Draft Monograph), dated November 6, 2015. I am not writing on behalf of my employer and all opinions are my own. I am writing on my own behalf as a toxicologist and risk assessment practitioner who has been following the NTP process for cobalt metal to enhance my understanding of cobalt toxicity.

The purpose of this letter is to request that the NTP consider additional revisions to the Draft Monograph, elaborating on the differences in oral vs respiratory bioavailability for cobalt compounds that are insoluble in water and poorly soluble in low pH (gastric phase) fluids. This can be an important issue in environmental cleanup decisions as the oral ingestion pathway is the larger source of exposure from impacted soil and settled dusts in human health risk assessments. Furthermore many or most human health risk assessments assume that all airborne chemicals are bioavailable, even if not of respirable size, since the fate of larger particulates is swallowing and hence gastrointestinal absorption.

Currently the Draft Monograph discusses the literature on the evidence for the uptake and release of cobalt ions from insoluble fine or nano-sized particulate matter through a number of mechanisms relevant to the respiratory system (Sections 1.2.2 and 6.1), including direct passage of nanoparticulate matter through alveolar membranes, clathrin-mediated endocytosis of very fine particulate matter into alveolar epithelial cells, and phagocytosis of particulate matter by alveolar macrophages and dissolution in the lysosome. As discussed in the Draft Monograph, particulate matter taken up into cells in the lung can be solubilized in lysosomal fluid in lung epithelial cells and macrophages. The rate of that release was slow, but was found to be higher in studies of smaller submicron particles, and given sufficient time might result in complete dissolution of insoluble particulates. The importance of this evidence to the conclusions provided in the Draft Monograph is stated…."Thus cobalt particles with low solubility (e.g., cobalt oxides) are retained in the lungs for long periods and represent a continuing source of exposure" (Section 7.1.1). The relevance to gastrointestinal absorption would appear limited.

The potential for uptake from the gastrointestinal tract for cobalt compounds insoluble at low pH is not discussed in the same detail as the respiratory tract in the Draft Monograph (literature on gastrointestinal absorption is reviewed in Section 3.1.1 and 3.1.2). Table 7-1 lists Co_3O_4

oxide (Co(II,III)oxide) as bioaccessible in gastric fluid and thus presumably orally bioavailable despite the low bioaccessibility results (2%) reported in Table 1-1. The reason for this are the observations from Kreyling *et al.,* 1990 that given sufficient time, even very insoluble particulate matter can still be dissolved in the lysosome of alveolar macrophages. This does not appear to be a major concern for the ingestion route of exposure. In regards to the possibility of particulate uptake from the gastrointestinal tract, uptake by any mechanism may only be relevant for some fraction of ingested particles of about 1 micron in size or less including nanoparticles which may vary also based upon size and surface characteristics of the particles in question (Schleh *et al.,* 2012, Hinkley *et al.,* 2015, Walczak *et al.,* 2015 and references contained therein). For the remainder of any cobalt gastrointestinal exposures, the dissolution of cobalt compounds in the stomach would be the concern for cobalt bioavailability. As reviewed in the Draft Monograph, *in vivo* oral bioavailability of Co(II,III)oxide fine particulate matter is low. For instance one study found that the oral bioavailability of fine particulates of Co(II,III)oxide ranged from 0.8-5.1% in 5 mammalian species (Bailey *et al.,* 1989, referenced in the Draft Monograph). Human oral bioavailability testing for Co(II,III)oxide was unable to measure increased plasma or urine levels in a set of 12 male and 11 female volunteers, although this study had limited sensitivity to measure small increases over background cobalt physiological levels (Christensen *et al.,* 1993a, referenced in the Draft Monograph). According to the July 8, 2015 comments provided by Cobalt Development Institute, in a 90-day repeat dose toxicity study in rats, the only observed toxic effects (hematocrit/erythropoiesis) was at the highest dose of Co(II,III)oxide tested of 1000 mg/kg-bw day; this is in comparison to historical studies of soluble cobalt in rats and mice with a LOAEL of 2.5 mg/kg-bw day and NOAEL of 0.6 mg/kg-bw day for polycythemia in rats following 8-weeks of exposure (ATSDR Toxicological Profile for Cobalt, 2004). In summary, *in vivo* evidence in animals and humans demonstrates limited oral bioavailability of a cobalt compound poorly soluble at gastric pH.

As the Draft Monograph is currently written, the potential gastrointestinal uptake of cobalt compounds of low solubility in gastric fluids is discussed in less detail although it is indicted that lower soluble compounds have lower oral bioavailability (Section 3). There are a variety of cobalt compounds known to be or expected to be of low solubility in gastric fluids besides Co(II,III)oxide including some cobalt pigments, cobalt stellite alloy, alloys of steel, or nickel based superalloys. These uses make up a high percentage of total cobalt consumption in the Unites States (Draft Monograph Appendix, Page P-7). There is a discussion of releases to the environment and potential public exposure referencing sources such as the Toxics Release Inventory which includes exposure to these insoluble forms of cobalt. Potential exposure to the general public includes the ingestion of soils and airborne or settled dusts impacted by such releases. When discussing the potential uptake of cobalt compounds with different physical properties including solubility and size, the focus is on the inhalation route of exposure to fine

particulate matter. There would be benefit to risk assessors if the discussion provided would distinguish the bioavailability of different cobalt compounds that are poorly soluble at gastric pH through the oral routes of exposure from the inhalation route of exposure.

I would also like to suggest some modifications to the Draft Monograph. One alteration would be to alter Table 7-1 (Comparison of chemical and biological properties of cobalt metal and cobalt compounds) to show that Co3O4 oxide is minimally bioaccessible at gastric pH, or in some other way indicate that it would be expected to be highly accessible only following cellular uptake. Another suggestion would be to modify Sections 1.2.2, 1.4 and 7.1 to indicate that the concern over the applicability of *in vitro* bioaccessibility testing due to the uptake of particulate matter and release in cellular compartments is specific for the inhalation of fine particulates due to the only available clearance mechanisms for particles deposited in the deep lung. In my understanding of the literature, the evidence from *in vivo* studies with Co(II,III)oxide indicates that cobalt compounds which are of low solubility at gastric pH will have reduced oral bioavailability compared with soluble cobalt and thus reduced toxicity at equivalent doses.

I appreciate this opportunity to provide comment.

Efrem Neuwirth, Ph.D.

References:

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