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14-day toxicity studies of tetravalent and pentavalent vanadium compounds in Harlan Sprague Dawley rats and B6C3F1/N mice via drinking water exposure

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ABSTRACT

Background: The National Toxicology Program (NTP) performed short-term toxicity studies of tetra- and pentavalent vanadium compounds, vanadyl sulfate and sodium metavanadate, respectively. Due to widespread human exposure and a lack of chronic toxicity data, there is concern for human health following oral exposure to soluble vanadium compounds.
Objectives: To compare the potency and toxicological profile of vanadyl sulfate and sodium metavanadate using a short-term in vivo toxicity assay.
Methods: Adult male and female Harlan Sprague Dawley (HSD) rats and B6C3F1/N mice, 5 per group, were exposed to vanadyl sulfate or sodium metavanadate, via drinking water, at concentrations of 0, 125, 250, 500, 1000 or 2000 mg/L for 14 days. Water consumption, body weights and clinical observations were recorded throughout the study; organ weights were collected at study termination.
Results: Lower water consumption, up to −80% at 2000 mg/L, was observed at most exposure concentrations for animals exposed to either vanadyl sulfate or sodium metavanadate and was accompanied by decreased body weights at the highest concentrations for both compounds. Animals in the 1000 and 2000 mg/L sodium metavanadate groups were removed early due to overt toxicity. Thinness was observed in high-dose animals exposed to either compound, while lethargy and abnormal gait were only observed in vanadate-exposed animals.
Conclusions: Based on clinical observations and overt toxicity, sodium metavanadate appears to be more toxic than vanadyl sulfate. Differential toxicity cannot be explained by differences in total vanadium intake, based on water consumption, and may be due to differences in disposition or mechanism of toxicity.

1. Introduction

Vanadium is a naturally occurring metal, found in several minerals and fossil fuels and exists in various oxidation states from −1 to +5 [5]. It has been estimated that natural processes, such as volcanic eruptions and weathering, result in the release of 65k tons of vanadium into the environment, while anthropogenic sources add an additional 200k tons per year [30]. Anthropogenic sources of vanadium include various industrial processes, e.g. coal-fired power plant production of fly ash and intermediates of iron smelting. In addition to domestic generation of vanadium-containing industrial waste, the United States imports additional waste and intermediates for use in steel production; in 2013 it was reported that more than 9000 t of vanadium (as ferrovanadium, vanadium pentoxide or ash and residues) were imported by the U.S. [22]. Likely due to its industrial uses, vanadium has been detected at several Superfund sites (at least 319 out of 1699) as classified by the EPA [2].

The National Toxicology Program (NTP) has previously studied the carcinogenic potential of vanadium pentoxide in male and female F344/N rats and B6C3F1/N mice following chronic whole-body inhalation exposure, up to 2 mg/m^3. Exposure to vanadium pentoxide is primarily occupational. Chronic exposure to vanadium pentoxide resulted in increased incidences of several non-neoplastic lesions of the respiratory tract (lung, larynx, nose)

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and increased incidences of alveolar/bronchiolar neoplasms in male and female mice, and to some degree in male rats [16]. Vanadium pentoxide has since been listed on California's Proposition 65 list of chemicals "Known to the State [of California] to Cause Cancer or Reproductive Toxicity" and classified as "Possibly Carcinogenic to Humans", category 2B, by the International Agency for Research on Cancer [9,20].

While occupational exposure to vanadium primarily occurs via inhalation, environmental exposure occurs largely via ingestion of soluble vanadium compounds. Ingestion of vanadium may include dietary supplements, food or contaminated drinking water. Due to being a ubiquitous element, food is considered the primary source of exposure to vanadium. Many foods naturally contain some vanadium, with the amounts varying based on soil/water conditions and the extent of vanadium absorption into the food product. Background consumption of vanadium from food is estimated to be between 10 and 60 μg daily [2,15]. Anthropogenic sources of vanadium have the potential to increase human exposure to vanadium through food and drinking water. Vanadium (V) levels in soil from non-contaminated areas range from 3 to 310 μg V/g soil and have been measured up to 400 μg V/g soil in fly ash contaminated areas [30]. Fly ash contamination of drinking water may also contribute to vanadium exposure; in North Carolina, the Department of Environmental and Natural Resources (NC DENR) has led efforts to assess vanadium drinking water contamination due to fly ash leakage. The Coal Ash Management Act of 2014 enforced by the NC DENR has required Duke Energy to test any well within 1500 feet of all Duke Energy coal-fired facilities in the state. Data released in August 2015 list vanadium as an analyte exceeding the N.C. interim maximum allowable concentration (0.3 μg/L) at 10 of the 11 sites, with 2–118 wells tested at each site [19]. In addition, vanadium has been studied by numerous investigators due to reported effects on glucose metabolism; a summary of the therapeutic history of vanadium and diabetes can be found in [28]. Outside of potential pharmaceutics, vanadium is also sold as a dietary supplement with purported benefits including cholesterol metabolism and enhancing athletic performance.

Due to industrial releases of vanadium into the environment and consumption of dietary supplements, there is concern for human health following chronic oral exposure to soluble vanadium salts. The uncertainty regarding safety of vanadium consumption is further complicated by the existence of multiple oxidation states with the potential to interconvert via oxidation-reduction, both in the environment and following ingestion. Highly oxidized forms of vanadium, +3 to +5, are considered the most stable forms and are most likely to be found as environmental contaminants; vanadyl sulfate, a tetravalent form (V^{IV}), is one of the dietary supplements advertised for diabetes and cholesterol. Studies in the literature suggest that oral exposure to vanadium (V^{IV} or V^{V}) may have immunological, neurological, reproductive/developmental and general toxicity effects [12,6,7,11,21,24,25]. Despite the numerous studies published on vanadium compounds, the available literature lacks an adequate comparison of V^{IV} and V^{V}, under a standard study design, in two rodent species exposed in drinking water, the most relevant route of exposure.

Vanadium has gained recognition on the federal level by being included in the U.S. Environmental Protection Agency's (U.S. EPA) draft drinking water Contaminant Candidate List 4, or CCL4, which will be finalized in 2016 [29]. Tetravalent and pentavalent vanadium compounds were nominated to the NTP for testing, in part by the U.S. EPA, and the critical data gaps have been outlined by the Agency for Toxic Substances and Disease Registry [2]. Some data gaps highlighted by ATSDR include comprehensive subchronic and chronic toxicity testing, with an adequate comparison of different oxidation states under similar testing conditions. To help address uncertainties regarding the safety of oral exposure to vanadium, the NTP conducted 14-day drinking water studies of vanadyl sulfate (V^{IV}) and sodium metavanadate (V^{V}) in adult Harlan Sprague Dawley rats and B6C3F1/N mice at exposure concentrations of 0, 125, 250, 500, 1000 or 2000 mg/L. These exposure concentrations were selected based on a critical review of the literature and consideration of the following: palatability, adult versus perinatal toxicity, the relative toxicity of pentavalent versus tetravalent compounds and an overall lack of toxicity data in mice. Vanadyl sulfate was selected as the V^{IV} test article due to its use in dietary supplements. Sodium metavanadate was selected as a representative V^{V} species following characterization of drinking water formulations of sodium orthovanadate and sodium metavanadate [13]. The designs of the two studies were essentially identical to facilitate comparisons between the two valence states. These data will help inform the design and exposure concentration selection for subchronic and chronic toxicity assessments of vanadium, which will provide essential data for the risk assessment of vanadium following oral exposure.

2. Methods

2.1. Chemical and dose formulation

Vanadyl sulfate (CASRN 27774-13-6, Lot No.0210324/1.1) was purchased from Noah Technologies Corporation (San Antonio, TX). Sodium metavanadate (CASRN 13718-26-8, Lot No. 8579K) was purchased from MP Biomedicals (Santa Ana, CA). The identity of each vanadium compound was determined by infrared spectroscopy, X-ray diffraction, and proton-induced X-ray emission spectroscopy. The overall purity, determined by high performance liquid chromatography (HPLC) with charged aerosol detection, was 100 and 99.5% for vanadyl sulfate and sodium metavanadate, respectively.

Drinking water formulations (0, 125, 250, 500, 1000, 2000 mg/L) of sodium metavanadate (~pH 7) and vanadyl sulfate (~pH 3.5) were prepared using tap water from the City of Columbus, Ohio municipal supply. Formulations were analyzed with HPLC with ultraviolet detection prior to study start and following the last exposure; all samples were within ±10% of the target concentration and 0 mg/L formulations were below the limit of quantitation of the analytical method (vanadyl sulfate 0.43 mg/L, sodium metavanadate 0.46 mg/L).

The stability of formulations was established prior to study start. Sodium metavanadate and vanadyl sulfate formulations were stable for up to 42 days; at day 42, sodium metavanadate was 99–101% of day 0 and vanadyl sulfate was 92–100% of day 0 when stored protected from light at ~5 °C or room temperature. In some vanadyl sulfate formulations, a small peak was observed ≥day 7, in the HPLC chromatogram at the retention time of vanadate, suggesting slight oxidation of vanadyl to vanadate [13].

2.2. Animals and animal maintenance

The studies presented here were conducted at Battelle Memorial Institute (West Jefferson, OH). Harlan Sprague Dawley (HSD) rats were purchased from Harlan (Indianapolis, IN). B6C3F1/N mice were supplied from the NTP colony at Taconic Biosciences (Germantown, NY). Upon arrival, animals were quarantined for at least 10 days and provided *ad libitum* access to irradiated NTP-2000 wafer feed (Zeigler Brothers, Inc., Gardeners, PA) and tap water. Following quarantine, 5–7 week-old animals were randomized into exposure groups by body weight. Animals were uniquely identified by tail tattoo upon randomization. On study day one, animals were placed on control or dosed-water (125, 250, 500, 1000, 2000 mg/L)