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Letter to the Editor

Comment on Strupp Papers on Beryllium Metal Toxicity

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I read with great interest the research articles by Strupp (2011a,b) 'Beryllium Metal I' and 'Beryllium Metal II' which were funded by the REACH Beryllium Consortium (2008), an industry group led by Brush Wellman Inc., the largest producer of beryllium in North America. I would like to comment on the author's conclusion that beryllium metal is not a skin sensitizer and to caution readers on the certainty of this conclusion. First, I will address the method used for the skin sensitization test (Strupp, 2011a) and then comment on the discussion of the data that omitted key papers in the published literature to support the finding (Strupp, 2011b).

Organization for Economic Cooperation and Development (OECD) Guideline 406 is a guinea pig maximization test that calls for intradermal injection and subsequent topical application of the test agent (OECD, 1992). Using this protocol, Strupp (2011a) reported that none of the test animals became sensitized following exposure to beryllium metal. As noted in OECD Guideline 406, testing mice using objective validated tests such as the mouse ear swelling test (MEST) and the local lymph node assay (LLNA) is also sufficient to designate a substance as a sensitizer. Tinkle *et al.* (2003) topically applied beryllium sulfate to ear skin of mice and demonstrated that mice had become sensitized as quantified using the LLNA assay. In a second experiment, Tinkle *et al.* (2003) topically applied beryllium oxide in petrolatum to bare skin on the backs of mice for 1 day and 1 week later challenged the same mice with beryllium sulfate. Ear thickness, as measured by the MEST, was significantly increased at 48 h after challenge, indicating that beryllium oxide particles had induced sensitization when applied topically to the skin.

To induce sensitization, Strupp used intradermal injection of metal powder suspended in polyethylene glycol (PEG) though topical application is an effective pathway for poorly soluble beryllium compounds (Tinkle *et al.*, 2003). Metal powder in PEG was also used for delivery of booster and challenge exposures. PEG has near neutral pH in aqueous solution and dissolution of metal is a factor of 10–40 slower than soluble beryllium chloride at neutral pH (Strupp, 2011a). In contrast, previous studies used soluble beryllium sulfate for challenge to ensure that sufficient ions were available to provoke a reaction in sensitized animals (Marx and Burrell, 1973; Zissu *et al.*, 1996; Tinkle *et al.*, 2003). Hence, reasons why Strupp's data for metal differ from previous studies using beryllium sulfate and oxide may be due to the method of exposure delivery and/or lack of beryllium ions in the challenge exposure.

In discussing the results of the skin sensitization test, Strupp (2011b) omitted several key studies (Marx and Burrell, 1973; Tinkle *et al.*, 2003) and discounted the relevance of studies by Curtis (1951) and Zissu *et al.* (1996) as these latter two studies were deemed to 'not give a satisfactory answer for metal'. Such incomplete compilation and assessment of the literature do not provide a full perspective on existing knowledge with regard to the skin-sensitizing potential of beryllium compounds, including the metal. Curtis (1951) demonstrated 60 years ago that beryllium salts were skin sensitizers in humans and subsequent animal studies confirmed this observation (Marx and Burrell, 1973; Zissu *et al.*, 1996; Tinkle *et al.*, 2003). Additionally, hypersensitivity reactions can be elicited from sensitized guinea pigs upon challenge with beryllium metal (Zissu *et al.*, 1996). Importantly, Tinkle *et al.* (2003) demonstrated that topical application of poorly soluble beryllium oxide powder was sufficient to induce skin sensitization in mice. This finding is especially noteworthy as Cummings *et al.* (2007) reported that when an occupational preventive program, which included protecting skin, was instituted in an oxide machining facility, the

prevalence of beryllium sensitization among new workers declined, despite similar air exposure levels before and after the program.

Strupp (2011b) correctly refers to the availability of beryllium ions as an important factor in development of sensitization, but attempts to differentiate salts from metal and oxide on the basis of water solubility values (salts $>>$ metal $\approx$ oxide). Water solubility refers to how much of a material will dissolve in a solute until reaching equilibrium. In biological systems, solubility is used to describe the rate at which a material will dissolve in a fluid relative to the rate of mechanical clearance. A poorly soluble material is one that dissolves at a rate slower than the rate of mechanical clearance and a highly soluble material is one that dissolves at a rate faster than mechanical clearance. In the occupational environment, workers sweat and it was just recently demonstrated that beryllium metal was much more soluble than beryllium oxide in artificial sweat (Stefaniak *et al.*, 2011). Thus, we now know that beryllium compounds possessing a wide range of (biological) solubilities, from highly soluble salts (Curtis, 1951; Marx and Burrell, 1973; Zissu *et al.*, 1996; Tinkle *et al.*, 2003) to poorly soluble beryllium oxide (Tinkle *et al.*, 2003), have skin-sensitizing potential. The fact that solubilities of beryllium salts and oxide bracket that of beryllium metal provides indirect evidence of the biological plausibility that beryllium ion release from particulate metal in contact with the skin can induce sensitization.

Strupp (2011a) used OECD Guideline 406 to test the skin-sensitizing potential of beryllium metal to comply with REACH. A limitation of Guideline 406 is that it was adopted in 1992, more than a decade prior to demonstration that topical application of beryllium oxide could induce sensitization. Strupp (2011a) justified use of the guinea pig maximization test rather than LLNA because 'beryllium metal cannot be dissolved in a vehicle suitable for this assay'. Interestingly though, Strupp (2011a) used artificial lung fluids to extract metal ions for genotoxicity testing. Hence, the study design employed by Strupp (2011a) is insufficient to definitively conclude that beryllium metal is not a skin sensitizer. To properly address this question, beryllium metal powder should be tested with mice using (i) topical application of metal powder, challenge with soluble beryllium, and quantitative assessment by the MEST (following the protocol of Tinkle *et al.*, 2003) and (ii) topical application of beryllium ions extracted from metal powder into artificial sweat (to better represent workplace exposure) followed by quantitative assessment by the LLNA assay. Ideally, the guinea pig maximization test would be performed side-by-side with the mouse LLNA.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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