Differences in Estimates of Beryllium Aerosol Size Distribution for Toxicity Studies Using Phase Contrast Microscopy, Scanning Electron Microscopy, and Liquid Particle Counter Techniques

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INTRODUCTION

Relating the physicochemical properties of an aerosol to its toxicity following inhalation is of specific interest for beryllium (Stefaniak et al., 2004) as well as of general interest for a wide range of particles, e.g., nanomaterials (Oberdörster et al., 2005). Various hypotheses have been put forth regarding particle mass, surface area, and number as metrics of toxicity of inhaled particles. Detailed control and physico-chemical characterization of aerosols generated for inhalation toxicology studies is essential for obtaining meaningful study results.

A high priority of research is characterization of the exposure material as encountered by workers and as administered in an inhalation toxicology study. Characterization of bulk material as produced or supplied may not accurately reflect the properties of the particles in the workplace atmosphere or delivered by inhalation, installation, or pharyngeal aspiration to laboratory animals. For example, current methods for characterizing dry material using microscopy can reveal general morphology and particle size, but are not capable of determining whether material consists of solid particles or clusters, or whether material will deagglomerate in lung surfactant. Similarly, current methods for characterizing material in suspension can reveal "equivalent" particle diameter, but are not capable of determining particle morphology. Thus a suite of techniques are needed to understand the multiple particle properties that may influence toxicity.

The purpose of this study was to characterize the morphology and investigate the size distributions of four beryllium materials using three standard sizing techniques as they may reveal important characteristics of the study material.

MATERIALS AND METHODS

We studied finished product metal powder (product type I-400, Brush Wellman Inc., Elmore, OH); finished product beryllium oxide (BeO) powder (product type UOX-125, Brush Wellman Inc.); process-sampled particles from the BeO powder production line; and process-sampled aerosol from a copper-beryllium alloy production line.

Bulk metal and BeO powders were aerosolized and aerodynamically size-separated using a 5-stage aerosol cyclone operated at 24 L min^{-1} and 20 °C; aerodynamic cutoff diameters (D_{ae}) were >6, 2.5, 1.7, 0.9, and 0.4 μm for stages 1 to 5 of the aerosol cyclone, respectively (Hoover et al., 1989). Process-sampled particles were collected from ventilation ductwork using a 5-stage aerosol cyclone operated at 28 L min^{-1} and 23 °C; D_{ae} were >5.7, 2.3, 1.5, 0.7, 0.4 μm for stages 1 to 5 of the aerosol cyclone, respectively (Stefaniak et al., 2004).

Material collected in stages 2, 3, and 4 of the aerosol cyclone were studied. Morphology was determined using transmission electron microscopy (TEM). The geometric mean (GM) particle size and geometric standard deviation (GSD) particle size was determined using phase contrast microscopy (PCM), a liquid particle counter (LPC), and computer-controlled scanning electron microscopy (CCSEM).

PCM was used to determine particle Feret diameter (distance between tangents drawn from the extreme left and right edges of a particle measured perpendicular to a reference line) using a Filar ocular micrometer; the theoretical limit of resolution was 0.4 μm. For each material, 1000 particles were sized from slides of particles dispersed in mounting medium on a slide.

A LPC (Multisizer II, Coulter Electronics, Inc.) operated with 30 μm diameter aperture tube (0.6 – 18 μm actual cutoff range) was used to determine particle spherical equivalent diameter (size equivalent to a spherical particle that produces the same voltage pulse when drawn between two electrodes). For analysis, a dilute suspension of each material in physiologic saline was subjected to ultrasonic agitation for 30 sec to mix then an aliquot of each suspension added to electrolyte solution (Coulter Electronics, Inc.); tens of thousands of particles were sized for each material. Data were background corrected for particle counts in fluids.

CCSEM (R.J. Lee Group, Monroeville, PA) was used to determine particle projected area diameter using the rotated chord technique (length of 16 chords traced across the particle at equiangular intervals through the centroid of the particle) and ZepAPA computer software program algorithm. For analysis, a dilute suspension of each beryllium material in distilled and deionized water was deposited onto a polycarbonate filter using vacuum filtration and coated with a thin layer of gold/palladium; 1000 particles were sized from a representative section of each filter.

RESULTS

From TEM analysis, metal powder was compact with smaller sub-micrometer size particles attached to the surface of larger particles. BeO powder and process-sampled particles were clusters of primary particles. Alloy was also clusters of primary particles.

The GM (and GSD) particle size of each beryllium material is summarized by sizing technique in Table 1.

Table 1. Geometric mean diameter of beryllium powders and process-sampled particles

		Geometric mean, μm (GSD)[A]		
Material	Stg	PCM	CCSEM	LPC
Be powder	2	3.0 (1.8)	NP	1.5 (1.8)
	3	2.7 (1.7)	NP	1.3 (1.5)
	4	2.0 (1.5)	1.1 (1.9)	1.1 (1.6)
BeO powder	2	1.0 (1.3)	NP[B]	1.1 (1.3)
	3	1.2 (1.5)	NP	1.0 (1.3)
	4	1.0 (1.4)	0.9 (1.5)	1.0 (1.3)
Process BeO	2	1.2 (1.6)	NP	NP
	3	1.1 (1.6)	NP	NP
	4	1.2 (1.6)	1.0 (1.9)	NP
Process alloy	2	2.1 (1.9)	NP	1.1 (1.3)
	3	1.9 (1.7)	NP	1.0 (1.3)
	4	1.6 (1.7)	--[C]	0.9 (1.3)

[A] GSD = geometric standard deviation
[B] NP = analysis not performed for this size-fraction
[C] Greater than 60% of these particles were 0.4-0.7 μm precluding graphical determination of the GM

DISCUSSION

As expected, the GM Feret diameter of metal powder decreased with aerodynamic size-fraction; however, when suspended in liquid, the GM particle size often decreased by half. A small portion of this decrease in particle size is attributed to differences in the definition of size among the three analytical techniques. Additionally, our inability to size small particles using PCM could explain a small portion of this decrease in size. Stein (1968/1969), in studies using polystyrene latex spheres found that sizing using PCM at the theoretical limit of resolution of the objective lens (0.4 μm) missed just 10% of particles relative to SEM. A more likely source of variability is the sample preparation protocol. Ultrasonic agitation of each suspension prior to analysis with the LPC might be expected to break apart particles, although for CCSEM analysis, particle size distribution also shifted smaller, despite the fact that the samples were not subject to ultrasonic agitation. Thus, for beryllium metal suspended in liquid, the smaller submicrometer size particles attached to the surface of larger particles probably detach, thereby shifting the particle size distribution toward a smaller GM particle size. Detachment of metal particles could significantly change the expected aerosol size distribution when deionized water or electrolyte solution are used as vehicles for particle delivery to an exposure subject.

The size of each BeO material was nearly constant regardless of sizing technique. Thus, for BeO, any small differences in GM particle size may be attributed to differences in particle size definition. For these materials, aerodynamic cluster size will dictate deposition in the lung, but primary particle size will influence biological activity.

The GM Feret diameter of particles sampled from the alloy production line did not show an appreciable shift in size with PCM; however, when suspended in liquid for CCSEM and LPC analyses, GM particle size decreased by 50 to 100%. Thus, these alloy particles probably also detach in liquid, thereby shifting the size distribution smaller. This detachment of particles could have significance for the expected versus actual size distribution of aerosol delivered to an exposure subject.

CONCLUSIONS

Careful attention is needed when characterizing exposure aerosols for inhalation toxicology studies. When estimating size distribution, consideration must be given to the use of a liquid vehicle which may alter the expected aerosol size distribution.

Keywords: *Aerosol generation, Respiratory deposition*

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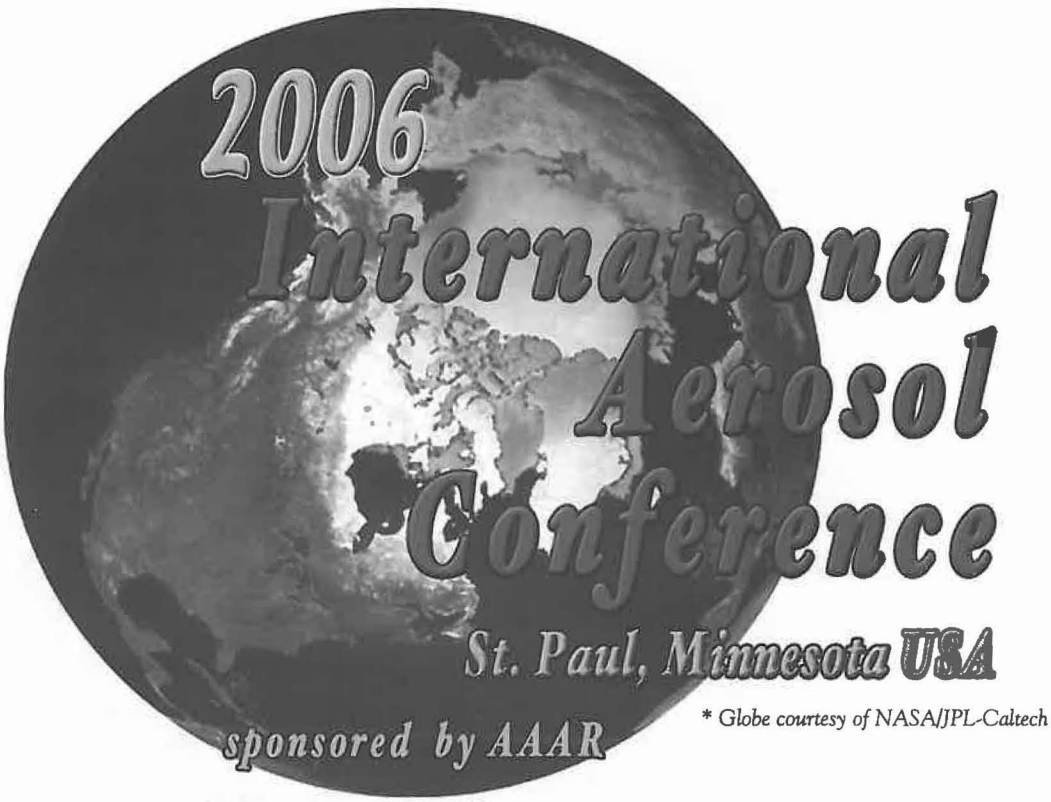

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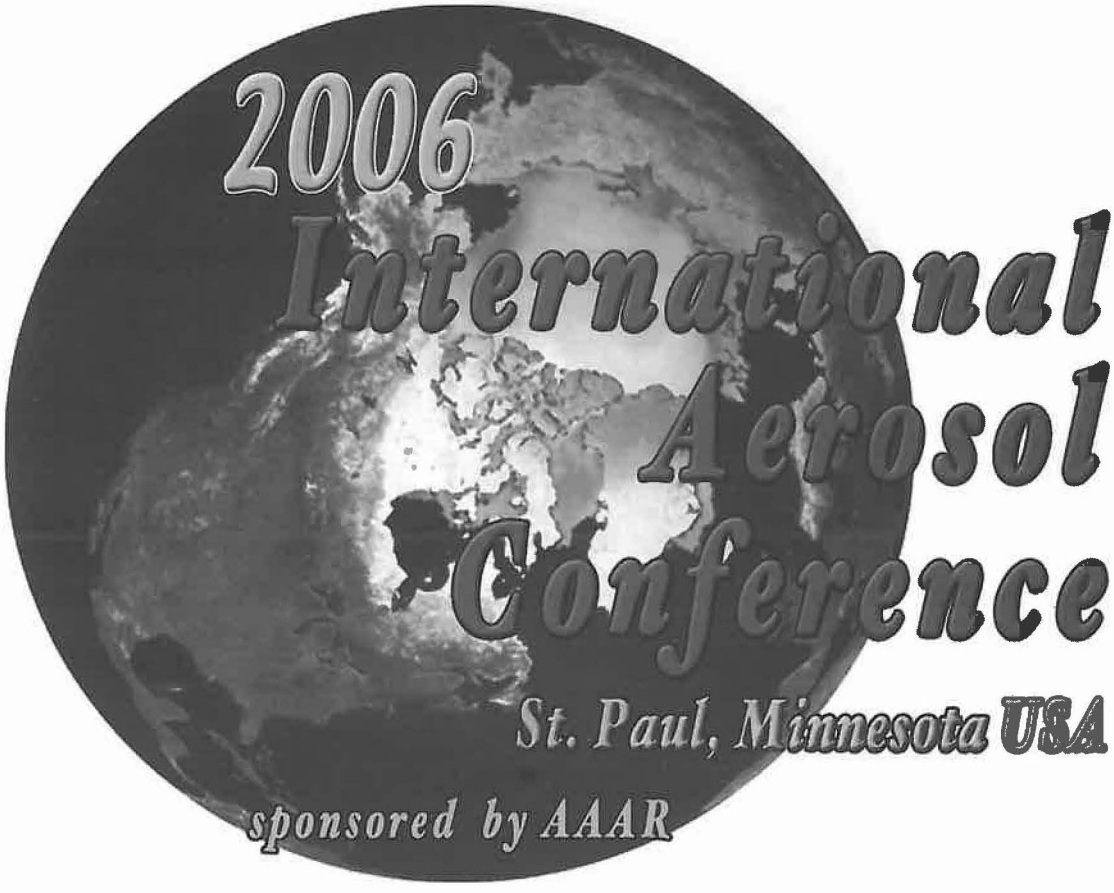

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