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Research paper

Occupational exposure during handling and loading of halloysite nanotubes – A case study of counting nanofibers

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ABSTRACT

Halloysite nanotubes (HNTs) are abundant naturally-occurring hollow aluminosilicate clay mineral fibers with a typical diameter < 100 nm and an aspect ratio of up to 200. Here we assessed the potential inhalation exposure to HNTs in an industrial research laboratory. Inside a fume hood, ten times 100 g of HNTs were poured at rate of 0.5 kg min^{-1}, which increased concentrations from the background level up to 2900 cm^{-3} and 6.4 μm^2 cm^{-3}. Inside the fume hood, the respirable mass concentration was 143 μg m^{-3} including background particles. Outside the fume hood we did not measure elevated concentrations. We classified 1895 particles according to their length and aspect ratio. Five particles were in aspect ratio > 3 and in length > 2 μm. These particles were agglomerated and/or aggregated particles where the longest individual fiber was 2 μm in length. The occupational exposure limits for refractory mineral fibers vary from 0.1 to 2 fibers cm^{-3}. Following standard protocols for fiber analysis, detection of 0.1 fibers cm^{-3} would require analysis on 4×10^4 images when the filter loading is good. Thus, the fiber sampling and quantification procedures needs to be improved significantly if nanofibers < 100 nm in diameter are included in regulatory exposure assessment. Due to very limited toxicological information of HNTs we recommend avoiding inhalation exposure.

1. Introduction

Halloysite nanotubes (HNTs) are a low cost and naturally occurring abundant clay mineral of the kaolin group (Joussein et al., 2005). HNTs are hollow insoluble mineral fibers with lengths of up to 30 μm and aspect ratio values of up to 200 (Makaremi et al., 2017). They are characterized by high mechanical strength and modulus, and due to their hollow nanostructure they are widely used for loading and controlled release of functional compounds (Makaremi et al., 2017; Yang et al., 2016; Huang et al., 2016; Saif and Asif, 2015; Lvov et al., 2016a). HNTs are used in, *e.g.*, self-healing anticorrosive coatings (Wei et al., 2015), hydrogen production and storage (Sahiner and Sengel, 2017; Jin et al., 2017), pharmaceutical excipients (Hanif et al., 2016; Lvov et al., 2016b; Yendluri et al., 2017), biomedical applications (Liu et al., 2016; Bonifacio et al., 2017), cosmetics (Saif and Asif, 2015), active food packaging materials (Shemesh et al., 2016; Tas et al., 2017; Krepker et al., 2017), water treatment (Yu et al., 2016), and for improvement the mechanical properties and thermal stability of polymer composites (Liu et al., 2014). Globally, HNTs annual production is over 50,000 metric tonnes (Lvov et al., 2008), which is similar to that of carbon fibers (40,000 t/y; Gutiérrez and Bono, 2013), and approximately 10 times higher than the production of carbon nanotubes which is only *ca.* 4000 t/y (De Volder et al., 2013).

Pulmonary exposure to long and poorly soluble fibers is associated with a high risk of serious adverse health effects (*e.g.* Lippmann, 1988). It has been shown that mesothelioma and pleural plaques are caused by biodurable fibers thinner than ~0.1 μm and longer than ~5 μm while cancer and pulmonary fibrosis are caused by fibers thicker than ~0.1 μm and longer than ~20 μm (Lippmann, 1988). This phenomenon of inhalable, long and biodurable fibers is denoted the fiber paradigm, and fibers fulfilling the criteria are defined as WHO fibers (Lippmann, 1988, 2014; Harrison et al., 2015). Another important hazard indicator

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for HNTs is their high aspect ratio and large specific surface area. For mineral fibers with a diameter in the range of 0.15 and 2 µm and a length above 2 µm, fibrosis correlated with the surface area of the fibers (Lippmann, 1988, 2014; Stayner et al., 2008, 2013; Hwang et al., 2014). While airway exposure to short carbon nanotubes, ~0.3 µm in length, was shown to cause fibrosis and long lasting inflammation (Pauluhn, 2010; Poulsen et al., 2015, 2016, 2017).

The occupational exposure limits (OELs) for refractory mineral fibers fulfilling the fiber paradigm generally vary from 0.1 to 2 fibers cm^{-3} (Harrison et al., 2015; Nielsen and Koponen, 2018). For high aspect ratio nanomaterials (HARN), such as non-entangled carbon nanotubes, carbon nanofibres, and nanocellulose, with fiber lengths over *ca.* 5 µm, a more stringent OEL of 0.01 fibers cm^{-3} has been proposed for precautionary reasons (Mihalache et al., 2017). Gebel et al. (2014) proposed that biodurable HARN that do not meet the fiber paradigm may be classified as granular biodurable particles (GBPs). GBPs are classified as low toxicity particles, which may however cause inflammation and acute phase response (Moreno-Horn and Gebel, 2014; Saber et al., 2014), which in turn are risk factors for cardiovascular disease (Saber et al., 2014).

Stanton et al. (1981) showed that pleural dose to 40 mg of two different types of HNTs in hardened gelatin implanted on to the pleural surface resulted in formation of pleural sarcomas in 9 of 53 rats (17%) 2 years post-exposure as compared to 3 of 488 sham-treated controls (0.6%). Kaolin, a platy sheet silicate with similar chemical composition as HNTs, has been shown to induce cytotoxicity and genotoxicity in isolated rat alveolar macrophages (Gao et al., 2000). Unmodified HNTs have shown to induce very low cytotoxicity in the following human cell types: carcinoma cells, peripheral blood lymphocytes, primary umbilical vein endothelial cells, intestinal cells, and epithelial cells (Ahmed et al., 2015; Vergaro et al., 2010; Nan et al., 2008; Lai et al., 2013). However, as HNTs have similar dimensions as short carbon nanotubes (CNTs), they may potentially cause pulmonary inflammation and acute phase response following pulmonary exposure as previously reported for CNTs (Saber et al., 2013, 2014; Poulsen et al., 2015, 2017; Jaurand, 2017).

Here, we studied HNTs release and exposure during a two-step loading process where HNTs are mixed with essential oil. The product is used for example in active food packaging materials, which have been reported to reduce bacterial growth by up to seven orders of magnitude, thereby increasing the shelf life of perishable foods (Shemesh et al., 2016; Tas et al., 2017; Krepker et al., 2017). Here we assessed workers inhalation exposure by measuring air concentrations using diffusion chargers and by sampling airborne particles for gravimetric and electron microscopy analysis. Subsequent risk assessment was performed based on HNTs exposure levels in fiber number, surface area, and mass concentrations. Finally, we discuss the potential challenges in fiber counting when the fiber diameter is < 100 nm.

2. Experimental section

2.1. Measurement strategy

Particle concentrations were measured from different locations named here as fume hood (30 cm above the mixing bowl), near-field at a height of 1.5 m, breathing zone, and incoming ventilation air using four miniature diffusion size classifiers (DiSCmini, Matter Aerosol AG, Wohlen, Switzerland) and four respirable particle samplers (Fig. 1). The DiSCminis were equipped with 0.7 µm pre-separators and *ca.* 50 cm Tygon sampling hoses. The DiSCminis readings were compared before the measurements started by sampling room air aerosol for 240 s within with 10 cm radius. The DiSCminis showed good agreement between all four instruments when measuring at the same location (Fig. 2) as has also been observed in previous studies (Bau et al., 2017). The correlations as compared to average values measured by the DiSCminis were for *N* from 0.63 to 0.87, *LSDA* from 0.69 to 0.90 and $D_{p,DM}$ from 0.60 to

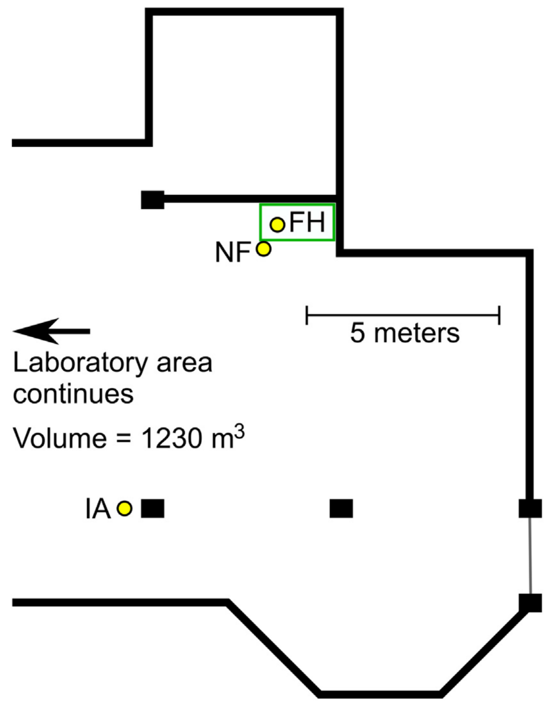

Fig. 1. Layout of the laboratory and sampling locations of Incoming Air of mechanical ventilation air inlet (IA), Near-Field (NF), and Fume Hood (FH).

0.77 (Table S6, Supporting Information).

Respirable particles were collected on a pre-weighted 37 mm Teflon filters with a 0.8 µm pore size (Millipore, Billerica, MA, USA) using a BGI Model GK2.69 (Q_s = 4.2 L min^{-1}) Triplex cyclones (BGI Inc., Waltham, MA, USA; Stacey et al., 2014). Three control blind filters were used to correct for handling and environmental factors. Filter weighing was completed in a climate controlled weighing room at 50% relative humidity and 22 °C after at least 24-hour acclimatization. The fume hood air flow was measured using a hot wire anemometer (BL-30 AN, Voltcraft, Hirschau, Germany).

2.2. Particle sampling and characterization

The sample was collected at a flow rate of 0.5 L min^{-1} (model NMP 830, KNF Neuberger, Germany) using a Micro Inertial Impactor (Kandler et al., 2007), consisting of three stages, each equipped with Nickel TEM grids with a Formvar carbon foil. This sampling technique has been used successfully in environmental (Lieke et al., 2011; Kandler et al., 2011; Nguyen et al., 2017), occupational (Jensen et al., 2015; Kling et al., 2016; Koivisto et al., 2018), and combustion particle studies (Lieke et al., 2013). The Micro Inertial Impactor samples particles up to *ca.* 30 µm in diameter depending on the sample flow's iso-axial behavior (Kandler et al., 2007). The calculated d_{50} cut-off diameters by inertial impaction are 1.3, 0.5, and 0.05 µm for the impaction stages. The particle collection size ranges by inertial impaction are:

- Stage 1: 1.3 µm < d_p < ~30 µm
- Stage 2: 0.5 µm < d_p < 1.3 µm
- Stage 3: 0.05 µm < d_p < 0.5 µm

Here we took overview images of the samples from each stage which was used to locate the impaction spots which were analyzed in further detail at higher magnifications, sufficiently high for resolving the nano-scale dimensions suspected in the sample.

The analysis was made at the Center for Electron Nanoscopy, Technical University of Denmark, using a FEI Nova NANO Scanning Electron Microscope 600, which was used in scanning transmission