A Review of the Toxicity of 0.1 to 1 Micron Aerodynamic Diameter Airborne Particles

J.A. Pickrell[1], L.E. Erickson[2], M Dhakal[1], Sigifredo D. Castro [2], K. J. Klabunde[3], R G. Maghirang[4] and F.W. Oehme[1]

[1]Comparative Toxicology Laboratories, [2]Chemical Engineering, Chemistry[3] and [4]Biological and Agricultural Engineering .

Kansas State University, Manhattan, KS 66506-5705 USA

EXTENDED ABSTRACT

Nanoparticles have been defined as particles having one or more dimensions $\leq$ 100 nanometers [nm] or 0.1 microns. They are so small they have different physical properties than conventional crystals of the same chemical. They have promising applications; a benefit is that airborne nanomaterials (NM) can condense with and clean smoke from the air more quickly than the smoke would settle without assistance because their surface is very active. Because of their nanocrystalline structure, a higher portion of their molecules are on the surface and may interact with airborne particles.

Parallel to this application are growing concerns about NM, If NM are poorly soluble particles (PSP) NM may enter the body, show biopersistence, travel to target tissue and cause brain, lung, or heart effects at low concentrations. As an example, at extremely small dimensions (< 0.2 microns [ultrafine or nanoparticles] or 0.2 to 1 micron [accumulation particles]), effects are thought to be related to particle numbers and surface activity. In nature, these particles originate mostly from transportation aerosols and are at higher concentrations in urban situations. At 1-2.5 microns (intermodal particles) aerodynamic diameter, solubility and biological effects were thought to be related to total surface area. Finally, particles between 2.5 and 10 microns or larger are mostly related to particle mass. These particles originate mostly from the earth's crust. Alternatively, particles readily dissolve, have minimal biopersistence, and represent minimal to negligible danger to exposed animals or people. For NM that are

manufactured and have been shown to be of great technological benefit, we have a great responsibility to consider their potential to produce environmental adverse effects.

Metal oxides have a gradient of solubilites. For example, when 1 g (1,000 mg) of magnesium oxide (MgO) was dissolved in 1 liter of deionized distilled water, it had only moderate solubility over a considerable time period (300 hours) (Figure 1); titanium dioxide (TiO_2) was reported to have almost no solubility in aqueous media.

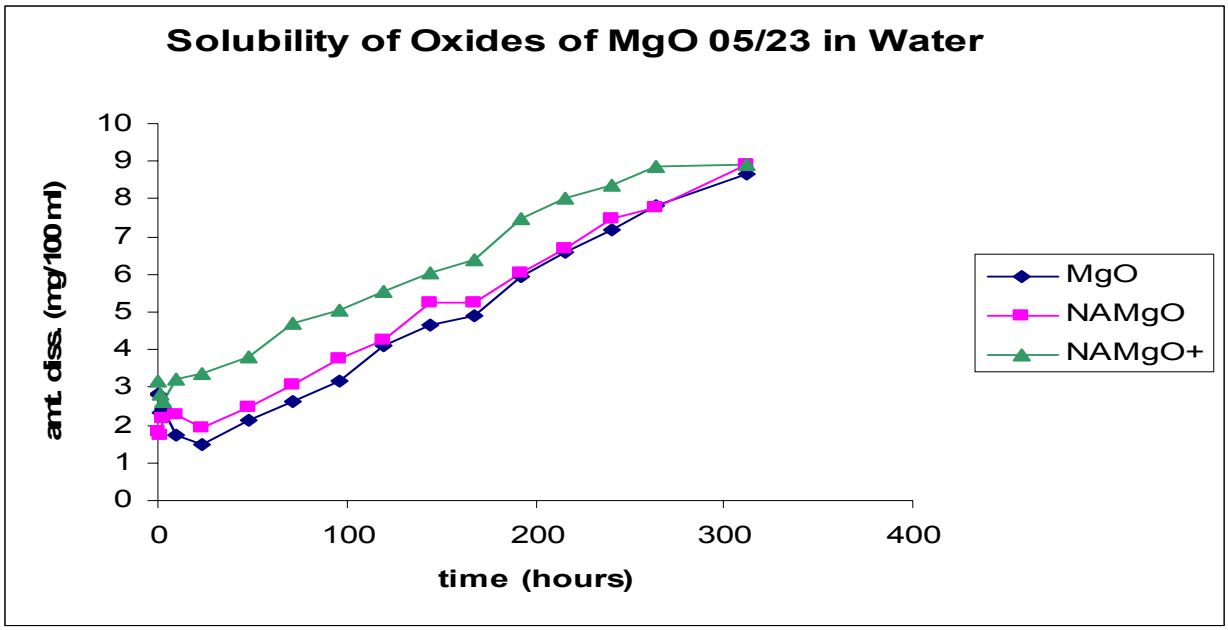

Figure 1.

Amount of MgO dissolved is shown as a function of time in hours. MgO (1 g/L; 1000 mg/L) added to deionized distilled water used as the initial simulant epithelial lining fluid (ELF). Materials examined were conventional macrocrystalline magnesium oxide (MgO), Nanoactive ™ MgO (NA ™ MgO) and NA ™ MgO Plus (NA ™ MgO+). Each point represents a mean of 3 replicates. Samples were from 3 minutes to Total MgO dissolution increased from 1.6 to 3 mg/100 ml at 3 minutes to nearly 9 mg/100 ml by 330 hours.

When the same amount of MgO compounds were dissolved in Hanks Balanced Salt Solution (HBSS), media that contained 35 mg bicarbonate per 100 ml of media to simulate lung

epithelial lining fluid (ELF) the solubility of each MgO was increased 3-fold early and 2-fold at 300 hours (Figure 2). We did not find any reports of soluble titanium carbonates in nature.

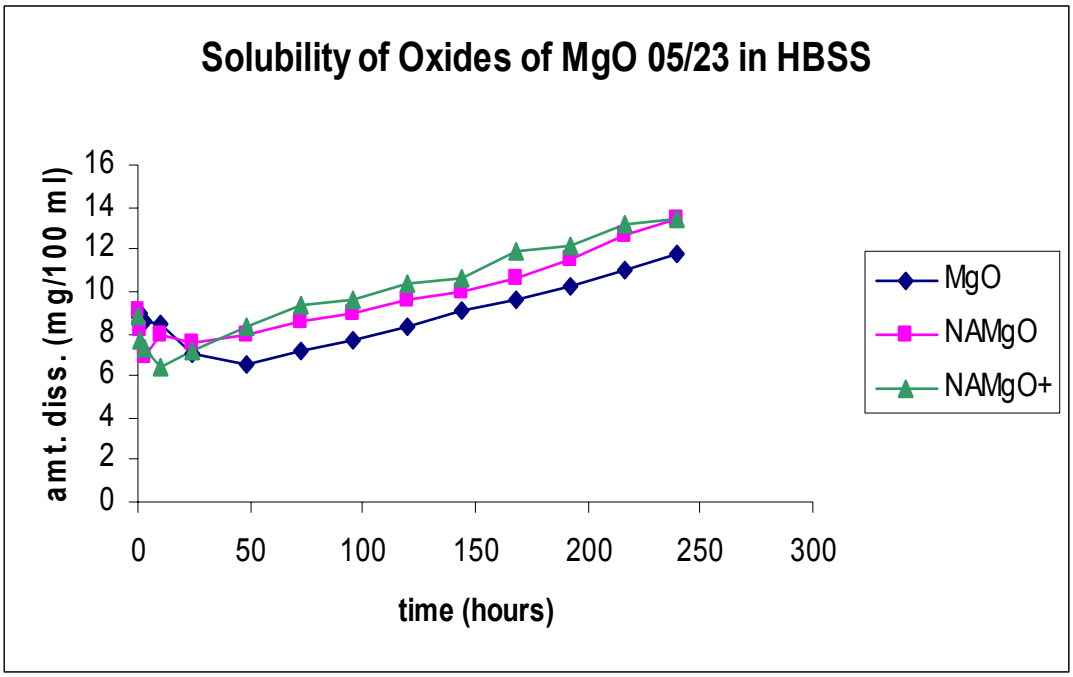

Figure 2.

Amount of MgO dissolved is shown as a function of time in hours. MgO (1 g/L; 1000 mg/L) added to Hanks Balanced Salt Solution (HBSS) deionized distilled water used as the simulant epithelial lining fluid (ELF). Materials examined were conventional macrocrystalline magnesium oxide (MgO), Nanoactive ™ MgO (NA ™ MgO) and NA ™ MgO Plus (NA ™ MgO+). Each point was a mean of 3 replicates. Total MgO dissolution increased from 7 to 9 mg/100 ml at 3 minutes to nearly 11 to 12 mg/100 ml by 240 hours.

The MgO solubility was proportional to the bicarbonate concentration in ELF simulant fluid ($p<0.001$). After 2 weeks dissolution, higher percentages of MgO were dissolved as total amounts of MgO were reduced from 500 mg MgO/liter to 50 mg MgO/liter using HBSS to simulate lung ELF (Figure 3). Dulbeccos Modified Eagle's Medium (DMEM) produced more rapid and more complete solubility.

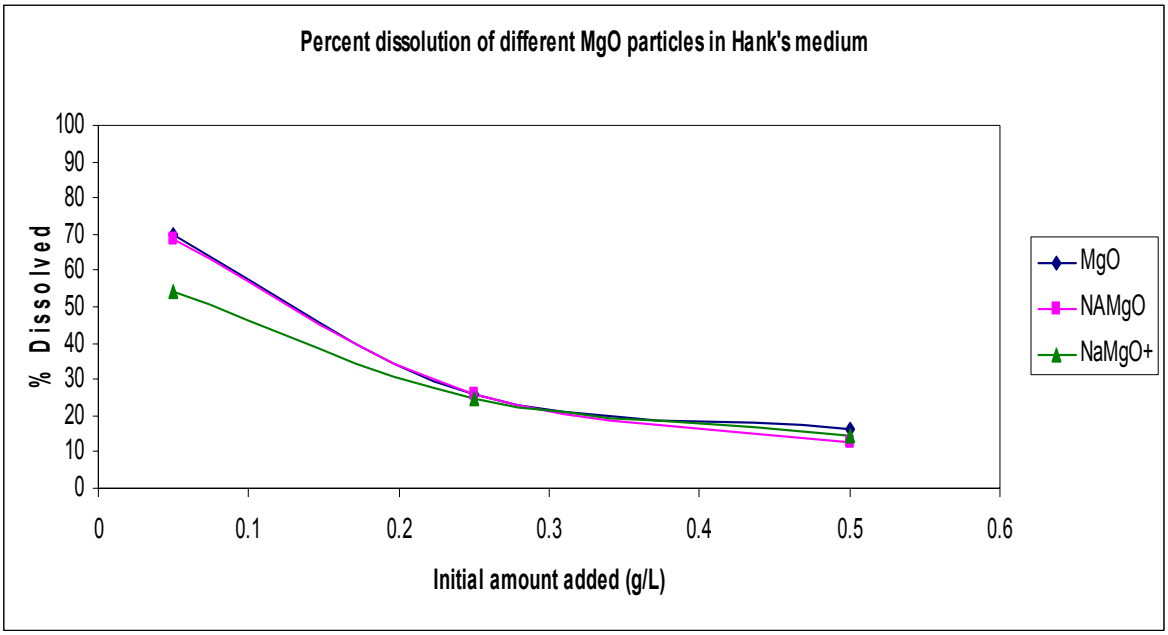

Figure 3.

Amount of MgO dissolved is shown as a function of amount of MgO added (50-500 mg/L). MgO was added to Hanks Balanced Salt Solution (HBSS) deionized distilled water used as the simulant epithelial lining fluid (ELF). Materials examined were conventional macrocrystalline (MC) magnesium oxide (MgO), Nanoactive ™ MgO (NA ™ MgO) and NA ™ MgO Plus (NA ™ MgO+).Total MgO dissolution (%) increased from 20 to 26 to 62%, respectively for 500, 250 and 50 mg/dl. Each point was a mean of 2 replicates (range was 15-18% of the mean; sample was peak solubility in a 14 day dissolution study in this experiment.

To evaluate the nature of the compound formed when MgO was dissolved in aqueous bicarbonate a pH was also measured when MgO concentrations were measured for each of the MgO particles. The pH of conventional macrocrystalline MgO and both NA MgOs increased as increasing concentrations of MgO to be dissolved increased for bicarbonate containing solutions (Figure 4). The pH increased from 7.4 to 8.4 with DMEM which contained a stronger bicarbonate buffer with the same MgO compounds (data not shown).

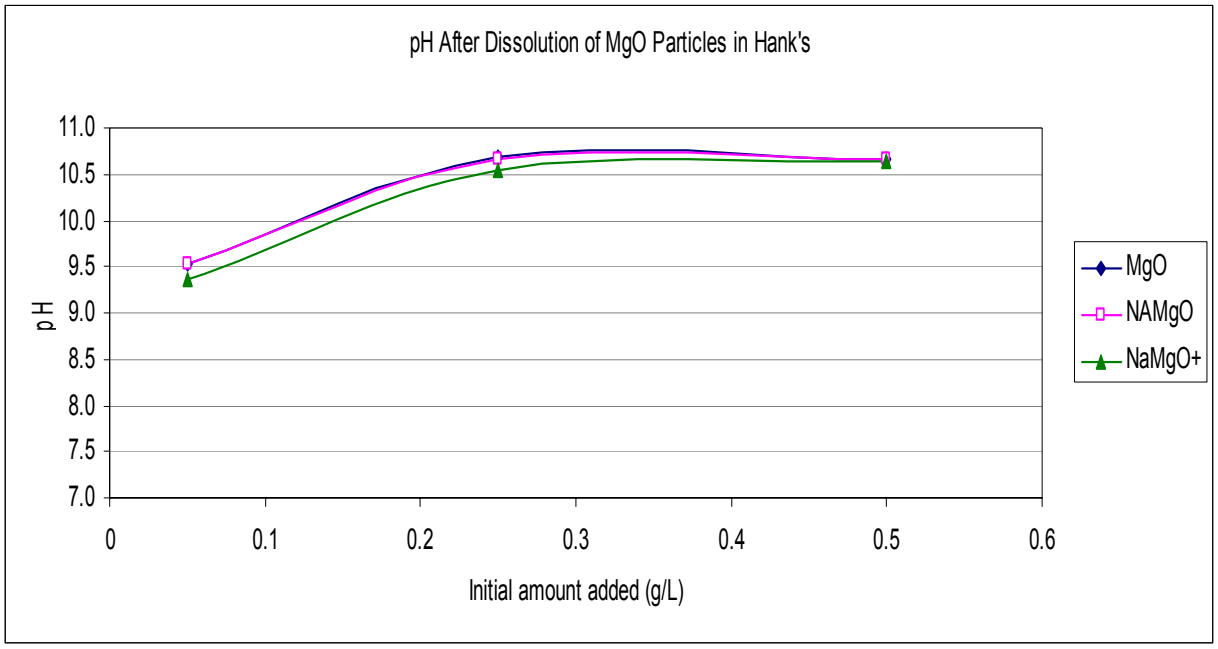

Figure 4.

The pH is shown as a function of MgO added to Hanks Balanced Salt solution (HBSS) used as a simulant epithelial lining fluid (ELF). Materials examined were conventional macrocrystalline magnesium oxide (MgO), Nanoactive ™ MgO (NA ™ MgO) and NA ™ MgO Plus (NA ™ MgO+). The pH increased from 9.4 at 50 mg MgO/L to 10.6 at 250 mg/L MgO. No change was noted when the concentration was increased from 250 to 500 mg/L. The increase in pH suggests the formation of a compound with a higher pH than the lung stimulant fluid whose buffer was bicarbonate. Each point was a mean of 2 replicates; sampling was at the time of peak solubility in a 14 days dissolution study in this experiment

Increased pH and the relation of bicarbonate to solution suggest that the compound causing the increase in pH might be a carbonate. Magnesium carbonate trihydrate, nesquehonite fulfills the requirements for stability at human body temperature (37^{o}C) and body pCO_2 (~40 torr). Magnesium oxide, magnesium hydroxide, magnesite, Landsfordite, and hydromagnesite all lack sufficient solubility to account for the data, or stability at either human body temperature or P_{CO2}.

Nanoparticles that enter the brain through the olfactory bulbs have been shown to lie along the optic tracts; as yet there are no reports of their entry into the deep brain. Metal oxides which are soluble may enter deep brain structures as metal ions. At low concentrations metals such as magnesium will act as essential metal ions.

Metal oxide NM particles can be inhaled into the lung; a small fraction of them will be sufficiently small to go to deep lung (eg. NA MgO; 8-30% by mass). An even smaller fraction will avoid phagocytosis (~1-5% by mass). We attempted to determine the extent to which NA MgO is rapidly dissolved in a lung simulant fluid containing physiological amounts of bicarbonate (Figure 2). The same MgO compounds were incubated in HBSS alone, or in HBSS in the presence of 5% CO_2. When both were incubated at 37^{o}C with gentle agitation (Figure 5, there were only modest differences (1.5- to 2-fold) between solubilities of MgO in HBSS or DMEM at room temperature (22 to 25^{o}C) and their respective solubilities at human body temperature (37^{o}C) (Figure 5). At 250 mg/L MgO, solubility of all particles in HBSS + 5% CO_2 exceeded solubility in HBSS alone after 3 hours (Figure 5). The MgO particles incubated with DMEM alone or incubated with 5% CO_2 were similar to HBSS and 5% CO_2, implying an upper limit of solubility from bicarbonate (Data not shown).

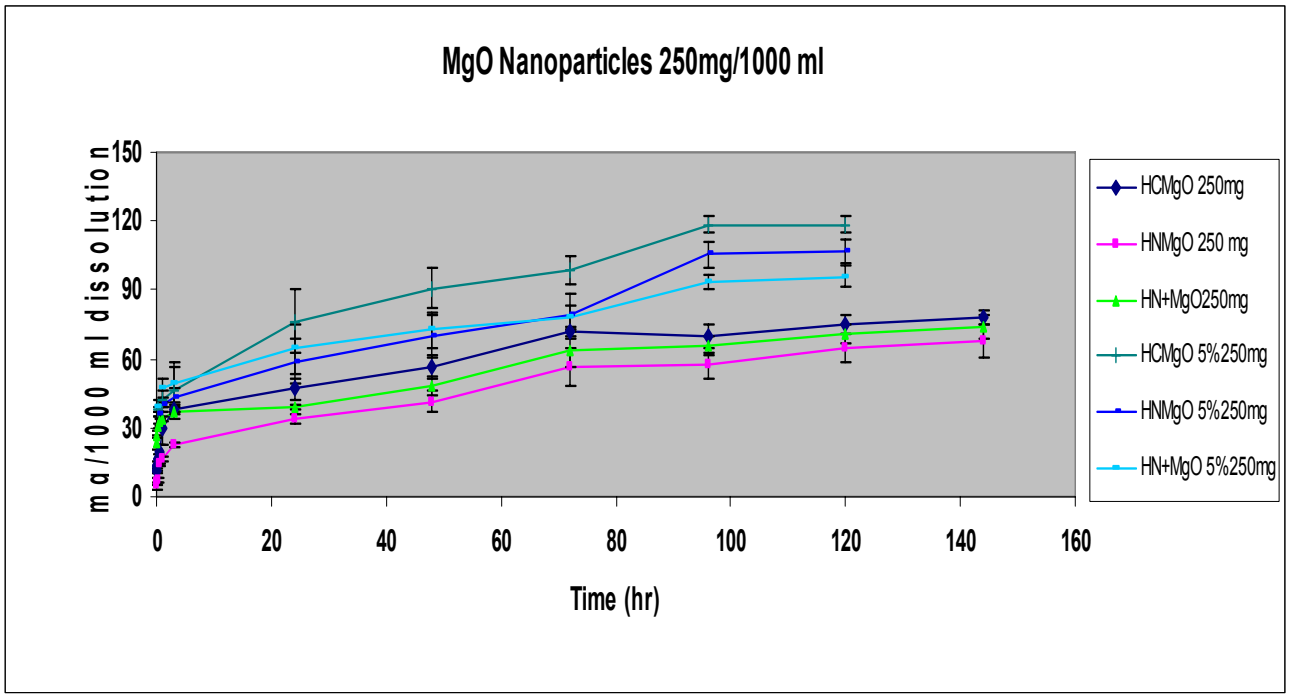

Figure 5.

Solubility of MgO in mg/L is shown as a function of time of dissolution (hr) (144 hours for HBSS alone and 120 hours for HBSS + 5% CO_2 to minimize media contamination) for macrocrystalline (MC), nanoactive (NA) ^{TM} MgO and NA ^{TM} MgO Plus. Comparison was made between Hanks Balanced Salt Solution alone (HBSS alone; HN) and HBSS incubated in the presence of 5% CO2 (H 5% CO_2) both conditions simulating epithelial lining fluid (ELF). Data are shown as means of 3 samples $\pm$ standard errors of means. Solubility for HBSS incubated in the presence of 5% CO_2 was greater than that of HBSS alone at 37^{o}C.

MgO nanorods (length/diameter ~30/1; diameter 0.2 microns) were developed to physically obscure light when generated as an aerosol. They were evaluated for solubility at room temperature alone, human body temperature (37^{o}C) alone and 37^{o}C with added 5% CO_2 for 50 mg MgO nanorods/L. The latter 2 incubations were performed for 250 mg MgO nanorods/L (Figure 6).

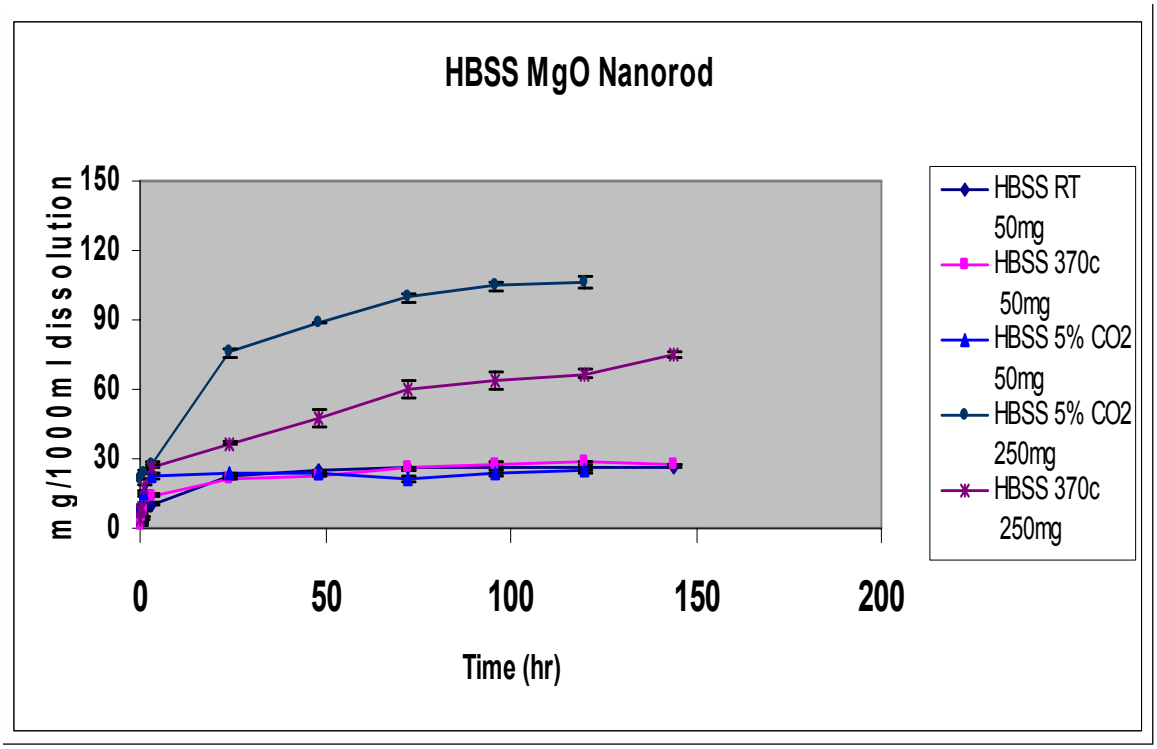

Figure 6.

Solubility of MgO in mg/L (1000 ml) and at 37°C is shown as a function of time of dissolution (hr) (144 hours for HBSS alone and 120 hours for HBSS + 5% CO2 to minimize bacterial contamination) for MgO nanorods. Comparison was made between Hanks Balanced Salt Solution alone (HBSS alone; HN) and HBSS incubated in the presence of 5% CO_2 (H 5% CO_2) for 50 and 250 mg/L. In addition, samples at 50 mg/L were incubated room temperature for comparative purposes. Both conditions simulated epithelial lining fluid (ELF). Only marginal early differences were seen when 50 mg/L concentrations were incubated for the three conditions of incubation simulating ELF. However, at 250 mg/L concentrations of nanorods, 37°C in the presence of 5% CO_2 to simulate ELF caused 150-200% of the dissolution of HBSS alone. Data are shown as means of 3 samples $\pm$ standard errors of means. Where only means appear to be present, the range of $\pm$ the standard error of the means was less than the width of the dots.

All particles studied were rapidly soluble in bicarbonate fluid simulating ELF. There appears to be little difference between the dissolution of MC MgO, MgO nanoparticles and MgO nanorods in these media. However, they appear to have different physical properties. Dissolution of each particle, the complex agglomeration and disagglomeration of particles and shortening of nanorods appear to equalize dissolution. Future work will be necessary to define these processes further. However, the important feature is that rapid dissolution minimizes potential health effects. Thus, if nanoparticles are to be used to clean atmospheres, or nanorods to exclude light, it is convenient that they are soluble in epithelial lining fluid because of its bicarbonate buffer.

NM particles that do not undergo phagocytosis are small enough to leave the lung, enter the vascular system and cause inflammation of previously damaged atherosclerotic arteries; coronary arteries (CA) are a likely target site. Irritating PSP 24 nm copper nanoparticles [2] are small enough to escape phagocytosis (< 500 nm to 1 micron; 0.5 – 1 microns). They have the potential to create such inflammation because CA are directly downstream from the pulmonary artery and aorta. Oral soluble cupric chloride nanoparticles caused no damage to the CA vasculature, even though they were present at high enough concentrations to damage liver, kidney and spleen. Ionic copper and micro copper (17 micron diameter) caused no damage.[2] Alternatively, MgO particles or nanorods similarly soluble in bicarbonate buffers such as HBSS or DMEM would be expected to dissolve rapidly with minimal or no potential for injury. Ionic magnesium serves as essential cofactors for biochemical processes.

Nanocrystalline size, reflecting surface activity, can lead to respiratory tract injury. [1, 3, 4] By way of comparison, 21 nm TiO_2 microcrystals added to tracheal minces, induce increased procollagen production, while 120 nm microcrystals have no effect. Agglomerates of the 21 nm TiO_2 particles are ~3,000 nm in diameter, while those of 120 nm monomers are 600 nm in diameter, suggesting that it is nanocrystalline, not agglomerate size that induces biochemical fibrosis (increased procollagen and collagen). [1, 3, 4] Natural crystals of silica (silicon dioxide) induced a similar increase in inflammation relative to the amorphous silica with increased aqueous solubility and less biopersistence. [1, 3, 4] MgO particles reported in our earlier paper would be expected to show even less persistence. Under conditions of rapid early dissolution nanocrystalline MgO plus increases the advantage by 1.5- to 3-fold. In the absence of

bicarbonate (deionized distilled water), NC MgO Plus dissolved more rapidly than MC MgO (Figure 1).

Risks of production of metal oxide nanoparticles were compared to that of organic PSP such as carbon black and to other common industrial processes such as battery production and oil refining. Manufactured metal oxide nanoparticles had the lowest potential risk. Carbon black was estimated to have significantly more risk for respiratory disease than preparation of metal oxide nanoparticles. This research was partially funded through the award of a contract from the Marine Corps Systems Command to M2 Technologies, Inc.

Key words: nanoparticles, microcrystals, single-walled carbon nanotubes, pneumonitis, pulmonary fibrosis

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