

# THE DISSOLUTION AND BIOAVAILABILITY OF ZINC OXIDE IN SIMULATED LUNG FLUID

John Kannas, University of Utah  
Rod Larson, University of Utah  
Han Kim, University of Utah  
Leon Pahler, University of Utah

johnkannasjr@hotmail.com

## ABSTRACT

Zinc oxide is an odorless, amorphous white powder whose fume is suspected to cause metal fume fever, a short-term flu-like illness. The main route of exposure is through inhalation of fumes during the welding, cutting or brazing of galvanized metal. Studies have suggested that intracellular and extracellular lung fluids are able to dissolve particles and translocate them to other parts of the body. Dissolution rates for 1 to 5  $\mu\text{m}$  zinc oxide were determined in simulated lung fluid at two different pH levels. A modified Gamble's solution with a pH of 7.2 was chosen to simulate extracellular lung fluid and a modified Gamble's solution with a pH of 4.5 was chosen to simulate alveolar macrophage (intracellular) fluid. The two simulated lung fluid samples were continuously agitated along with quality assurance and quality control samples for 14 days. A ten milliliter sample was removed from each solution on days 0, 1, 3, 5, 7, and 14. The samples were analyzed using atomic absorption to determine the concentration of zinc in solution. Results indicate that the zinc oxide dissolved at a rapid rate in the 4.5 pH solution with essentially all significant dissolution occurring in the first day. After that day the concentration of zinc was observed to level off and proceeded to decrease on days 5 through 14. This was most likely due to the precipitation of zinc with the rise in pH over time. Dissolution in the 7.2 pH solution occurred at a slow rate throughout the experiment. Statistical analysis of the results from the 4.5 and 7.2 pH solutions using a paired t-test indicated a significant difference ( $p = 0.0021$ ) in the concentration of zinc in solution at the  $\alpha = 0.05$  level. The results indicate that the probable pathway employed by the zinc oxide to induce the systemic effects of metal fume fever is through dissolution in the alveolar macrophages.

## INTRODUCTION

Zinc oxide (ZnO) fume produced during the welding, cutting, smelting, and brazing of galvanized metal is most commonly associated with metal fume fever, a self-limiting, short-term, flu-like illness. This illness is characterized by nasal and throat irritation, and a sweet or metallic taste in the mouth soon after exposure. Within 3 to 12 hours

after exposure, symptoms may progress into headache, chills, myalgia, cough, dyspnea, nausea, fever, chest tightness, dry mouth, lethargy, diaphoresis, and vomiting (Baker, 2001). There are several hypotheses as to the pathophysiology of these systematic effects; however a clear route from exposure to effect is not understood (Baker, 2001). This project investigated two potential mechanisms that could partially explain the route of the zinc oxide fume: dissolution of ZnO in extracellular lung fluid and dissolution in intracellular lung fluid. The dissolution rates of ZnO in simulated extracellular and intracellular lung fluids were experimentally determined to help discover its bioavailability.

## **BACKGROUND AND SIGNIFICANCE**

Metal fume fever was first described in metal guilders by Patissier in 1822 (Baker, 2001; Morgan, 1984). The illness has been thought to also be associated with exposure to magnesium, brass, copper, and many other metals. However, the most common cause of metal fume fever is exposure to zinc oxide fume. Zinc oxide fume is created when galvanized metal is vaporized by heating it to a temperature greater than 930°F (Baker, 2001). The vapor cools and condenses in the air to form a very small particle known as a fume (Antonin, 2003). These fumes are inhaled by workers and deposited in the gas exchange region of the lungs where they can induce metal fume fever.

The National Institute for Occupational Safety and Health (NIOSH) has estimated in the National Occupational Exposure Survey (NOES) that approximately 2 million workers were potentially exposed to zinc oxide in the U.S. workplace. Of this 2 million approximately 15,000 were exposed specifically to zinc oxide fume (NIOSH, 2006). Antonini et al. (2003) has reported that metal fume fever is the most frequent acute respiratory complaint among welders with approximately 1,500-2,000 cases annually in the U.S.

Even though zinc oxide is most commonly associated with metal fume fever, there are several issues that complicate this relationship. First, workers can gain a tolerance to inhaled zinc oxide. This tolerance is quickly gained and lost, which is why metal fume fever is also known as “Monday morning syndrome” because symptoms will be worse after a weekend away from exposure (Antonin, 2003; Fine, 2000). Secondly, zinc is an essential metal necessary for proper body function and may act as an anti-inflammatory agent in the lungs (St. Croix, 2005).

These complications make assigning of an exposure limit very difficult. The current standards are an Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) of 5 mg/m<sup>3</sup>, respirable fraction and an American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) time-weighted average (TWA) of 5 mg/m<sup>3</sup> (OSHA, 2005). Some studies have shown symptoms in participants at levels less than 5 mg/m<sup>3</sup> and others have shown no effects in participants exposed to levels much greater than 5 mg/m<sup>3</sup> (Fine, 1997; Gordon, 1992; Martin, 1999).

There are two main clearance mechanisms for inhaled matter. The first is clearance through the mucociliary escalator. This is a mechanical process in which special ciliated cells propel mucus up to the larynx where it is expelled or swallowed and deposited into the gut (Morgan, 1984; Jurinski, 2001). The second mechanism is the dissolution and translocation of particles to other

parts of the body (Kanapilly, 1973). There are two ways in which this can happen. First, matter can be dissolved by extracellular lung fluid and translocated to the blood (Morgan, 1984; Mattson, 1994). Secondly, matter can be engulfed by alveolar macrophages and dissolved in the intracellular fluid of these macrophages and then transported to the blood and other parts of the body (Morgan, 1984; Jurinski, 2001).

The potential dissolution of inorganic matter in the extracellular or intracellular fluid of the lungs has been approximated, in many studies, with the use of simulated lung fluid (Mattson, 1994; Christensen, 1994; Eastes, 2000; Kalkwarf, 1983; Scholze, 1987). A simulated lung fluid known as Gamble's solution has been shown to reasonably approximate lung fluid (Mattson, 1994; Eastes, 2000). The pH of the Gamble's solution is adjusted to more accurately represent the extracellular and intracellular lung fluids. The pH of extracellular lung fluid is typically between 7.2 and 7.6 (Marieb, 1989). The pH inside the alveolar macrophages is typically between 4.5 and 5.5 (Geisow, 1981; Kreyling, 1992; Nyberg, 1991). The lower pH in the macrophages may facilitate the dissolution of particles that will not be dissolved by the extracellular lung fluid.

## METHODS

This study was designed to measure the bioavailability of zinc oxide by assessing its rate of dissolution in simulated lung fluid. Rates of dissolution in modified Gamble's solution with a 4.5 pH and modified Gamble's solution with a 7.2 pH were compared to determine the most likely mechanisms for the translocation of ZnO out of the lungs. The two different pHs were used to simulate extracellular (7.2 pH) and intracellular lung fluid (4.5 pH). Gamble's solution blanks at each pH and reagent grade Type I water blanks were used as controls.

### Particle preparation

Zinc oxide powder with a particle size diameter of 1 to 5  $\mu\text{m}$  and a purity of 99.9% (Atlantic Equipment Engineers, Bergenfield, NJ) was used for this project. This size was chosen because it represents a respirable size. Respirable size particles are typically less than 10  $\mu\text{m}$  in diameter and are able to travel into the gas exchange region of the lungs. The particle size distribution of the ZnO was verified by the University Of Utah Department Of Metallurgical Engineering. They determined that approximately 65% of the powder is between 1 and 5  $\mu\text{m}$ , and approximately 90% is between 0.5 and 10  $\mu\text{m}$ . The distribution of the particle sizes can be seen in Figure 1.

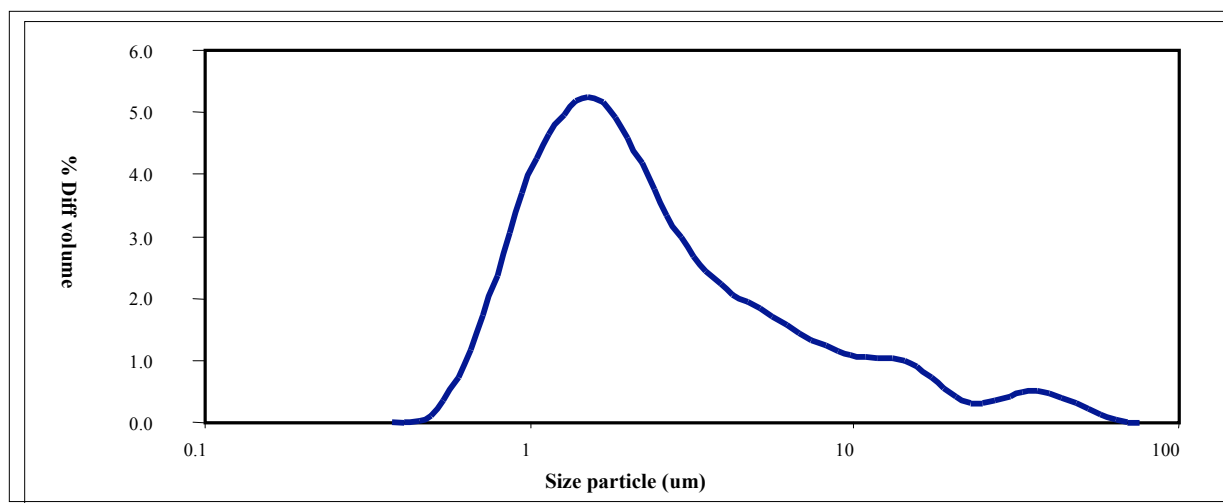


Figure 1. Zinc Oxide Powder Particle Size Distribution

### Lung fluid preparation

Modified Gamble's solution was prepared and used to analyze the dissolution of zinc oxide. The composition of the modified Gamble's solution is shown in Table 1. The solution is modified from the original Gamble's solution by removing the metal constituents per NIOSH guidance. This was done to reduce the effects of metal chelation and interference with the analytical method relative to the detection of zinc.

Species	Concentration (mM)
Na <sup>+</sup>	150.7
Ca <sup>+</sup>	0.197
NH <sub>4</sub> <sup>+</sup>	10
H <sub>2</sub> CNH <sub>2</sub> H (glycine)	5.99
H <sub>2</sub> CO (formaldehyde) methanol solution	67
Cl <sup>-</sup>	126.4
SO <sub>4</sub> <sup>2-</sup>	0.5
HCO <sub>3</sub> <sup>-</sup>	27
HPO <sub>4</sub> <sup>2-</sup> , H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	1.2
COH(CH <sub>2</sub> ) <sub>2</sub> (COO) <sub>3</sub> <sup>3-</sup> (citrate)	0.2

Table 1. Composition of simulated lung fluid (SLF) (Gamble's solution) in mM

Reagent grade type I water was used throughout this study in the preparation of the modified Gamble's solution and in sample blanks. The reagent grade type I water was obtained from the University of Utah Department of Metallurgical Engineering and was created using the Milli-Q water purification system (Millipore, Billerica, MA). The water was purified to a resistivity of 18.2 MΩ.cm at 25 °C and a total organic carbon (TOC) content of 5 parts per billion.

The modified Gamble's solution was prepared in the following manner: one liter stock solutions of ammonium chloride, sodium dihydrogen phosphate monohydrate, sodium citrate dehydrate, and calcium chloride dehydrate were prepared by adding 112.01 g of ammonium chloride (Fisher Scientific, #A687-500, Pittsburgh, PA), 116.40 g of sodium dihydrogen phosphate monohydrate (Fisher Scientific, #S369-500, Pittsburgh, PA), 23.60 g of sodium citrate (Fisher Scientific, #S279-500, Pittsburgh, PA), and 11.60 g of calcium chloride dehydrate (Fisher Scientific, #C69-500, Pittsburgh, PA) separately to 900 mL of reagent grade type I water. The solutions were gently agitated and heated until all solids had been dissolved. The solutions were made up to 1 L and left to cool to room temperature. After cooling the solutions were filtered through 0.45 µm Durapore® membrane filters (Millipore, #HVLP09050, Billerica, MA) and stored in dark high density polyethylene (HDPE) bottles (Fisherbrand, #03-084-1E, Fisher Scientific, Pittsburgh, PA). The modified Gamble's solution was made by adding 25 mL ammonium chloride solution, 33.9 g sodium chloride, 8.85 g sodium bicarbonate, 3.145 g sodium carbonate (Acros Organics, #419480010, Morris Plains, NJ), 12.5 mL sodium dihydrogen phosphate solution, 12.5 mL sodium citrate solution, 2.25 g glycine (Acros Organics, #12000010, Morris Plains, NJ), 12.5 mL formaldehyde/methanol solution (Mallinckrodt Baker, Inc., #5016, Phillipsburg, NJ), and 12.5 mL calcium chloride solution to 5 L of reagent grade type I water. After all solids had dissolved, pure carbon dioxide was bubbled through the mixture for 10 minutes. Carbon dioxide is bubbled through the solution to produce a mixture (likely to also form carbonic acid) similar to what occurs in the lungs. The modified Gamble's solution was then titrated with sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) (Fisher Scientific, #A300-500, Pittsburgh, PA) or sodium hydroxide (NaOH) (Mallinckrodt Baker, Inc., #7708-10, Phillipsburg, NJ) to obtain a pH of 4.5 and 7.2. The pH was measured using a pH 1100 bench meter (Oakton Instruments, Vernon Hills, IL) that was triple point calibrated.

### **Sample preparation**

Approximately 250 mg of zinc oxide powder was added to 400 mL of each of following: reagent grade type I water, 4.5 pH modified Gamble's solution, and 7.2 pH modified Gamble's solution in 500 mL HDPE bottles (Nalgene, #2199-0016, Rochester, NY). The samples were run in duplicate for quality assurance and quality control (QA/QC) purposes. The samples, along with the control blanks, were continuously agitated at 100 revolutions per minute for 14 days on an Orbit 1900 orbital shaker (Labnet International Inc., Edison, NJ).

### **Sample collection**

The solutions were mixed with magnetic stir bars that had been soaked overnight in 2 normal nitric acid and rinsed with reagent grade Type I water. Stirring for 2 minutes prior to and during the sample collection ensured proper mixing of the solutions. Aliquots of 10 mL were removed on process days 0, 1, 3, 5, 7, and 14. The aliquots were filtered through 0.2 µm mixed cellulose ester (MCE) membrane filters (Fisherbrand, #09-719-2B, Fisher Scientific, Pittsburgh, PA) to remove any undissolved ZnO particles. The filtered aliquots were stored in HDPE bottles (SKS, #0056-45, Watervliet, NY) with 1 drop (0.05 mL) of reagent grade nitric acid (Fisher Scientific, A200-500, Pittsburgh, PA) as a preservative. In addition, 2 extra aliquots were randomly selected from the batch and collected in the same manner.

## Sample analysis

All samples, including controls, were analyzed by flame atomic absorption (FS220, Varian Inc., Palo Alto, CA) at Data Chem Laboratories, Inc. in Salt Lake City, Utah to determine the mass per volume concentration of zinc in each aliquot. The concentration of zinc in each sample was used as a proxy for zinc oxide dissolution.

## Statistical analysis

The concentration of zinc in each aliquot from the atomic absorption analysis was used to determine the rates of dissolution in each of the modified Gamble's solutions. The concentrations were analyzed with STATA statistical software Version 8.2 (Stata Inc., College Station, TX) using a paired t-test, assuming a normal distribution, to determine if a difference exists. The test was one-tailed, with  $\alpha = 0.05$ .

## RESULTS

The mean concentration of zinc in each solution type on each sample day can be seen in Table 2. The mean concentrations were obtained by averaging the duplicate samples and when appropriate, the additional random sample. The results show that the control samples (solution blanks) did contain small amounts of zinc in solution. The background ZnO levels for the respective modified Gamble's solutions were subtracted from the study samples to correct for the underlying zinc in these solutions. These adjusted values can be seen in Table 3 and Figure 2.

Sample Description	Day 0	Day 1	Day 3	Day 5	Day 7	Day 14
DI water	48	265	240	370	605	725
DI water with ZnO	640	1633	2650	3867	4050	5300
4.5 pH Gamble	61	1400	1170	1350	1085	1485
7.2 pH Gamble	315	265	445	410	400	330
4.5 pH Gamble w/ ZnO	59333	413333	406667	365000	306667	253333
7.2 pH Gamble w/ ZnO	7867	23500	39000	53000	60667	69667

Table 2. Average Concentration of Zinc in  $\mu\text{g/L}$

Sample Description	Day 0	Day 1	Day 3	Day 5	Day 7	Day 14
4.5 pH Gamble w/ ZnO	59273	411933	405497	363650	305582	251848
7.2 pH Gamble w/ ZnO	7552	23235	38555	52590	60267	69337

Table 3. Adjusted Concentration of Zinc in  $\mu\text{g/L}$

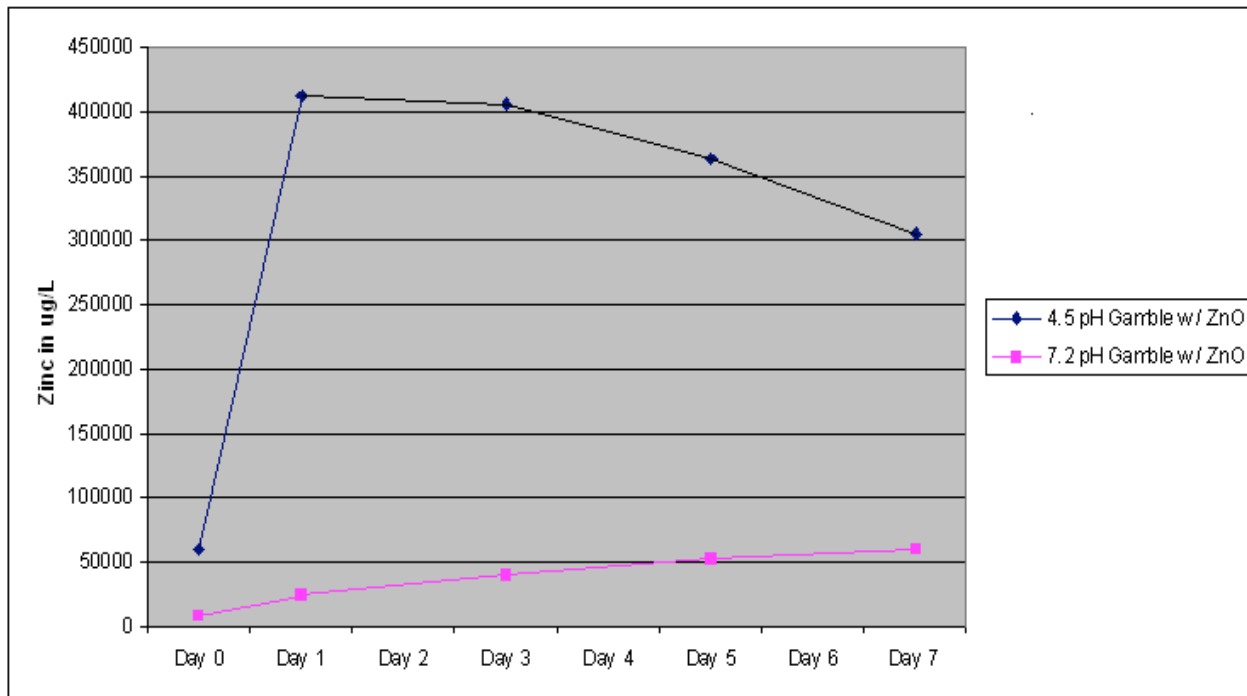


Figure 2. Adjusted Concentration of Zinc in µg/L for Days 0 to 7

The adjusted concentrations of zinc in solution are much higher in the 4.5 pH modified Gamble's solution than in the 7.2 pH modified Gamble's solution. A paired t-test was used to determine if there is a statistically significant difference. The mean concentrations of zinc in solution were found to be significantly different, with a p-value of 0.0021.

The rate of dissolution for the zinc oxide during the first day was determined using the adjusted concentration values. The rate was approximately 7490 µg/hour in the 4.5 pH modified Gamble's solution for the first 22 hours and 425 µg/hour in the 7.2 pH modified Gamble's solution for the first 22 hours. The rate was not calculated on subsequent days because the concentration in the 4.5 pH solution was observed to reach maximum on day 1 and then appears to decrease on days 3 through 14, most likely due to an increase in the pH of the simulated lung fluid.

## DISCUSSION

From the study results it is apparent that the dissolution and bioavailability of zinc oxide is much higher in the 4.5 pH modified Gamble's solution than in the 7.2 pH modified Gamble's solution. The concentration of zinc dissolved in the 4.5 pH solution is 17.7 times larger on day 1 than the concentration in the 7.2 pH solution on day 1. This is a statistically significant difference and suggests that dissolution in the alveolar macrophages is a likely mechanism for the translocation of zinc oxide out of the lungs and into the blood.

The difference in the rate of dissolution for the first day was also 17.7 times faster in the 4.5 pH solution than the 7.2 pH solution. This rapid rate is significant due to the pathology of metal

fume fever, because symptoms will typically appear 3 to 12 hours after exposure. The ability of the 4.5 pH simulated lung fluid to quickly dissolve the zinc oxide particles also points to the intracellular lung fluid as the mechanism for transport. A future study that collects samples every few hours over the first day of analysis may be able to more effectively determine the rate of dissolution during this important time period.

The concentration of dissolved zinc in the 4.5 pH modified Gamble's solution is not constant. Over the course of the first day the 4.5 pH solution rapidly dissolves the zinc oxide. The solution appears to become saturated within the first day of the experiment and remains that way until day 3. On day 3, the concentration of zinc in solution starts to decrease and continues to decrease steadily through day 14. This change is most likely due to the rise in pH of the modified Gamble's solution. During the course of the experiment the pH in the 4.5 pH modified Gamble's solution rose from an initial pH of 4.51 to a pH of 6.99. This is a significant change and would likely cause the zinc to precipitate out of solution as zinc hydroxide. Prior to the start of the experiment, daily pH measurements were decided to not be taken due to potential contamination from the pH probe. Since pH in the lung fluid of the body stays more regulated, measurement of the pH taken with every aliquot removal would be an area to investigate with future research, along with the effects of adjusting the pH to maintain a steady level throughout the experiment.

All of the results of this study point to the dissolution and bioavailability of zinc oxide in the intracellular lung fluid. Based on these results, the proposed mechanism is that workers inhale sufficient quantities of respirable size zinc oxide fume to cause them to experience metal fume fever. The fume is deposited in the gas exchange region of the lungs and quickly engulfed by the alveolar macrophages. These macrophages, with a pH between 4.5 and 5.5, are able to efficiently dissolve the zinc oxide fume. Once dissolved, the fume can be translocated to the blood and transported throughout the body, leading to the systemic effects common to metal fume fever.

## **CONCLUSION**

Metal fume fever is an occupational illness that affects thousands of workers every year. The pathophysiology of the illness is not clearly understood. Results of this study point to the dissolution of zinc oxide fume in the intracellular fluid of alveolar macrophages as the most likely mechanism for the translocation of the fume from the lungs to the blood. The zinc oxide particles dissolved at a rate approximately 18 times faster in the 4.5 pH modified Gamble's solution than the 7.2 pH modified Gamble's solution during the first day of the experiment. The concentration of zinc in solution over the course of the experiment was significantly different in the two solutions. This study implicates dissolution in the alveolar macrophages as a potential route for zinc oxide to induce systemic effects, however further research should be completed to verify these results.

## **ACKNOWLEDGEMENTS**

The authors would like to thank the DFPM Health Studies Fund for their financial support. They would also like to thank Jan Miller and the Department Of Metallurgical Engineering at the

University of Utah for their technical assistance with the particle size distribution analysis and the type I reagent grade water.

## REFERENCES

- Antonin, J.M., Lewis, A.B., Roberts, J.R., & Whaley, D.A. (2003). Pulmonary effects of welding fumes: review of worker and experimental animal studies. *American Journal of Industrial Medicine*, 43, 350-360.
- Baker, B.A. (2001). Metal fume fever. In M.D. Ford (ed.), *Clinical Toxicology* (pp. 699-703). Philadelphia, PA: W.B. Saunders Company.
- Christensen, V.R., Jensen, S.L., Guldborg, M., & Kampstrup O. (1994). Effect of chemical composition of man-made vitreous fibers on the rate of dissolution in vitro at different pHs. *Environmental Health Perspectives*, 102(5), 83-86.
- Eastes, W., Potter, R.M., & Hadley, J.G. (2000). Estimation of dissolution rate from in vivo studies of synthetic vitreous fibers. *Inhalation Toxicology*, 12(11), 1037-1054.
- Fine, J.M., Gordon, T., Chen, L.C., Kinney, P., Falcone, G., & Beckett, W.S. (1997). Metal fume fever: characterization of clinical and plasma IL-6 responses in controlled human exposures to zinc oxide fume at and below the threshold limit value. *Journal of Occupational and Environmental Medicine*, 39(8), 722-726.
- Fine, J.M., Gordon, T., Chen, L.C., et al. (2000). Characterization of clinical tolerance to inhaled zinc oxide in naïve subjects and sheet metal workers. *Journal of Occupational and Environmental Medicine*, 42, 1085-1091.
- Geisow, M.J., Hart, P.D., & Young, M.R. (1981). Temporal changes of lysosome and phagosome pH during phagolysosome formation in macrophages: studies by fluorescence spectroscopy. *Journal of Cell Biology*, 89, 645-652.
- Gordon, T., Chen, L.C., Fine, J.M., et al. (1992). Pulmonary effects of inhaled zinc oxide in human subjects, guinea pigs, rats, and rabbits. *AIHA Journal*, 53(8), 503-509.
- Jurinski, J.B., & Rimstidt, J.D. (2001). Biodurability of talc. *American Mineralogist*, 86, 392-399.
- Kalkwarf, D.R. (1983). Dissolution rates of uranium compounds in simulated lung fluid. *Science of the Total Environment*, 28, 405-414.
- Kanapilly, G.M., Raabe, O.G., Goh, C.H.T., & Chimenti, R.A. (1973). Measurement of in vitro dissolution of aerosol particles for comparison to in vivo dissolution in the lower respiratory tract after inhalation. *Health Physics*, 24, 497-507.

Kreyling, W.G. (1992). Intracellular particle dissolution in alveolar macrophages. *Environmental Health Perspectives*, 97, 121-126.

Marieb, E.N. (1989). *Human Anatomy and Physiology*. Redwood City, CA: Benjamin/Cummings Publishing.

Martin, C.J., Le, X.C., Guidotti, T.L., et al. (1999). Zinc exposure in Chinese foundry workers. *American Journal of Industrial Medicine*, 35, 574-580.

Mattson, S.M. (1994). Glass fiber dissolution in simulated lung fluid and measures needed to improve consistency and correspondence to in vivo dissolution. *Environmental Health Perspectives*, 102(6), 87-90.

Morgan, W.K.C., & Seaton, A. (1984). *Occupational Lung Diseases* (2<sup>nd</sup> ed.). Philadelphia, PA: W.B. Saunders Company.

National Institute for Occupation Safety and Health (NIOSH). *National occupational exposure survey: 1981-1983*. Retrieved January 24, 2006 from <http://www.cdc.gov/noes/noes5/x7921ctr.html>.

Nyberg, K., Johansson, U., Johansson, A., & Camner, P. (1991). Phagolysosomal pH and location of particles in alveolar macrophages. *Fundamental and Applied Toxicology*, 16(3), 393-400.

Occupational Safety & Health Administration (OSHA). *Occupational safety and health guideline for zinc oxide*. Retrieved October 24, 2005 from <http://www.osha.gov/SLTC/healthguidelines/zinc/zincoxide/recognition.html>.

Scholze, H., & Conradt, R. (1987). An in vitro study of the chemical durability of siliceous fibres. *Annals of Occupational Hygiene*, 31, 683-692.

St. Croix, C.M., Leelavaninchkul, K., Watkins, S.C., Kagan, V.E., & Pitt, B.R. (2005). Nitric oxide and zinc homeostasis in acute lung injury. *Proceedings of the American Thoracic Society*, 2, 236-242.