Introduction to nano-composites and toxicity concerns

Though the common use of the prefix “nano” is relatively recent, the idea of mixing nanomaterials with plastics to create nanocomposite materials is not new. A polymer (vulcanized rubber) is mixed with particles (carbon black, zinc oxide and magnesium sulfate), a portion of them in the nano-size range in the manufacturing of tires addition—a process begun over 150 years ago. Over the past few decades, with the ability to produce particles of precise dimensions and morphologies, more applications have been developed for nanocomposites primarily in the electronics, aerospace and automotive industries. In particular, carbon nanotube (CNT) composites have received increasing attention due the enhanced properties that they can contribute including electrical and thermal conductivity, strength, and stability. CNTs are much smaller than carbon fibers— on the average one thousand times smaller. Single walled carbon nanotubes (SWCNT) have diameters of 1-2 nm whereas multi-walled carbon nanotubes (MWCNT) have diameters ranging from 8-12 nm. Their length can range from 10-100 μm.

The main toxicological effect of CNT is the inhalation of airborne CNT. Carbon nanotubes on the macro scale are too big to be respirable. Unless they are anchored on a surface, CNT tend to agglomerate into small bundles or ropes, and macroscopic quantities of CNT are generally found as clumps of size 10 μm or less. The "microscopic ropes" of nanotubes can become airborne through the handling and processing of the CNT; simply emptying a container of macroscopic CNT can yield an airborne hazard.

Both single- and multi-walled carbon nanotubes have been shown to elicit toxicity responses in vivo. Based on their relative similarities in size, carbon nanotubes are presumed by some to be the next asbestos. The responses included peribronchial inflammation and T-cell activation in mice, inflammation, granulomas and fibriosis in rats, and pulmonary lesions in guinea pigs. In one study, mice exposed to 0.5 mg exhibited 55% mortality within 7 days of exposure [Handy, R.D. & Shaw, B.J, Health, Risk and Society, June 2007; 9(2): 125-144] Other effects observed in rodents include immunosuppression and increased sensitivity to infection; In 2009 female workers in China exposed to polyacrylate nanoparticles exhibited similar responses (pulmonary inflammation, fibrosis and granulomas), and were hospitalized [Shvedova, A. Kagan, V.E., Journal of Internal Medicine, 2009,267; 106-118].

Case study scenario 1: Use of Carbon Nanotubes in Packaging

A small, start-up company (X) has recently patented a technology that utilizes a mixture of multi-walled and single-walled carbon nanotubes(MWCNT and SWCNT) in a novel plastic matrix. The composite plastic material provides significant advantages due to its strength, durability, and low weight, in addition to extending the shelf life of products by up to 100 times. The company has patented a process that allows for the composite to be manufactured at lower costs than traditional packaging materials for high-end products. Their new composite can replace glass, and most traditional plastics currently used to manufacture containers. The process of manufacturing the composite involves uniformly dispersing the CNTs as fillers within the plastic matrix. The CNT's do not become bonded with the matrix, but rather encased within it.

Company X has been approached by a large manufacturing firm (Y) to commercialize the plastic. However, the manufacturing firm has expressed their interest in pursuing this option under the stipulation that it can be proven to be non-toxic. Their goal is to manufacture containers for high-end consumer products such as wine, beer, juices, and cosmetics. Company (Y) could choose to go another route on a new non-nano technology by another company for a similar cost, but that wouldn't offer the same benefits as company (X)'s nanocomposites with carbon nanotubes.

Company X did some toxicity testing, but very primitive work. Company X does not have toxicology or biology expertise. All three partners have a chemistry/physics backgrounds. A friend in another lab let them culture some rat fibroblasts cells on a sample of their nanocomposite: they found that 90% of the cells remained viable; the 10% death may not have been due to the nanocomposite, but rather a myriad of other possible influences.

The three partners, and scientists, in company X are excited about the prospects of finally getting the company from being in the red, to a company flush with cash. One of the partners has been tracking the literature on the toxicity of MWCNT and SWCNT. Recently EPA made a determination that CNTs may be hazardous to human health and the environment. A new requirement is now in place to notify the agency of intended uses of CNTs.

Each partner brings to the meeting an option to pursue. Which should they choose?

- **Option A:** Hire a toxicologist to research the effects of carbon nanotubes on living tissue. Also do more research to see if the carbon nanotubes can become detached from the plastic over a long period of time.
- **Option B:** Hire a law firm to circumvent EPA and FDA policies. This way, their application can be classified as non-toxic.
- **Option C**: Find an alternative application for carbon nanotubes, since the current application is probably toxic.

<u>Case Study #2: Influence of Funding on Publication</u>

This scenario involves a fictitious lab that is funded by a corporation, Biological Imaging Inc., which manufactures and sells medical imaging supplies. Biological Imaging Inc. has developed their own coated nanotubes based on the ideas developed at the University of Arkansas, where coating carbon nanotubes with a thin layer of gold allowed for better contrast with near-infrared waves and significantly reduces toxicity [Kim, J.W. et. al. Nature Nanotech. 4 (2009) 668-694]. They have funded Dr. Jones' lab to study the use of their coated nanotubes for imaging. The funding contract between Biological Imaging Inc. and the University states that the company may review all papers prior to publication, but cannot prevent Dr. Jones' lab from publishing their findings. Casey is a graduate student working in Dr. Jones' lab and has been assigned to the project. This is the first project Casey will be in charge of.

Casey administered these coated nanotubes to five mice with tumors. In four of these mice, Casey was easily able to image the tumor with great contrast. However, in one mouse the tumor was not visible using the same technique. Casey writes up these results in a paper including all five experiments. Dr. Jones approves the paper and forwards it on to Biological Imaging Inc. for review.

Dr. Jones receives a letter back stating that there was clearly an error in the experiment involving the anomalous mouse and this data point should be removed. Casey reviews the experimental notes but finds no differences between the anomalous experiment and the others. Casey speaks to Dr. Jones who states that it is an irregular point and if Biological Imaging Inc. says to discard it, it should be discarded. Although uncomfortable, Casey publishes the paper excluding the experiment involving the anomalous mouse.

Questions: Did Casey and Dr. Jones act appropriately? Are there other options they should pursue? What other information might you need to make an informed decision?

CASE STUDY 3: Lab Safety Culture

The research group is researching the synthesis of CNT for the formation of macroscopic CNT cables. The lab PI, Kevin, is aware that the longest CNT cable synthesized was only 18 centimeters and he is determined to best this record by improving synthesis and formation.

The lab is well equipped with the proper equipment for the study of CNT. The big hazard is the airborne CNT, so there are up to date fume hoods and chambers to manipulate the CNT under/inside. Ming, who did his masters degree in China, is not used to using such equipment and feels such practices are inefficient and unnecessary. Instead Ming frequently performs his processes on a bench top where he has more space to spread out.

Within her first month in the lab, Julia notices Ming handling the CNT outside of a ventilated area. As a visiting student from Germany, where such behavior is not acceptable, Julia is shocked and informs Ming right away about the potential airborne and inhalation hazards of having no ventilation when handling CNT. Julia also notices containers of uncovered and unlabeled CNT waste and demands Ming make changes to his processes and waste management. Ming nods his head, says, "Yes. Okay." and starts moving things around in his workspace. Ming only pretends to fix the hazards because he does not accept Julia's credentials as a European graduate student for setting lab safety procedures. Later that week Julia sees Ming's workspace has not changed. Julia confronts Ming about this. Ming said he was unaware of any hazards he was creating because the lab PI never told him about any hazards or any changes to make as a result.

Julia complains to Kevin, the lab PI, about the hazards Ming is creating. Kevin agrees to look into it and talks to Ming about changing his procedures. Ming tells him that there is not enough room in the hood, and that the new process won't work there, since the air flow messes things up. Kevin also contacts the National Institute for Occupational Safety and Health (NIOSH) and finds there are no definite regulations for CNT. Kevin attributes this to CNT being a relatively new idea to the world. Kevin also finds suggested research that CNT can become airborne, inhaled, and have adverse effects on human lungs. However, Kevin decides that since there are no regulations, the lab situation does not have to change. Kevin does not want to change anything involving the process because it is working well, forming some of the world's best CNT cables. A change Kevin does make is having Ming manage his CNT waste more appropriately; he does not mention any changes about his process.

When Julia finds out about Kevin's changes, she is outraged and leaves the lab. Conditions and regulations in the lab do not change until 11 months later, when Ming develops a chronic cough and a shortness of breath. Ming's physician thinks that there could be a connection between the research and his health condition. Kevin acknowledges it may be a result of inhaled CNT and begins making changes in the lab to reduce the CNT safety hazards.

Discussion: Should CNT be better regulated? Should Kevin have acted sooner?