NIOSH Public Meeting
Millennium Hotel Cincinnati

NIOSH Current Intelligence Bulletin
Occupational Exposure to Carbon Nanotubes and Nanofibers
February 3, 2011

CHARLES GERACI: Thank you for coming through the lovely weather. I know it's a real challenge. We have several people who wanted to be here this morning who couldn't make it because of the weather. We'll probably have some others joining us throughout the meeting because of delays in getting here, but I wanted to thank you for participating in this phase of our development of a very important piece of work that NIOSH is doing, our Current Intelligence Bulletin on carbon nanotubes and carbon nanofibers. My name is Chuck Geraci and I am the coordinator of our NIOSH Nanotechnology Research Center. We have a panel of NIOSH experts with us here today who will be making presentations throughout the morning, and we'll follow the agenda that is available in the front but also posted on our Website. Part of the meeting logistics I want to mention is the exit door that leads out to the hallway that will also lead you to exits for the building should an emergency occur but it also leads you out to the restrooms out by the coat check. Our agenda today will bring us up to lunchtime and depending on the length of the discussions and presentations; we do have several public commentators that are here today and we'll see if we can get those in before lunch, or we'll see if we want to or need to continue on after lunch. The other part of the logistics is that we are recording this meeting so that people who can't be here can see the transcript of the proceedings of this meeting so the presentations and the transcript of our discussion will be available on the docket, the NIOSH docket site, for this document. So if you do have a question or a comment please use the microphone, identify yourself with your name and your affiliation because this transcript will be made available to the public on the NIOSH docket.

For today our objective is to go through a summary of the document which has been out for public review since December 10. We also have official peer review underway as well as stakeholder review. Those comments and reviews will also be a part of the docket at the NIOSH Website. We want to review the key elements of this document and get into some public comments that people have requested to make during the meeting too, and then we'll also have some discussion and a description of what are the next steps for the development of this document. So this is your agenda for the day. As you look at the agenda you can see that we are going to have a summary of some of the key parts of the document so presenting within these we'll have our NIOSH experts. We have Dr. Vincent Castranova from our nanotoxicology group, Dr. Eileen Kuempel who performed the risk assessment for the document, Mr. Ralph Zumwalde who will give us a summary of the work that we are looking into and have done on exposure assessment and controls, and Dr. Doug Trout to do a summary of the medical recommendations that have been made. We also have an additional panelist, Dr. Eileen Birch, who is here to discuss the analytical method; and I'll say the portal of methods that have been use and we have been looking into for further refinement of the exposure assessment approach for this document. We had a request for two speakers to make comment today. One of those speakers, Dr. Mike Ellenbecker from UMASS Lowell discovered that you really can't get

through 3 feet of snow in your front yard to get to the airport which was shutdown anyhow. So Mike Ellenbecker will be sending his comments into the docket. We also have Jay Feitshans who will be here to make public comment.

Because we have an open slot in the agenda, and because we are now recording this session and it will be available on the docket, Dr. Juergen Pauluhn has asked to make comment as well so we will have that added to our agenda, and then we will adjourn.

So what we wanted to do is give some of the background and some of the rationale of why is NIOSH doing this work? What was the rationale behind undertaking this project, and it goes back to a number of different inputs throughout the literature and through a variety of research investigations in the early part of, and I say early, not all that long ago; somewhere between four and six years ago, investigations of the biologic activity of a variety of nano materials, including carbon nanotubes and carbon nanofibers. Because of their accelerated development and move toward more commercialization, they were receiving a lot of attention in the toxicology arena so several of the animal studies caught NIOSH's attention along with work that NIOSH was doing to build some experience base on the possible impact of worker exposure. So these effects were seen associated with a variety of carbon nanotubes and nanofibers and were occurring at relatively low doses. And so the ability of these materials to possess biologic activity and to be persistent and other adverse effects that were being reported in the toxicology literature with in vitro and in vivo studies led NIOSH to investigate further and take on the responsibility of looking into the possible workplace exposure and hazard that existed with these materials. The additional rationale is that a number of people were asking questions about the similarities between carbon nanotubes and asbestos and other fibrous materials, and there was also an increasing frequency of the production and use of these materials. So a number of pieces were running together that provided NIOSH with the rationale for undertaking this project. We also do indicate both in the document and in a number of our research investigations that to date we are not aware of any studies that have demonstrated any adverse health in workers, but our position is we want to be proactive in preventing the possibility of adverse health effects.

So the conclusion of these early reviews was that there were a lot of information gaps, a number of them there is work being done to close those information gaps, but based on animal data to date, experimental laboratory animal data to date, there is justification for taking a precautionary approach to controlling carbon nanofiber exposure. Precautionary meaning that it is precautionary; it's proactive. It's a protective and prudent approach to developing a good product stewardship program for the handling of these particular materials, and so in order to enable this part of the process is to look at the possibility of developing guidelines for good risk management, good control. Part of that being the development, if there is sufficient data, to develop a recommended exposure limit and to conduct a quantitative risk assessment for those materials. And so to help facilitate this, what we want is to foster and recommend good work practices as part of the whole program. That's the embodiment of all of this information into our current intelligence bulletin. What is the current state of knowledge, what is our current assessment of that body of knowledge, what kind of recommendations would we make to help improve risk management practices for these materials and so our conclusion was to develop a current intelligence bulletin document. The eye chart for you today is the next two slides.

This is a history of the development of that particular document. We were officially given approval to undertake this project within NIOSH in March of 2009, so it wasn't all that long ago in the grand scheme of things that happen within government agencies. This was not all that long ago so this is pretty good evidence that this is a high priority and a high profile product for the institute. In April 2009 we properly showed notice in the federal register requesting information on the topic. One of the things you will note here at the bottom of that particular entry is we didn't receive any submissions which is not unusual. When the federal government requests information it's not very often that we get a lot of information. We get a lot of our information through a process we're going through right now. We formulate an opinion, we formulate a position, and then ask for input and go through a very extensive review and vetting process with stakeholders across the entire community.

In November 2010 we got approval to release a draft document for peer and public review. Also that same month, November, we forwarded that document to the National Nanotechnology Initiative, Environmental Health Implications Working Group. That group represents all of the agencies that participate in the National Nanotechnology Initiative and so it was our way of getting that document in draft form out to all of the other government agencies who are key participants and stakeholders in the nanotechnology arena.

December 2010, the document was placed for public review and comment on the NIOSH Website, and we're still in that period because in that period we invited public comment, announced this meeting, and the comment period is open until February 18 of this year. A publication summary, meaning a face-to-face meeting, was presented to the NNI, the working group I mentioned earlier, to provide the agencies with a little more detail on the content of the document, the rationale behind the document, and our timetable for development of the document, and that brings us up to today, our public meeting. To do just that for the public is to give a summary of the document, invite comments, and describe what the next steps will be for this document so the goal of this document, as I have already mentioned, is to review the evidence that is available to us, conduct a risk assessment, and develop the rationale for a recommended exposure limit; first to determine if we have sufficient data to recommend an exposure limit and second to do a risk assessment that would support that limit. Next is to disseminate that information to employers and employees and then provide good risk management practices to those employers. Part of this is an ongoing activity within the institute. Not everything we are doing and everything we know is currently captured in the document because we're doing work today that we want to incorporate into this document so it will be a very active and living document as we go forward through its development. A very key part of it is, as I said, what we're doing here today: good review and public comment and vetting of the document.

Focus presentations: I've already introduced our panelists today. We'll get into the individual components of the document. This is the flow we'll follow throughout the day today. I will remind you if you have a comment or question please use the microphone, identify yourself and your affiliation so that we can capture it for the transcript of today's meeting. So my summary and wrap up comes later so what I'll do now is invite Dr. Castranova. I think we're settled in; it's also a good small group so there's an opportunity for us to be fairly informal and interactive

which is great. I'll be your master of ceremonies but I'll also make sure that we have good dialogue occurring here and if I do this right we'll get Vince's slides up here.

VINCENT CASTRANOVA: Good morning. I'm Vince Castranova from NIOSH, and I'm going to look at some of the toxicology data that are available currently. Some of those data were, in fact, used to formulate the Current Intelligence Bulletin. The data that were used to formulate the Current Intelligence Bulletin were the pulmonary toxicology studies and they involved two species of animals, rats and mice. The carbon nanotubes looked at were both single and multi-walled carbon nanotubes in two forms both the raw form which contains the metal catalyst and the purified form where the catalyst has been removed. The structures looked at were studies that actually had poorly dispersed structures so large agglomerates, micro meter size agglomerates, as well as studies where there was much better dispersion and so the structures were much smaller. We have looked at various methods of exposure; bolus exposure using pharyngeal aspiration and intratracheal instillation as well as inhalation exposure both short and long term from 1 to 90 days. What we found is there is a commonality in pulmonary response across these studies. If you look at most of the studies they showed rapid elevation and bronchoalveolar lavage markers of pulmonary inflammation and damage. The pulmonary inflammation marker would be recruitment of polymorphonucleocytes from the blood to the airspace. The damage marker would be release of cytosolic enzyme and release of plasma proteins into the airspace. These markers in general increase very rapidly, 1 to 7 days post-exposure, and then slowly decline towards control over a 3-month period post-exposure. Commonality again was the rapid and persistent formation of inflammatory granulomatous lesions at the deposition sites of the agglomerates, and then lastly rapid and persistent interstitial fibrosis associated with migration of more dispersed structures into the alveolar septa or alveolar walls. And this is an example of a granulomatous lesion. These are from data from NIOSH Porter, et al. It was aspiration of multi-walled carbon nanotubes. This is a field emission SEM showing a granulomatous lesion. These finger-like cells here are elongated macrophages surrounding a mass of multi-walled carbon nanotubes. If you took this lesion in cross-section you would see the lesion was filled with rather large agglomerates of multi-walled carbon nanotubes. These lesions are usually in the proximal alveoli.

This is a study by Mercer, et. al at NIOSH that looked at two aspirations; one the top panel was to poorly dispersed suspension of single-walled carbon nanotubes and the lower draped figures are much better dispersed suspension of the single-walled carbon nanotubes. Looking at 1, 7, and 30 days postexposure you see at 7 days postexposure we can see in the poorly dispersed preparation large granulomas forming in the proximal alveoli and those granulomas persist through 30 days postexposure. In contrast, if we have smaller structures, more dispersed single-walled carbon nanotubes we get this response where we don't see any granulomas but we see interstitial fibrosis. Those are these white structures here. This is luciferin yellow stain for collagen and you see in the alveolar walls very early 7 days postexposure we're starting to see interstitial fibrosis which progresses at 30 days. You see the collagen in the interstitial walls much greater and so dispersed structures are giving you interstitial fibrosis, large structures are giving you granulomas. There are other pulmonary responses in response to a carbon nanotube exposure and I thought I would review those as well. There is a study out of North Carolina State where they did inhalation exposure, 6-hour inhalation, relatively high dose, and then followed 2- and 6-weeks postexposure and found that multi-walled carbon nanotubes can reach

the subpleural tissue. A study out of our lab, Mercer, et. al, has looked at aspiration of multi-walled carbon nanotubes 2 months postexposure and has actually found that some of the material that was in the subpleural tissue can migrate into the intrapleural space. That is the space between the lung wall and the chest cavity, and that would be the space where mesothelioma would occur. This study was a morphometric study. They actually counted the number of multi-walled carbon nanotubes that reached the intrapleural space and at an 80 μg dose per mouse there were 12,000 multi-walled carbon nanotubes in the intrapleural space.

In this field emission scan EM of that this is the outer surface of the lung so this is the intrapleural space here. This is the outside surface of the lung so this is the visceral pleura. These very curly cells are the mesothelial cells. The mesothelial cells; this is the subpleural tissue and here is the single multi-walled carbon nanotube that is piercing into the intrapleural space, and so what Mercer did is he counted the number of structures that were out here and got 12,000 after 2 months' exposure.

Other pulmonary responses; Shvedova, et. al in our lab has looked at aspiration of single-walled carbon nanotubes. Animals were pretreated with single-walled carbon nanotubes for 3 days and then the animals were exposed to listeria bacteria; 10 days later the animals were sacrificed, the lung tissue was taken, and the colony-forming units of bacteria were measured. Long and short of it is that the single-walled carbon nanotube animals were susceptible to infection and had 5 times the amount of bacteria in the lung than the control animals. Other pulmonary responses of concern are disruption of mitosis. If you take broncho epithelial cells, normal broncho epithelial cells, in vitro, and look at normal mitosis you form 2 poles from which mitotic spindles form which attach to the chromosomes and divide the chromosomes in half during division. Well when you expose these cells to single-walled carbon nanotubes you get instead of a bipolar mitosis you get a multipolar mitosis and if you expose to multi-walled carbon nanotubes you get a monopolar cell division and these are data from Sargent, et. al. And this shows single-walled carbon nanotube-exposed broncho epithelial cells and instead of seeing 2 poles you see 4 poles here. There's a pole here, here, here, and here. Another in vitro study out of our group, again exposed cells to the carbon nanotubes. These are broncho epithelial cells. These exposures were very low dose, 0.2 $\mu g/cm^2$ surface area cells for a very long time, 25 weeks, and what happens is during this time the carbon nanotubes cause the cells to transform. They have now, uncontrolled cell proliferation, cell invasion, and cell migration. They lose contact inhibition and they grow in soft auger which has caused transformation which is indication that cells have gone from a normal cell type to a cancer cell type. If you take these cells that were transformed and inject them into the abdomen of a normal mouse you get a tumor, and studies in our lab have shown that the single-walled carbon nanotubes are more potent in causing this transformation than the multi-walled carbon nanotubes.

Another issue of pulmonary concern is the question about mesothelioma. Because carbon nanotubes are long, thin fibers they have been compared to asbestos which is also a long, thin fiber, and asbestos is known to cause mesothelioma or cancer of the pleural lining of the lung. There is a study by a Japanese group that injected multi-walled carbon nanotubes into the abdomen and several months later they found mesothelioma in the lining of the abdomen that was equal in magnitude to their asbestos-positive control. The issue with this study is that the dose was very, very high. Poland, et. al used a much lower dose in mouse; again an abdominal

injection of multi-walled carbon nanotubes. They had 3 groups; 1 had long multi-walled carbon nanotubes, another short multi-walled carbon nanotubes, and a third asbestos. Two weeks later they looked at granulomatous lesions in the lung and they found that the long multi-walled carbon nanotubes gave granulomatous lesions to the same extent as asbestos but the short ones did not so that the long thin morphology was important. The last study out of Japan was intrascrotal injection of multi-walled carbon nanotubes. Those multi-walled carbon nanotubes migrated to the abdomen where they caused mesothelioma and so the question about mesothelioma is an issue, and remember the study by Mercer, et al. we have shown, in fact, that the multi-walled carbon nanotubes can reach the intrapleural space. We are also looking at cardiovascular effects of pulmonary exposure to multi-walled carbon nanotubes. Here we are aspirating into the lungs single-walled carbon nanotubes, 20 μg/mouse for each exposure and exposing every 2 weeks for 2 months. The mouse model we use is susceptible to atherosclerosis and we found that the animals exposed to single-walled carbon nanotubes had more aortic plaques and that is shown here. These are the control animals and you see the red lesions here on the artery. Those are pleural plaques, this is the control, and here is the animal that was exposed to the single-walled carbon nanotubes. You see a much greater area and extent of the pleural plaque formation and this is quantification of that showing single-walled carbon nanotubes cause more pleural plaques than control.

We also did an inhalation study, 1-day inhalation, to26 μg/cm of multi-walled carbon nanotubes to give a burden of 22 μg in the lung. Then we sacrificed the animals, looked at the coronary arterials and their ability to respond to a dilator, acetylcholine. What happens is animals exposed to the multi-walled carbon nanotubes did not respond normally to acetylcholine. That is, the vessels did not dilate so if the animal were under stress and had to exercise the vessels would not dilate normally and so you would have less oxygen delivery to the heart muscle.

We are also looking at central nervous system effects. Here was aspiration of mice to multi-walled carbon nanotubes. We sacrificed the animal 1-day postexposure, took the brain and found that messenger RNA for inflammatory chemokines, cytokines, and selectins, selectins are markers of blood-brain barrier damage, were elevated in selective areas of the brain; the olfactory bulb, the frontal cortex, the midbrain, and the hippocampus. So what was the risk assessment that was in the current intelligence bulletin? Well the first thing was a calculation of the lung burden in the rodent models. The second thing was to normalize the lung burden to equivalent alveolar epithelial surface area. They took the data from Stone, et. al; this is a morphometric study of surface area of the alveolar epithelium in the mouse, rat, and human and, therefore, they can now compare lung burdens in the mouse model to the rat model, and then extrapolate it to what burdens would be in the human model.

The risk analysis looked at 2 endpoints, inflammatory granulomas and interstitial fibrosis, and they calculated the lung burden which would give a 10% risk of that endpoint and that was called the benchmark dose. From that benchmark dose in the rodents they calculated what would be the workplace airborne concentration that would give you that benchmark dose in a worker in a working lifetime and the working lifetime exposure was 8 hours a day, 5 days a week, 50 weeks a year, 45 years of work. And this table summarizes some of that calculation. The endpoints in the rodent models were either granulomatous inflammation or fibrosis, the benchmark dose calculated was the dose that would cause the 10% risk of that endpoint in the rodent model and

then use an equivalent surface area, burden per surface area, they calculated what the airborne concentration in the workplace would have to be to give you that risk in the worker. You noticed that the studies with the multi-walled carbon nanotubes involve exposure by intratracheal instillation, aspiration, and short- and long-term inhalation, they involve both rat and mouse models, and here are the airborne concentrations predicted in the workplace that in a lifetime would give you that lung burden and you see they are relatively close among these 4 studies. This study is an outlier having a much higher level so much lower effect and the question is why is that the case. Well this study they took multi-walled carbon nanotubes and ground the material and during the grinding they shortened the material substantially; in fact, the mean length of the material they used was 0.7 micrometers. If you remember the Poland study, there was a big difference in the bioactivity between long multi-walled carbon nanotubes and short multi-walled carbon nanotubes so I believe that this study showed less potency because their material is very, very short.

In summary, what were the studies looked at in formulating the analysis for the Current Intelligence Bulletin. Well, the studies involve both single and multi-walled carbon nanotubes, both mouse and rat models, both raw and pure forms of the nanotubes, both agglomerate and well dispersed materials were in these studies and both bolus dose exposure and inhalation exposures were done, and qualitatively all of these studies, no matter what the differences were, gave you very qualitatively similar results, rapid and persistent inflammatory granulomas and rapid and persistent interstitial fibrosis. Other endpoints of concern that NIOSH is actively studying are lung cancer because we are seeing changes in mitosis and cell transformation, we are doing in vivo exposures and carrying those out for a year, looking at lung cancer development, mesotheliomas since we have evidence that the particles actually get to the interpleural space. The question is, will that develop into the mesothelioma. Again, we're doing an inhalation study and following up 1 year. Cardiovascular dysfunction and CNS dysfunction, we're following up on the data I have presented before to see what the time course in those dependents of that is. Thank you very much.

CHARLES GERACI: Are there any questions?

RALPH FROEHLICH: Have you done studies with other material, other nano materials such as non carbon fibers? I realize that it's somewhat outside of the focus of this group but…

VINCENT CASTRANOVA: Yes we have looked at silicon nanowires and we have also looked at titanium dioxide nanowires. The titanium dioxide nanowire study we compared those results after pulmonary exposure to titanium dioxide spheres of the same diameter and the same composition and what we're finding both in vitro and in vivo is that the wires are more inflammatory and more cytotoxic than the spheres but still the wires are less potent than the carbon nanotubes, the TiO2 wires. The silicon nanowires show some level of inflammation but again less potent than the carbon nanotubes.

JUERGEN PAULUHN: Germany. I have 1 question in regard to your cardiovascular study. Did you examine the degree of lung inflammation relative to the vascular changes?

VINCENT CASTRANOVA: Yes. We've done this type of study with residual oil fly ash fine size, titanium dioxide fine size, titanium dioxide nano size, and the multi-walled carbon nanotubes. With the residual oil fly ash, the titanium dioxide fine, and the nano titanium dioxide fine, we can get down to a dose which by BAL does not cause gross inflammation and still gave us the cardiovascular effects. Now if you do histology at deposition we do see a little localized inflammation in the alveolus but the next alveoli are clean so that's why we don't see the BAL change. With the multi-walled carbon nanotubes we keep lowering the dose and we haven't found the dose yet that caused no inflammation in the lung but we're continuing to lower that but what we have done is the lowest dose caused 100% inhibition of the ability of the vasculature to dilate.

PHIL SAYRE: EPA. Nice presentation. Did you mention the carbon nanofiber data at all or is that coming later?

VINCENT CASTRANOVA: Anna Shvedova is presenting her carbon nanofiber data at this year's Society of Toxicology. She is seeing an inflammatory response with the carbon nanofibers. She just got a paper accepted looking at genotoxicity with the carbon nanofibers looking at multinucleation of epithelial cells and what she found when she compared the carbon nanofibers to single-walled carbon nanotubes to asbestos and the carbon nanofibers were actually the most potent of the 3 in causing multinucleation of the cells. Thank you very much.

CHARLES GERACI: Dr. Eileen Kuempel will now summarize the work that was done during the development of this document on the risk assessment that was performed. You heard the toxicology data from Dr. Castranova and NIOSH felt there was sufficient toxicology data globally to undertake a risk assessment and that's the subtopic of our next focus presentation.

EILEEN KUEMPEL: Thank you everybody for coming. I'd like to start with acknowledgments. There were a number of people who contributed to this risk assessment including researchers both within NIOSH and outside NIOSH who provided data from their toxicology studies and information and without that we would not have been able to perform these risk analyses and we appreciate that. We also had a number of contributors within NIOSH in terms of statistical support and review and input which was very valuable. The NIOSH role in this is to develop recommended occupational safety and health standards which was authorized in the Occupational Safety and Health Act of 1970 and to do that we perform a number of research studies and risk assessments and then develop criteria for the recommended standard and then we transmit these recommendations to OSHA which is the regulatory agency.

I wanted to give a brief overview of a risk assessment paradigm that was established in 1983 by the National Research Council in the US and that really includes 3 pillars of research, assessment, and management and we heard just now the toxicology data which contribute to information in the hazard assessment and is also used in the dose response assessment. There are a number of other areas if research are available that we can use including epidemiology and exposure measurements which are pretty limited at this point. The 4 steps in the risk assessment process include hazard identification, dose response assessment, exposure assessment or exposure scenario assessment when we don't have actual data, and then putting those steps together into a risk characterization and any data from any of these steps can contribute to

information to making risk management decisions and important in all of this is risk communication and our meeting today is an example of that process.

Recently the NRC was asked by the US Environmental Protection Agency to reevaluate the 1983 paradigm with the view of how to make risk assessment more useful as practiced and something that came from that was that they continued to recommend the basic steps in the risk assessment but they also added some additional steps in the problem formulation and scoping and also in the interaction between the risk characterization and risk management decision making and they asked what options are there to reduce the hazards and exposure and how can risk assessment be used to evaluate the various merits or options. And this was really the guidance that we used in proceeding with our risk assessment. How can the risk assessment be useful to evaluate the various risk management options. Here's a taxonomy of the carbon nano objects that comes from the International Standards Organization and it describes the single-walled and multi-walled carbon nanotubes for which we have most of the toxicology data that we just heard about. As part of the carbon nanotube which together with the carbon nano rod constitute carbon nanofibers and there could be more discussion about the taxonomy but this is the ISO version.

The data that we used to evaluate, that were evaluated in the risk assessment, were as we just heard the toxicology studies, there are no epidemiology studies at this point. The focus is to prevent occupational respiratory diseases in workers and that includes with working lifetime exposure. So the animal dose response data available included a number of single or short-term exposure studies. Those may be dosed one day by intratracheal instillation or pharyngeal aspiration and then the animals followed for 1 month to 3 months. There were 2 subchronic inhalation studies; that's 90 days of exposure. One of them had up to a 6-month followup and the responses that we looked at were early-stage inflammation, granuloma and fibrosis and these were shown in the studies that had followup to persist or even progress after the end of exposure. So we consider that these responses are relevant to humans. There are similar types of lung pathology that's observed in workers that are exposed to dusty jobs and these can be functionally or clinically significant.

One of the questions was how can we use these short-term data? The data are pretty sparse for carbon nanotube that we would typically like to have but we want to use what data we can to help inform our risk management decisions. So there are a number of studies that were short term. There were 2 that would typically be considered the best data for risk assessment; the subchronic studies but those were for 2 types of multi-walled carbon nanotubes and we had all of these other studies of different types of single-walled carbon nanotubes with different metal contents and different multi-walled carbon nanotubes so the data also showed that the carbon nanotubes were persistent in the lungs. They were not cleared very effectively so that would lead to the suggestion that even though there was an exposure for 1 day and the effects were followed up from 1 to 3 months later, the effects were seen to persist so that suggested that those data were relevant and that there was not very good clearance but because of the different experimental designs, the different animals, the different exposure routes, the different durations, the different endpoints of these early lung effects, it was very hard to compare directly between them but we were very happy to see that there were a set of studies from 1 laboratory; in fact, Dr. Pauluhn is here today we're happy to see. And the 1 study was a 1-day inhalation followed 13 weeks later and then a 13-week inhalation. So this allowed us to look at the dose response

relationship in terms to see what effect the dose rate had and as you can see based on the data available, whether the… When you estimate the amount of carbon nanotubes deposited in the lung it didn't matter whether the dose was given in 1 day and the response was looked at 90 days later or whether there was an exposure for 90 days, the relationship with the focal septal thickening was dependent on the deposited dose. So this gave us increased confidence that it was relevant to look at the shorter term studies.

The next question is what sort of effect level or endpoint should be used. The no-observed adverse-effect level and the lowest-observed effect level have long been used in toxicology and these are well recognized. There are some limitations to this and, in fact, the NRC risk assessment guidelines that I introduced to you recommend using the benchmark dose estimation. The no-observed and lowest-observed adverse-effect levels do depend on the dose spacing and the sample size in the study. They are not risk based. The approach to using these in risk assessment involves extrapolation to lower doses by various adjustment or uncertainty factors. Some advantages of the best benchmark dose estimates is that the model is able to use most or all of the dose response data. The risk estimates include both the maximum likelihood estimates and confidence limits around that estimate and takes appropriate statistical account of the sample size and variability and finally it provides a standardized point of departure within the range of the data. For example, 10%, and from that you can do lower dose extrapolation to predict doses associated with lower levels of risk. This illustrates the risk assessment scheme that we did which Dr. Castranova overviewed and this is similar to approaches we have used previously for other types of inhaled particles and it's also consistent really with the concepts used in various risk assessment approaches. Basically you start with the animal data and you have to have some way to extrapolate that dose to humans. So we started with the exposure response data and then you can either measure it you have that or model the estimated lung dose and then calculate through the dose response modeling a benchmark dose and that's defined as the dose associated with the specified level of risk. And then we extrapolate that dose in animals to humans to estimate the equivalent lung dose. For example, we adjusted for the species differences in lung surface area in the alveolar region the site where the granulomatous inflammation and fibrosis occurred. And then the assumption is that if you can estimate an equivalent dose then the risk would be equal in humans to that observed in rats if you have no other data concerning the sensitivity across species. And then we estimate the exposure over an 8-hour day for a 45-year working lifetime that would result in that equivalent lung dose and then once we have the human equivalent exposure concentration we use that information with coming up with different risk estimates or applying various uncertainty factors, etc. and also consider technological feasibility in measuring and controlling exposures to recommend an exposure limit.

This first slide shows an example of the benchmark dose estimation for 1 type of data. As I mentioned, the database across the toxicological studies was quite varied and one factor was we had either continuous response endpoints or dichotomous and the continuous is that you can see as you go along with the estimated deposited lung dose you have an increase in the amount of alveolar connective tissue thickness which was a measure of the pulmonary fibrosis and because there's no… In a continuous data if you don't have a biological basis for saying where on this continuum, at what point is this biologically adverse, we didn't have that information and so we used a statistical criterion for that. And then using an example of the dichotomous data and that's defined as if you have say a group of ten animals and you have a criterion, in this case the

granulomatous inflammation was the criterion and they had categories of minimal or mild, moderate... Different grades of the granulomatous inflammation and then they scored the proportion of animals which had that. So here we have a response of 30% of the animals responded at this dose and then you can see and this is an issue that we had with these data is you quickly get to 100% response which this type of information made it challenging for doing benchmark dose modeling and it's pretty much at the edge of the amount of data that you have for that. In fact, we found that of the various benchmark dose models only the multistage was able to converge and provide an adequate fit but we did look, and in this case actually I'm showing you the exposure concentration rather than the lung dose because I want to illustrate that from this study the lowest observed adverse effect level reported in the study was 0.1 mg/m^3 and when we did the benchmark dose estimation the most likelihood, the maximum likelihood estimate, was 0.06. So it wasn't very much different from the lowest-observed adverse-effect level. We prefer to use the lower 95% confidence limit on that benchmark dose which in this case is 0.02 and the reason is because that accounts for variability in the data and it gives us 95% confidence that the true benchmark dose is above that.

And the second subchronic inhalation study that was available looks at the alveolar interstitial thickening of minimum or greater severity and again we had a similar pattern in the exposure response with sparse data in the area that we're interested in estimating a benchmark dose. But again, looking at the lowest-observed adverse-effect level and the no-observed adverse-effect level in this study the lowest adverse-effect level reported was 0.4 mg/m^3 and our best estimate for the benchmark dose was 0.1. The no-observed adverse-effect level in that study was 0.1 and our lower bound estimate was 0.05 so we're not too far from the sort of traditional lower or no-observed adverse-effect level estimates. What we did with our benchmark dose estimates because they're risk based they represented the dose associated in this case with a 10% excess risk of these early stage lung effects. This chart shows the lung burden estimated in the rat or mouse and then the human equivalent and then the working lifetime, 8-hour time weighted average concentration, as I had shown you on the chart, and this includes again various types of carbon nanotubes with different amounts of metal and different types of exposure to the rats or mice and also I point out in this Mercer study which Dr. Castranova mentioned, another reason why there is the higher level probably is because this used a hydroxyproline amount which is much less sensitive to detecting fibrosis than these other measures which could detect it at much lower levels so I think a difference in the endpoint is another factor. So there are a number of reasons for the differences in study designs, endpoints, routes of exposure, rodent species, etc. A number of reasons to contribute to the variability in these estimates but one thing that comes across is they are relatively low in terms of a mass concentration compared to some of the other types of poorly soluble types of particles that we are familiar with.

This chart summarizes the results for the dichotomous exposure response data again looking at different types of single-walled or multi-walled from different study designs. Again, the working lifetime, 8-hour time-weighted average is not too different actually in terms of the relatively low mass concentrations. Finally, we have the best data available for risk assessment because of the most information in terms of doses and a more similar exposure as workers in terms of the daily inhalation but still just 13 weeks. So these studies from Ma-Hock, et. al and Pauluhn, same approach and these are the working lifetime risk estimates and we would use the lower 95% confidence interval. These tables are all in the document.

So to summarize these results the subchronic inhalation studies of 2 types of multi-walled carbon nanotubes in rats showed that a human equivalent exposure from about 0.2 to 2 $\mu g/m^3$ which is the lower 95% confidence limit of the 8-hour TWA for a full working lifetime would be estimated to be associated with the 10% excess risk of these early-stage pulmonary inflammation and fibrosis and several short-term studies of multi-walled and single-walled carbon nanotubes in rats and mice had relatively similar estimates of 0.08 to 12 $\mu g/m^3$. Again, the lower bound estimates.

So these risk estimates were used in part for the basis for the recommended exposure limit but we found that we were faced with a technological feasibility of measuring the airborne exposures at the limit of quantification of the analytical method that NIOSH recommends of 7 $\mu g/m^3$ and NIOSH would prefer to have lower risk estimates and recommend lower exposure limits but we were faced with this limitation in the ability to measure the airborne carbon nanotubes. So this value of 7 $\mu g/m^3$ is supported by the studies from the multi-walled and single-walled carbon nanotubes from the 2 subchronic and several short-term studies in rats and mice to different types of materials, purified or unpurified, and there were quantitatively similar lung effects from carbon nanofibers which is reported in 1 study in mice. There are a number of uncertainties in any risk assessment and in this risk assessment, in particular, in terms of extrapolating the short term or subchronic data in animals to predicting the dose and effects in humans from long-term exposure. We have limited information on the human clinical significance of these early stage lung effects in animals. We have uncertainty in terms of the generalizability of these findings to other types of carbon nanotubes or carbon nanofibers that have not been studied. There is uncertainty about the physical and chemical properties of the carbon nanotubes used in the animal studies and what's encountered in the workplace. There are a number of uncertainties. Research needs to reduce this uncertainty including studying the chronic effects in animals. In particular, we really have no information that we have used at this point concerning the possibility of cancer effects. It's important to compare the biological effects of different types of carbon nanotubes and carbon nanofibers. They have different physical chemical properties that can affect their biological response and we need to develop improved models to predict the carbon nanotube or carbon nanofiber deposition and retention in the respiratory tract of animals and humans. Currently the models out there are for spherical particles or fibers and the carbon nanotubes are a mix between those structures and so we have uncertainty and actually that was a reason why we estimated risk either assuming that the amount of material that deposited in the lung wasn't cleared or that there was normal clearance as in spherical particles and we felt that gave a bound on the risk estimates and it's important to characterize what exposures are actually occurring in workers whether workers are exposed and to what extent. Also, it's important to examine the alternative exposure and dose metrics. We're using mass, that's the metric that's reported in most of the toxicology studies and which is also used to measure airborne exposures in the workplace but there may be other metrics such as fiber, particle number, surface area, or volume which give better prediction of the effects and if we can associate these with the effects that we are trying to prevent and if we can measure these in the workplace we would want to use the best metric available. I invite any questions or there will be time later if you want to think about it a bit. I'll be available at another time as well.

CHARLES GERACI: We're right on our agenda and we do have a break scheduled so we'll take our break and it will be an opportunity for those of us who had to get here late and assembly ourselves and we'll move from toxicology into the risk assessment prior to our break into some of our experiences in the field in measuring, evaluating, and controlling releases and exposures and also recommendations for the medical screening and surveillance activity that's in the document. We'll break until 10:30.

We do have an active field activity in nano materials research. We're talking today specifically about carbon nanotubes and carbon nanofibers so that's been a great success for us to be able to partner with companies to understand this even better. Ralph Zumwalde will give us an overview on the background on exposure, assessment, and control with the focus on carbon nanotubes and carbon nanofibers.

RALPH ZUMWALDE: Good morning. I'm one of several people who contributed to the development of this document. In addition to those seated at the table today there are many other NIOSH researchers who were contributors. There are three things that I'd like to cover; the first one The Measurement of Occupational Exposure to Engineered Nanoparticles is not per se a discussion in the document but I thought it would be appropriate to provide some background on the types of instruments and the types of exposure metrics that are being used for characterizing exposures to nanoparticles. The second point is on occupational exposure, the measurement of occupational exposure to carbon nanotubes. I'll say a little bit about that and then I'll end with saying what we know about the ability to control occupational exposures to carbon nanotubes.

One of the challenges that face investigators today is how to best characterize exposures to nanoparticles in the workplace. Here are 2 examples of instruments that are probably the more frequent instruments currently being used to monitor exposures to nanoparticles. They're one of the few portable instruments that can be used to give real time measurements. The Condensation Particle Calendar and Optical Particle Calendar Sizer provide information on particle size distribution and at the same time provide information on particle concentration. These instruments have been around for a while and were primarily used for measuring fine and ultrafine particles. More recently these instruments were modified to enable the user to measure airborne particles at the nanoscale.

The second illustration is another group of instruments that are being used to measure nanoparticles in the workplace. Unfortunately, many of these instruments as you can see, are not readily portable. They're not the kind of instruments that would be used, from an industrial hygiene perspective, for routine monitoring. But in fact these instruments are the ones that are being used for research studies to gain a better understanding of particle size distributions. In one case, the instrument located at the bottom left, will give you information on active particle surface area. A couple of the other instruments here are different types of impactors that allow the collection of various size distributions of particles that can be further analyzed such as metal analysis, or for determining mass concentrations. So again, I just want to illustrate that there are a number of instruments that are being used in the workplace. Many of these instruments are being used for research purposes, and only very few of them would I consider to be easily used for doing measurements in the workplace on a routine basis. Likewise, in terms of exposure metrics there are a number of different metrics that are currently being used. One of the more

common metrics is particle count concentrations and mass concentrations of particles including respirable and total mass. If you use some of the more sophisticated instruments; you can do surface area measurements or you can combine the collection of different samples to get better particle size information and particle identification. And, of course, as a fallback you can perform more comprehensive analysis of samples using electron microscopy which can provide information on particle morphology, size, and composition.

With all these exposure metrics there are advantages and disadvantages. A mass exposure metric is one that historically has been used for occupational exposure limits. Unfortunately, a mass concentration, if it's just a mass of material, lacks specificity on particle identification. However, if you're doing mass measurements you can also determine the mass of a particular metal or in the case of carbon nanotubes you can do a mass based on elemental carbon. Particle size distribution, again there are instruments that will give you information on the size of particles, but again most of those instruments don't provide sufficient information for identifying particles. Surface area measurements, as I mentioned earlier, can be used to determine the surface area of particles. Particle surface area may be relevant in the future depending on the results of toxicology studies, but again the measurement of a particle's surface area from airborne exposures can't be easily accomplished with real-time instruments. The instrument technology for particle surface area needs to be improved before it can be routinely used. And again, the use of electron microscopy has the advantage of being able to analyze samples for particle morphology, size, and identification.

So what do we know about occupational exposure to carbon nanotubes and nanofibers? For the most part the data that has been collected and reported in the literature have been studies that have looked at the extent of exposures at particular job tasks and operations. Very few of the data have reported personal exposures. The intent of many studies was to determine whether or not at a particular task or job handling practice resulted in a release of carbon nanotubes. And what have these studies reported? Most of the exposures that do occur seem to be short-term in duration. What we don't know, at least from the literature, are exposures to carbon nanotubes during their downstream use. Most of the data that's been reported to date have been in a research laboratory application, a pilot study, or in the manufacturing of carbon nanofibers or carbon nanotubes. In terms of reported exposure measurement data, what has been reported has been primarily particle count and mass concentrations with little identification on the characteristics of exposure.

Here are a couple of examples of photomicrographs of airborne carbon nanotubes and nanofibers that I thought would be informative as to what is being found in the workplace. I want to bring your attention to the picture on the right which is an agglomerate of multi-walled carbon nanotubes and what's interesting here is this particular airborne sample was collected during the sonication of multi-walled carbon nanotubes in a liquid medium. During sonication there was the release of fine droplets and within those droplets there was an agglomeration of multi-walled carbon nanotubes. The photomicrograph on the left is interesting in that both carbon nanotubes and nanofibers were released into the air during the manufacturing of carbon nanofibers. This manufacturing process not only generated carbon nanofibers but also generated exposures to carbon nanotubes which wasn't the intent. The implications from these finding are at some point there may be interest to have recommendations that are based on tube type including tubes that

have been functionalized. From an analytical standpoint there may be a need to have some kind of analytical tool that's going to be able to make distinctions between tube types.

So what is NIOSH recommending? As you heard this morning NIOSH is recommending 7 $\mu g/m^3$. It is an 8-hour TWA to correspond to the risk assessment and that the TWA is for a 40-hour workweek. The exposure recommendation is for a respirable mass sample, which corresponds to what we know about the animal toxicology data. As you heard earlier the exposure recommendation is at the limit of quantification of the analytical method. Actually I would refer to it as the upper limit of exposure. NIOSH method 5040 for elemental carbon is the analytical method. In Appendix C of the document, we provide guidance on how to optimize the collection of samples and how to use method 5040. In Appendix C it provides guidance and how you can achieve a low limit of detection. It also provides guidance on how to do personal sample monitoring of workers, how you can optimize the collection of samples by changing the flow rate, and how you can optimize the amount of sample you collect to be able to achieve a sample at the LOQ or above.

One of the concerns that has come up with controlling exposures to carbon nanotubes is whether or not traditional engineering control methods that are used for capturing particulates can be used for capturing carbon nanotubes and nanofibers. In principle, based on the physical characteristics of particles including aerodynamic size and density, there is every reason to believe that traditional methods should be able to control exposures to nanoparticles in the same manner as larger size particles. In fact, what we know from the literature, and again this is a small database, carbon nanotubes exposures can be controlled when local exhaust ventilation is applied or when the process is enclosed. Based on some of NIOSH's field studies the introduction of engineering controls does help to reduce exposures. The guidance that we give in the document is to inform the user that under certain conditions in which carbon nanotubes are being handled or used that the type of control used will differ depending on the nanomaterial. If you're using a free fiber form then enclosure may be the only option that you have in terms of maintaining exposures below the NIOSH recommended exposure limit. If it's encapsulated in a material then the potential release of exposure might be low unless you're drilling or sawing on the composite material.

Here's an example where there was a clean out of a reactor furnace in which exposure measurements were made during the clean out of the catalyst material. Some of the material that was left in the reactor was generating an exposure, and people doing that clean out were being exposed. NIOSH worked with this particular industry and was able to help them implement local exhaust ventilation at the source that almost totally eliminated exposures. Here is another example of workers mixing carbon nanofibers and I don't know if you can make it out but what they did was enclose the work area with plastic sheeting and used overhead exhaust ventilation that created a negative pressure within the work area and brought clean air from the outside of the room. This simple type of control measure was able to prevent exposure to the outside of the work area. Unfortunately, because they're venting the exposures from the ceiling it's probably not the best design in terms of capturing carbon nanotubes exposures since you're drawing the contaminated air across the front of workers to the ceiling. It would have been preferred to use either local or downdraft exhaust to prevent worker exposure. At least in this particular case these workers were wearing respirators.

So in summary we believe that the respirable mass sampling being recommended by NIOSH using method 5040 will provide reasonable estimates of worker exposures and help to provide guidance in establishing appropriate risk management practices. This is based on knowledge we have in the use of engineering controls from NIOSH's studies and from studies reported in the literature including studies on controlling exposures to fine and ultrafine aerosols. We believe that putting in engineering controls will provide a reduction in exposures to carbon nanotubes but will require systematic evaluation to ensure that exposures are maintained below 7 $\mu g/m^3$. There still remains a lot of research that needs to be done. There needs to be more work in quantifying personal exposures to workers who are handling carbon nanotubes. In fact, one of the research efforts that NIOSH is doing is to provide a better understanding of personal exposures to carbon nanotubes and nanofibers. There's room for improvement in terms of the analytical method not only getting a better understanding of how method 5040 works in the field but also thinking about other sampling and analytical methods that might be more reflective of the toxicology data. You heard previously there are other exposure metrics that may turn out to be better for monitoring exposures to carbon nanotubes and nanofibers. For example, is there a need for an analytical method that would allow you to quantitate exposures to carbon nanotubes based on their size or on surface area or volume. Again, through NIOSH's studies we will be able to get a better understanding of exposure metrics best suited to carbon nanotubes and the types of engineering controls that will be effective in reducing exposures below 7 $\mu g/m^3$.

And then lastly, I just wanted to mention that while there's some limited data in terms of dermal exposure and dermal penetration of carbon nanotubes there still needs to be more research into the effectiveness of gloves and other types of barrier materials, such as clothing, used to prevent dermal exposures. I'll leave it open to any questions.

TIMOTHY FEELEY: Tim Feeley with Bayer MaterialScience in Pittsburgh. Do you have much of a sense I mean elemental carbon since you're going to be monitoring a lot of it could just be background, other sources obviously do you have a sense from the work you've done already how much would be not related to or… I guess the concern would be you do monitoring on someone, you're above the REL, your exposure controls could be good for the person that is handling the task but yet that elemental carbon could be coming from other sources and you think you need to improve your control methodology so just a sense of that.

RALPH ZUMWALDE: No that's a good question and it's a question that I think we anticipated and that's why we have Dr. Birch at the end of this table and I'm going to ask her to answer that question. I know it's one that we've talked about in terms of being able to differentiate other types of elemental carbon when measuring exposures for carbon nanotubes and nanofibers.

EILEEN BIRCH: Yes, well elemental carbon alone doesn't differentiate between a background elemental carbon for instance from a diesel vehicle so the differentiation is really based on having very low backgrounds generally. Typical environmental backgrounds are 0.5 $\mu g/m^3$ so if you're measuring 7 you're obviously so far above what that background is it's understood that you are measuring that material in question. Now taking a background you have to have a judicious choice of what you're calling your background. If you sample inside a facility what is that HVAC system, in the ventilation what filtration? You may well in an office area or

administrative area way from the process get a background that's lower than outside. If outside you happen to station samplers in a loading dock area and a diesel truck comes in to deliver the mail or package so a combination of backgrounds is used to gage what the background is and then how elevated that concentration is relative to background is an indicator. We don't just use elemental carbon I should say. We also do microscopy so we have more qualitative assessment of what the nature of the material is.

TIMOTHY FEELEY: Okay.

RALPH ZUMWALDE: Thank you.

RALPH FROEHLICH: Ralph Froehlich again. I thought I saw on the first speakers Dr. Castranova's slides that he used a variety of different methods or units of measurement to describe what the exposures were for the test animals upon which toxicology is based. Have all those measurements been also evaluated by the NIOSH 5040 so that we can compare directly what you're recommending we use to measure to the animal toxicology data?

RALPH ZUMWALDE: Good question. First let me say the risk assessment and what Dr. Castranova was presenting in terms of a dose metric for the animal studies is based on a mass dose metric. Some of the other data that he presented looks at dispersed fibers as they migrate into the different areas within the lung. We haven't really quantitated those kinds of exposures although I know Vince mentioned the number of tubes that have been observed in the lung of animals. Now one of the research efforts that we have underway, since animals in the NIOSH toxicology studies were dosed based on a mass metric, is to generate exposures at the same concentrations that were used for the animal studies. These exposures will be evaluated using other metrics and of course looking at the morphology and size of the tubes would clearly be one of those exposure metrics. So the outcome of that effort would give us some kind of correlation between the mass and a fiber or tube number concentration. I don't know to what extent we can characterize those exposures to the extent where we can come up with some kind of size distribution based on dose but maybe that's possible too. But I think the point I want to make is that we understand that other dose metrics may be better and while mass has been the primary dose used in animal studies I think we're trying to make an effort to better understand whether or not there are other appropriate dose metrics that might be a better indicator of health endpoints.

JUERGEN PAULUHN: My question is; is there any scientific basis to lump those CNT's together which are present? They are thin walled so they are present as assemblages that way agglomerated structures. The more ridged ones could be relatively easily dispersed fibers so the question I have is do you think that all aggregated or agglomerated structures may be ending up as fibers; therefore, you have 1 unique value or would it be better to have 2 values? One value for agglomerated structures and a value for more fibril structures.

RALPH ZUMWALDE: Well this may be more of a toxicology question than exposure. I mean obviously in the workplace we're seeing both. We're seeing the agglomerated and clearly we know that for many of the carbon nanotubes and nanofibers that are being used right now that there seems to be this high affinity to agglomerate. But we also know that there are processes where they're trying to produce these fibers where they're trying to decrease their ability to

agglomerate because they have different commercial applications by being these free tubes. So we're seeing both so maybe the question is from a toxicology standpoint can we make any kind of a differentiation between the agglomerated versus any free fibers?

VINCENT CASTRANOVA: Well in the NIOSH studies when we looked at the same material and either used a poorly dispersed or a well dispersed sample of that same material and that holds true for the carbon nanotubes as well as other nanoparticles. We've done the same thing with titanium dioxide and carbon... There is, I think strong evidence that more dispersed material gave a more potent effect. I think I'll let Eileen speak on this but I think that the risk analysis in the Current Intelligence Bulletin looked across agglomerated versus dispersed and came up with ballpark similar responses. That may more accurately reflect that agglomerated weren't super agglomerated and that dispersed weren't super dispersed but I'm going to let Eileen comment more on that.

EILEEN KUEMPEL: Yes as mentioned the toxicology studies showed that the dispersed material had a different effect particularly in Dr. Mercer's study. The well dispersed particles caused more interstitial fibrosis where the more agglomerated particles caused more granulomas. One question and an uncertainty that we'd like to have more data is what happens to the more agglomerated structures? Do they stay that way? To what extent do they separate to release some individual materials in the lungs over time? That is a question. In terms of the risk assessment there was variability across these different studies for a number of reasons, and differences in the estimates but for all the studies the estimates were such that they all gave low mass concentrations. So we were still limited regardless of the type of material or its agglomeration state. We were still limited by the issue of the limit of quantification of the measurement method.

VINCENT CASTRANOVA: And if I could just speak a little further on whether an agglomerate would disperse once it was in the lung the theory behind that is that there is alveolar lining fluid in the lung. That alveolar lining fluid has surfactant basically disaturated phosphatidylcholine and many of our disbursements that we do outside of the lung take advantage of that alveolar lining fluid component. In fact, that does break up agglomerates for many materials not only carbon nanotubes but carbon black, titanium dioxide, cerium oxide, etc. fairly effectively. The question is how much of this agglomeration occurs in vivo? To tell you the truth there are no good data currently out there on how extensive that is. We can only give you very, very subjective data. If we looked at structures immediately after exposure to single-walled carbon nanotubes and then looked at those animals a year later qualitatively it looked like we were having more structures without more exposure. The only thing we could figure out is that some of the agglomerates were releasing smaller structures over time by action of the alveolar lining fluid or some other action. But again this was very, very subjective; it's not a complete study so certainly that's an issue that has to be resolved.

BARBARA CUMMINGS: Barbara Cummings also with Bayer MaterialScience; just a little bit of a clarification and you said you've done some work on measurement for baseline or background measurements with this elemental carbon method, was that done same day where you could get an estimate of what those values were or were you doing this over some time or just within a certain type of manufacturing? I'm just trying to understand the relationship.

EILEEN BIRCH: The same day, multiple surveys but in addition to those measurements there's a large body of data available for background elemental carbon concentrations just from air pollution studies. So yes we do take backgrounds in different locations and outdoors but there are so many surveys we've done but to compliment those results we have a large database full of environmental background concentrations of elemental carbon.

BARBARA CUMMINGS: Okay would they also take into account the variations you would have of day to day within that particular operating environment based on whatever background versus what you might find in the ambient traditionally and so forth?

EILEEN BIRCH: They would take in account variations over the time weighted whatever the sampling period was and whether or not we have real time instrument or direct reading instruments that show whether or not a diesel truck had passed and so you had a transient or that just a constant background concentration was present. So between the two we'll have a time weighted average over the sampling period and some information on whether or not there was a transient that occurred.

BARBARA CUMMINGS: So you're relying also on some real time instrumentation during the course of that day to try to measure variation within a workplace?

EILEEN BIRCH: We do and in the case of one of the personal exposures it was clear based on the direct reading instruments that, that exposure occurred over a period of 10 minutes rather than the 5-hour sampling period so that transient concentration level was much higher than what was implied by the time weighted elemental carbon concentration.

BARBARA CUMMINGS: Okay so it's really a combination then of methods because you have the time weighted average sampling that you are using with the filter but also perhaps coupling that with the real time instruments and kind of getting a sense of where you're seeing these variations and how it is attributable to that task.

EILEEN BIRCH: Yes and practice when we do the industrial hygiene surveys. Yes but the REL is applying to an 8-hour time weighted average.

BARBARA CUMMINGS: Just in my experience I've seen variations through the day and in any workplace just a door opening and closing or something that's starting up or shutting down that could impact a measurement particularly on a particulate level. And so I'm just wondering how you…?

EILEEN BIRCH: That certainly is true for a particle number.

BARBARA CUMMINGS: Right and the elemental carbon again not being specific perhaps to the carbon nanotube per se but just to anything of that nature that's in the environment. So it's a challenge.

EILEEN KUEMPEL: Yes although I'd say at 7 $\mu g/m^3$ that's not a typical environmental background. So it definitely would flag dispersion of the material.

BARBARA CUMMINGS: I see, thank you.

CHARLES GERACI: Thank you Ralph. Before we head into Dr. Trout's presentation to finish the formal part of our program this morning, I feel compelled to remind us that the first word in the title of our document is current; our Current Intelligence Bulletin because these really are our current understandings but it is also a statement of our current challenges and you've heard a pretty good discussion here this morning of a very active agenda of research that NIOSH has undertaken. Those of you who have taken advantage of NIOSH's field presence and field activity would see that it is a challenge; it's a very multimetric approach. A NIOSH field team showing up at a facility is equipped with traditional industrial hygiene methods and some rather progressive and research level methods. So, in followup to the discussion this really is a challenge; it's an ongoing challenge and it's an evolution of the technology that we need to address. All of these very good questions, and we'll get into that at the very end of the session when we get into what we believe are the research needs and is actually a description of our current and fairly aggressive research agenda on nano materials within the Institute. That's the lead into our final presentation by Dr. Doug Trout.

DOUGLAS TROUT: Good morning. As Chuck mentioned I will be over the next couple of minutes summarizing the medical screening and surveillance recommendations in the Current Intelligence Bulletin. As Ralph mentioned, this effort regarding the surveillance and screening recommendations is a group effort. Lots of people have had input and we look forward to the input that we will be receiving during this review process. So the evidence summarized in the document and summarized also this morning leads to the conclusion that workers occupationally exposed to carbon nanotubes and carbon nanofibers may be at risk of adverse respiratory effects. These workers may benefit from inclusion in the medical screening program recommended as a prudent means to help protect their health. A screening and surveillance program is among the steps recommended in the CIB to minimize potential health risks associated with exposure to these substances. Workers who could receive the greatest benefit from medical screening include these bulleted here: workers exposed to concentrations of carbon nanotubes or carbon nanofibers in excess of the REL, the recommended exposure limit, that we've just been discussing and workers in areas or in jobs, activities, or processes involving contact with carbon nanotubes or carbon nanofibers who have the potential for intermittent elevated airborne concentrations to these substances. For example, workers involved in the transfer, weighing, blending, or mixing of bulk nanotubes or nanofibers; workers involved in the cutting and grinding of composite materials containing these substances or workers in areas where others may be performing such activities.

Oversight of the medical surveillance program should be assigned to a qualified healthcare professional who is informed and knowledgeable about potential workplace exposures, roots of exposures, and potential health defects related to carbon nanotubes and carbon nanofiber exposure. The screening should consist of an initial evaluation, periodic evaluations, and written reports of medical findings. The initial or baseline evaluation should consist of an occupational and medical history, a physical examination with an emphasis on the respiratory system,

spirometry testing, a chest x-ray interpreted by a NIOSH certified *B reader, and other examinations or medical tests deemed appropriate by the responsible healthcare professional. Periodic evaluations should be conducted at regular intervals; for example, annual, or at other times for example after some incident or unusual exposure has occurred as deemed appropriate for the individual worker by the responsible healthcare professional based on data gathered in the initial evaluation, ongoing work history, changes in symptoms such as new or worsening respiratory symptoms, or when processes change in the workplace. These periodic evaluations should include an occupational and medical history including an update of respiratory symptoms, physical examination, and consideration of specific medical tests such as repeating spirometry and or x-rays. A written report of medical findings is part of this screening program. The healthcare professional should give each worker a written report containing the individual worker's medical examination and test results, and opinions or recommendations concerning end relationship between the individual worker's medical condition and occupational exposure. Any special instructions warranted concerning the individual's workplace exposures and/or the use of personal protective equipment.

Recommendations may also involve recommendations concerning further evaluation or treatment. For each examined employee the healthcare professional should also give the employer a written report specifying any work or exposure restrictions based on the results of the medical evaluations and opinions and/or recommendations concerning work relatedness and the use of personal protective equipment or other workplace preventive measures. Findings from the medical evaluations having no bearing on the worker's ability to work with carbon nanotubes or carbon nanofibers should not be included in any reports to employers and confidentiality of the worker's medical records should be enforced in accordance with all applicable regulations and guidelines.

Worker training is an important component of prevention and should be included in the screening and surveillance program. Worker training should include information sufficient to allow workers to understand the nature of potential workplace exposures, roots of exposures, and instructions for reporting health symptoms. Workers should be provided with information about the purposes of medical screening, the health benefits of the screening program, and the procedures involved.

Periodic evaluation of both the data collected in the screening program and the screening program itself are important parts of the program. Medical screening data should be periodically aggregated and evaluated to identify patterns or trends in exposure or health-related data that may be linked to work activities or practices that require additional primary prevention efforts. So this is really the surveillance aspect of the screening and surveillance program. This analysis should be performed similarly by a qualified health professional or some other knowledgeable person. And as with all activities involving these data confidentiality of worker's medical records should be enforced in accordance with all applicable regulations and guidelines.

And lastly, in terms of this summary of the recommendations; employers should periodically evaluate the elements of the medical screening program to ensure that the program is consistent with current knowledge related to exposures and health effects associated with occupational exposures to carbon nanotubes and carbon nanofibers. This reflects the fact that this is

essentially a living document and as Chuck mentioned the word current is in the document and we do expect that things will change over time. So that's the summary of the recommendations that are in the Current Intelligence Bulletin. I'll be happy to take any questions or comments.

RALPH FROEHLICH: Ralph Froehlich again. With similar medical surveillance of people occupationally exposed to asbestos fibers there are some well-defined endpoints; lung cancer, mesothelioma, and pleural plaques, etc. Are there any specific endpoints that based on the toxicology that physicians should look for in people occupationally exposed to these carbon nano materials?

DOUGLAS TROUT: Well we don't have… That's a very good point, we do not have… We essentially have no human data concerning such endpoints. However the concern based on the animal data would be interstitial fibrosis, fibrotic changes, and other similar nonmalignant changes.

RALPH FROEHLICH: Can you see them on an x-ray?

DOUGLAS TROUT: Sure, you can see just going to your comparison you can see asbestosis the fibrosis associated with asbestosis on a chest x-ray. Now it's not as sensitive as a pleural plaque associated with asbestos exposure so there is no evidence to support the fact that we have similar markers such as pleural plaques for these substances. So the testing recommended is part of standard respiratory health clinical screening. So let me just backup; the question that you really are asking is what is the basis of these recommendations and we're looking for input on this but currently the basis for these recommendations really are standard of care for screening for known occupational respiratory hazards. That's essentially what it boils down to. We recognize that there are no human data concerning clinical endpoints for occupational exposure to these carbon nanotubes or nanofibers.

VINCENT CASTRANOVA: If I could just followup on that. NIOSH is currently sponsoring an active program looking for more sensitive biomarkers, if you will. I mentioned in my talk that we were seeing some cardiovascular changes; it is possible that those cardiovascular changes are occurring at lower pulmonary doses than the pulmonary changes in which case some biomarkers of cardiovascular effect might be predictive of an exposure and potential adverse effect. We are looking at measures of vessel dilation. There are human correlates to that looking at the brachial artery constriction and the release of that cuff and then measuring return of flow. There are some human studies that were diesel exhaust exposure return to flow is delayed and that's because the vessels aren't dilating as normally as they should. So there are, we are looking into more sensitive tests because we realize just as you say the current tests for fibrosis aren't very, very sensitive.

DOUGLAS TROUT: And there are others as well in addition to what Vince mentioned others have proposed tests to look at pulmonary inflammation using for example exhaled breath analyses of various substances. So it's a research area; an active research area.

CHARLES GERACI: We're going to do an agenda check. We're a little bit behind but not terribly on our agenda. I'm going to do a quick wrap up and summary and then I'm going to put

it to you the people who have made the sacrifice and the commitment to be here to see if we want to break for lunch and come back for our two requested presentations or continue on with our presentations and then we will be done for the day. I'll do my summary first and then we can get to that question.

I really don't want to do a lot of repeating of what you've heard today and so I wanted to summarize what is in the document in rather rapid fashion. And what it's going to sound like is a very basic industrial hygiene risk management, good product stewardship approach to managing these materials. And that's exactly what our intent is. We want to provide you with the science behind our recommended exposure limit but also a clear statement of what we see the current activity to be based on interacting with a lot of our partners, getting input from various constituents around the world and our own field experience and so the summary of our recommendations are pretty basic, and to identify and characterize where you may have the potential for releases and exposure to these materials and train your workers about what's known right now about what the discussion is with these materials. Train them about the sources where they may have some releases and exposures but also on the use of the control. So it's fairly good effective industrial hygiene practice that we are recommending in the document. Some of these may look very standard and that is, in fact, they are. We want to make use of all the history that's been developed over the years in good occupational health and safety practice but also present to you our current understanding of why it's important to put these practices in place.

On exposure assessment is probably a good point of discussion today but also a very active pursuit within the institute and many other places, on how do we do exposure assessment and how do I know that I need to institute controls, get good practices in place, and how will I know when I have achieved my objective of controlling exposure and managing the material. And we are focusing very heavily on a variety of research topics within the institute to address those questions and also to give the businesses the tools they need to identify where there's some attention that's needed for different tasks and processes. In research environments, in nano material manufacturing environments and a very extensive population where nano materials are being used to create these great nano intermediates, nano-enabled products that are going to be in commercial application if not already.

And the question of engineering controls in the document we give a pretty good description of what we know so far for certain basic applications such as the use of HEPA filtration in exhaust systems and I'll say exhaust meaning both the control but also the containment. We recognize that these materials are the key ingredient or the material of interest in your processes not contaminants or side reactants within processes. The nano materials are the material of interest and so there's a combination of both containment and control that is needed. And the selection of those exposure control devices. We're going to draw upon a long history of dealing with fine powder materials from a variety of industries to evaluate and assess the effectiveness of controls. And then on work practices fairly good but fairly basic work practices that are recommended in the document for personal and industrial hygiene, and also personal protective equipment and clothing. We've mentioned that there are health effects that are being evaluated for dermal exposure; we don't know if there will be significant health effects for dermal exposure but it also seems prudent to provide workers with dermal protection and other barrier materials when they do have instances where there is either evidence of or suspicion of exposure.

And then respiratory protection; one of the early pieces of research that NIOSH conducted in our nanotechnology program was to evaluate the effectiveness of various filter types for nanometer size particulate and within the document we repeat a number of research findings and recommendations we've made in our other documents which is the effectiveness of various types of NIOSH certified respirators for nanometer sized particulate.

And you just heard Dr. Trout discuss the medical screening and surveillance content that's in the document and the takeaway message with that is a good standard of care for occupational health protection when there are possible respiratory irritants or respiratory hazards involved is what we're following and what we're recommending. Worker training is also important. This is a real challenge for all of us right now because we're trying to understand what are the hazards and what are the potential risks based on the materials, the processes, the frequency, all of the factors we have to put in there. And now a real challenge is how we now communicate that to the workers, to the employers, to the general nano business community. And so we recognize that this is also a challenge for us in an area that needs attention as far as additional work on how to effectively communicate what we're all talking about here today.

And then limitations of the REL;limitations of the recommended exposure limit. We've already identified a number of research needs and it does play out and describe a rather active program of research within the institute but also within the occupational health and safety community worldwide. We recognize that mass may not be the best metric to use but right now it's the one that we're using as one of several metrics that are being used to evaluate worker exposure. We are investigating the potential of either a fiber or a particle or a structure approach to make it more similar to particle and fiber approaches that have been used for other materials in the past, and we also know that based on what limited data that we have not only from our studies but from field studies done by other organizations that it appears that these exposure limits or these control limits appear to be achievable in those processes where it looks like it has the greatest potential for not only exposure but application of the controls, and that would be those instances in either a production or a use of the material where you have the material in a physical state where it's dry or it's powdery or it's in a highly dispersed state. We need to do more investigation to verify that but based on what we have seen so far it appears that good containment and control, and I do want to emphasize those two aspects of it, containment and control techniques, have been successful and should continue to be successful. We'd like to be more focused and more specific on that. And what we don't know though is because of that is we don't know how achievable this recommended exposure limit will be in all workplaces. There will always be those situations where we need to do further study and further investigation to validate our statements. The other thing that I think would be important to see worldwide is how are people coming out when they do similar exercises as we've done here at NIOSH, a risk assessment, a recommended exposure limit which then gives us guidance for our risk management practices. This particular slide shows various occupational or recommended occupational or internal exposure limits or guidelines that have been published over the last couple of years, and you can see that we're all pretty much within a similar order of magnitude of each other. So we're recognizing that we cannot treat these materials as simple nuisance dusts. Now the question is, what is the appropriate approach and what are the appropriate guidelines that will come out of this? I'll go back to the statement I made a little earlier which is

this is our current understanding and a couple of years from now we'd like to say we know even better and we'll even be able to refine the picture. But you can see that based on published data from various parts of the world that we're all kind of definitely interested in this topic and we're coming in that range of certainly not mg/m^3 quantities but down in $\mu g/m^3$ quantities.

Research needs; I want to spell out here we've already talked quite a bit about continuing on our work with chronic inhalation studies to look at the different types of carbon nanotubes and carbon nanofibers and certainly mechanistic studies trying to understand mechanistically the basis for their biologic activity. And as Vince said are there any early markers of exposure that could be used? Within the human studies just assessing the potential for gathering more exposure data but even in creating a registry of workers, or employers, or companies, or organizations who can track nano material handling processes and tasks. This is a big undertaking, NIOSH's belief and we've stated this in several of our publications, is that this is probably a global issue because of the scope of the number of workers involved with nano materials and just being able to have enough workers involved to say we have some strength in our investigation of this. This is a very high level research proposal within the institute right now andit will be something that as we go forward with this and if we are successful in the concept you'll certainly hear more about that. And then our epidemiologic and surveillance research it's a real cornerstone of what we do at NIOSH and we're trying to look at ways to do as much progressive and proactive and prospective epidemiology as we can.

Workplace exposure needs; we've had a pretty good discussion on that today. Suffice to say we know that we need to do a lot of work in refining the metrics that are used. It'll help everything we're doing both worker exposure studies but also demonstrating effectiveness of controls. Being more specific with the nano material and, in this case the nanotubes and nanofibers and the development of improved approaches in methods. It's all a part of putting together a good effective risk management strategy and determining the effectiveness of engineering controls. And those controls I will repeat myself again, must include both containment technology as well as control technology. Having said that NIOSH is convinced and we have some active work underway to borrow from the pharmaceutical industries, the dry powder cosmetics industries, and dry powder laundry and detergent, any industry that's had experience of good practice with dry powder materials. That's a statement of our belief that the greatest area of concern is any of those processes manufacturing use of these materials where you do have the material in a dry powder state are the highest area of concern. That's another opportunity for the nano materials industry and that is, how can we engineer materials that don't have to be handled in a dry powder state or techniques or processes where it does not have to be in a dry powder state.

With that I want to thank all of you for your participation, and your attendance today. Please if you haven't done so take a look at the document itself and the opportunity to comment into the docket office. We have until February 18, 2011, to make comments into the docket. What will happen is today's proceedings; both the transcript and the presentations that are being made, will be a part of that docket as well as comments that come in from the public on that docket. Our next step will be to receive a report from our peer reviewers; their reports will also be on the docket and then NIOSH will take all of those comments and review them, address them, look at the impact. Impact meaning improvements that we will make on the document and then our next step would be to go for additional review internally within the institute. And then decisions will

have to be made on whether we need additional external review or whether we need to make more content within the document. That's a very quick sprint through what would probably be a very extensive process for us at NIOSH.

So now we can exercise our democratic process here today. We're at a point in our agenda where we can do one of two things. We can break for lunch and come back for our two presenters who have requested to make presentations today and we've asked them to hold to 15 minutes. Or we can do it now and just break a little bit later than we expected for lunch and we'll be done for the day. Show of hands on plan A which is we do it now and then we break for a late lunch. Okay it's almost unanimous that we'll just proceed on now with our presentations. How about a 5-minute restroom break because one of our panelists would like a break and then we'll come back for our two invited presentations.
CHARLES GERACI: Okay, welcome back. We're going to follow our democratic process and go with our vote which was, I think, unanimous to wrap up here with our two requested presentations. One of the questions asked me on the way out for the break is we're such a small group; if willing, would people like to introduce themselves? I don't see anybody shaking their heads in violent objection so I think it would be good. I think many of us know each other already in the room but it would probably be good for the rest of us who don't to get an idea of who is from where and I think help build some more of our community here. So if we can start over here.

BEBE RAUPE: I'm Bebe Raupe. I'm reporter with BNA. We publish occupational safety and health reports.

PHIL SAYRE Hi, I'm Phil Sayre I'm with the EPA Office of Pollution, Prevention and Toxicology.

RALPH FROEHLICH: Ralph Froehlich. I'm a Certified Industrial Hygienist with Helix Environmental in Dayton, Ohio.

JANET CARTER: Janet Carter; I'm a Health Scientist with OSHA.

ROGER ABER: Roger Aber with Ohio Bureau of Workers' Compensation.

LAURA HODSON: I'm Laura Hodson; I'm a Certified Industrial Hygienist and I'm working as Assistant Coordinator of NIOSH NTRC.

CHARLES GERACI: The NTRC is the acronym for our Nanotechnology Research Center.

JAMES DAVIS: Jim Davis. I'm with Owens Corning.

WILLIAM PRICE: Bill Price, University of Dayton Research Institute.

JIM HARTINGS: Jim Hartings from NanoSperse in Kettering.

MIKE WEISMAN: Mike Weisman from NanoSperse also.

BARBARA CUMMINGS: I'm Barbara Cummings with Bayer MaterialScience in Pittsburgh.

TIMOTHY FEELEY: I'm Tim Feeley with Bayer in Pittsburgh also.

JUERGEN PAULUHN: Juergen Pauluhn, toxicology, Bayer Schering Pharma AG.

JAY FEITSHANS: I'm Jay Feitshans. I'm from Philadelphia. I'm representing my mom, Ilise Feitshans on behalf of the International Safety Resources Association. I'll talk in a few minutes.

ANDREA OKUN: Andrea Okun. Deputy Director of the Education and Information Division at NIOSH.

LAURALYNN McKERNAN: I'm Lauralynn Taylor McKernan. I'm an Industrial Hygienist in the Education and Information Division at NIOSH.

JOHN PIACENTINO: I'm John Piacentino in the Office of the Director at NIOSH.

KEVIN HANLEY: Kevin Hanley. I'm a Certified Industrial Hygienist with the Industry-Wide Science Branch at NIOSH.

PAUL MIDDENDORF: I'm Paul Middendorf in the Office of the Director of NIOSH.

THOMAS BLOOM: I'm Tom Bloom. I'm with the Ohio Bureau of Workers' Compensation; I'm an Industrial Safety Consultant Specialist; former NIOSH employee.

MATTHEW DAHM: Matthew Dahm. I'm an Industrial Hygienist at NIOSH.

MARY SCHUBAUER-BERIGAN: I'm Mary Schubauer-Berigan. I'm an Epidemiologist with the Division of Surveillance, Hazard Evaluations and Field Studies, and I'm working with Matt on the carbon nanotube study hoping to do an epidemiology study.

TROY OCHS: My name is Troy Ochs. I'm an Industrial Hygienist at the local GE Aviation facility here in town.

CHARLES JENKINS: I'm Chuck Jenkins. I'm a Certified Industrial Hygienist. I have the GE Aviation Business Global Leadership role.

CHARLES GERACI: Okay, thank you all for that. We've already had our panel up here introduce themselves. You will all see the attendance sheet for this meeting on the docket but I thought it would be helpful for you to know who each other were.

We have our two requested commentators. We have two individuals who have requested to make a comment here as a part of the meeting so we'll invite Jay up to be our first speaker.

JAY FEITSHANS: I'm presenting this on behalf of Ilise Feitshans. Although this is scripted it's not memorized. There are copies available online in the docket, some paper copies over there, and some flyers for the International Safety Resources Association so please pick one up. The International Safety Resources Association is headquartered in Fullerton, California with offices in Houston, Texas. It's a nonprofit organization that develops and produces high quality affordable occupational safety and health training programs in various media, on the web and via life coaching throughout the world. Their Website is safetyforjobs.org, all one word. Professor Ilise Feitshans is a bilingual lawyer with a Masters of Science in Public Health from the Johns Hopkins University and currently a candidate for a doctoral program at the Geneva School of Diplomacy. She is the author of Designing an Effective OSHA Compliance Program and Bringing Health to Work. She can be contacted at ilise@prodigy.net. Also, an ISRA Board of Directors Member, Dr. Michael Riediker of the International University in Lausanne, Switzerland, also provided detailed comments regarding the NIOSH methodology. ISRA supports the proposed NIOSH REL and Worker Protection Governing Occupational Exposure to Carbon Nanotubes and Nanofibers. We offer three recommendations and sample text for legal justification of NIOSH actions in our written comments. Due to the time constraints, our oral comments focus only on the first recommendation concerning legal justification for NIOSH action. Our written comments also offer specific proposed language.

This testimony is prepared in response to the question presented by NIOSH:
"Whether the hazard identification, risk estimation, and discussion of health effects for carbon nanotubes and nanofibers are a reasonable reflection of the current understanding of the evidence in the scientific literature."

The sound freedom that resonates from civil and political rights rings hollow to a newborn who has low birth weight, because the baby's mother had no access to a clean and safe workplace, good nutrition or adequate prenatal care. And what good are political and civil rights to a different baby, who has lost a parent due to an occupational accident, or whose parents are debilitated by an occupational disease such as lung cancer, or to the baby who may suffer personal injury due to the effects of a parent's workplace exposure due to mutagens or unchecked but foreseeable harms caused by unregulated applications of nanotechnology, at home or in their parent's workplace?

The National Institute of Occupational Safety and Health (NIOSH) was created under the Occupational Safety and Health Act of 1970 to perform a very special job. The US Congress actually created three agencies: OSHA with power to promulgate and enforce standards regarding safety and health in millions of USA workplaces; the standards regarding Occupational Safety and Health Review Commission with the power to adjudicate and review OSHA enforcement decisions; and NIOSH, the research arm of OSHA, which was given a very special job because the USA Congress did not trust anyone else with this job.

The job of NIOSH is to protect workers by providing the latest, cutting-edge robust scientific research to support the law.

By its mission and its outstanding reputation for path-breaking science, NIOSH defines the meaning of the terms prevention, risk and recognized hazards among the scientific community

concerned with industrial hygiene, safety engineering, occupational medicine, risk management and public health.

NIOSH has been assigned the unique role by the US Congress that created it in the OSH Act to look ahead and forecast occupational safety and health problems to figure out how to prevent disasters before they happen.

Congress understood, when it wrote the OSH Act that gave birth to NIOSH, that there were things it could not understand. Congress trusted NIOSH, only NIOSH, to have an independent budget and independent thinking to engage in robust, path-breaking science to solve problems that impact workplace safety and health.

OSH Act requires that each employer shall furnish to each of his employees employment and a place of employment which are free from recognized hazards that are causing or are likely to cause death or serious physical harm to his employees.

Congress intended an expansive view of this term when determining the adequacy of safety and health programs. The term "recognized hazard" has long been construed to include hazards one can "taste, hear, see or smell," as well as hazards less easily recognized by conventional testing or monitoring. Even if an employer determines that the specified means of compliance is infeasible, it must affirmatively investigate alternative measures of preventing the hazard and implement prevention to the extent feasible.

In the four decades since the passage of the unmodified OSH Act, this statutory mandate to look out as the vanguard of protection from recognized hazards has placed NIOSH in the role of world class opinion leader in industrial hygiene, occupational medicine and occupational safety and health risk management and supporting research.

The NIOSH Current Intelligence Bulletin which is the subject of this public docket regarding carbon nanotubes and nano materials discussed at public hearings states: "Currently there are no studies reported in the literature of adverse health effects in workers producing or using carbon nanotubes (CNT) or carbon nanofibers (CNF). The concern about worker exposure to CNT or CNF arises from results of animal science."

The question whether such hazards have been described in the scientific literature and whether potential risks are sufficiently recognized or understood to trigger statutory protections is therefore vital first step towards planning the research-to-practice phase of nanotechnology applications and for preventing risks that wise people believe exist, even when those risks are not well understood.

Consistent with NIOSH concerns, according to the nanotechnology implementation strategy authored by the Swiss National Science Foundation, "Physically confining materials at the nanoscale alters the behavior of electrons within them, which in turn can change the way they conduct electricity and heat, and interact with electromagnetic radiation." Moreover, materials engineered at the nanoscale can enter into places that are inaccessible to larger materials, and can

therefore be used in new ways. These behaviors also have potential consequences on the abilities of synthetic nano materials to cause harm in novel ways.

Also consistent with NIOSH concerns, according to the Royal Commission on Environmental Pollutions of the UK, the governance of emerging technologies pose serious constraints on any regulator. First is the condition of ignorance about the possible environmental impacts in the absence of any kind of track record for the technology. Second is the condition of ubiquity – the fact that new technologies no longer develop in a context of local experimentation but emerge as globally pervasive systems which challenges both trial-and-error learning and attempts at national regulation.

NIOSH is, therefore, asking the right questions, consistent with its role by statutory mandate, in the opinion of ISRA, and when the agency, using its resources and expertise concludes that the state of the art of scientific research promoting safety and health in the workplace lags behind the implementation of new technologies in commerce, therefore, raising grave concern if not alarm, NIOSH is in good company.

Given the profound importance of the NIOSH mandate from the US Congress, combined with an international scientific consensus recommending precautionary approaches in a state of potential risks with great uncertainty, ISRA feels that NIOSH has been too weak in its statement of the statutory justification for its recommended practices and RELs regarding carbon nanotubes and nano materials, discussed here.

ISRA Recommendation One:

NIOSH has been timid in its assertion of the justification for the use of its powers regarding nanotechnology. Instead, NIOSH must be bold in its assertion of this statutory mission once the agency has discovered that there remain logical and clear risks to human health from the implementation of a new generation of technology, and concluded that although potentially very important through its diligent research and ongoing discourse with stakeholders, private sector partners and peer organizations in Europe, the United Kingdom, and international governance around the world, additionally, ISRA requests that NIOSH use the following language to fill this important regulatory void regarding the justification for the final NIOSH RELs:

"Justification for use of NIOSH authority to craft carbon nanotube RELs and to propose methods of risk management to protect workers facing occupational exposure to carbon nanotubes and nanofibers."

It is NIOSH and no one else that Congress entrusted with the OSH Act, to take the lead in crafting methods for risk management and protection when the risks are on the horizon but the precise parameters of risks remain unquantified by science and, therefore, unknown at the present time.

NIOSH is not a regulator or enforcer; it is the voice of reason safeguarding the lives and health of everyone who works from the boardroom to the mailroom, from the CEO to the domestic worker using harsh chemicals to clean his house, from the diplomat to the foreign migrant,

possibly undocumented who needs accurate chemical information and urgent care in the event of accidental spills that jeopardize their health; from the Director of Coca Cola to the factory worker in the bottling plant who comes into contact with food additives and new plastics that use nanotechnology, from the Nobel Laureate scientist to the lab technician at the bench using carbon nanotubes to research and develop new nanomedicines that will benefit all mankind. In essence, this research by NIOSH is essential to protecting the public health as much as it is about looking at the narrow scope of preventative measures for risk management among workers. The true value of NIOSH's mission, therefore, concerns preserving the work, health and survival of all civilization.

End of Justification to quote.

NIOSH has failed to so state in its Current Intelligence Bulletin, and has left the Preface blank in the draft that was provided to ISRA. We are prepared to work with NIOSH to draft language that can best fill that void. ISRA welcomes NIOSH to quote all or part of our written testimony regarding the statutory justification in its final document.

Thank you for allowing the public comment and thank you for your time.

CHARLES GERACI: Thank you, Jay. The printed document that was provided for this will be available on the docket as well as transcript of the oral presentation. Our second commentator is Dr. Juergen Pauluhn; I'm always saying your name wrong. I apologize. He has prepared for us to assist him with his comments a quick PowerPoint show which if I'm skilled enough I'll bring up on my computer here.

JUERGEN PAULUHN: Thank you very much for the opportunity to summarize the highlights of our studies to better understand the mode of action of carbon nanotubes. I think the most important step in risk assessment is to understand what is the key mode of action with what is making the effect? It appears from all the studies we have performed the two modes of actions have to be considered. The first mode of action is shown in the left-hand side panel that means the deposition of such structures into the lower respiratory tract and based on the surface activity or surface area the interaction with the environment of the lower respiratory tract, and as eluded to by Dr. Castranova already the interaction with surfactants may cause transient acute dysfunctions in the alveolar area. What is of more concern for chronic occupational exposure is that these materials can accumulate in the lung and some kind of retention occurs? The specificity of these types of particles is at that stage these particles lose their particle properties. Namely, they are mopped up by alveolar macrophages and the macrophages are activated to a certain extent or increase in number. As shown in the right-hand side panel that means we have no particle-like toxicity anymore. The toxicity is mediated by the key mediator cell, the alveolar macrophage and associated inflammatory cells. So that might be changing the metric to such an extent that we have to think what is making the activation of the macrophage? And as already articulated by Dr. Morrow 20 years ago the volume displacement within the macrophage caused by the particles may be the principle and key effect, and we tried to analyze our carbon nanotubes and we exemplified everything with BI-tubes and are they really different to other particulates? And on the left-hand side slide we compared a number of different insoluble or

poorly soluble dusts in relation to the accumulative dose during the course of 4- and 13-week inhalation studies and the changes in the elimination halftime.

As you can see on the left-hand side panel very interestingly there is some systematic change in all these particles. That means they have some common denominators and whether you have titanium dioxide or something else they appear to follow the same rules. Most interestingly if you look at the accumulative mass which is on the left-hand side and the accumulative volume you see that the volume metrics appears to be the best metric. If you compare kinetic changes to inflammatory changes that means elimination halftime on the left-hand side and inflammatory cells on the right-hand side you see kinetic changes precede the inflammatory changes. That means the most sensitive endpoint indicating any change is the change in kinetics and not necessarily in inflammation. In developing a universal formula to predict the outcome of inhalation studies based on the common generic kinetics of such particles mediated by alveolar macrophages you can predict the NOELs, the no-observed effect levels, in these types of studies just based on kinetics.

Assuming everything acts as a granular type poorly soluble particle. As you can see from carbon nanotubes in the left-hand side to iron oxide to the right-hand side predicted NOELs and experimentally or empirically the right NOELs are essentially identical. You see the real difference in metrics is the density. It appears as iron oxide has the lowest toxicity, the highest NOEL because it has a density of 5 g/cm^2 whereas multiple carbon nanotubes are the most toxic ones with the lowest NOELs because they have the lowest density which fits exactly with Morrow's hypothesis, the lower the density the higher the volume you generate in a fixed system the alveolar macrophage as a result of the high volume. Based on this you can formulate or you can make formula simulating the kinetics of these types of particles. We have designed our inhalation studies that way that we have performed kinetic designer studies to verify anticipated responses. In the left-hand side, it's barely visible; you have at the lower part a dotted line. This is the theoretical steady state in the lung following exposure at a level where you have no significant influx of additional alveolar macrophages, phagocytizing these types of particles. So by theory the lowest level should just touch the steady state of particle incorporation. The next higher step was designed to elicit some kind of inflammatory effect alveolitis; however, it should be fully revisable within a postexposure period of 6 months. If you go higher you overload the system and this is always accommodated with increase of a lowering of clearance. That means the elimination time increases significantly and as you see in the highest level you are in the range of 800. That means it almost exceeds the entire lifetime of the rat. That means based on kinetic behavior these particles may be cleared or not cleared in the lifetime of a rat.

This has been shown on the right-hand side we have verified these kinetic endpoints and when we look at the findings suggested by increases in collagen you see after cessation of exposure everything follows a kinetic paradigm. If you are in the non-overload range everything is NOEL or rapidly reversible. If you are above the kinetic threshold of clearance it stays in the lung; however, relatively stable or with the tendency of regression. However, not with progression. And this is true for collagen and inflammatory collagen. Collagen is usually considered to be a marker of fibrosis; however, there are two types of collagens: inflammatory collagen and the fibrotic collagen. The inflammatory collagen is soluble and it's called collagen type 4 to type 6. It's always associated with inflammation and it is transudative from the vascular system into the

lung. The fibrotic collagen in turn is produced and synthesized locally by myofibroblasts. So it's extremely important to distinguish whether the collagen you see in a histopathology slide our any type of measurement whether it's a transudative collagen or a locally synthesized collagen. And here you see a picture; this is phosgene. This is a gas which reacts momentarily, has no halftime in the lung so it's immediately destroyed. And you see collagen follows exactly the kinetic of protein that means it comes from the vascular system and is treated that way as proteins. If you look at bleomycin this is for instance an agent which is extremely fibrogenic you see the black bar which its collagen is zero. That means no change to the control at all. That means the commonly measured collagen in the lung is not indicative of fibrosis at all. The left-hand side panel where the carbon nanotubes we have examined unfortunately these types of subtle septal thickenings as a result of inflammation have the same pathologic terminology septal thickening as you see on the right-hand side where you see focal interstitial thickening as a result of bleomycin-induced fibrosis and; however, you see a lot of inflammation in cells in the septa. That means you have local production of collagen which is clearly indicative of fibrosis. However, the subtle types of septal thickenings we believe are not indicative of fibrosis.

The BI-tubes we have examined were as shown before reversible during the course of exposure and as a result of decreased kinetics we have seen sustained inflammation only at levels of frank overload conditions. However, even at these exposure levels changes were regressive and not progressive during a course of a 6-month postexposure period.

As a usual procedure, when the lung has some kind of inflammation you have increased clearance of particulates from the alveolar space to the lymph nodes. This is normal physiology. This has been measured by determining the catalyst associated with the carbon nanotubes and you see at those two levels which have an increased persistence in the lung as a result of delayed clearance caused also physiologically an increased transition of particles from the alveolar space to the lymph node. On the right-hand side you'll see just simple lymph node weights and this is just indicative of some kind of inflammatory response of the lymph node as a result of the particle load. That means we could assume based on these findings if you overload the lung that you get sustained inflammation you will have some kind of transitions of particles from the alveolar space to the lymph node. So the question is what is happening pathologically? And this is shown in this slide here the left-hand side is pathological findings at these 4 exposure groups and you have a grading system from 1 to 5 which is indicated by different colors. The lowest grading that means minimal to slight just barely visible is black. Here in these studies green means free moderate change.

You see real changes did not occur from end of study after 13 weeks and after a 6-month post exposure period. That means despite the fact that particles are moving from the alveoli to the lymph node through the septal structures of the lung the pathology at high level overload conditions with sustained inflammation did not change at all. That means no evidence of progressive changes at overload conditions and inflammation.

So overall we think these types of changes start or are initiated in the alveolar space and not in the septal structures of the lung. Any risk assessment therefore should be started on the inflammatory situation of the alveolus and not secondary responses thereof. This has been done in this analysis and in the middle panel you see we have done a benchmark analysis and based on

this benchmark analysis of inflammatory cells and bronchoalveolar lavage we would arrive at a benchmark lower confidence interval 95% of 0.16 mg/m^3 of NOEL benchmark.

The empirical NOEL was definitely 0.1 mg/m^3. You see this type of dose response reflects the biological behavior of inflammation better than a more dichotomous response of a categorical change. We have utilized this piece of information to arrive at a risk assessment to make an extrapolation from rats to humans taking into consideration these modes of actions. Two aspects were considered: the ventilation and disposition assuming that humans would inhale the same types of particles as we have generated during the course of our 90-day inhalation study. This correction corrects the respiratory minute volume of rats to humans and the differences in disposition patterns. Based on this we arrived at a factor of 0.9 for lung burden ventilation adjusted. The clearance that means the way particles are handled within the lung is slightly different between rats and humans and this is addressed by addressing the so-called volume of distribution. This is the total volume of all alveolar macrophages at steady state and the kinetics of these macrophages as long as they are not laden with particles that the kinetics would change.

If you address these two points and key issues of kinetics then most astonishingly the outcome is the same number. That means the rat appears to be as sensitive as the human. Based on this an occupational exposure level could be calculated NOEL A means the animal NOEL obtained in the 13-week inhalation study. Addressing these ventilation and clearance dependant species differences as well as an additional factor because we are addressing and focusing on chronic lifetime exposure and not 90 days exposure. This is accommodated by an additional factor. If we would be utilizing this relationship based on the benchmark NOEL we would be arriving at an OEL of 100 mcg multiple carbon nanotubes respirable by cubic meter of air. We have also developed a kinetic generic approach to develop OELs for purely soluble particles. That generic approach is defined to be a volumetric approach and that would be 0.5 microliters particles per cubic meter of air. If we would be using this philosophy for this generic approach then the generic NOEL would be 0.15 mg/m^3 of air showing that the empirical approach fits the generic approach.

So overall I would like to conclude it appears that based on this comparison the multiple carbon nanotubes investigated in this study do not behave differently than all other poorly soluble particles. The only really real difference of these types of structures is the extremely low density. That means in comparison to iron oxide they consume 50 times more volume in the macrophage as compared to the larger density particles. Lung to symmetry must be accompanied by respiratory tract histopathology involved; however, histopathology should be refined by monoclonal antibodies for instance to better distinguish the various collagen species for instance type 1, type 3 collagen to distinguish between locally produced collagen and transudative inflammatory collagen. The metric of those appears to be the key issue; however, for the retained long-term dose it appears that the volume is more important than the mass or any other metric. So overall I would like to emphasize the best inhalation studies appear to be those who have a clearly articulated starting hypothesis and this appears to be best when starting with a volume-based hypothesis trying to prevent overload like inflammatory responses in the lung to derive a kinetic-based conservative OEL for these types of agents. Thank you very much for your attention.

CHARLES GERACI: That concludes our meeting for the day. A reminder that proceedings from this meeting the transcript and the presentation materials will be posted on the NIOSH docket. We'll also be proceeding on with the rest of the document review agenda that I laid out for you earlier in my presentation. So once again thank you all for your participation today. NIOSH certainly values your partnership in making this the best product possible. Thank you.