Interview with Dr. Günter Oberdörster, D.V.M., Ph.D.
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PART ONE: Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-walled carbon nanotube

ICON: What is the most important thing that readers should take away from this work?

Günter Oberdörster: The most important finding coming out of this study is that multi-walled carbon nanotubes (MWCNT) administered into the peritoneal cavity of mice induced a tumor response similar to what has been seen in many earlier studies with asbestos fibers. It is well known that asbestos-exposed workers developed cancer of the lung and pleural cavity, the latter, malignant mesothelioma, is almost exclusively caused by exposure to asbestos fibers. In fact in the late 1970’s and early 1980’s an area in Anatolia, Turkey (which I visited as a student) became infamous for very high incidences of endemic malignant mesothelioma in villagers from Karain and other villages due to the presence of erionite, tremolite and actinolite asbestos in mined powdery material, which they used as plasters and white-wash on their houses and even as substitutes for baby powder. Genetic disposition running in families was later discovered as a susceptibility factor.

In this study, Takagi et al. used the technique of intraperitoneal (i.p.) injection of MWCNTs into mice because the peritoneal cavity is lined with the same cell type as the pleural cavity.

ICON: Were the techniques used in the experiment standard, or was there something unique or special about them?

GO: Injection into the peritoneal cavity, as well as into the pleural cavity, has been used for years by many researchers to test whether unknown fibrous materials can act like asbestos. Although it is an artificial unphysiological route of administration, it has become an acknowledged test in Europe for determining whether or not certain fibers should be classified as carcinogens. However, there are serious caveats that apply to this methodology. European regulations dictate that this test should be used in rats, which are bigger than mice. Moreover, fibrous materials should be well-dispersed when injected. The basis for this regulation is that it is known from in vivo studies that, when inhaled, asbestos fibers migrate and arrive at the pleural space and can induce this type of lethal cancer.
Normally, effective defense mechanisms will eliminate inhaled harmful materials depositing in the respiratory tract. These include mobile macrophages in airspaces of the lung, which remove cell debris and foreign materials from the lungs in a process called phagocytosis. However, if the fibers are too long, more than about 15-20 micrometers, these cells cannot completely engulf them, a process called frustrated phagocytosis. These are the fibers that appear to be the most carcinogenic. They can get into lung tissue and migrate to reach the pleural cavity and cause both lung and mesothelial tumors. Shorter fibers translocate as well, in particular if there is a high fiber load in the lung. The big question is how many fibers does it take to get to pleural sites and induce tumors. For example, at best only a minimal fraction of inhaled MWCNTs depositing in the deep lung is likely to translocate to pleural sites, heavily agglomerated structures, i.e., larger clumps, will not. At inhaled concentrations of 50 µg/m³ – a concentration found in a simulated carbon nanotube workplace scenario by Dr. Maynard and colleagues – the i.p. doses of 3 mg/mouse used in the Takagi et al. study would never have been achieved in the pleural cavity, even under continuous exposure conditions. Thus, we have to be very careful in interpreting results of i.p. injection studies. This is a particularly important question for MWCNT’s. We have no data. We need to know more about their biokinetics following inhalation, specifically if and to what degree they will migrate across cell barriers and reach the pleural cavity. We should keep this in mind.

Thus, an important caveat is the extremely high dose administered. Three milligrams i.p. is a huge amount for a mouse as well as a rat. Regarding the injected numbers of 10⁹ fibers, given the uncertainty of the method that was used to count fibers, it is very difficult in my view to even estimate the number of fibers per milligram MWCNT. The material that was injected was highly agglomerated, unlike what the European regulations dictate, i.e., that it should be highly dispersed material, with the highest i.p. dose in a rat of 10⁹ fibers. In this case, it appears there were only a few non-agglomerated individual fibers; most were big clumps of materials, and it's hard to discern how many fibers were in those clumps. So what we are dealing with is a mix of very large agglomerates and a few individual fibers. That raises another issue, namely – as is well-known from earlier studies where investigators injected simultaneously asbestos fibers and non-fibrous particles (even benign ones) – that even benign spherical particles together with a carcinogenic fiber cause an increase in tumor induction. They act synergistically. Moreover, even a biosoluble fiber – which is not carcinogenic by inhalation – when injected i.p. into rats at an extremely high dose – comparable to the present mouse study – induced mesothelial tumors in an earlier study.

The peritoneal cavity where the MWCNTs were injected has lymphatic clearance pathways through which particles are eliminated from this site. These are important for shorter fibers because phagocytes can pick up short fibers and clear them via these lymphatic channels. We know from earlier studies, particularly from Dr. Kane at Brown University, that for long fibers and with larger amounts of short fibers, the lymphatic channels are clogged up and no clearance is possible, resulting in persistent retention and inflammation.

**ICON:** Does using genetically-modified mice affect the outcome of this study?

**GO:** The p53 heterozygous mouse model is by many regarded as too sensitive, although I think it is in general a very useful model. It is, indeed, very sensitive and can give you an answer in a much shorter period of time than a regular mouse model would do. In this context, and more controversial, is the i.p. injection for fiber testing. At the time it was used in the 1970’s, 1980’s and on, it raised great scientific
controversies as to its appropriateness to identify a carcinogenic fiber. When European regulations were introduced to accept this test in 1998, it was not accepted in the United States as a valid model. Anyway, it is out there and useful as a “proof of principle” methodology in my view.

ICON: Did these findings surprise you or were they expected given the current knowledge in the field?

GO: They didn’t surprise me too much given the high dose and a certain similarity of individual MWCNT fibers with asbestos, and given that elemental carbon is not soluble and very biopersistent like asbestos. These are decisive factors for fiber toxicity and carcinogenicity and are part of the fiber carcinogenicity paradigm of the 3D’s: Dose, Dimension, and Durability. It’s a critical paradigm – like a dogma for identifying a tumorigenic fiber.

ICON: Is there anything else you would like to add?

GO: This is a “Proof of Principle” study: MWNT under these conditions can induce effects. The question is: Will it happen in vivo following inhalation exposure? We need to know that before we come to a final judgment. The mesothelial response to MWCNTs that was observed here appears to be mesothelioma. Clearly, mesothelioma were induced in this study with crocidolite, an amphibole asbestos used as a positive control. With respect to the MWCNTs, doubts have been raised as to whether genuine mesothelioma were induced or some other kind of tumor. I am not a pathologist and cannot judge this from the published histological slides. Because this is an important question, I suggest that a panel of independent pathologists be convened who are provided with slides and tissue samples collected from the mice of that study. The charge to the pathologists would be to issue a statement following thorough examination as to whether or not these lesions are mesothelioma or another type of response or tumorous tissue.

PART TWO: Carbon nanotubes introduced into the abdominal cavity display asbestos-like pathogenic behaviour in a pilot study

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ICON: What do you think is most important about this study?

GO: This study in mice was set up to test whether the fiber length paradigm established for long asbestos fibers also applies to long MWCNTs. The result shows, in extension to the Takagi et al. study, that if i.p. injected, long MWCNTs induced inflammatory responses, including granuloma formation; these responses were similar to those of long fiber amosite, another amphibole asbestos used as positive control. Different manufacturers provided the two long and two short carbon nanotube samples, and one of the long fiber MWCNT samples seemed to be the same as the one used in the Takagi et al. study. Here again, we have a “Proof of Principle” study that MWCNTs – provided they are long – can induce responses similar to asbestos, in this case inflammation, in a sensitive target cell type in vivo following the same mode of i.p. administration. No mesothelioma were induced; the study didn’t last long enough and was not designed for this.
**ICON: What do you think of the techniques used in this experiment?**

**GO:** The dose administered to the mice - 50 micrograms/mouse - was considerably lower than in the Japanese study, where the dose was 3 mg/mouse, although also in this experiment the dose has still to be considered as very high. However, the nanotubes were well dispersed without the large clumps shown in the Takagi et al. study. It would have been useful to provide information about the dose expressed as number of fibers that were injected, not just the mass. Fiber regulations are based on fiber numbers. Although the use of different suppliers of nanotubes might be considered as making it more difficult to interpret the results because of differences in material properties, the consistency of inflammatory response within the long fiber samples and the lack thereof within the short fiber samples supports the authors’ conclusion that the fiber length paradigm is applicable for MWCNT toxicity. A limitation of the study is the very low number of mice, yet the statistically significant differences between long and short MWCNT responses diminish this limitation.

**ICON: What do you think of the findings of this experiment?**

**GO:** This pilot study by Poland et al. represents an important contribution to the rapidly expanding field of nanotoxicology. The authors recognize that they need to obtain more information about the biokinetic behavior of the MWCNTs. This experiment was done by a group that has vast experiences related to asbestos fiber toxicology and fiber-induced lung tumors and mesotheliomas.

However, such a short-term study does not allow us to make a definitive prediction about the long-term outcome. It needs to be followed up with well designed additional studies, as pointed out by the authors.

**PART THREE: COMPARISON OF THE TWO PAPERS**

**ICON: Taken together, what do these papers tell us?**

**GO:** For one thing, until we know better, we need to be very careful in handling these materials. We should avoid exposure by inhalation and contamination of skin and clothing, as it happened in the early days of heavy asbestos exposures when workers used to bring fibers home on their clothes. Clearly, this and inhalation exposure can be avoided by wearing personal protective equipment and handling these materials in appropriately ventilated hoods. These are, of course, precautionary measures based on the limited data we have. Once we know better, one way or the other, final regulatory measures need to be adopted.

Scientifically, more research is needed to find out more about potential toxic and carcinogenic effects and underlying mechanisms. Scientists need to work together to resolve that as soon as possible. Nanotubes have great potential, but we need to assess their risks appropriately.

In addition, I think both studies should serve as a wake-up call for governmental agencies, to provide more resources, monetary and otherwise, for research to speed up a science-based risk assessment process; and for industry, to practice and develop good product stewardship programs and to be doubly
careful and cautious when providing information about nanomaterials, for example, in Material Safety Data Sheets.

**ICON:** How should people interpret these findings within the larger context of nanotechnology environment, health and safety research?

**GO:** Here we are dealing with nanoparticles of a fibrous shape with associated specific concerns. As for other nanoparticles, the issues will be different. I think that for most of them a risk does not exist or is not as high as many think it is, given that there will be no or only very low exposures. The MWCNT doses in these two studies were high or extremely high and clearly have identified a significant hazard. In the real in vivo world, doses reaching the mesothelial target cells are likely to be orders of magnitude lower, perhaps even zero. In order to find out for sure, however, we need to do more research; we need more research funding.

In this context I would like to add a general comment regarding the terms hazard and risk. Unfortunately, these terms are often understood by lay people as meaning the same, and published results of studies demonstrating significant toxicity of a nanomaterial – particularly from unrealistic high dose in vitro studies – are naively interpreted as showing a high risk of adverse health effects. Such misunderstanding of risk may be a real “danger” for the future of nanotechnology, and researchers should make every effort and emphasize the importance and need to consider exposure as a key element of the risk assessment process.

**ICON:** Please comment on the likely impact of these works on other researchers or policymakers.

**GO:** Based on the results of these two studies, researchers will probably be stimulated to try even more determinedly to validate the observed tumorigenic and inflammatory MWCNT effects with realistic in vivo experiments and to resolve questions of mechanisms operating under in vivo conditions. Policymakers may consider precautionary measures regarding the handling and usage of nanotubes, but at the same time also promote necessary scientific research. Some others may even call for a moratorium on their manufacture, although this would be extreme.

**Bio**

Dr. Oberdörster earned his D.V.M. and Ph.D. (Pharmacology) from the University of Giessen in Germany and is Professor in the Department of Environmental Medicine at the University of Rochester, Director of the University of Rochester Ultrafine Particle Center, PI of a Multidisciplinary Research Initiative in Nanotoxicology and Head of the Pulmonary Core of the NIEHS Center Grant.

His research includes the effects and underlying mechanisms of lung injury induced by inhaled non-fibrous and fibrous particles, including extrapolation modeling and risk assessment. His studies with ultrafine particles influenced the field of inhalation toxicology, raising awareness of the unique biokinetics and toxicological potential of nano-sized particles.