Interview with Drs. Jun Kanno and Akihiko Hirose

ICON: What is the most important message that readers should take away from your publication?

Jun Kanno & Akihiko Hirose: That a certain form of multi-walled carbon nanotubes (MWNT) causes mesothelioma when administered to genetically modified mice. Mesothelioma is a cancer associated with long-term asbestos exposure. Our results indicate that these particular MWNT, which share some physical features with asbestos, show a similar biological response in an animal model for foreign body carcinogenesis. Another important message of this paper is to propose a new paradigm of risk assessment/management. When new materials appear, there is always a time period where hazard identification data comes out before any exposure assessment is done. Classically, no risk assessment is done without exposure assessment. Two questions arise: 1) is the generation of hazard identification data without exposure data intended to scare people into believing that a material is dangerous? and 2) Should such hazard data be suppressed until there has been exposure to humans and exposure assessment is possible? The answers are ‘no’ and ‘no’. This type of data can inform the manufacturers so that they can produce a safer product and, in the long run, create a win-win situation for both the consumers and the producers. We have to build up a system where new high-tech materials are swiftly checked for chronic toxicity by real studies and/or by receiving opinions from highly trained/experienced toxicologists, with the results swiftly feeding back to the developers and manufacturers so that they can reflect such information to new product planning.

ICON: What was the source of your multi-walled carbon nanotubes and how did you apply them to the mice?

JK/AH: We received the MWNT from Mitsui Corporation as a bulk carbon powder; we needed to create a suspension of these powders in an appropriate biological solution in order to perform the experiments. To do that we placed them in a buffer and sonicated; what resulted was a black suspension. We injected these suspensions into the abdominal cavity of mice in a single dose. This is called an intraperitoneal (IP) abdominal injection.

ICON: What dose of MWNT did you use and how did you decide on that dose?

JK/AH: We used a dose of 3 mg/mouse, which works out to be about 100 mg per kg of animal. This is a high level, near the maximum amount recommended by the draft guideline for man-made mineral fibers, which is per animal 1,000,000,000 fibers longer than 5 micrometer. We wanted to make sure that we optimized the experiment to create the most favorable conditions for observing mesothelioma if indeed that was a biological endpoint.

ICON: Why did you use genetically modified mice instead of normal mice?

JK/AH: Mesothelioma is a difficult disease to study scientifically. It can take decades to develop in humans, and near full life span in normal rodents, which is two years. The p53 knock out mice (heterozygous) have a gene (one allele of p53 gene) removed that makes them develop cancer more quickly when they encounter a cancer-causing agent, especially of genotoxic type; this means that we can get an answer about the effects of a new material within about six months. Using normal rodents we might have to wait years. We have been breeding p53 knock out mice for various toxicological studies including usage in short-term carcinogenesis studies. Recently, p53 knock out mice have been shown to be sensitive to oxidative stress-mediated carcinogenesis, and Kane’s group has reported that asbestos, for which oxidative stress is believed to be the carcinogenic mechanism, effectively and
quickly induces mesotheliomas in these mice when intraperitoneally injected. Since we postulated from its shape, size and durability that MWNT would be able to induce mesothelioma by the same mechanism as asbestos, we adopted this animal model in order to gain results fast. (Note: p53 heterozygous knock out mice spontaneously develop certain types of tumors, but mesothelioma has never been found nor, to our knowledge, reported in this animal.)

**ICON: How did you know that the mice developed this disease?**

JK/AH: The first sign was that the mice started to emaciate (lose weight) because of the development of multiple tumors in the abdominal cavity. Autopsy of the animals reveal multiple peritoneal neoplastic (cancerous) lesions in most of these mice. We diagnosed these lesions macroscopically and microscopically. Both features were compatible with mesotheliomas.

**ICON: What controls did you run and what was their purpose?**

JK/AH: To make sure our experimental conditions were able to monitor mesothelioma induction, we used crocidolite (blue asbestos) as a positive control. As mentioned above, the use of intraperitoneal abdominal injection of crocidolite into p53 mice has been established via prior peer-reviewed publications as a good system to study mesothelioma. This positive control did work as reported. As a negative control we ran granules of fullerene (C60), a spherical non-rod-shaped nanoscopic form of carbon. Our hypothesis was that this material would not induce mesothelioma since it was not fibrous. Histologically, the foreign body reaction itself was also different. The study indicated that injection of just any shape of non-degradable foreign particulate matter is not sufficient to induce mesotheliomas in this model. To reinforce this finding, we would plan to add a few studies on non-fibrous materials with different irritation properties.

**ICON: What experiments are you running now to expand upon this result?**

JK/AH: We are repeating our intraperitoneal injection study at dosages that are lower than this study by 10 times (high-dose group), 100 times (medium-dose group), and 1000 times (low-dose group). After more than 300 days, peritoneal tumors were induced in nearly 100% of the high-dose group, about 60% in medium-dose group and about 20% in low-dose group. We will terminate the study at one year. We have run a microarray study of peritoneum exposed to crocidolite to investigate the detailed mechanism during the early stage of carcinogenesis (See also here and here.) We are also studying rodents that are not genetically engineered so as to determine the generality of our observation; this takes longer since they are less susceptible. We are also planning to run a chronic intraperitoneal exposure study of fullerene using wild type mice.

**Bios**

Dr. Jun Kanno (left) received M.D. from School of Medicine, Tokyo Medical and Dental University (TMDU) and Ph.D. in Pathology from TMDU Graduate School for Medicine. From 1986-1997 he served as lecturer at the Department of Pathology, TMDU. From 1991-1993 he was Visiting Scientist at the US National Institute of Environmental Health Sciences (NIEHS) in North Carolina. In 1997, he was engaged as a section chief of the Division of Cellular and Molecular Toxicology, National Institute of Health Sciences (NIHS), Japan, where he has served as division head since 2002. Dr. Kanno specializes in general pathology, toxicology and experimental pathology. His research includes molecular toxicology on Endocrine Disrupting chemicals (receptor...
mediated toxicity), carcinogenesis, nanomaterial toxicology and toxicogenomics. In 2002, he initiated Toxicogenomics Project of NIH & Japanese pharmaceuticals and has led the NIH Percellome Toxicogenomics Project since 2003.

Dr. Akihiko Hirose (right) is a Head (since 2008) of the Division of Risk Assessment at National Institute of Health Sciences, Japan, where he has worked since 1990. He graduated from Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, and received Ph.D. in Physiology from Tohoku University Graduated School of Medicine (1990). He is a diplomate of the Japanese Society of Toxicology (2002). He specializes in hazard/risk assessment of industrial chemicals, contaminants in drinking water and food packaging. Current emerging works are research on the development of (Q)SAR system for industrial chemicals and the methodology for evaluating health effects of manufactured nanomaterials.