Title of Research:

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Establishment of mechanism based assay protocol for hasard and carcinogenic risk of carbon based nanomaterials

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Summary of Research:

Purpose: To establish a method of evaluating the toxicity and carcinogenicity of respirable nanomaterials, especially carbon nanotubes, that is less expensive and easier to perform than conventional high-cost inhalation exposure. We developed transtracheal intrapulmonary spraying (TIPS) to administer test materials to the lungs of test animals. We tested several types of multi-walled carbon nanotubes (MWCNT): MWCNT-7 (Hodogaya, 40 layers), needle-like aggregates of MWCNTs; MWCNT-N (Nikkiso, about 30 layers), another type of MWCNT that forms needle-like aggregates; MWCNT-A (Company C, 150 layers), a third type of MWCNT that forms needle-like aggregates; MWCNT-B (Company C, 15 layers), which because of its much smaller number of layers is flexible and produces cotton-like aggregates. We have shown that these four types of MWCNTs are carcinogenic to the lung. We are currently testing two-layered carbon nanotubes (DWCNT) to determine if this type of carbon nanotube is also harmful to the lung and the pleural tissues as observed with the MWCNTs composed of several layers.
Methods: We conducted a preliminary dose setting trial using DWCNT from Sigma-Aldrich and from Company A. We determined that 0.5 mg per rat was an appropriate dose. We administered DWCNT (1-3 nm diameter) (Company A) to 10-week old male rats. The test materials were suspended in a solvent (saline + 0.5% dispersant PF68 PF copolymer) to give final concentrations of 0.125 mg/ml and 0.0625 mg/ml, and 0.5 ml of the suspended material was administered to the rats a total of 8 times, for final doses of 0.25 and 0.50 mg per rat. The control groups were (1) untreated and (2) administered vehicle alone. MWCNT-7, whose lung carcinogenicity was demonstrated using both a whole body exposure inhalation test and our TIPS administration procedure was used as the carcinogenic positive control. Rats were killed at experimental week 3 (1 week after the end of administration) and week 8 (6 week after the end of administration). Histopathological analysis of the lung and pleura and biochemical analysis of the lung tissue, tracheopulmonary lavage (BALF), and thoracic cavity lavage (PLF) was performed for each rat. Inflammatory markers in supernatants and lavage cell pellets were also evaluated.
Results: Real time PCR and ELISA analyses of the lung tissue, pathological and histopathological observation (distribution of sample, shape and degree of inflammation), and biochemical analysis (inflammatory cytokines, DNA damage, oxidative stress markers) indicated that damage of the

Research on the safety of new chemicals such as “Nano materials” etc.

lung tissue and visceral pleural mesothelium was absent or weak in the DWCNT treated rats. No grossly recognized neoplastic lesions were observed in rats killed at one year (52 weeks) after the start of administration. Tissue samples from these rats are currently being prepared for histopathological analysis.

New Studies: Using TIPS administration, we have started a study of the pleural toxicity of two new types of materials. Both materials, like single walled carbon nanotubes, are constructed from a single carbon sheet. The 8-week interim sacrifice is scheduled for this April 15-16.

Timeline:

March 1, 2018 – February 28, 2019

Topics:

The 6th Research Achievement Presentation Meeting
Date:September 9, 2018
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Publications:

Elgazzar AM., Abdelgied M., Alexander D., Alexander W., Numano T., Iigo M., Naiki A., Takahashi S., Takase H., Hirose A., Kanno J., Elokle OM., Nasem AM., Tsuda H. Comparative pulmonary toxicity of a DWCNT and MWCNT-7 in rats, Arch. Toxicol., Oct. 10, https ://doi.org/10.1007/s0020 4-018-2336-3, 2018

Abdelgied M., Elgazzar AM., Alexander D., Alexander W., Numano T., Iigo M., Naiki-Ito A.,Takase H., Abdou KB., Hirose A., Taquahashi Y., Kanno J., Tsuda H. Potassium octatitanate fibers induce persistent lung and pleural injury and are possibly carcinogenic in male Fischer 344 rats, Cancer Sci., May 2018, https://doi.org/10.1111/cas.13643