Study shows that inhaling a common manufacturing material could inadvertently injure the brain

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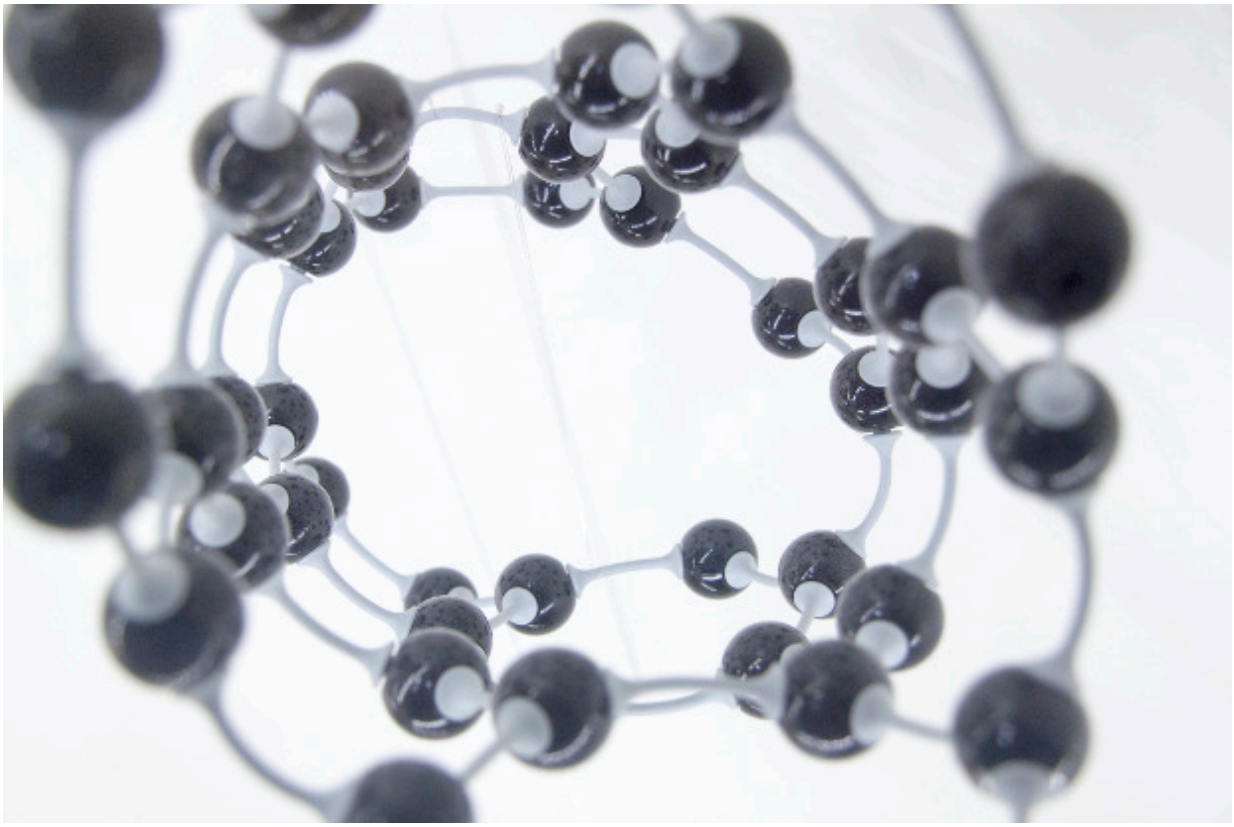


Model of the structure of carbon nanotubes, which are small fibers with multiple uses in manufacturing. Inhalation of carbon nanotubes could negatively impact the brain. Credit: Virginia Commonwealth University

Virginia Commonwealth University researchers in a multi-institutional collaboration are uncovering the degree to which inhalation of carbon nanotubes—a novel manufacturing material used to make anything from tennis rackets to spacecraft parts—could unintentionally cause neurological disease.

Carbon nanotubes are smaller than a human hair, but they are stronger than steel and are shown to effectively conduct electricity and heat. While these fibers have many practical applications, they should be handled with care by workers in the manufacturing sector, according to recent findings by Andrew Ottens, Ph.D., an associate professor of anatomy and neurobiology in the VCU School of Medicine; the Ottens Group research lab; investigators from the University of New Mexico; and the National Institute for Occupational Safety and Health.

With assistance from a $1.9 million four-year grant from NIOSH divided between VCU and UNM, researchers from both institutions have found that inhalation of carbon nanotubes causes inflammation in the brain. Previous research has shown that chronic neuroinflammation is linked to neurological diseases such as Alzheimer's, dementia and hemorrhagic strokes.

"Inhalation-induced neuroinflammation is presently a hot area of study as a causal factor in the development of neurodegenerative disease, leaving open the possibility that working with these compounds and inhaling them may contribute to later neurological ailment," Ottens said.

The study's most recent findings were published in a paper this winter by the *Proceedings of the National Academy of Sciences*.

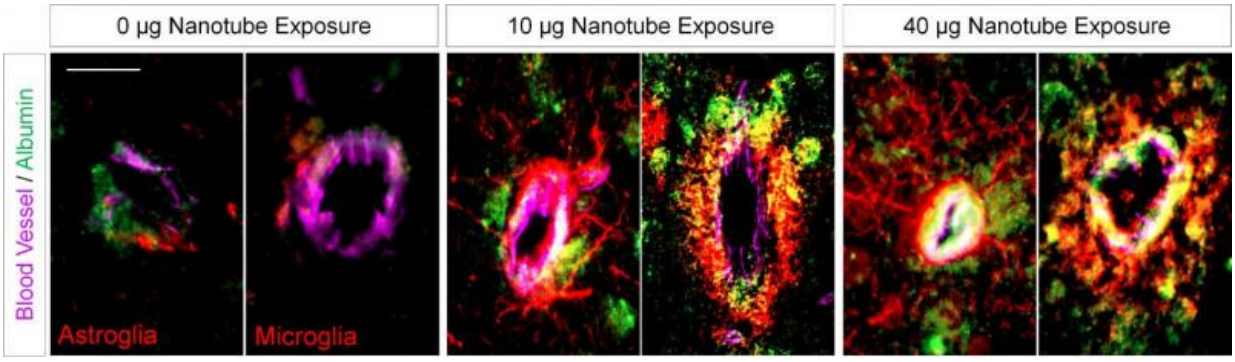
Breaking the blood-brain barrier

The neuroinflammatory effects of inhaled carbon nanotubes show close

links between the respiratory and vascular systems, and the brain. Ottens and his partners concluded carbon nanotubes indirectly cause neuroinflammation by negatively impacting the lungs and blood.

When carbon nanotubes enter the lung, the smallest fibers bury deep into the tissue. Researchers saw that similar to other irritants, the embedded fibers cause lung inflammation. What is novel about the study is that it expands knowledge of how lung inflammation caused by small particulates leads to neuroinflammation.

"There are many studies out there that conclude that you can get lung inflammation from breathing in particulate. It could be from the smoke of burning wood or consuming cigarette smoke," Ottens said. "The mystery was how this affects other organ systems such as the brain. That's what wasn't clear."



Pictured is the induced leakage of the blood-brain barrier and the consequential neuroinflammatory activation of the glial cells in brains exposed to varying amounts — from 0 to 40 micrograms (µg) — of nanotubes released into the lungs. Serum albumin (green) is shown having leaked from the blood vessels (purple) into the brain. At top, astroglia (red) react by forming a scar-like barrier around the leaking vessel, controlling further leakage. At bottom, microglia (red) — a hallmark of neuroinflammation — are also activated and recruited to the leaking vessels where they are cleaning up the leaked albumin. As the nanotube

exposure is increased, more albumin leaks and a greater number of glial cells become active, extending further out from the vessel and impacting more brain tissue. Credit: Virginia Commonwealth University

Ottens said other researchers proposed the particulate escapes from the inflamed lungs into the blood. It was thought this would damage blood vessels, leading to a break in the blood-brain barrier (a blood vessel lining that protects the brain from outside substances), allowing particulates into the brain.

But this isn't completely the case.

Ottens and his partners demonstrated the breakdown of the blood-brain barrier in animal test cases, but it wasn't caused by the particulate directly invading the brain. The researchers found the lung inflammation triggered a biochemical change in the blood, which caused the blood-brain barrier to open.

"The lung serves as a barrier, with our NIOSH colleagues showing that only 0.001 percent of inhaled nanotubes make it to the brain." Ottens said. "This raised the hypothesis that inflammation in the lung alternatively causes the release of bioactive factors into the blood, which then impact the blood-brain barrier."

Normally, very few substances apart from sugar and oxygen permeate the blood-brain barrier. When the barrier broke during the test cases, substances inherent in blood leaked into the brain, such as albumin—the most common protein found in blood.

With the barrier disrupted, the brain's immune responses kicked into overdrive. Glial cells, which make up the brain's primary defense against

biological threats, gathered around the leaky blood vessels to neutralize the threat.

While clean-up by immune cells is necessary, the associated neuroinflammation may become detrimental, Ottens said. Investigators have shown that such inflammation can prime the brain's immune cells to be more easily activated in the future, possibly leading to chronic neurodegeneration. It is this substantial inflammation that has researchers questioning the degree to which exposure to carbon nanotubes may lead to neurological disease.

From science to policy

To get a better idea about how carbon nanotubes impact workers, investigators are working to determine airborne levels of the particulate in manufacturing facilities. The team is also developing blood-based biomarkers that would gauge the biological response that an individual may have after inhaling the particulate matter.

"We hope that this study can contribute to thresholds and guidelines for the safe use of carbon nanotubes in the industry, and provide diagnostics to assess worker's health, for example, in case of an accident," Ottens said. "As a neuroscientist whose particular interest is toxicity pathways, it is very exciting to see the potential impact in terms of the safe commercialization of these materials and understanding the risk factors associated with different levels of exposure."

More information: Mario J. Aragon et al. Serum-borne bioactivity caused by pulmonary multiwalled carbon nanotubes induces neuroinflammation via blood–brain barrier impairment, *Proceedings of the National Academy of Sciences* (2017). DOI: 10.1073/pnas.1616070114

Provided by Virginia Commonwealth University