Carbon-Nanotube Toxicity Test Tricks Scientists

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Recent research has revealed that a standard cell-viability test may be causing carbon-nanotubes to “fake” toxicity. This work may explain why some studies have concluded that carbon nanotubes – which are being studied for their potential to improve building materials, drug-delivery systems, and electronics, to name a few applications – are dangerous to human health while others have not.

Researchers from the Institute of Toxicology and Genetics at the Karlsruhe Research Center in Karlsruhe, Germany, exposed human lung cells to single-walled carbon nanotubes (SWCNTs) – large cylindrical carbon molecules – and conducted several tests to determine the nanotubes' effect on the cells' viability. Three tests showed the nanotubes to be non-toxic, but a fourth curiously produced the opposite result.

“Each of the four tests gauges the toxicity of the SWCNTs in a different way, using different indicators, but we would expect them to yield the same result,” said the study's lead scientist, Harald Krug, to *PhysOrg.com*. “The fact that one test appears to produce a 'false positive' in terms of toxicity suggests that past carbon-nanotube toxicity studies may be flawed.”

The first test, known as the MTT assay, works by measuring how a salt, methylthiazol tetrazolium (MTT), is chemically converted to formazan, a purple dye, after being applied to nanotube-exposed cells. This conversion only takes place when certain cell mitochondria enzymes are active – that is, if the cell is alive and well. According to the results of

the MTT assay, the nanotubes compromised cell viability.

Krug and his colleagues attempted to verify the results of the MTT assay using another salt-based viability test, the water-soluble tetrazolium (WST) assay. According to this test, the nanotubes had no negative effect on the cells. Two other tests also showed no reduction in cell viability.

Why the discrepancy between the MTT assay and the other tests? The answer seems to be due to the non-soluble nature of MTT and formazan. Using an electron microscope, the researchers saw that MTT-formazan crystals had covered the nanotubes, clumping everything together. The nanotubes were reacting with the MTT, causing the formazan to withdraw from the assay. This made the formazan undetectable and, as a result, made the nanotubes appear toxic. Several attempts to dissolve the crystals, as well as heat treatments, were unsuccessful.

“In these studies, the viability assay of choice really needs to be double-checked, since interferences and disturbances are likely,” said Krug. “Further, we think our work demonstrates that standards should be established when testing the toxicity of carbon nanotubes and other nanomaterials.”

An in-depth paper on this work can be found in the June 2006 edition of *Nano Letters*.

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