

NANOTECHNOLOGY

Can High-Speed Tests Sort Out Which Nanomaterials Are Safe?

A flood of strange new substances based on ultrasmall particles is forcing researchers to reinvent toxicology

If you've ever marveled at a painter mixing colors on a palette, you have a taste of what nanotechnologists feel when designing materials at the smallest scale. Nanoscientists today mix collections of different atoms to create a multitude of novel pint-sized particles with a vast array of new electronic, optical, catalytic, and chemical properties. Iron oxide particles, for example, are proving exceptional as contrast agents for medical imaging. Bits of titanium dioxide harvest sunlight in solar cells. And on and on. "These are materials with wonderful properties," says Fanqing Chen, a biomedical scientist at Lawrence Berkeley National Laboratory in California. "Their contribution to science, technology, and the economy is going to be huge."

But the remarkable diversity of nanomaterials is also one of the field's biggest headaches. With potentially thousands of novel materials under investigation, health

and safety regulators are left scratching their heads over which are safe and which are potentially toxic to humans or other species. That uncertainty threatens to undermine public confidence in nanotechnology and stymie the development of what is expected to be a major global economic engine. What's worse, traditional toxicology studies that test one



Benign or harmful? Rat lung cell attempts to ingest a carbon nanotube. Clumping nanotubes can stymie the animals' immune systems.

Bright invaders. Nanosized quantum dots pervade cells, tinting each structure a different color.

material at a time appear woefully inadequate to the task of sorting the dangerous from the benign. "If we continue with the classical toxicological assessment [for nanomaterials], we will never catch up with this work," says Andre Nel, a nanomaterials toxicologist at the University of California, Los Angeles.

In hopes of picking up the pace, Nel and a handful of other toxicologists around the world are creating batteries of high-speed assays for testing the toxicity of hundreds and even thousands of different nanomaterials at the same time. The assays involve exposing different types of cells to nanoparticles and monitoring the cells for changes, such as DNA damage, alterations in gene expression, cell death, and impacts on cell growth and proliferation. It's still

too early to say whether these hopes will pan out. But at a minimum, nanotoxicologists hope high-throughput studies will help them prioritize nanomaterials that should undergo conventional toxicology tests.

Clearly, there is cause for concern about the toxicity of some nanomaterials. Early studies with soccer-ball-shaped carbon fullerenes revealed, for example, that the particles can damage brain cells in fish. Conventional toxicology studies also raised yellow flags about the straw-shaped cousins of fullerenes called carbon nanotubes. When the lung tissue of rats was flushed with high levels of nanotubes, researchers found that the tubes clumped together, making it difficult for the animals' immune systems to clear them from their bodies. Still other studies have raised concerns about particles mostly considered harmless at larger sizes, such as titanium dioxide nanoparticles.

Unfortunately, such studies are slow and expensive. A single in vivo animal study can cost \$50,000. In addition, it's often difficult to extrapolate how a material tested in one tissue will affect others. It's even harder to extend that one study to determine how other nanomaterials will behave. The study that showed fullerenes can damage the brain cells of fish, for example, says little about exposure to particles of iron oxide, silicon dioxide, or carbon nanotubes.

To confound matters further, particles can also come with different coatings that alter their properties, and they can even acquire new coatings upon entering the environment or the human body. Chen notes that some of his team's early studies reveal that the toxicity of nanoparticles can change even when they change size or shape but not composition. "They show different effects, even if they are the same material," Chen says. "It looks like there is an additional dimension ... when you are dealing with nanomaterials."

Faced with such complexity, researchers have begun looking to high-throughput testing techniques as a route to better, faster, and cheaper toxicology studies. Drug companies have long used similar methods to test compounds for curing and preventing disease. But because toxicologists must look for not just one positive outcome but potentially dozens of negative ones, they face a much tougher task: developing a battery of in vitro assays using a variety of cell types to assemble a fingerprint of ways a particular nanoparticle affects different tissues.

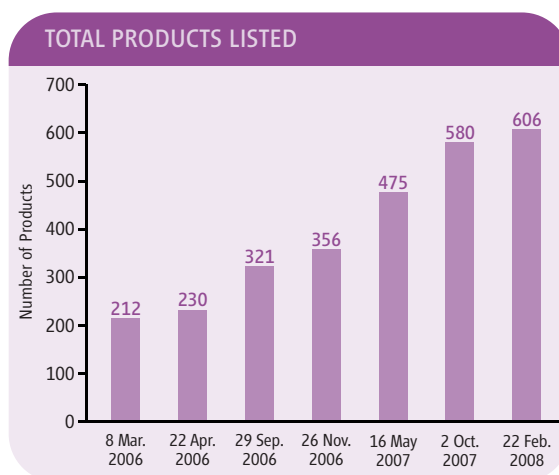
One of the biggest such efforts to date is ToxCast, a 5-year initiative by researchers with the U.S. Environmental Protection Agency in Research Triangle Park, North Carolina. Launched in 2007 to speed up toxicity testing on chemicals in general, ToxCast is nearing the end of its pilot phase. Researchers are examining 320 different chemicals, such as pesticides, that all have previously undergone extensive conventional toxicological screens, says Keith Houck, an EPA toxicologist who is helping to develop the program. Houck says he and his colleagues are testing each chemical in a wide variety of cell-based assays, looking for 400 different "endpoints" that could signal danger to living organisms.

"What we are trying to do is get a broad bioactivity profile for each compound and correlate those with toxicology studies we have," Houck says. In the program's second phase, set to begin in 2009, Houck says ToxCast researchers will evaluate probably about another 1000 compounds, which will likely include several varieties of nanomaterials. Houck notes that the number of different starting nanomaterials, coupled with possible coatings and other molecules that could interact with them, makes it impossible to test every combination. "But we can use computational approaches to hopefully test the right ones and make some predictions about which materials are of most concern." The compounds determined to be the riskiest will then be given top priority

for conventional toxicity screening.

Although the results from ToxCast won't be known for years, smaller scale efforts are already giving a sense of what the approach can offer. In a paper published in 2006 in *Nano Letters*, for example, Nel and his colleagues compared the effects of ambient ultrafine particles such as carbon black—produced in diesel exhaust—with the effects of manufactured nanoparticles such as chemically functionalized fullerenes and polystyrene. Using a series of assays that monitored levels of oxidative stress and cytotoxicity, they found clear differences in cellular responses to different particles. Functionalized polystyrene particles topped the list of materials causing the most oxidative damage to mouse macrophage cells.

"In nature, there are only a dozen or so major pathways that lead to 90% of toxico-



In demand. According to the Woodrow Wilson International Center for Scholars, the number of consumer products containing nanoparticles nearly doubled in 14 months.

logical outcomes," Nel says. So Nel and his colleagues are currently working to set up a broader set of screens to systematically monitor these pathways, conducting up to about 1000 cell-based assays a day. The screens, he says, will look for changes in addition to oxidative stress and cytotoxicity, such as DNA damage and changes in cell growth and proliferation. As ToxCast is doing, he then intends to gauge which nanomaterials are the likeliest to be harmful and put them first in line for more rigorous tests. "Instead of going through the haystack handful by handful, we've come up with a procedure to get rid of the hay," Nel says.

Chen has been developing a related approach. In another 2006 *Nano Letters* paper, he and colleagues looked at the genetic response of human skin fibroblasts when exposed to different nanoparticle-

based imaging compounds. For their tests, Chen's team used gene chips that tracked how gene expression changed in about 22,000 genes in response to different types and doses of nanoparticles. Only about 50 of the genes showed significant changes. From the pattern, the Berkeley team concluded that—unlike several of the naked nanoparticles—particles coated with a polymer called polyethylene glycol induced minimal impact on exposed cells.

In a more recent study, Ralph Weissleder, a molecular imaging expert at Harvard Medical School in Boston, and colleagues have also turned to high-throughput analysis to sort out which nanoparticles have the best shot at succeeding as medicines. Nanoparticles are currently being developed as imaging agents, drug carriers, and, in some cases, therapeutic drugs themselves. A multitude of such compounds are under development, many of them coated with different small molecules to help target them to different tissues in the body, Weissleder says. But drugmakers want to avoid sinking too much time and effort into nanoparticles that have no shot at being approved for in vivo use because of their toxicity.

So Weissleder and his colleagues set out to flag potential troublemakers. They evaluated 50 different nanomaterials at four concentrations on four cell types with four different assays. In the 27 May issue of the *Proceedings of the National Academy of Sciences*, they reported that they had found

broad, consistent patterns of activity, although different cell types behaved very differently. "There is not a single test that will predict how nanomaterials will behave in vivo," Weissleder says. But the batteries of cell assays can help researchers decide which ones are likely to be safest for human studies, he says.

Taken together, the early studies suggest that high-throughput assays could vastly speed up toxicological screening, Weissleder and others say. They can't do everything, Houck cautions: "In vitro assays won't ever replicate a whole body." Still, if they can begin to sort out which types of nanoparticles pose the biggest risk, they could encourage targeted testing of those compounds and thereby help shore up faith in nanotechnology's future.

—ROBERT F. SERVICE