"People have plunged headlong in without properly assessing the health impacts"

Dominique Lison is a Professor of Toxicology at Belgium's Catholic University of Louvain. He has headed a number of research projects on carbon nanotube toxicity in recent years. We quizzed him on current scientific knowledge about the risks of nanomaterials to humans.

Interview by
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Editor

Your research team exposed rats to carbon nanotubes and found asbestos-like effects...

DL – We injected multiwalled carbon nanotubes (CNT, *ed.*) directly into the animals' airways. We found inflammation and fibrotic reactions in the lung that displayed the same pathogenic effects as asbestos fibres.

We also did a genotoxicity study. Here again, we found that, like asbestos, CNT can cause damage to the genetic information written into the cells.

Having identified inflammatory, fibrotic and genotoxic effects like those of asbestos fibres, we naturally wondered whether CNT might be carcinogenic. So, we did a two-year study in which we injected carbon nanotubes into the peritoneal cavity of 250 rats. While the same experiment done with asbestos fibres produces tumours, we were quite surprised to find that none of the CNT exposures caused tumours in rats. The most likely explanation for that is that the CNT we used were too short. Where asbestos is concerned, the length of the fibres is known to be decisive in the development of mesothelioma. An article published in *Nature* magazine after our study was completed singled out the length of fibres[1]. So it may be that the CNT we used were too short to demonstrate carcinogenicity, because research teams elsewhere, notably in Japan, have shown the carcinogenic effect of some CNTs.

Working together with a Turin laboratory and the University of Orléans, we then set about better identifying the properties of these carbon nanotubes. We knew that CNT produced by iron-cobalt catalysis retain less than 1% of these metals, which are trapped as it were inside the CNT. It is known that the iron content of asbestos fibres is a key factor of their toxic effects. We therefore sought to eliminate these potentially toxic metals by heating our CNT to 2 400 C. That worked remarkably well, but also resulted in a "cleansing" of CNT bond defects. The thing is that industrially produced CNT are never chemically perfect. You will occasionally find a bond that we call "unsatisfied". Reintroducing "bond defects" in later experiments eventually led us to conclude that the defects in the crystalline structure of the nanotubes are what give them their toxic effect. Nanotubes cleansed of their imperfections are no longer inflammatory or genotoxic, but the "fibrosis effect" in the lung persists.

What would you recommend to producers based on what you discovered?

Well, clearly that the quantity of CNT defects, which is measurable by simple, cost-effective techniques, can be a good predictor of the toxicity of their nanotubes. The length and defects of CNT are two key factors to be looked at closely.

Industry argues that these animal exposures are not the same as real-life human exposure...

Everyone in the toxicology community has worked by instilling CNT in mice because of the great technical difficulty of generating a carbon nanotube aerosol. This is because nanotubes have a tendency to clump together to form agglomerates, large balls that some have described as looking like "tangled spaghetti".

1. Carbon nanotubes introduced into the abdominal cavity of the mice show asbestos-like pathogenicity in a pilot study, *Nature Nanotechnology* 2008, 3, p. 423 – 428.

A lot of people with little in the skills department have gone into this new "gold rush for toxicologists".

Now, industry will say, "the only things you find in the air are these agglomerates which cannot be breathed in, so there is next to no chance of nanotubes making their way into the airways." This fosters the idea that while nanotubes may be dangerous in and of themselves, the health risk to humans is low because of this propensity to form agglomerates of non-inhalable size. But this tells only part of the story. There is not a shred of evidence that all of any particles you find in the air are agglomerates; you may also find individual nanotubes. The reason is that these nanotubes will be used in materials that will be machined and sawn, and so are bound to be in the air. At this stage, we know nothing about how these aerosol releases will behave.

Also, a recent inhalation toxicity study done in a BASF laboratory suggests a need for caution (see box). The study's authors confirm that the lesions observed in the animal after intratracheal instillation are found after inhalation. But they go on to show that you have to go down to very low airborne concentrations of nanotubes before you see no observed adverse effect. This is quite concerning, because it means that the No Observed Adverse Effect Concentration is probably very low.

Using the standard method of calculation for extrapolating animal results to humans – a coefficient at least equal to 100 – the BASF study findings suggest that the No Observed Adverse Effect Concentration for humans should be set at least below 1 microgram per cubic metre of air, which is very low.

But how do you measure levels as low as that in workplaces? How do you assess the risks of substances that are around a billionth of a metre in size?

The sudden emergence of nanos in the world of work brings in a number of new issues compared to standard practices for identifying exposure in workplaces. The traditional way is to measure the number of fibres in the air, but this type of measuring instrument is not precise enough for nanos. They are a world unto themselves, and the tools we have are not fitted to it. We cannot define occupational exposure limit values with the scientific knowledge we have. So all we can do at the moment is to take the usual preventive measures. And because substitution is not possible in this particular case, it means working in a closed system, limiting the number of exposed workers, putting extraction and ventilation systems in place, and, as a last resort, using personal protective equipment. It also means providing health surveillance for workers.

Products containing very different kinds of nanoparticles, whose toxicological properties we still know next to nothing about, are already on supermarket shelves. How can the scientific community help consumers and law-makers distinguish between the "good" and "bad" products?

Science can't really inform policy properly at this juncture about the implications of nanotechnology development for society. To grossly over-simplify, the scientific literature falls into two types of publication. One is articles published by those who want to use or sell nanoparticles, who don't want to miss the boat of this technological breakthrough, who rush out small-scale *in vitro* tests on a cell with the corner lab and publish – usually in chemical industry, technology or similar journals – that their nanoparticles are not dangerous. And then, there are the toxicologists who are finding out that in some cases the self-same nanoparticle, looked at more forensically, does have dangerous properties. Where nanos are concerned, it has to be said that a fair few toxicology studies are only fit for scrapping. A lot of people with little in the skills department have gone into this new "gold rush for toxicologists" because of the many public research budgets around.

As far as putting nanotechnology products on sale goes, my feeling is that people have plunged headlong in without properly assessing the impacts on human health and the environment. The many varieties of titanium oxide powders used in sun block are a particular case in point – at least a dozen serious scientific publications suggest that these powders can elicit genotoxic responses. ●

More on the work done by Professor Lison's team

– Genotoxicity of nanomaterials: DNA damage and micronuclei induced by carbon nanotubes and graphite nanofibres in human bronchial epithelial cells *in vitro*, *Toxicology Letters*, vol. 186, Issue 3, 8 May 2009, p. 166-173.
– Absence of carcinogenic response to multiwall carbon nanotubes in a 2-year bioassay in the peritoneal cavity of the rat, *Toxicol Sci.*, 2009 Aug, 110(2), p. 442-8.
– Structural defects play a major role in the acute lung toxicity of multiwall carbon nanotubes: toxicological aspects, *Chem Res Toxicol.* 2008 Sep, 21(9), p. 1698-705.