With the mass production of engineered nanoparticles, risk assessment efforts are in need of platforms that offer predictive value to human health and environment, and also possess high throughput screening capacity. Scientists, when turning to a model-organism to help answer genetic questions that cannot be easily addressed in humans, often chose the zebrafish (Danio rerio). Zebrafish share the same set of genes as humans and have similar drug target sites for treating human diseases. They are small, easy to maintain, and well-suited for whole animal studies. Furthermore, their early embryonic development is completed rapidly within five days with well-characterized developmental stages. The embryos are transparent and develop outside of their mothers, permitting direct visual detection of pathological embryonic death, mal-development phenotypes, and study of real-time transport and effects of nanoparticles in vivo.

However, the current screening process in zebrafish involves mostly counting the survival rate, hatching and developmental abnormalities etc. through visual examination of each embryo and/or larvae under a dissecting microscope. Such process is time-consuming, labor-intensive and has limitations on data acquisition as well as statistics analysis. Researchers say that advanced imaging technology and automated testing are key challenges toward developing high content screening of nanomaterials, chemicals, and drugs in zebrafish.

"With the development of high content imaging, one can acquire hundreds to thousands of microscopic images with high throughput and the images would allow further in silico image analysis and hazard ranking tools for engineered nanoparticles to greatly speed up the screening process," says Sijie Lin, a postdoctoral researcher at the University of California's Center for Environmental Implications of Nanotechnology (CEIN).

Lin is first author of a paper in ACS Nano ("High Content Screening in Zebrafish Speeds up Hazard Ranking of Transition Metal Oxide Nanoparticles") where an international team of collaborators successfully demonstrated two high content imaging platforms to enhance the ability to screen the toxicological effects of nanoparticles in zebrafish embryos.

"Our study provides, for the first time, the use of high content imaging – which includes bright-field and fluorescence based imaging – to compare the toxicological effects of transition metal oxide nanoparticles in zebrafish embryos and larvae," says Lin. "We have demonstrated the embryo hatching interference exerted by nanoparticles due to their intrinsic material characteristics, i.e. shedding of heavy metal ions, through bright-field high content imaging."

The team describes the use of an imaging platform for bright-field imaging analysis, capable of capturing phenotypic and developmental abnormalities in embryos and larvae. For larvae, the team also captured the stress induced by different types of nanoparticles on the larval stage of a transgenic zebrafish line (hsp70:eGFP) by fluorescence based high content imaging.

"In order to apply these imaging tools to new engineered nanomaterials not previously assessed, we utilized a series of transition metal oxide nanoparticles – CuO, NiO, and Co₃O₄ – to compare to ZnO (positive control)" explains Lin. "Our results demonstrate profound interference in embryo hatching in parallel with hsp70:eGFP expression in larvae by CuO and NiO but not Co₃O₄."

The researchers assume that the profound hatching interference and stimulation of hsp70 gene expression by CuO, ZnO, and NiO nanoparticles could be attributed to nanoparticle

Continued on page 3
PERSONAL NANOPARTICLE RESPIRATORY DEPOSITION SAMPLER STREAMLINES EXPOSURE ASSESSMENT

Until more information becomes available on the mechanisms underlying nanomaterial toxicity, it is uncertain what measurement technique should be used to monitor exposures in the workplace. Current research indicates that mass and bulk chemistry may be less important than particle size and shape, surface area, and surface chemistry (or activity) for some nanostructured materials.

Many of the sampling techniques that are available for measuring airborne nano aerosols vary in complexity but can provide useful information for evaluating occupational exposures with respect to particle size, mass, surface area, number concentration, and composition. Unfortunately, relatively few of these techniques are readily applicable to routine exposure monitoring. NIOSH, the National Institute for Occupational Safety and Health in the United States, has initiated exposure assessment studies in workplaces that manufacture or use engineered nanoparticles.

"We have developed a new sampler that collects nanoparticles separately from larger particles in a way that mimics their deposition in the respiratory system" explains Peters. "By capturing only nanoparticles on the sampling media, cost-efficient bulk analysis techniques (e.g., inductively coupled plasma mass spectrometry) could then be used to estimate the amount of deposited nanomaterials without the need for electron microscopy. For TiO$_2$, we anticipate that the cost per sample will be about $30."

Central to their new sampling device is a new sampling criterion for nanoparticles – the nano-particle matter criterion (NPM). This criterion is analogous to other sampling criteria (i.e., respirable, thoracic, and inhalable) in that it ties sampler performance with respiratory deposition.

"In the 1990s, I was involved with development of regulations and samplers for environmental use – particulate matter smaller than 2.5 µm" says Peters. "This experience provided the perspective that new sampling criterion are sometimes needed before new samplers are developed."

Peters notes that, "rather than attempting to collect nanoparticles with 100% efficiency, the team's sampler was designed to collect nanoparticles with efficiency matching how they deposit in the respiratory tract to provide a physiologic relevance to sampler’s performance. The NPM provides the target collection efficiency, by particle size, for the sampler."

"That means that our NRD sampler is fundamentally different from commonly used samplers – e.g., respirable and

The components and schematic drawing with airflow paths of the Nanoparticle Respiratory Deposition sampler. (Reprinted with permission from American Chemical Society)
As more and more data is generated on the toxicological effects of engineered nanoparticles, scientists are no longer satisfied with identifying which nanoparticles are toxic, and to what degree, but they are also trying to boost the throughput capacity of their screening set-ups as well as understand the exact underlying toxicity mechanisms. The challenges that remain to be overcome are how to link the specific physicochemical properties of nanoparticles to their toxicological outcomes both in vitro and in vivo.

He points out that, with chemical analysis of the diffusion media, the NRD sampler can be used to directly assess exposures to nanoparticles of a specific composition apart from other airborne particles. "It also provides a streamlined way to conduct quantitative, routine exposure assessment for metallic nanoparticles."

The immediate application is for sampling of metal-based nanoparticles in the workplace. The team are already working on the analytical technique for titanium dioxide and other metals. Other analytical techniques will be explored in future research to broaden the applicability.

"We plan to develop the sampler further to enable greater airflow rates for lower detection limits" says Peters. "We also plan to deploy the sampler in field tests."

This image represents a 96-well microtiter plate used for the screening of nanoparticles in zebrafish. Each well of this plate was filled with one embryo and the bright-field based high content imaging automatically acquired one image per well. The images were then combined to show the screening results as well as layout of the plate. (Image: Sijie Lin, CEIN)
**SWITZERLAND UPDATES PRECAUTIONARY MATRIX FOR SYNTHETIC NANOMATERIALS**

The precautionary matrix for synthetic nanomaterials makes it possible for trade and industry to adopt a structured approach to recognizing the possible risks in dealing with synthetic nanomaterials. The Federal Office of Public Health (FOPH) and Federal Office for the Environment (FOEN) launched the introductory phase in December 2008. The precautionary matrix was revised on the basis of users' experience at the beginning of 2010.

**An important tool for trade and industry**

The precautionary matrix provides a structured method to assess the "nanospecific precautionary need" of workers, consumers and the environment arising from the production and use of synthetic nanomaterials.

**WHY CARBON NANOTUBES SPELL TROUBLE FOR CELLS**

It's been long known that asbestos spells trouble for human cells. Scientists have seen cells stabbed with spiky, long asbestos fibers, and the image is gory: Part of the fiber is protruding from the cell, like a quivering arrow that's found its mark.

But scientists had been unable to understand why cells would be interested in asbestos fibers and other materials at the nanoscale that are too long to be fully ingested. Now a group of researchers at Brown University explains what happens. Through molecular simulations and experiments, the team reports in *Nature Nanotechnology* ("Cell entry of one-dimensional nanomaterials occurs by tip recognition and rotation") that certain nanomaterials, such as carbon nanotubes, enter cells tip-first and almost always at a 90-degree angle. The orientation ends up fooling the cell; by taking in the rounded tip first, the cell mistakes the particle for a sphere, rather than a long cylinder. By the time the cell realizes the material is too long to be fully ingested, it's too late.

"It's as if we would eat a lollipop that's longer than us," said Huajian Gao, professor of engineering at Brown and the paper's corresponding author. "It would get stuck."

The research is important because nanomaterials like carbon nanotubes have promise in medicine, such as acting as vehicles to transport drugs to specific cells or to specific locations in the human body. If scientists can fully understand how nanomaterials interact with cells, then they can conceivably design products that help cells rather than harm them.

"If we can fully understand (nanomaterial-cell dynamics), we can make other tubes that can control how cells interact with nanomaterials and not be toxic," Gao said. "We ultimately want to stop the attraction between the nanotip and the cell."

Like asbestos fibers, commercially available carbon nanotubes and gold nanowires have rounded tips that often range from 10 to 100 nanometers in diameter. Size is important here; the diameter fits well within the cell's parameters for what it can handle. Brushing up against the nanotube, special proteins called receptors on the cell spring into action, clustering and bending the membrane wall to wrap the cell around the nanotube tip in a sequence that the authors call "tip recognition." As this occurs, the nanotube is tipped to a 90-degree angle, which reduces the amount of energy needed for the cell to engulf the particle.

Once the engulfing — endocytosis — begins, there is no turning back. Within minutes, the cell senses it can't fully engulf the nanostructure and essentially dials 911. "At this stage, it's too late," Gao said. "It's in trouble and calls for help, triggering an immune response that can cause repeated inflammation."

The team hypothesized the interaction using coarse-grained molecular dynamic simulations and capped multiwalled carbon nanotubes. In experiments involving nanotubes and gold nanowires and mouse liver cells and human mesothelial cells, the nanomaterials entered the cells tip-first and at a 90-degree angle about 90 percent of the time, the researchers report.

"We thought the tube was going to lie on the cell membrane to obtain more binding sites. However, our simulations revealed the tube steadily rotating to a high-entry degree, with its tip being fully wrapped," said Xinghua Shi, first author on the paper who earned his doctorate at Brown and is at the Chinese Academy of Sciences in Beijing. "It is counter-intuitive and is mainly due to the bending energy release as the membrane is wrapping the tube."

The team would like to study whether nanotubes without rounded tips — or less rigid nanomaterials such as nanoribbons — pose the same dilemma for cells.

"Interestingly, if the rounded tip of a carbon nanotube is cut off (meaning the tube is open and hollow), the tube lies on the cell membrane, instead of entering the cell at a high-degree-angle," Shi said.

The matrix is a tool to help trade and industry meet their obligations of care and self-monitoring. It helps them to recognize applications which may entail risk and to take precautionary measures to protect human health and the environment. In the case of new developments, the matrix can contribute to the development of safer products. It enables users to conduct an initial analysis on the basis of currently available knowledge and indicates when further investigations are necessary.

The precautionary matrix is available to a broad circle of users at home and abroad. It will be further developed in close cooperation with trade, industry and science as well as with consumer and environmental organizations.
The Danish Environmental Protection Agency (DEPA) has initiated the study "Survey on basic knowledge about exposure and potential environmental and health risks for selected nanomaterials". The objective of the study is to provide an overview of the applications of the most commonly used or widespread nanomaterials and to identify areas most likely to have health or environmental problems associated with their use.

There is no single source of information that provides an overview of the use of nanomaterials and products in Denmark or in the EU for that matter. Pieces of information are, however, available from databases and previous studies initiated by DEPA. This information has in this project been reviewed together with results from other studies carried out in the Nordic countries and including estimates on relevant consumer applications and uses of the selected nanomaterials. A considerable part of the nanomaterial-containing products are found to be sold from web shops in Denmark and abroad but an increasing part is sold from ordinary shops.

Limited Industry Survey

A limited industry survey on the industrial use of the selected nanomaterials in Denmark has been conducted. The objective of this survey was to confirm the use of the nanomaterials in question in Denmark, and to develop a rough estimate of the consumption.

The survey was carried out among identified actors dominating the markets for the selected nanomaterials and their typical applications. The relevant actors were asked about the uses and the amounts of the nanomaterials. Focus for the survey was on obtaining information for the most dominant field of application and not to cover all different use areas.

The outcome of our survey can be summarized as follows:

- Titanium dioxide, nanoclay and silicium dioxide are all materials used in most significant quantities in Denmark.
- The use of nanosilver has not been confirmed, but indications exist that some products/brands may contain nanosilver.
- The use of cerium dioxide has not been confirmed either. It is not used by leading marked actors in Denmark.
- No information was available on fullerenes and zero-valent iron.

Individual Nanomaterial Profile Developed

A profile for each of the selected materials was then developed. For each material the focus has been on the general characteristics and manufacture of the nanomaterials, their current uses (mainly focused at consumer products), and hazard profiles (ecotoxicity and human toxicity). Furthermore the profiles include sections discussing relevant exposures from consumer products and considerations regarding the related risk.

Each nanomaterial profile is summarized in a 'summary sheet' containing the key findings and also emphasizing areas where information is lacking. The general picture is that the specific knowledge base is limited and that more information is needed for sufficient characterization of the nanomaterials and for illustration of the relevant (eco)toxicological endpoints. In addition more information is required with regard to fate, behavior and kinetics of the different nanoparticles and crucial to the assessment of the relevant risks is an agreed methodology for risk assessment.

No Conclusive Risk Assessments Possible

Conclusive risk assessments were therefore not possible to develop within the framework of the present project. Based on the reviewed literature the seven selected nanomaterials were not found to exhibit new and completely unknown risks to the consumer or to the environment in the current application. Products in the form of liquids or free particles are expected to give rise to the highest exposures in the environment and to humans, particularly those liquids that are intended to come in direct contact with the body, and the potential risk is likely to increase with increased exposure. However, as the applicability of the existing exposure and risk assessment methodology has been challenged in relation to nanomaterials, there are still areas that need to be explored - especially for engineered nanomaterials.

A key question in relation to risk and safety assessment of nanomaterials as raised in Stone et al. (2010) is to which extent the existing knowledge base about toxicity and risk related to the bulk counterparts can be used in the evaluation of the nanomaterials. In other words, it is the question of whether the risk information can be scaled from bulk substances to the nano-form taking the size of the nanoparticles into account or whether it is the small size that triggers the nano-specific behavior and effects.

Based on the reviewed literature there are some indications that scaling of toxicity could be relevant for the more chemically inert materials as TiO₂ and SiO₂ whereas e.g. carbon-based materials like fullerenes where surface modifications are introduced are more likely to acquire nano-specific properties. This is an area that needs further clarification before firm conclusions can be made. Relevant for this discussion is also the fact that many nanoscale particles (e.g. silver, nanoclay, TiO₂ and SiO₂) are naturally occurring particles that have been used for decades. However, these materials may also be modified with different surface coating, which can alter their physical-chemical properties and toxicity.

Further Research Required

In order to answer the many questions regarding nanomaterials and risk more information and research is required in the future. Some of the gaps can be summarized as follows:

- Characteristics sufficient for toxicity testing
- Fate, behavior and kinetics of different nanoparticles
- Agreement regarding risk assessment methodologies to comply with regulatory regimes
- More information on chronic effects of nanomaterials
- Effect of surface functionalization on toxicity of the nanomaterials

A press release from DEPA (in Danish) can be found here. The full report (in English) is available for download here (pdf).
EU'S INSTITUTE FOR HEALTH AND CONSUMER PROTECTION CALLS FOR NANOMATERIAL DEFINITION

European Commission's Joint Research Centre (JRC), Institute for Health and Consumer Protection defends the need to define engineered nanomaterials for regulatory purposes in the opinion published in the latest edition of Nature magazine ("Risk factors: Nanomaterials should be defined").

This is a reply to an article by Andrew Maynard, in which he argues against it ("Why we don't need a regulatory definition for nanomaterials").

JRC concludes that there is an urgent need for a definition of nanomaterial, more specifically "particulate nanomaterial", that can be used in a regulatory framework. Such a definition should not seek to identify hazardous materials, but should assist industry and regulators in identifying where specific safety assessments might be necessary.

NICKEL NANOPARTICLES MAY CONTRIBUTE TO LUNG CANCER

All the excitement about nanotechnology comes down to this: Structures of materials at the scale of billionths of a meter take on unusual properties. An interdisciplinary team of scientists at Brown University finds that nanoparticles of nickel activate a cellular pathway that contributes to cancer in human lung cells.

"Nanotechnology has tremendous potential and promise for many applications," said Agnes Kane, chair of the Department of Pathology and Laboratory Medicine in The Warren Alpert Medical School of Brown University. "But the lesson is that we have to learn to be able to design them more intelligently and, if we recognize the potential hazards, to take adequate precautions."

Kane is the senior author of the study published in the journal Toxicological Sciences ("Bioavailability, intracellular mobilization of nickel, and HIF-1α activation in human lung epithelial cells exposed to metallic nickel and nickel oxide nanoparticles").

Nickel nanoparticles had already been shown to be harmful, but not in terms of cancer. Kane and her team of pathologists, engineers and chemists found evidence that ions on the surface of the particles are released inside human epithelial lung cells to jumpstart a pathway called HIF-1 alpha. Normally the pathway helps trigger genes that support a cell in times of low oxygen supply, a problem called hypoxia, but it is also known to encourage tumor cell growth.

"Nickel exploits this pathway, in that it tricks the cell into thinking there's hypoxia but it's really a nickel ion that activates this pathway," said Kane, whose work is supported by a National Institutes of Health Superfund Research Program Grant. "By activating this pathway it may give premalignant tumor cells a head start."

The research team, led by postdoctoral research associate and first author Jodie Pietruska, exposed human lung cells to nanoscale particles of metallic nickel and nickel oxide, and larger microscale particles of metallic nickel. A key finding is that while the smaller particles set off the HIF-1 alpha pathway, the larger metallic nickel particles proved much less problematic.

UPCOMING EVENTS LOOKING AT THE RISKY SIDE OF NANO

NanoImpactNet Training School on the Impact Assessment of Nanomaterials: In vivo and in vitro testing strategies
October 12-14, 2011, Bratislava (Slovakia)
This training school will provide the participants with the necessary knowledge and skills to assess the impact of manufactured nanomaterials on human health and the environment.

Nanoparticles Metrics and Exposure: From Exposure to Effects
October 17-18, 2011, London (UK)
The main aim is to bring together experts in these different research fields to build a bridge from exposure to health effects for manufactured nanomaterials. The discussions will start with a presentation of the state of the art and of our future needs and will move onto cover all the relevant aspects bridging the fields of release and exposure with toxicological studies.

Governance and Ethics of Nanosciences and Nanotechnologies
October 20-21, 2011, Warsaw (Poland)
The conference will focus on the EC Code of Conduct for responsible nanosciences and nanotechnologies research, and activities of Member States concerning implementation of the Code will be presented and discussed.

BioNanoTox International
November 17-18, 2011, Little Rock, AR (USA)
BioNanoTox (Biology, Nanotechnology, and Toxicology) is a novel field of research that investigates biological systems (plants, human, and animals) and the environment in conjunction with nanomaterials.

Joint Workshop "Risk Governance of Manufactured Nanoparticles"
November 21, 2011, Brussels (Belgium)
This Workshop is co-organized by the European Parliament STOA Panel and the European Commission.

Trends in Nanotechnology International Conference
November 21-25, 2011, Tenerife (Spain)
The topics of this high-level scientific meeting series include risks, toxicity, and regulations.

International Conference on Nanoscience and Technology 2012 (ICONSAT)
January 20-23, 2012, Hyderabad (India)
ICONSAT is primarily motivated by the desire to promote scientific exchange between experts in India and abroad in the area of nanoscience and technology. One theme of the event is nanotoxicology.
IN SHORT – PAPERS, INITIATIVES & UPDATES

PAPER: Graphene oxide - A nonspecific enhancer of cellular growth
This study conclusively demonstrates that graphene oxide does not have intrinsic antibacterial, bacteriostatic, and cytotoxic properties in both bacteria and mammalian cells. Furthermore, graphene oxide acts as a general enhancer of cellular growth by increasing cell attachment and proliferation.
doi: 10.1021/nn202699t

WORKSHOP: Presentations from US-EU nanoEHS workshop available online
On March 10-11, 2011 The US and EU jointly held a workshop to
• engage in an active discussion about environmental, health, and safety questions for nano-enabled products
• encourage joint programs of work that would leverage resources
• establish communities of practice, including identification of key points of contact/interest groups/themes between key US and EU researchers & key US and EU funding sources.
The presentations from this workshop are now available online.

REPORT: NGO warns that nanosilver products are breeding superbugs
Overuse of antibiotics has contributed to the problem, by promoting the development of more powerful bacteria that are resistant to treatment. Now, in a new Friends of the Earth report (pdf), leading microbiologists have warned that the rapid rise in household antibacterial products containing nano-silver could put more lives at risk.

PAPER: Cellular uptake and cytotoxic impact of functionalized and polymer-coated CNTs
A modified cytotoxic (lactate dehydrogenase (LDH)) assay is developed in an attempt to offer a valid and reliable methodology for screening carbon nanotube toxicity in vitro. Two of the most widely used types of surface-modified multiwalled carbon nanotubes are tested. These findings indicate the reliability of the modified LDH assay as a screening tool to assess carbon nanotube cytotoxicity and illustrate that high levels of carbon nanotube cellular internalization do not necessarily lead to adverse responses.
doi: 10.1002/smll.201101004

RESEARCH: Smart nanotoxicity testing for biodiversity conservation
Researchers in The Netherlands suggest that an approach based on ecological traits could be extremely helpful for interpreting nanoparticle toxicity test results and efficiently extrapolating them to living ecosystems. Ecological traits” usually refer to measurable morphological and physiological characteristics and ecological attributes of species that are comparable within and across species. Given the specific interactions between nanoparticles and organisms in ecosystems, a trait-based framework can be established that comprises environmental characteristics, particle properties, and ecological traits.

PAPER: Full assessment of fate and behavior of quantum dots in a model organism
The researchers evaluated the in vivo fate and physiological behavior of quantum dots (QDs) in Caenorhabditis elegans by GFP transfection, fluorescent imaging, synchrotron radiation based elemental imaging, and speciation techniques. The in situ metabolism and degradation of QDs in the alimentary system and long-term toxicity on reproduction are fully assessed. This work highlights the utility of the C. elegans model as a multiflexible platform to allow noninvasively imaging and monitoring in vivo consequences of engineered nanomaterials.
doi: 10.1021/nl201391e

PAPER: Interactions between magnetic nanowires and living cells: Uptake, toxicity, and degradation
The surprising result revealed in this work concerns the fate of the wires once taken up by the cells. As shown by TEM after a 24 h incubation, the cells were able to degrade the nanowires and to cut them into smaller aggregates, with typical size 200 nm. These results suggest that the degradation is likely to occur as a consequence of the internal structure of the wires which is that of a composite material characterized by noncovalent (electrostatic) forces. The researchers anticipate that in the long term, all the wires should be transformed, avoiding a potential asbestos-like toxicity effect related to high aspect ratio morphologies. From these investigations, it is concluded that the iron oxide based nanowires can be used safely with living cells, for example, as microtools for in vitro and in vivo applications.
doi: 10.1021/nn201121e

REPORT: Researchers find potentially worrisome effects of carbon nanoparticles in kidneys
A study by researchers from the schools of science and medicine at Indiana University-Purdue University Indianapolis examines the effects of carbon nanoparticles (CNPs) on living cells. This work is among the first to study concentrations of these tiny particles that are low enough to mimic the actual exposure of an ordinary individual. The research, published in the journal Nanotoxicology ("Effect of carbon nanoparticles on renal epithelial cell structure, barrier function, and protein expression"), focuses on the effect of low concentration CNP exposure on the cells that line the renal nephron, a tubular structure inside the kidney that makes urine. The investigators found the role of the CNPs in this part of the body to be significant and potentially worrisome.

PAPER: Distribution of fullerene nanomaterials between water and model biological membrane
Biological membranes are one of the important interfaces between cells and pollutants. This work presents the first quantitative study on the distribution of fullerene engineered nanomaterials between lipid bilayers, used as model biological membranes, and water. Comparisons with existing aquatic organism bioaccumulation studies suggested that the lipid bilayer-water distribution is a potential method for assessing the bioaccumulation potentials of engineered nanomaterials.
doi: 10.1002/smll.201101004
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