



# Gradient CORPORATION

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## Recent Government Briefs

### US EPA Announces Research Strategy to Study Nanomaterials

Link: [http://www.epa.gov/nanoscience/files/nanotech\\_research\\_strategy\\_final.pdf](http://www.epa.gov/nanoscience/files/nanotech_research_strategy_final.pdf)

The US EPA's Office of Research & Development recently announced the release of its revised *Nanomaterials Research Strategy*. According to a September 29<sup>th</sup> press release, the strategy aims to promote "focused research to inform nanomaterial safety decisions that may be made under the various environmental statutes for which EPA is responsible."

Research described in the report will be conducted in both US EPA laboratories and by grant recipients as part of a collaborative effort with federal organizations and the international community. The proposed research is focused on four areas of nanomaterials science and environmental remediation, including: identifying sources, fate, transport, and exposure; understanding human health and ecological effects to inform risk assessments and test methods; developing risk assessment approaches; and preventing and mitigating risks.

The draft version of the research plan, released in March 2008, was mentioned in [a previous issue of EH&S NanoNews](#).

More information about US EPA nanotechnology research can be found at:

<http://www.epa.gov/nanoscience/>

### US EPA Launches Effort Requiring Increased Reporting on Nanoscale Materials

Link: <http://www.epa.gov/oppt/nano/>

On September 29, 2009, US EPA Administrator, Lisa P. Jackson, announced a set of "core principles" that may both strengthen the existing chemical management programs within the agency, and increase efforts to address specific chemicals that "pose a risk to the public." Included in these efforts is an increase in the reporting requirements for nanoscale chemical materials. As Gradient described in [our last issue of EH&S NanoNews](#), the US EPA is also reviewing how nanoscale materials (specifically carbon nanotubes P-08-177 and P-08-328) are managed under the Toxic Substances Control Act (TSCA).

More information about the US EPA's Chemical Management Program can be found at:

<http://www.epa.gov/oppt/existingchemicals/pubs/enhanchems.html>

## Reports, Reviews, White Papers, and Books

### The Known Unknowns of Nanomaterials: Describing and Characterizing Uncertainty within Environmental, Health and Safety Risks

K. Grieger, S. Hansen, A. Baun

Link: <http://dx.doi.org/10.1080/17435390902944069>

Dr. Grieger, in collaboration with colleagues in the Department of Environmental Engineering at the Technical University of Denmark, recently reviewed 31 scientific papers examining the potential risks of nanomaterials. The review, recently published in *Nanotoxicology*, includes a qualitative uncertainty analysis of their results, reporting that there were deficiencies in all aspects of basic environmental health and safety knowledge of nanomaterials (e.g., testing procedures, instruments, effect and exposure assessments, and characterization). The authors suggest that research should be prioritized towards the assessment and development

of test procedures and equipment, as well as the full characterization of nanomaterials.

### Essential Features for Proactive Risk Management

V. Murashov, J. Howard

Link: <http://www.nature.com/nnano/journal/v4/n8/abs/nnano.2009.205.html>

A list of essential elements to effectively manage occupational health risks are proposed in an article in the August 2009 issue of *Nature Nanotechnology*. The authors, Dr. Vladimir Murashov, Special Assistant to the NIOSH Director, and Dr. John Howard, a former NIOSH Director, propose a proactive approach to health risk management based on six features: “qualitative risk assessment; the ability to adapt strategies and refine requirements; an appropriate level of precaution; global applicability; the ability to elicit voluntary cooperation by companies; and stakeholder involvement.”

The paper focuses on voluntary risk management approaches that complement existing regulations; however, the authors stress that these types of approaches may not be as protective for workers as government risk management regulations. Alternatively, they emphasize that these voluntary approaches offer a critical interim measure of protection until risks can be accurately quantified and addressed.

### Potential Health Impact of Nanoparticles

T. Xia, N. Li, A. Nel

Link: <http://arjournals.annualreviews.org/doi/abs/10.1146/annurev.publhealth.031308.100155?journalCode=publhealth>

Drs. Xia, Li, and Nel of the Division of NanoMedicine, in the Department of Medicine at UCLA, recently published a review of the health impacts of nanoparticles in the Annual Review of Public Health. They extensively reviewed the generation of reactive oxygen species, which, according to the authors, is the principal mechanism of cellular injury from nanoparticle exposure in both human and animal models. Xia and colleagues suggest that existing research in oxidant injury, such as the injury resulting from ultrafine particle exposure (e.g., diesel exhaust, quartz and mineral dust), can serve as a useful resource for understanding the possible mechanisms by which nanoparticle exposure causes injury. The authors comment that toxicology screening tests should be developed to be consistent with both the National Academy of Sciences 2007 recommendations for toxicology testing (see: [Toxicity Testing in the 21<sup>st</sup> Century: A Vision and a Strategy](#)) and the European Union REACH legislation. They argue for “a paradigm shift from a predominant observational science in animals to a screening paradigm that could be used to prioritize *in vivo* testing.”

### Review: Study of Chinese Print Workers Claims to Provide the First Human Evidence of the Clinical Toxicity of Long-term Nanoparticle Exposures

CM Long, BD Beck

Link: <http://www.internano.org/content/view/306/1/>

Drs. Chris Long and Barbara Beck of Gradient have reviewed a study of Chinese workers, published in *European Respiratory Journal*, that claims to have found a link between nanoparticle exposure and mortality due to severe respiratory effects. Due to these claims, the study has received a lot of publicity, despite having insufficient exposure data for the workers.

The abstract of the original study can be found at: <http://erj.ersjournals.com/cgi/content/abstract/34/3/559>

### Upcoming Meetings and Conferences

#### NNI Workshop: Nanomaterials and Human Health & Instrumentation, Metrology, and Analytical Methods

November 17-18, 2009, Arlington, VA (USA)

Link: <http://nano.gov/html/meetings/humanhealth/index.html>

Through a combination of presentations and breakout sessions, the National Nanotechnology Initiative’s workshop aims to bring together researchers and decision makers in order to discuss current nanotechnology issues and to identify emerging trends in nanotechnology health and safety research. Plenary sessions will cover the characterization of nanomaterials and the *in vitro* and *in vivo* interactions of engineered nanomaterials. Also, invited speaker Tom Kalil, from the White House Office of Science and Technology Policy, will present the White House’s perspectives on nanotechnology health and safety.

#### 5<sup>th</sup> International NanoRegulation Conference

November 25-26, 2009, Rapperswil, Switzerland

Link: [http://www.nanoeurope.com/wEnglisch/messen/nanoeurope/01\\_besucher/NanoRegulationW3DnavanchorW261110065.php](http://www.nanoeurope.com/wEnglisch/messen/nanoeurope/01_besucher/NanoRegulationW3DnavanchorW261110065.php)

The 5<sup>th</sup> International NanoRegulation Conference will focus on how nanotechnology information is transferred along the value chain. Topics include the global political and regulatory background of nanotechnology, stakeholder’s needs and expectations, and the information needed by nanomaterial manufacturers, processors, and consumers. Attendees will include scientists, politicians, the media, the public, and representatives from international regulatory bodies, industry,

and associations. Workshops will also focus on labeling consumer products, the information needs of downstream users and authorities, and the development of Material Safety Data Sheets for nanomaterials.

### **Webinar: Why Thinking About Small Things is Important: How Engineered Nanomaterials May Present Health & Safety Risks in Your Workplace and How to Prepare Your Business**

December 9, 2009

Link: <http://www.daypitney.com/news/news-results.aspx?type=13>

Day Pitney, LLP and Gradient will give a joint presentation on the occupational health and safety risks posed by nanomaterials and will offer solutions on how businesses can both prevent worker exposure and reduce liability. The webinar will cover the state of knowledge for engineered nanoparticles, challenges to exposure and risks assessment, potential health effects for workers exposed to nanoparticles, and what can be done to increase workplace safety. Gradient and Day Pitney will also discuss nano-specific guidance documents that can help businesses stay up-to-date on the evolving scientific understanding of nanotechnology and human health.

### **2<sup>nd</sup> NanoImpactNet Conference**

March 10-12, 2010, Lausanne, Switzerland

Link: [http://www.nanoimpactnet.eu/object\\_class/nano\\_lausanne2010conference.html](http://www.nanoimpactnet.eu/object_class/nano_lausanne2010conference.html)

Hosted by a consortium of 24 leading European academic and government laboratories, the NanoImpactNet Conference will consist of five sessions. Topics for presentations include the interactions between nanomaterials and biological, environmental, and physical barriers; quality control in nanomaterial research; tools for impact assessment; and development of policy based on research results. The conference is aimed at representatives from academia, industry, regulatory bodies, and the public.

## **Hot-off-the-Presses Peer-Reviewed Research Articles of Note**

**1. Sargent, LM; Shvedova, AA; Hubbs, AF; Salisbury, JL; Benkovic, SA; Kashon, ML; Lowry, DT; Murray, AR; Kisin, ER; Friend, S; McKinstry, KT; Battelli, L, Reynolds, SH. 2009. "Induction of Aneuploidy by Single-Walled Carbon Nanotubes." *Environ. Mol. Mutagen.* 50:708-717.**

**Abstract:** <http://www.ncbi.nlm.nih.gov/pubmed/19774611>

### **Synopsis:**

- Single-walled carbon nanotubes (SWCNT) are currently used in a variety of both consumer and industrial products, and could potentially cause respiratory health effects, particularly for workers exposed during either production of SWCNT or manufacture of products containing SWCNTs. SWCNTs can generate reactive oxygen species, and cause oxidative stress, inflammation, cytotoxicity, cell proliferation and DNA damage. SWCNTs can also enter cells, where they can bind to the cytoskeleton and to DNA. *In vivo*, exposure to SWCNTs can result in macrophages lacking nuclei and dividing macrophages connected by nanotubes. Exposure to multi-walled carbon nanotubes (MWCNTs), which are more rigid and have a larger diameter than SWCNTs, can produce micronuclei and also cause loss of whole chromosomes. Both micronuclei and loss of chromosomes can be caused by disruption of the mitotic spindle, which in turn may increase the risk of developing cancer. The objective of this study was to examine whether exposure to SWCNTs can disrupt mitotic spindles and induce aneuploidy, in human respiratory epithelial cells.
- SWCNTs, consisting of 99% elemental carbon and 0.23% iron, were generated using the high pressure carbon monoxide disproportionation process. The ability of SWCNTs to disrupt mitotic spindles was evaluated in immortalized human bronchial epithelial cells (BEAS-2B); and ability of SWCNTs to induce aneuploidy was evaluated in primary human respiratory epithelial cells (SAEC) isolated from the small airways of a normal human donor. The BEAS-2B and SAEC cells were exposed in parallel to either SWCNTs, or to the spindle poison vanadium pentoxide, as a positive control. Vanadium pentoxide fragments the centrosome and inhibits microtubule assembly, which can disrupt mitotic spindles and induce aneuploidy. Both SWCNTs and vanadium pentoxide were suspended in cell culture media and sonicated over ice. Both cell types were exposed in parallel for 24 hours to 24, 48 or 96  $\mu\text{g}/\text{cm}^2$  SWCNTs; or 0.031, 0.31 or 3.1  $\mu\text{g}/\text{cm}^2$  vanadium pentoxide.
- Following exposure, cells were assessed for viability, apoptosis, morphology of the mitotic spindle and centrosome, and number of chromosomes. Mitotic spindles and centrosome number were detected using dual-labeled immunofluorescence for tubulin and centrin. Morphology of the mitotic spindle and centrosome, and their relationship with aggregated SWCNTs (referred to as carbon nanoropes) in the BEAS-2B immortalized cells were visualized using laser scanning confocal microscopy. Centrosome integrity in the SAEC cells was visualized using transmission electron microscopy. Aneuploidy in the SAEC cells was assessed using fluorescence *in situ* hybridization (FISH) for human chromosomes 1 and 4.

- Viability of the BEAS-2B cells was reduced in a dose-dependent manner at all three SWCNT treatment levels, with statistically significant reductions at the two higher treatment levels (48  $\mu\text{g}/\text{cm}^2$  and 96  $\mu\text{g}/\text{cm}^2$ ). In the SAEC cells, viability at the 24  $\mu\text{g}/\text{cm}^2$  SWCNT treatment level was comparable to control viability, with statistically significant reductions in viability at the two higher treatment levels. SWCNTs had a greater effect on viability in the BEAS-2B cells than in the SAEC cells. In contrast, vanadium pentoxide did not significantly affect viability of the BEAS-2B cells, but reduced viability by more than 50% in the SAEC cells. There was no evidence that SWCNTs or vanadium pentoxide induced apoptosis in either cell type.
- There were treatment-related increases in mitotic spindle abnormalities in the BEAS-2B cells, and treatment-related increases in aneuploidy in the SAEC cells, with statistically significant increases at all SWCNT treatment levels. SWCNTs displaced portions of the mitotic spindle, and were observed in the bridge between the dividing daughter cells and associated with the DNA. Treatment-related increases in centrosome fragmentation were observed in both BEAS-2B and SAEC cells, with statistically significant increases at all treatment levels. SWCNTs were also observed in association with the centrosome.

#### Implications:

- This study demonstrates that SWCNTs suspended in an aqueous media can enter respiratory epithelial cells and become associated with the mitotic spindle, centrosomes, and DNA. SWCNTs can also induce disruptions of the mitotic spindle, centrosome fragmentation and aneuploidy. These are all characteristics of cancer cells, thus indicating that SWCNTs may have carcinogenic potential. While providing suggestive evidence that SWCNTs may have carcinogenic potential, definitive evidence that SWCNTs can cause lung cancer requires evaluation in long-term *in vivo* studies.
- It is important to consider potential *in vitro/in vivo* differences, in terms of behavior and characteristics of both the SWCNTs and the cellular environment. Specifically, the SWCNTs may behave differently, and hence interact differently with the cells, in an aqueous cell culture media vs. in the more viscous mucous lining of the respiratory tract. Because the SWCNT surface is hydrophobic, SWCNTs form bundles in aqueous environments, and the formation of such bundles may occur to a different degree in the respiratory epithelial mucous lining. The viscous nature of the mucous might also limit access of the SWCNTs to the epithelial cells.
- This study also demonstrated that the two cell types differed in their response to the SWCNTs and the vanadium pentoxide. Whereas the BEAS-2B immortalized cells were more sensitive, in terms of reduced viability, to the SWCNTs, the primary human epithelial cells were more sensitive to the vanadium pentoxide. This highlights the difficulty in drawing conclusions regarding potential effects of nanoparticles based on results from individual studies.

#### 2. Domingos, RF; Baalousha, MA; Ju-Nam, Y; Marcia Reid, M; Tufenkji, N; Lead, JR; Leppard, GG; Wilkinson, KJ. 2009. "Characterizing Manufactured Nanoparticles in the Environment: Multimethod Determination of Particle Sizes." *Environ. Sci. Technol.* 43(19):7277–7284.

**Abstract:** <http://pubs.acs.org/doi/abs/10.1021/es900249m>

#### Synopsis:

- The characteristics of nanoparticles, including their size, structure and surface chemistry (e.g., charge), are important for assessing their fate, transport, and interaction with biological systems in the environment. In the environment, nanoparticles undergo dynamic changes in particle size and surface properties due to aggregation and dissolution. Determination of the "true" size of nanoparticles is therefore a challenging exercise due to: (1) changes in particle sizes in different environments (e.g., solution vs. aerosol); and (2) the effects of sample concentration and solution chemistry. In addition, differences in the fundamental principles of the various analytical techniques may also introduce variability in the reported particle sizes.
- Most researchers and nanoparticle manufacturers use state-of-the-art analytical techniques such as Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and Fluorescence Correlation Spectroscopy (FCS) to report "true" particle sizes. Unfortunately, the use of a single technique under a set of specific analytical conditions (e.g., acidic pH, low concentrations) may introduce a bias in the results. For example, nanoparticle sizes measured using the DLS technique may be skewed in the presence of dust, as the measurement intensity is proportional to the 6<sup>th</sup> power of the particle size. In order to obtain a useful picture of the overall properties of nanoparticles, as well as the magnitude of their variability, it is essential to accurately determine the particle size, and thereby the characteristics of both single discrete and large assemblies of nanoparticles, using more than one analytical technique.
- The objective of this study was to determine the particle size of nano – TiO<sub>2</sub>, ZnO and quantum dots (CdSe) using 6 different analytical techniques: (1) Transmission Electron Microscopy (TEM); (2) Dynamic Light Scattering (DLS); (3) Fluorescence Correlation Spectroscopy (FCS); (4) Atomic Force Microscopy (AFM); (5) Nanoparticle Tracking

Analysis (NTA); and (6) Flow Field Flow Fractionation (FFF). The results from each analytical technique under specific conditions were compared and the advantages/disadvantages were discussed.

- For the size determination experiments, metal oxide solutions of 1, 30 and 100 mg/L were prepared using TiO<sub>2</sub> and ZnO. The manufacturer-specified sizes of TiO<sub>2</sub> and ZnO are 5 and 20 nm, respectively. A quantum dot (QD; 6 to 10 nm) solution of 1.92 µg/L was also prepared. Small amounts of fulvic acid were added to most samples to: (1) test the ability of the analytical technique to distinguish background (*i.e.*, fulvic acid) *versus* the nanoparticles; and (2) minimize aggregation of the particles. Sodium nitrate (10 mM) was used as the background electrolyte. The pH of nanoparticle solutions was maintained at either 4.0 or 8.0. For each analytical technique, the sample preparation followed specific protocols commonly used for nanoparticle size measurements. Special care was taken to minimize the effect of contamination and to prevent aggregation of particles during sample preparation.
- Size determinations using the TEM technique revealed some disadvantages in the sample preparation and analytical techniques. Sample preparation using air-drying of particle solutions and inorganic salts (*i.e.*, sodium nitrate) created some artifacts in the analysis, thus making it difficult to distinguish among the actual nanoparticles, impurities, and inorganic salts. The effects of high solution pH and concentration were also pronounced, as the formation of larger aggregates made it difficult to distinguish between individual smaller particles. Particle sizes for the oxide particles (*i.e.*, TiO<sub>2</sub> and ZnO) measured using TEM were almost 2-fold higher than the manufacturer's specifications. The size of QD particles measured using TEM were within the manufacturer's specifications.
- Size analysis for metal oxide particles using FCS was similar to that using the TEM technique. However, measured particle sizes of the QD were approximately one order of magnitude higher than the manufacturer specified value of 6 – 10 nm. Due to the photophysical properties of the QDs, single particle detection measurements (under fluorescent light) are episodic (*i.e.*, measurements are not based on long periods of time), resulting in a bias in the size measurement of QD particles.
- Absorption of nanoparticles to the mica substrate is essential for size measurement using AFM. Metal oxide particles were not detected using the AFM technique in the presence of fulvic acid, suggesting that the metal oxide nanoparticles were not absorbed to the substrate material. In select cases (*i.e.*, low pH), small amounts of particles were detected, but only in the presence of much larger aggregates. Size analysis of QDs using AFM was more effective. Similar to the TEM analysis, the size of QD particles measured using AFM was within manufacturer

specifications.

- In general, nanoparticle particle sizes measured using the DLS, FFF and NTA techniques were one to three orders of magnitude higher than manufacturer specifications largely due to: (1) onset of sedimentation; (2) bias in the tracking of particles (*i.e.*, larger particles are tracked more frequently than smaller particles); and (3) differences in intensity measurements (*e.g.*, measurement intensities of the smaller particles are masked by those of the larger particles).

#### Implications:

- Measured particle sizes are expected to vary with analytical technique and sample conditions. For most particles, aggregation was a function of the particle concentration, pH, and ionic strength of the solution. Conforming to coagulation theory, larger particles are observed at: (1) high particle concentration; (2) high pH; and (3) greater sample preparation (*i.e.*, equilibration) time. The presence of naturally occurring material such as fulvic acid also affected particle aggregation.
- Each analytical technique has its own advantages and disadvantages. Polydispersity (*i.e.*, presence of both small and large particles) induced a large bias in flow-based measuring techniques, as seen with FFF, DLS and NTA analyses. Even though these techniques are simpler to use, accurate size measurement were not generally possible due to sample aggregation. The use of smaller particle concentrations (*i.e.*, 1 mg/L) to circumvent aggregation issues does not provide accurate results due to low measurement intensity. Other techniques such as FCS and AFM are able to provide size measurements at low concentrations, with fewer aggregation issues compared to flow-based analytical techniques. However, they may not be suitable for size analysis of select nanoparticles, either due to their absorptive characteristics (*e.g.*, metal oxide particles on mica) or photophysical properties (*e.g.*, QDs).
- In general, the measured individual particle sizes using the various analytical techniques were higher than those reported by the manufacturer. The particle size measured by each analytical technique was compared to the size specified by the manufacturer. In general, the measured particle size varied in the order: TEM ≈ AFM < FCS ≈ FFFF < DLS < NTA, with particle size measured using TEM and AFM similar to manufacturer specified size. Theoretically, the particle sizes should be similar for all monodisperse, or single size, particle suspensions using any analytical technique. However, flow – based analytical techniques such as DLS and FFF – suffer from analytical accuracy due to dynamic particle aggregation in solution.
- Overall, engineered nanoparticles present particular characterization challenges because of their small size, complex structure, reactivity, time-dynamic

nature, and sensitivity to environmental matrices. This study highlights several challenges in accurate size measurement of nanoparticles. Even though some of the analytical techniques may be well established for a certain class of nanoparticles (e.g., AFM for QDs) and under certain measurement conditions, it is not yet possible to develop general guidelines for all types of nanoparticles. Since each analytical technique has its own advantages and disadvantages, it is recommended to use more than one analytical technique such as TEM, AFM or FCS to improve accuracy of the measurements. Particle size analysis using flow – based techniques (i.e., DLS, FFF) should be confirmed with the use of non – flow based techniques (i.e., TEM, AFM).

## Guest Contributor

By Robin Juni, Jones Day\*

### Development of Testing Protocols Key to Managing Nanotechnology Litigation Risk

Nanotechnology is currently being utilized in hundreds of products, with more coming on the market every day. However, few companies have comprehensively evaluated the risks that nanomaterials may present, and even fewer have addressed the potential for litigation surrounding alleged harms from products containing nanomaterials. It is important to be aware that significant litigation risk exists for companies not prepared to understand the underlying science and address the law of alleged harms stemming from nanomaterials.

One significant class of potential claims facing nanotechnology manufacturers, which has received little attention to date, is failure-to-warn claims under product liability law. Such claims seek to hold product manufacturers liable for “failure to warn” of risks – or even potential risks – that would have been identified had additional research been conducted. In other words, liability could be imposed even in the absence of information showing any likely harm, because a manufacturer failed to conduct research on a “reasonably foreseeable” risk and subsequently transmit relevant results to the consumer to be weighed in his or her purchasing decision.

A recent collaboration between the Food and Drug Administration (FDA) and the Alliance for NanoHealth (ANH), a consortium of health research organizations in and around Houston, Texas, may contribute to development of standards for research in nanotechnology development and, as such, bears careful watching. See, for example, 72 Fed. Reg. 10,927 (March 13, 2009) which notes that FDA and ANH will work together to assist in “[m]oving nanoengineered medical products from the preclinical stages of development through clinical stages and then to commercialization...” (see also <http://www.nanohealthalliance.org>).

Among other efforts, FDA and ANH expect, under the Memorandum of Understanding (“MOU”) entered among the parties, to implement programs that facilitate “[d]evelopment and validation of standards, risk/benefit analyses and other evaluative tools to identify risks and assess safety and efficacy in newly emerging nanoengineered products” (72 Fed. Reg. at 10930). Particularly since FDA is involved, such “tools” are precisely the sorts of metrics that might be adopted as “industry standards” for nanomaterials research and thus implicate liability risk for a manufacturer who does not follow the recommended practices, through ignorance or otherwise. In addition, formal regulatory guidance or binding administrative rules ultimately may stem from such endeavors.

Accordingly, at least in the medical product field, guidance is beginning to be developed regarding the sorts of testing that can (and, it may be argued, should) be performed before a device using nanomaterials is placed on the market. Attention should be paid to this and similar efforts, and early involvement in shaping the standards being created may be beneficial.

*\*Robin Juni is an attorney in the Washington, D.C. office of the international law firm Jones Day, where she focuses her practice on environmental and product liability matters, particularly those involving significant scientific issues. She received an undergraduate degree in physics from Hamline University and a J.D. from Harvard Law School. She can be reached at [rjuni@jonesday.com](mailto:rjuni@jonesday.com) or (202) 879-3850. The views expressed in this article are those of the author and not necessarily those of Jones Day or its Clients.*

## Coming In the Next Issue

Review of an inhalation exposure study of rats exposed to MWCNTs.

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