The Non-Government Organization (NGO) Perspective

On Nanomaterial Safety Testing and Policy Participation

Samantha Dozier, Ph.D.
Nanomaterials Policy Advisor
People for the Ethical Treatment of Animals

NGO Perspective and Issues

- Dialogue Across Stakeholder Sectors - Regulators, Industry, Academia, and NGOs
- Transparency/Accessibility of Information
- Safety Testing
- Protocol Sharing
- Precautionary Principle
- The "Nano Risk Framework"
- Voluntary Reporting Schemes
- Possible Dangers of Nanomaterials

PETA

Dialogue Across Stakeholder Sectors

Good practices

Requests for comments/input by various regulatory bodies

Willingness of regulators to meet with NGO representatives with relevant expertise

Public meetings with meaningful, open dialogue

Practices that need improvement

Transparency in regulatory agency expectations for safety testing

Academic publications that include authors that cross stakeholder sectors

Upholding EC 86/609 and making industry/researchers aware of regulatory preference for sensitive, reproducible, non-animal methods for testing nanomaterials

PETA

Transparency & Access to Information

Regulatory agencies should share data submissions (without disclosing confidential business information) and the types of tests required for market approval.

A database of nanomaterial-containing products and the tests that were performed to gain regulatory acceptance should be publicly available.

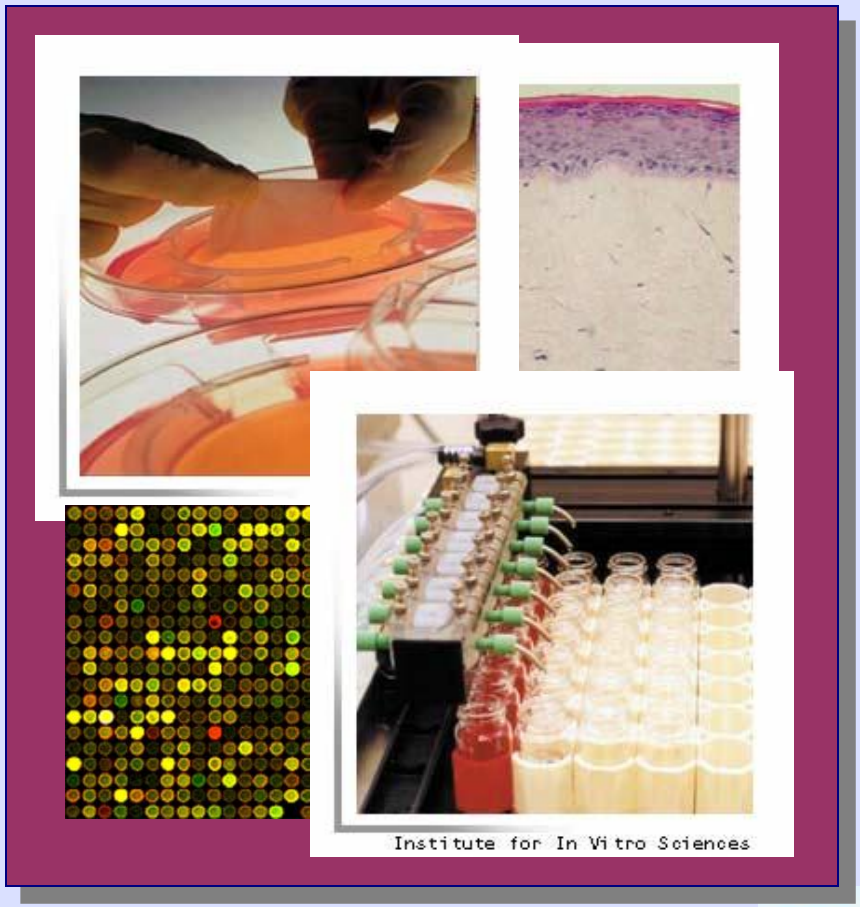
Until specific guidance is available, regulators should specifically encourage data submissions from the most modern, human-relevant test methods and discourage animal tests for this burgeoning field

PETA

Best Practices to Ensure Human Safety

Human-relevant, high-throughput methods are the most reliable and reproducible.

- Cell culture, microarray, lab-on-a-chip platforms and cell-based toxicity assays allow testing of up to 100,000 compounds a day.
- Controlled experimental conditions lead to less experimental variability
- Tests on human cells lead to human-relevant answers and treatments



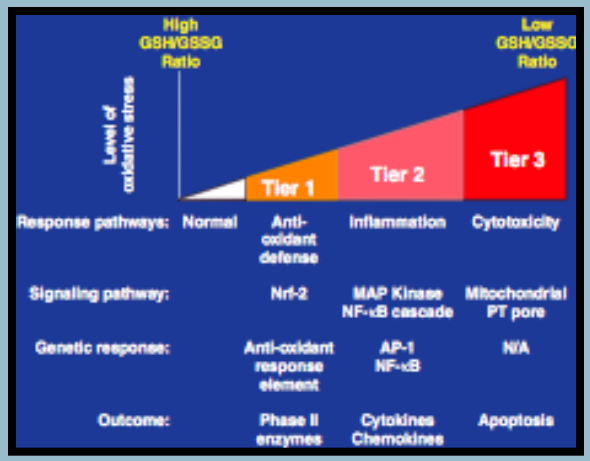
PETA

Cell-Based Assays Are Well-Suited for Nanomaterials

- Measurement of: reactive oxygen species (ROS), mitochondrial perturbation, membrane integrity, DNA and protein damage, glutathione production, and lipid peroxidation, etc. offer a glimpse into pre-apoptotic cellular distress signals and provide a set of early markers that *in vivo* animal experiments cannot offer
- Use of specialized target cell-lines assay effects of particle exposure
- Human cell-based assays avoid animal-specific artifacts that cause imprecise/incorrect interpretation of data for human health protection

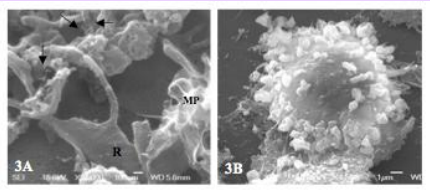
The ultimate goal of the predictive approach to toxicity testing "would be to develop a series of toxicity assays that can limit the demand for *in vivo* studies, both from a cost perspective as well as an animal use perspective"

From Nel *et al*. 2006. Science 311; 622-627



PETA

Cell Lines Focusing on Traditional Routes of Exposure are Used to Study the Toxicity of Nanoparticles



EM of lung epithelial cells contacting nanoparticles. From Panessa-Warren *et al*. 2006

Exposure routes:

- Colon cells (Caco-2) (e.g. Panessa-Warren *et al.* 2006)
- Lung epithelial cells (e.g. Panessa-Warren *et al.* 2006)
- Dermal fibroblasts (e.g. Sayes *et al.* 2005)

Movement through the system:

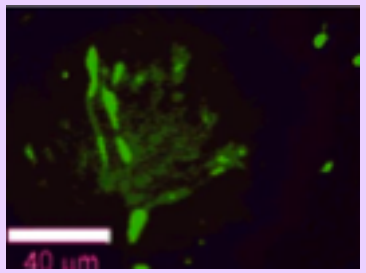
- Translocation through blood-brain barrier (e.g. Bourgougnon *et al.* 2006) or through the lung epithelia (e.g. Geys *et al.* 2006)

Target effects:

- Brain (astrocytes) (e.g. Sayes *et al.* 2005)
- Immune system: Macrophages (e.g. Porter *et al.* 2007); T-cells (e.g. Bottini *et al.* 2006)
- Liver cells (e.g. Sayes *et al.* 2005)
- Kidney cells (e.g. Cui *et al.* 2005)

Diseased tissues

- Liver carcinoma lines (e.g. Sayes *et al.* 2005)
- Human B2-microglobulin (e.g. Linse *et al.* 2007)
- Lung cancer cells (e.g. Magrez *et al.* 2006)



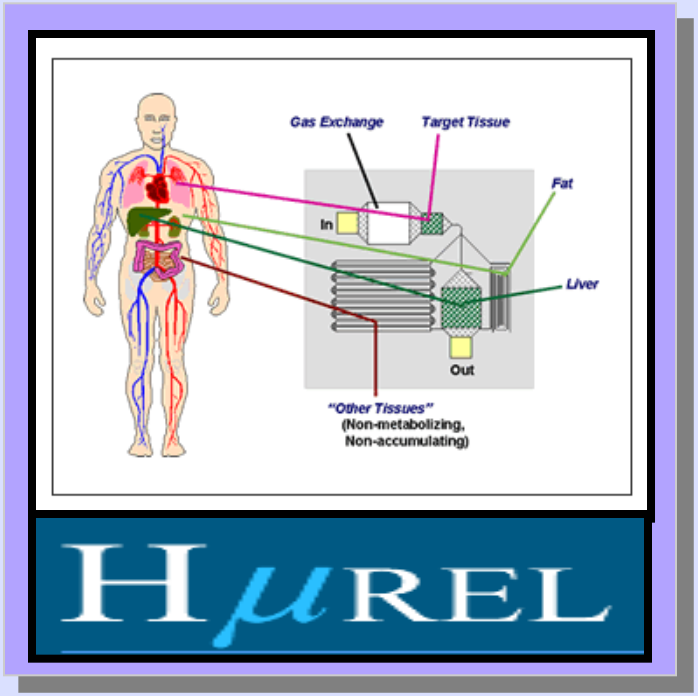
HDF cells dosed with 10 ppm Nano C_{60}. From Sayes *et al*. 2005.

PETA

Answering Questions Pertaining to Targeting and Toxicity in a Human-Relevant Manner

The advent and cost-effective availability of lab-on-a-chip platforms has allowed these questions to be answered.

The HuREL (Human Relevant) Platform allows testing of three different human cell-types linked via microfluidics so that questions pertaining to targeting and/or toxicity can be answered.



✔ **Compatible with plate readers and other traditional laboratory equipment.**

✔ **HuREL chips can be automated for increased throughput**

Details can be read in: Sin *et al. 2004. Biotechnology Progress: 20; 338-45.*

Or, seen on HuRELcorp.com

PETA

Using the Most Sensitive Species for Ecotoxicity Testing

Ecotoxicity testing focusing on relevant and sensitive plant, bacterial, algal, insect and worm species gives scientists an indication of how nanoparticles might affect the basis of ecosystems.

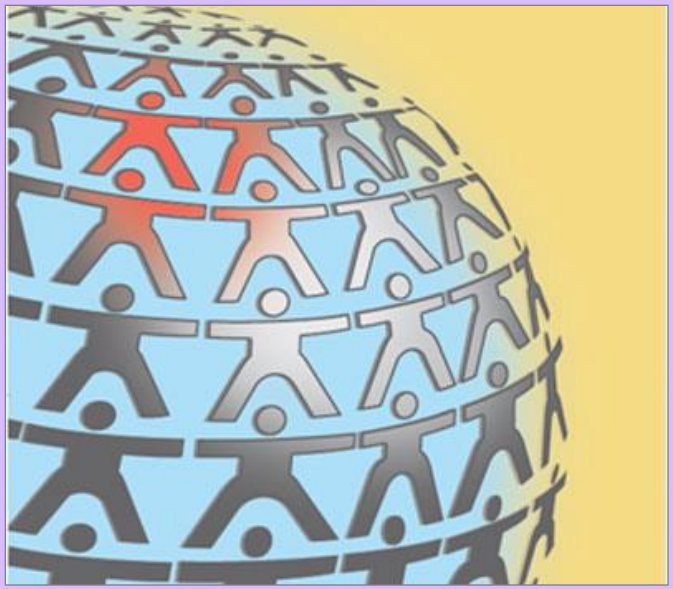
Because of the importance of these species in ecosystems, their reduced viability could be sufficient information to classify a nanoparticle as toxic to the environment.

•Velzeboer I,et al. Aquatic Ecotoxicity Tests of Some Nanomaterials. Environ Toxicol Chem. 2008 Apr 1:1.
•Hund-Rinke K, Simon M. Ecotoxic effect of photocatalytic active nanoparticles (TiO2) on algae and daphnids. Environ Sci Pollut Res Int. 2006 Jul;13(4):225-32.
•Bermúdez-Saldaña JM, et al.. Modelling bioconcentration of pesticides in fish using biopartitioning micellar chromatography. J Chromatogr A. 2005 Jan 21;1063(1-2):153-60.
•Weisbrod AV,et al. The State of In Vitro Science for Use in Bioaccumulation Assessments for Fish. Environ Toxicol Chem. 2008 Aug 21:1.

PETA

Protocol Sharing

Transparency and best-practices should be shared throughout industry and stakeholders



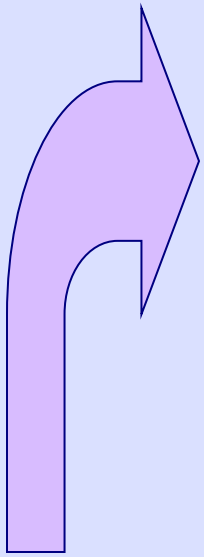
A database of the best general protocols (that would not conflict with CBI) should be shared

The Precautionary Principle and Nanotechnology

What is the Precautionary Principle?

- Taking precaution in the face of scientific uncertainty;
- Exploring alternatives to possibly harmful actions;
- Placing the burden of proof on proponents of an activity rather than on victims or potential victims of the activity;
- And using democratic processes to carry out and enforce the principle-including the public right to informed consent.

So, what does this mean from our perspective?



Use the most human-relevant, sensitive assays at our disposal! (for nanomaterials, these are cell-based assays).

Considering animals (as well as humans) as the potential victims of toxicity testing and seeking the best, humane ways to test nanomaterials

PETA

What Does the Precautionary Principle Really Mean

&

How Should it be Practiced for the Field of Nanotechnology?

- The most sensitive assays available/capable of detecting human health effects should be employed.
- Assays should be relevant to human rather than animal health
- Experiments on animals have shown that these assays often miss potential human-relevant health effects and are unable to show warning signs of health effects and instead rely on gross morphological changes.

PETA

Major Concerns with the "Nano Risk Framework"

Nano-Scaled Stakeholder participation

*One call for comments in a few trade news outlets

*After one round of comments, the Framework was published with very minimal changes

*Participating reviewers requested additional drafts and future opportunities to comment -- this did not happen.

*The purpose of this project was not clear -- was it DuPont's own internal guidance or is it prescriptive for others? Although it now appears that it is the latter, the objective for the project was not known and was a common question that commenters posed.

PETA

Major Concerns with the "Nano Risk Framework"

(continued)

The Nano Risk Framework is devoid of meaningful tiers

The first tests listed in the framework are animal tests -- **this is not tiered testing!**

*****The first tests listed (animal tests) are considered by most toxicity testing programs to be those reserved for the final steps of product testing -- if they are done at all.

***** The first tests in the Framework are not those tests that allow scientists to quickly screen and make reliable, cost-effective, and fast decisions about toxic potential.

***** The Framework does not allow researchers to efficiently use time and resources and does not save animal lives

PETA

Major Concerns with the "Nano Risk Framework"

(continued)

The Framework fails to use available *in vitro* test methods

* Many of the tests in the Framework's exhaustive list of animal tests were developed nearly a century ago and in no way represent modern, reliable test methods

* Framework authors refer to unvalidated animal tests as the preferred methods for nanotoxicity testing while stating that *in vitro* methods should be validated for nanomaterial use prior to incorporation into testing schematics.

* Results from *in vitro* methods have illustrated their greater sensitivity and predictive potential

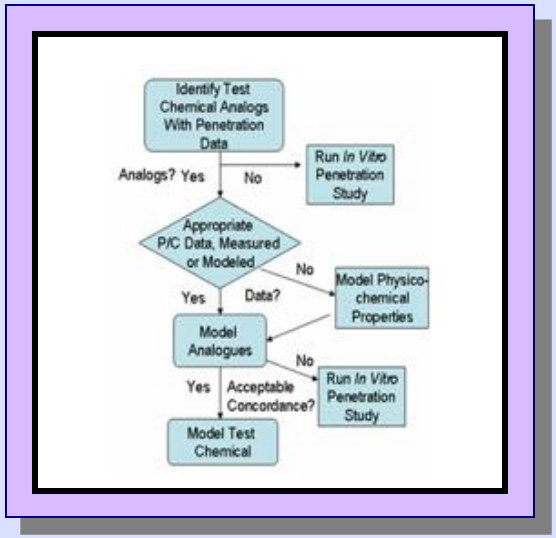
PETA

The Benefits of Meaningful Tiers

Tiered testing saves time, money, and animal lives and offers a logical decision-tree strategy which will allow the most promising products to move forward.

Regulators in the EU and US are encouraging tiered testing based on *in vitro* methods both for reasons of human relevance and animal welfare.

Tiered testing is the most efficient manner in which to develop and test new products.



The Pitfalls of Voluntary Testing Programs

- Lack of participation from industry and academia
- Researchers using old-fashioned test methods to test materials that have proven to be hard to detect in animals

→ Leading to inaccurate results in regulatory databases

- Lack of standardized assays results in useless information and sets the default testing methods to the lowest common denominator for those submitted

PETA

How Then Do We Test Nanomaterials to Best Ensure Safety?

- Employ the most relevant assays at our disposal -- human cells
- Follow a decision-tree/tiered testing schematic -- using *in vitro* screening and testing methods
- If the nanomaterial appears cytotoxic/ecotoxic in these tests → **STOP development**
- Protecting humans and the environment from nanomaterials should not mean testing these advanced chemicals in the most old-fashioned assays
- Animal testing has failed to protect humans and the environment from pharmaceuticals and industrial chemicals. Toxic chemicals continue to pollute our environment and harmful drugs are continually recalled from the market. Animal testing will continue to be a losing paradigm.

PETA

Nanomaterials: Dangers on the Macro-Scale?

NGOs advocate for the safety and well-being of humans and animals

- in that way, we should be united in bringing the best and most predictive testing methods to the field of nanotechnology
- in the few cases where we have epidemiological, human-cell-based data, and animal data -- where we can compare the results of each -- it has been shown that animal data has not predicted the health effects of nanomaterials.
- we hope to work with all stakeholders in putting together a scientifically-valid testing schematic for nanomaterials that takes their unique properties into consideration

PETA

Conclusions

- Societal concerns about nanotoxicity provide a ripe opportunity for the development of non-animal methods which are:
  - *Ethical
  - * Reliable
  - * Cheaper/faster
  - * Better suited to answer research questions

- Nanotoxicity testing provides us with a unique opportunity to
  - * use these modern methods
  - * promote their use in other applications
  - * change the paradigm of toxicity testing

- NGOs support non-animal methods and can help in their promotion

PETA

Thank You!

Special Thanks to NanoImpactNet Organisers for the invitation to present

SamanthaD@PETA.org

PETA US: 501 Front St., Norfolk, VA 23510, USA

PETA Europe: Lacon House, Theobald's Rd., London WC1X SRW, UK

Studies & Breakthroughs for Nanotoxicity Testing

Simultaneous structural measurement and chemical identification is now possible at the femtogram level. The combination of atomic force level resolution and the chemical identification of infrared spectroscopy.

•Park K, Lee J, Bhargava R, King WP., *Routine Femtogram-Level Chemical Analyses Using Vibrational Spectroscopy and Self-Cleaning Scanning Probe Microscopy Tips*. Anal Chem. 2008 Mar 27.

Studies show that a variety of nanomaterials are toxic to human cells:

•Monteiro-Riviere NA, Nemanich RJ, Inman AO, Wang YY, Riviere JE. Multi-walled carbon nanotube interactions with human epidermal keratinocytes. Toxicol Lett. 2005;155:377–384. doi: 10.1016/j.toxlet.2004.11.004.

•Monteiro-Riviere NA, Wang YY, Hong SM, Inman AO, Nemanich RJ, Tan J, et al. Proteomic analysis of nanooparticle exposure in human keratonicyte cell culture. The Toxicologist. 2005;84:2183.

•Shvedova AA, Castranova V, Kisin ER, Schwegler-Berry D, Murray AR, Gandelsman VZ, et al. Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. J Toxicol Environ Health A. 2003;66:1909–1926.

•Sayes CM, Gobin AM, Ausman KD, Mendez J, West JL, Colvin VL. Nano-C(60) cytotoxicity is due to lipid peroxidation. Biomaterials. 2005;26:7587–7595. doi: 10.1016/j.biomaterials.2005.05.027.

•Hetland RB, Cassee FR, Refsnes M, Schwarze PE, Lag M, Boere AJ, et al. Release of inflammatory cytokines, cell toxicity and apoptosis in epithelial lung cells after exposure to ambient air particles of different size fractions. Toxicol In Vitro. 2004;18:203–212. doi: 10.1016/S0887-2333(03)00142-5.

•Gurr JR, Wang AS, Chen CH, Jan KY. (2005) Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology 213:66–73.

Additional Test Methods of Interest:

•Particokinetics In Vitro: Dosimetry Considerations for In Vitro Nanoparticle Toxicity Assessments.

• J. G. Teeguarden, P. M. Hinderliter, G. Orr, B. D. Thrall, and J. G. Pounds (2007)Toxicol. Sci. 95, 300-312

•Nel A, Xia T, Madler L, Li N. (2006) Toxic potential of materials at the nanolevel. Science 311:622–627.

•Sin, A., et al., *The design and fabrication of three-chamber microscale cell culture analog devices with integrated dissolved oxygen sensors.* Biotechnol Prog, 2004. 20(1): p. 338-45.

PETA

Relevant Non-Animal OECD Test Guidelines Applicable to Nanomaterials

Environmental Hazard Data:
Microorganism Respiratory Inhibition/N-Transformation: OECD Test Guidelines209, 216
Algae Growth Inhibition: OECD Test Guideline 201
Daphnia Reproduction Test: OECD Test Guideline 211
Earthworm Acute Toxicity: OECD Test Guideline 207

Health Hazard Data:
Skin sensitization/irritation: OECD Test Guideline 429 (LLNA) is a partial replacement for the guinea pig tests often used to test skin sensitization and that EPISKIN™-SIT is a complete ECVAM-validated replacement for *in vivo* skin irritation tests for skin irritation.
Skin penetration: The *In Vitro* Skin Absorption Test is a full replacement for the *in vivo* skin penetration test under OECD TG 428.
Genotoxicity: The Ames test for bacterial mutation (OECD TG 471), testing for mutagenicity by *in vitro* chromosomal aberration (OECD TG 473), Unscheduled DNA synthesis (OECD 482), Sister Chromatid Exchange (OECD 479) each of which can be a partial or full replacement for *in vivo* methods. Negative results for these assays preclude the use of additional *in vivo* test confirmation.
Skin Corrosivity: Using CORROSITEX (OECD TG 435) as a full replacement for the *in vivo* skin corrosion test for acids, bases, and acid derivatives. EpiDerm and EPISKIN each serve as full replacements for the *in vivo* skin corrosion test (OECD TG 431).
Phototoxicity: The 3T3 Neutral Red Uptake Test is considered a replacement for the *in vivo* photoirritation tests (OECD TG 432) and has gained FDA preference.
Ocular Corneal Opacity-Permeability Test: ICCVAM and EU approved for use as a positive screen for ocular corrosivity and severe irritation and is acceptable for use based on the sequential testing strategy supplement to OECD TG 405.
Acute Neutropenia: In order to predict chemical-induced neutropenia, the CFU-MG assay has been validated for use by ECVAM and endorsed by ESAC.
Pyrogenicity: The PBMC/IL-6, WB/IL-1, CryoWB/IL-1, WB/IL-6, and MM6/IL-6 tests should be considered as replacements for the rabbit and LAL tests.
Embryotoxicity: Partial replacements for the *in vivo* developmental toxicity test exist and include tests such as: Embryonic Stem Cell Test, Rat Limb Bud Test, and Micromass Test.

PETA