Recent innovations in science and technology have allowed the development of human-relevant, predictive, non-animal assays for many biological endpoints. Since statutory requirements do not yet exist for nanomaterials testing, there is ample opportunity for scientists to seek out and use the best science available. Many in vitro models can out-perform the animal-based toxicity methods that have traditionally been used in the field of toxicology. Animal models are not only inhumane, but lack predictive value with regard to human responses in many cases.

For example, FDA figures show a 92% failure rate for drugs that pass preclinical trials which are based on animal experiments. The field of nanotoxicology can and should avoid this approach and incorporate the most recent science-based technology. This review examines many promising methods currently available to test for nanomaterial safety.

**Physical and Cells Relevant to Oxidative Stress**

Due to their unique properties, many nanomaterials cross cellular membranes and interact within DNA or damage proteins. Once the portal of entry for a specific nanomaterial is determined, subcellular cells from tissues can be assessed for DNA or protein damage. Several human cell-based assays exist that can be used to characterize genotoxic potential of nanomaterials.

**Methods currently available to test for nanomaterial safety.** For example, FDA figures show a 92% failure rate for drugs that pass preclinical trials which are based on animal experiments. The field of nanotoxicology can and should avoid this approach and incorporate the most recent science-based technology. This review examines many promising methods currently available to test for nanomaterial safety.

**Available In Vitro Methods for Nanotoxicology**

**Screening for Genotoxicity & Protein Damage**

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**In Vitro Methods Useful for Nanomaterials**

- Comet assay
- In vitro chromosomal aberration
- In vitro micronucleus assay
- In vitro DNA synthesis
- Apoptosis
- Chondroclast Exchange
- TUNEL

Each of these can be a partial or full requirement of an in vitro methodology. Negative results for these assays preclude the use of additional in vivo test confirmation.

**Additional Relevant References**


**Physical & Chemical Characterization**

**Oxidative Stress & Cytotoxicity in Cells Relevant to Portal of Entry**

Several methods exist for assessing activity of nanomaterials using cell types relevant to portal of entry. Cell-type specific assays exist that can measure cytotoxicity as well as gene expression, genotoxicity, or apoptosis.

**Assessing Uptake: Nucleus & Reticuloendothelial Systems**

Concerns about cellular uptake of nanoparticles related to human health as well as environmental safety has prompted many labs to assess the proclivity of nanomaterials to cross cell membranes into endocytic compartments and the nucleus. In order to determine potential risks from nanomaterials, it is critical to determine mode of exposure and assess whether a given nanoparticle will cross cell barriers.

**Inflammation and Human Blood Component Effects**

Monitoring the inflammatory effects of nanomaterials on in vitro human cell types is valuable for the prediction and assessment of the potential toxicities of nanomaterials. Resulting data are analyzed in order to predict a given nanomaterial’s proclivity for toxicity and to glean the mechanism of chemical action. The use of human-relevant cell types is of utmost importance as these cell types are comprised of human-specific binding sites and avoid the need for species-specific extrapolation.

**Model for Tiered Nanotoxicity Screening**

Advances in high-tech, analytical methods allow for human-relevant toxicity testing without relying on animals. Electron microscopy and cell culture coupled with diagnostic assays now afford scientists the ability to assess cells/organelles/DNA during toxicological studies and measure the chemical changes in these structures caused by a given nanochemical. The precision with which we are now able to monitor the cell’s cyclic changes over time is a more sophisticated diagnostic tool than animal-based toxicological experiments. It is imperative from a human and environmental safety standpoint that this field be built on a foundation of rigorous and predictive in vitro assays.