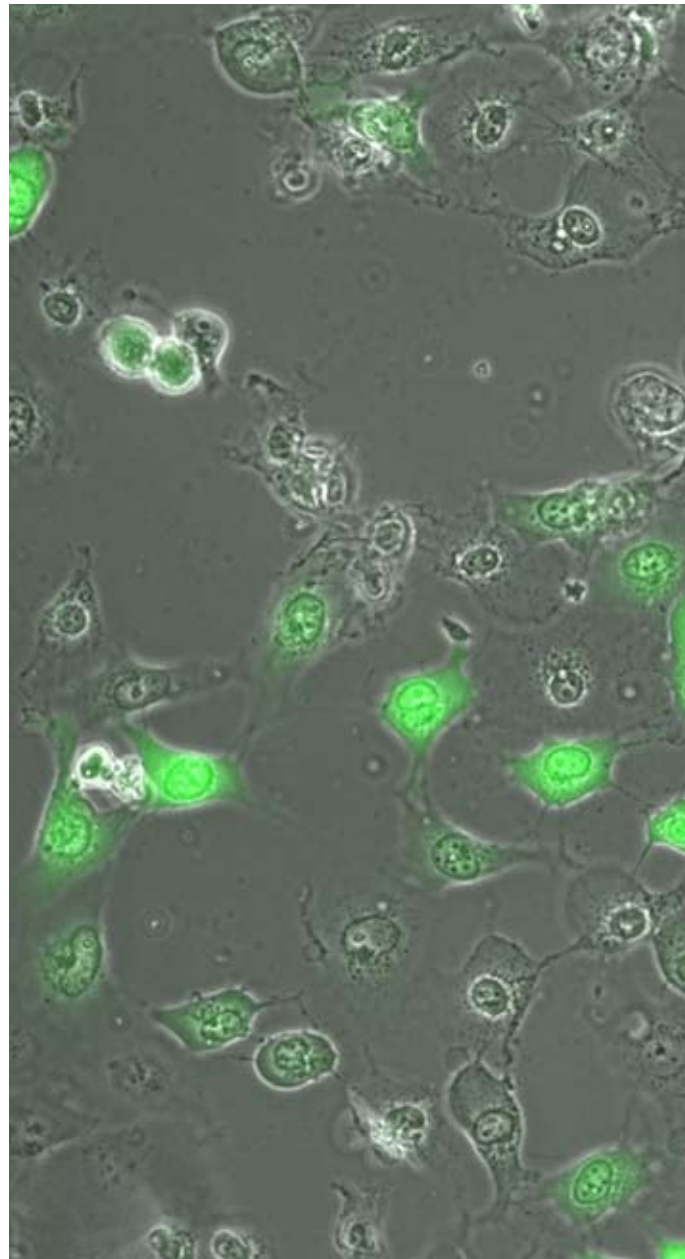


Assessment
of
Toxicological
Effects
by
in-vitro
and
in-vivo
Assays



Partners of the
EURO-NanoTox
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Introduction

Nanotechnology is one of the key technologies of the 21st century and is associated with high expectations. Products with completely new properties for application in medicine, science, industry and various techniques are designed. However, the larger surface area of nanoparticles makes them highly reactive compared to larger sized particles of the same chemistry resulting in both, desirable and undesirable effects. The need for toxicological data has become increasingly important, thus several international projects are ongoing throughout the European Union. The question concerning the risks for the health and environment should not be disregarded.

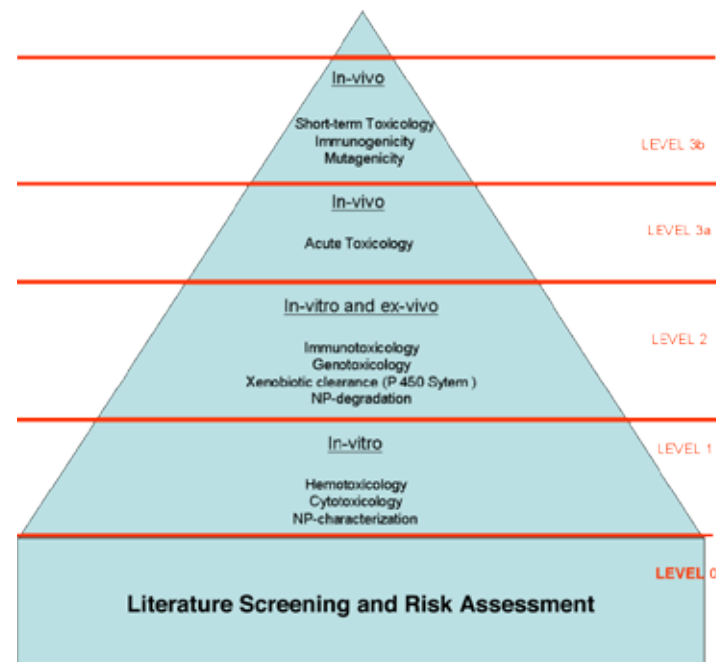
The European Center for Nanotoxicology (“EURO-NanoTox”) has been founded against this background as an Austria-wide and internationally-visible contact point to address all aspects of nanotoxicology. The center is active in the following areas:

1. Development and structuring of the field of nanotoxicology in Austria
2. The development, establishment and implementation of standardised in-vitro and in-vivo toxicological methods for nano-structured material
3. The development of national and international research projects on nanotoxicology
4. Provision to industry of a “tool kit” of methods for the in vitro and in vivo measurement of the toxicological potential of nano-structured materials and the carrying out and interpretation of these tests
5. The active establishment of international contacts with key players in the area of nanotoxicology
6. The active monitoring of relevant literature and provision of an “information point” for interested scientists and industry partners
7. Participation in and organisation of comparative studies including ring studies if necessary

Risk assessment for nano-structured materials

The toxicological profile of a given nano-structured material is determined by multiple parameters, including, amongst many others, size, payload, composition and geometrical structure. Because of this, it is essential to develop, in each case, an individual toxicology strategy tailored to each individual nano-structured material. This strategy should reflect current literature-based knowledge and enable an approach to this theme that is both cost-effective and well structured.

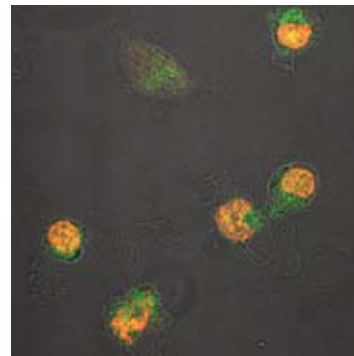
EURO-NanoTox prepares such testing strategies, accompanied by an overview of the relevant published information, on a service basis. This risk assessment, and the development of a strategy for the determination of the nanotoxicological profile, should constitute the first step in the toxicological testing of each novel nano-structured material.



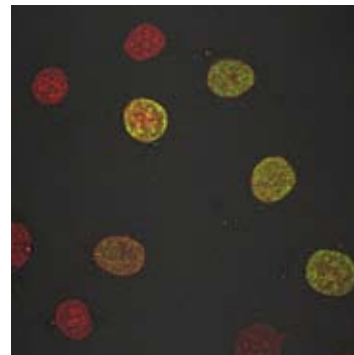
Cytocompatibility

Assays for screening on cell viability and cell number based on colorimetric, fluorescent and chemoluminescent detection are offered. Specific assays for membrane damage, generation of oxidative stress, mitochondrial damage, proliferation and apoptosis allow insight into the mechanism of the toxic effect. Various microscopic techniques (phase contrast, epifluorescence, confocal fluorescence microscopy) and automated image analysis in combination with radiometric and photometric read out are used.

Co-localization of cell damage and particle-uptake and mapping of particles to organelles is possible.

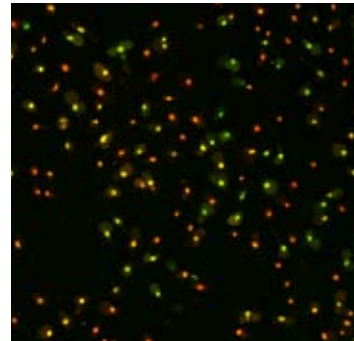


Cells damaged by 26 nm carboxyl-polystyrene beads (green) are identified by uptake of the nuclear dye propidium iodide which stained the nucleus of affected cells red.

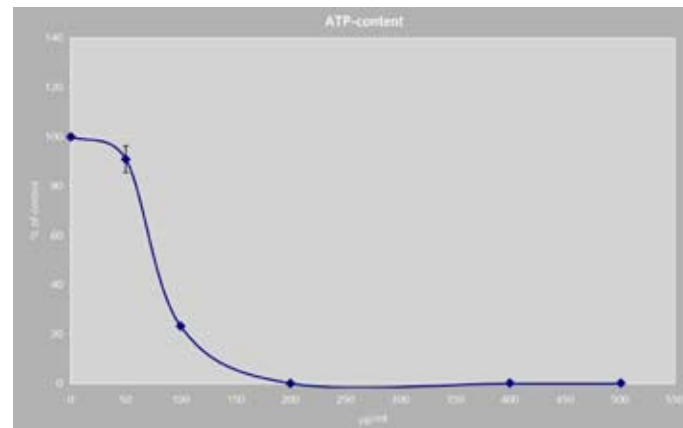


Identification of proliferating cells by BrdU incorporation and detection with anti-BrdU-antibody (green).

In-vitro Evaluation



Identification of cell damage with nuclear dyes: YO-PRO-1 (green nuclei) is taken up in apoptotic cells and propidium iodide (red nuclei) in necrotic cells.



Typical dose-response curve observed upon incubation with a noxious agent, in this case nanoparticles.

List of available assays: MTS, ATP content, LDH-release, AK-release, Neutral Red uptake, Thymidine uptake, BrdU-uptake, PI-exclusion, YO-PRO/PI-staining, DCF, DHE, Calcein AM/ethidium-staining, JC-1, ApoBrdU-staining, TMRM

In-vitro Evaluation

Hemocompatibility

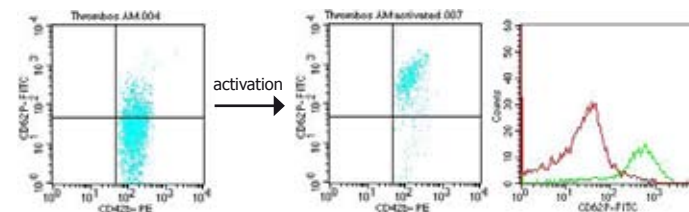
Assays for screening assess the ability to induce hemolysis and clotting in human blood. Assays elucidating the mode of action include thrombocyte activation, leukocyte activation and lymphocyte proliferation. Microscopy, photometric-, radiometric-, FACS- and ELISA-based assays are used.



Dose-dependent increase in the degree of hemolysis induced by nanoparticles.

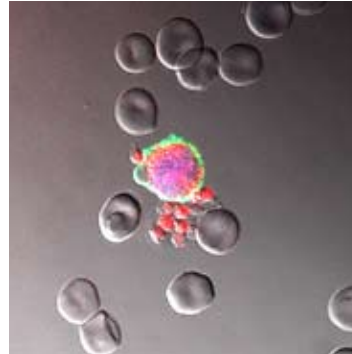


Quantification of fibrinogen by ELISA. Similar formats are used for other clotting assays and for complement activation.



Detection of thrombocyte activation by CD62P (activated thrombocytes)/CD42b (thrombocyte marker) staining. Upon activation CD62P-positive cells shift from the lower right quadrant to the upper right quadrant in the dot plot and from the red (no activation) to the green (activation) curve in the overlay.

In-vitro Evaluation



Monocytes identified by CD14 staining (green) are phagocytosing 26 nm carboxyl-polystyrene beads (red). Erythrocytes do not accumulate beads.

List of available assays: Hemolysis, F1.2, TAT, D-dimer, CD62P/CD42b labelling, CD11b/CD15-labelling, complement C3a, complement C5a, mitogen activated lymphocyte proliferation

Additional services

Endotoxin testing, live cell imaging of uptake of fluorescence-labelled substances/particles

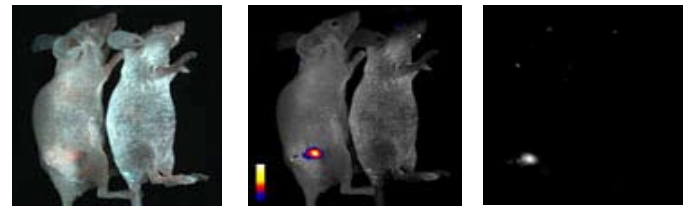
In-vivo Evaluation

In-vivo Imaging

The assessment of toxicological effects includes information on the bio-distribution of the substance. Especially particulate substances can be traced by their inherent physico-chemical properties or by the attachment of fluorescent tags.

Optical Imaging

Fluorescent nanoparticles are tracked with high sensitivity and quantitative analysis of the distribution by Maestro CRI. Multiple labels can be tracked in the same animal.



Conventional RGB
Image

Quantitative
Composite Image

Spectral Unmixed
Image

Accumulation of the label is seen in the hind leg. Autofluorescence is removed by spectral unmixing.

In-vivo Evaluation

Physiological measurements

Cardiovascular blood flow and blood pressure are assessed non-invasively by micro Ultrasound using equipment especially designed for imaging in small animals (Vevo 770).



Wall motion measurements of the left ventricle lumen are easily calculated (M-Mode).

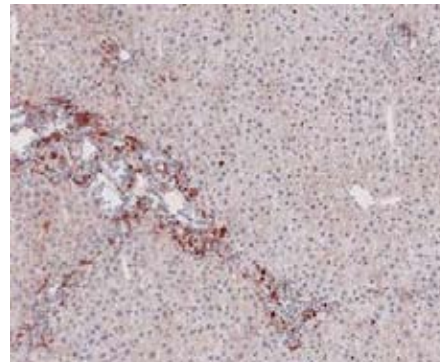


Quantification of blood flow in an adult mouse aorta using Pulsed Wave Doppler.

In-vivo Evaluation

Toxicology

Evaluations of the in-vivo effect of nanoparticles include blood count and clinical chemistry (serum parameters for liver damage, kidney function, inflammation, immune response), histopathology and immunohistochemistry addressing specific questions (proliferation, inflammation, oxidative stress etc.).



Immunohistochemical detection of activated granulocytes by anti-CD11b-staining.

Additional services

Embedding, sectioning and routine staining of tissue samples, assistance in various immunohistochemical techniques, automated analysis of immunohistochemical staining

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