The ToxTracker assay as a tool for mechanism-based (geno)toxicity screening of nanoparticles

G Hendriks[1], S McCarrick[2], F Cappellini[2], R Derr[1], J Hedberg[3], I Odnevall Wallinder[3] and H L Karlsson[2]

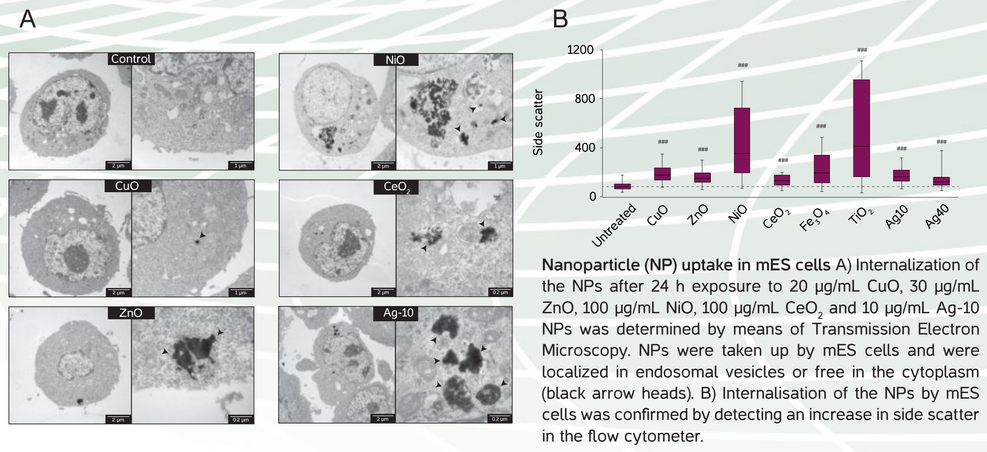
1 Toxys, Leiden, the Netherlands; 2 Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 3 KTH Royal Institute of Technology, Stockholm, Sweden

Introduction

There is a growing demand for fast and reliable assays to identify nanoparticle-induced toxicity and their underlying mechanisms. The ToxTracker assay consists of six mouse stem cell reporter cell lines that use GFP-tagged biomarker genes for detecting the activation of various cellular signaling pathways relevant for carcinogenesis. The assay can discriminate between induction of DNA damage, oxidative stress and protein damage.

We exposed the ToxTracker cell lines to 34 different metal-containing nanoparticles of different sizes and compositions to validate the applicability of the assay for toxicity testing for nanoparticles. We first verified that nanoparticles were taken up by the reporter cell lines. We then exposed the Toxtracker cell lines to a variety of nanoparticles, both metals and their oxides, as well as quantum dots of different sizes.

Nanoparticles are taken up by mES cells



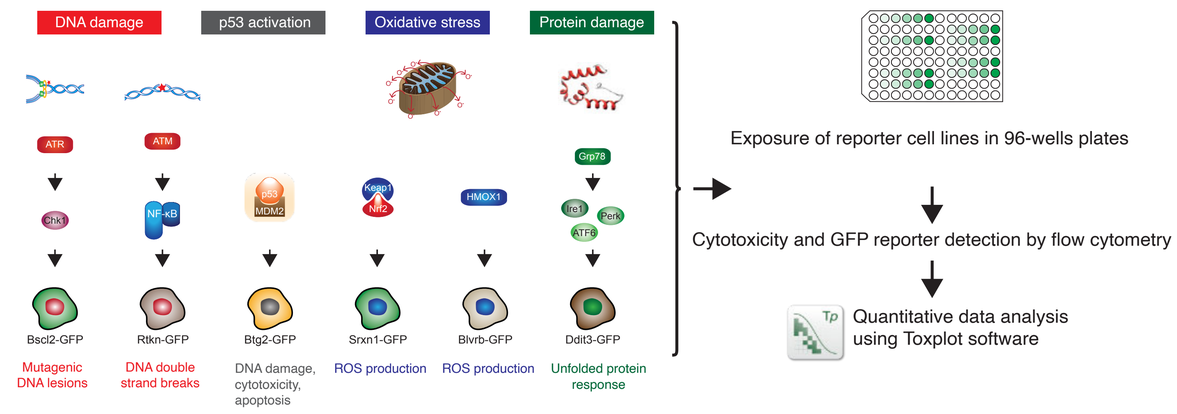
Nanoparticle (NP) uptake in mES cells A) Internalization of the NPs after 24 h exposure to 20 µg/mL CuO, 30 µg/mL ZnO, 100 µg/mL NiO, 100 µg/mL CeO_2 and 10 µg/mL Ag-10 NPs was determined by means of Transmission Electron Microscopy. NPs were taken up by mES cells and were localized in endosomal vesicles or free in the cytoplasm (black arrow heads). B) Internalisation of the NPs by mES cells was confirmed by detecting an increase in side scatter in the flow cytometer.

The ToxTracker reporter assay

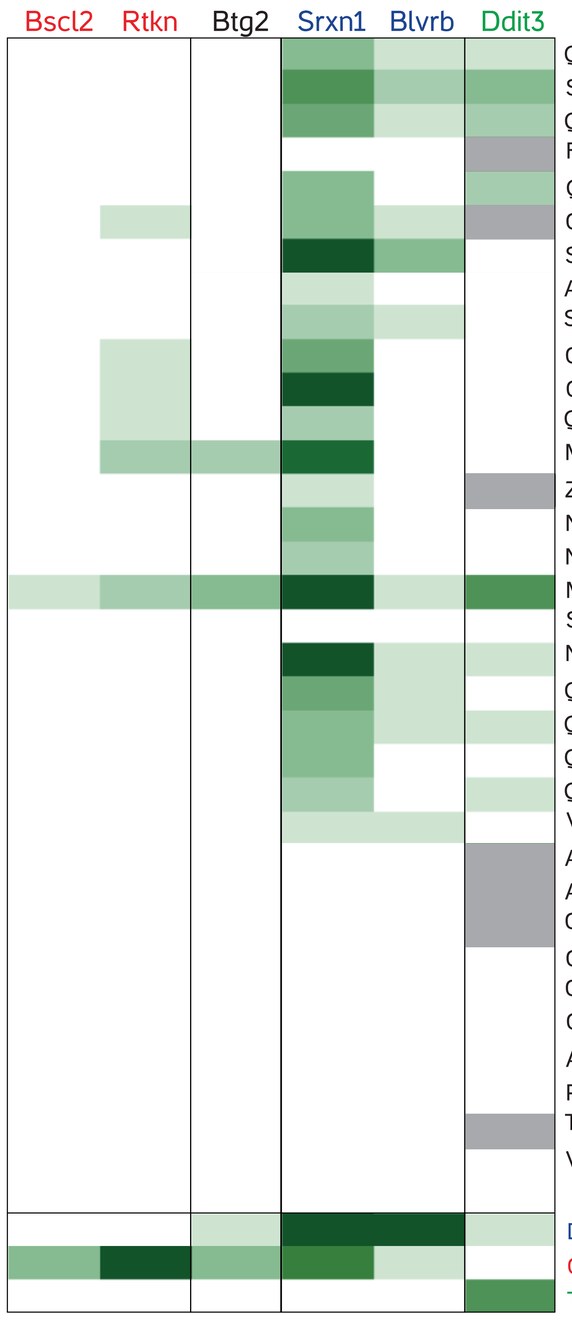
Stem cell-based reporter assay consisting of 6 GFP reporter cell lines

Developed for *in vitro* carcinogenicity hazard screening

Provides insight into mechanisms of genotoxicity



Genotoxicity of metal(oxide) nanoparticles



	Size (nm)	LD50 (µg/ml)
QD (780 nm)	8.6 nm	20.0
Sb	<100 nm	1.9
QD (510 nm)	1.5 nm	2.3
Fe_3O_4	20-40 nm	>100
QD (700 nm)	4.5 nm	8.2
CuO	20-40 nm	20
Sn	10-20 nm	23.3
Ag	5 nm	>50
Sb_2O_3	150 nm	80.0
CoO	30-50 nm	20
Co	20-35 nm	3
$QD\text{-}NH_2$	- nm	5.8
Mn_2O_3	<100 nm	>100
ZnO	20-200 nm	15
NiO	2-70 nm	20
Ni	50-100 nm	1.5
Mn	20-40 nm	89.2
SnO_2	20-40 nm	>100
$NiCl_2$	-	60.0
QD (550 nm)	2.6 nm	4.8
QD (750 nm)	6.5 nm	18.6
QD-PEG	- nm	4.5
QD-COOH	- nm	5.4
V_2O_5	80 nm	30.2
Ag	40 nm	>50
Ag	10 nm	>50
CeO_2	5-40 nm	>100
Cr	35-45 nm	>100
CO_3O_4	40-65 nm	>100
Cr_2O_3	10-30 nm	>100
Au	5 nm	>50
Pt	5 nm	>50
TiO_2	20-100 nm	>100
V	80-100 nm	>100

Control compounds:

Diethyl maleate

Cisplatin

Tunicamycin

DNA damage

Oxidative stress

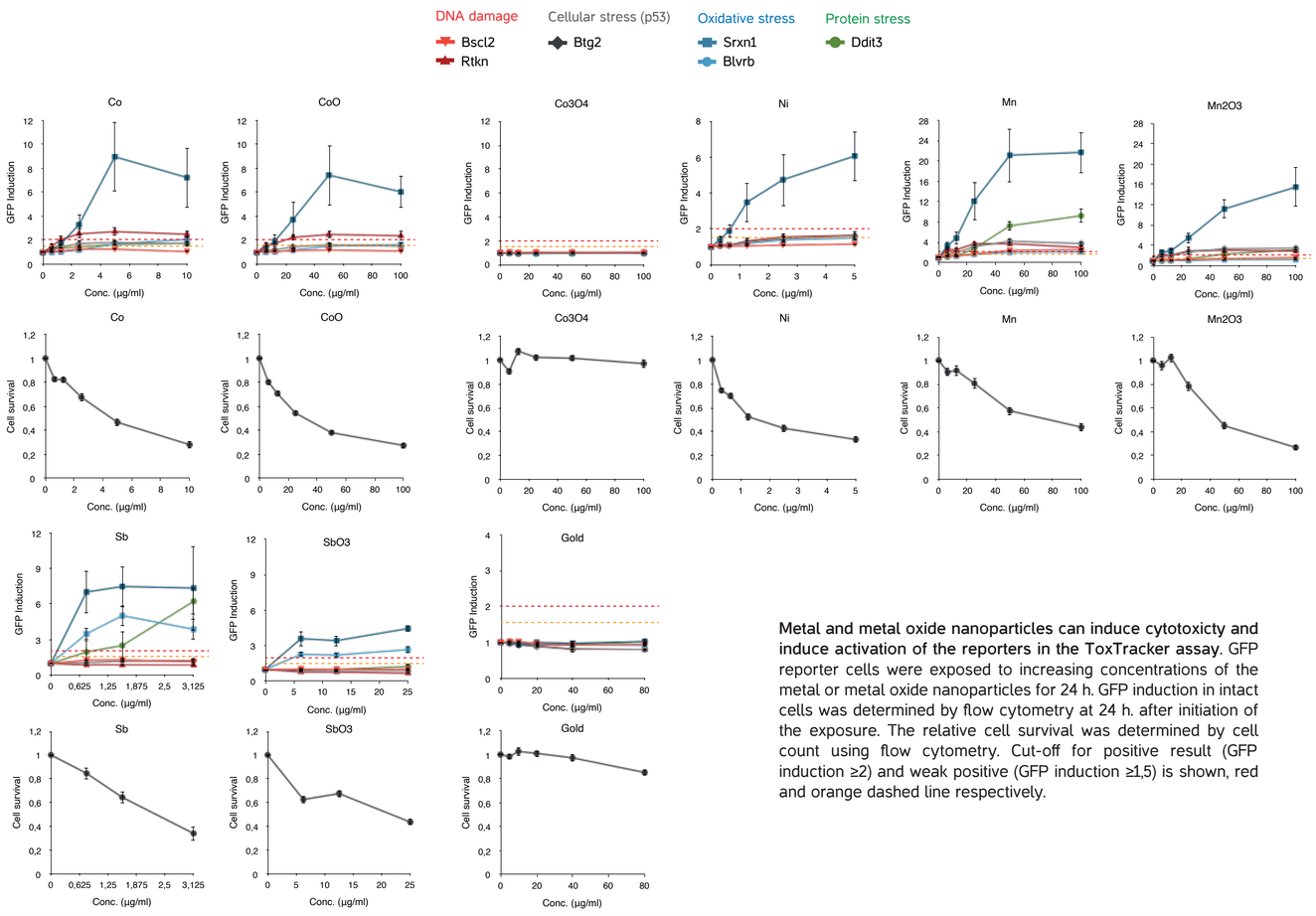
Protein damage

1 5 10

Fold change

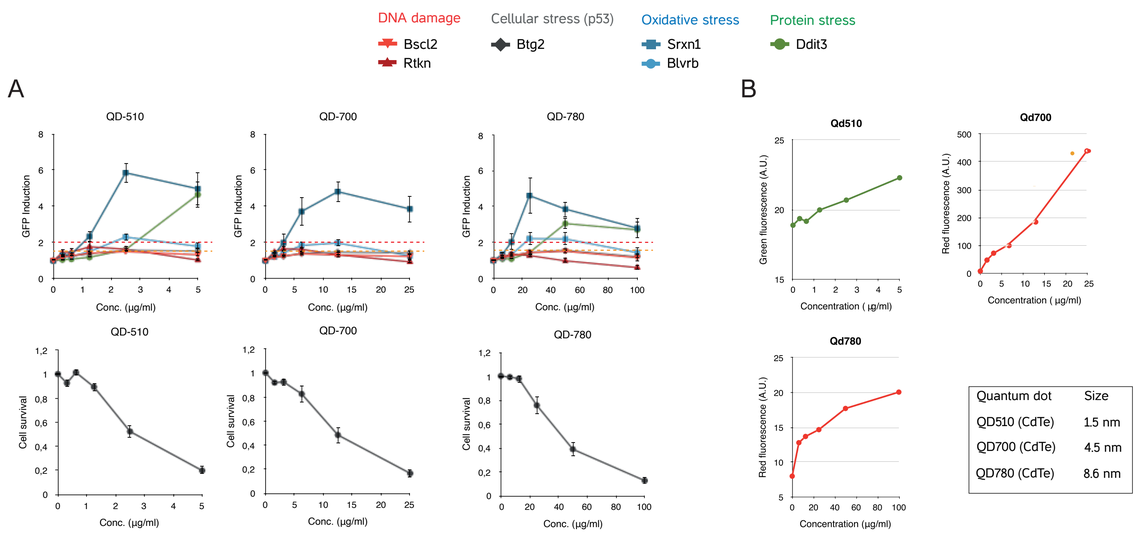
Selective activation of the ToxTracker reporter cell lines in response to exposure to a variety of nanoparticles at 50% cytotoxicity. ToxTracker GFP reporter cells were exposed to increasing concentrations of a variety of nanoparticles. GFP induction in intact cells was determined by flow cytometry at 24 h. after initiation of the exposure. The relative cell survival was determined by cell count after 24 h. exposure using flow cytometry. Ag: Silver, Au: Gold, Ce: Cerium, Co: Cobalt, Cr: Chromium, Cu: Copper, Fe: Iron, Mn: Manganese, Ni, Nickel, QD: Quantum dot, Sb: Antimony, Sn: Tin, V, Vanadium, Zn: Zinc. Grey: not determined.

Metal(oxide) nanoparticles induce cytotoxicity and activate reporters



Metal and metal oxide nanoparticles can induce cytotoxicty and induce activation of the reporters in the ToxTracker assay. GFP reporter cells were exposed to increasing concentrations of the metal or metal oxide nanoparticles for 24 h. GFP induction in intact cells was determined by flow cytometry at 24 h. after initiation of the exposure. The relative cell survival was determined by cell count using flow cytometry. Cut-off for positive result (GFP induction ≥2) and weak positive (GFP induction ≥1,5) is shown, red and orange dashed line respectively.

Quantum dots are cytotoxic and activate markers for oxidative damage



Quantum dot	Size
QD510 (CdTe)	1.5 nm
QD700 (CdTe)	4.5 nm
QD780 (CdTe)	8.6 nm

Quantum dots of different sizes induce cytotoxicty and activate the reporters for oxidative stress and protein damage in the ToxTracker assay. A) GFP reporter cells were exposed to increasing concentrations of the quantum dots for 24 h. GFP induction in intact cells was determined by flow cytometry at 24 h. after initiation of the exposure. The relative cell survival was determined by cell count using flow cytometry. Cut-off for positive result (GFP induction ≥2) and weak positive (GFP induction ≥1,5) is shown, red and orange dashed line respectively. B) Internalization of fluorescent quantum dots after 24h. of exposure was determined using flow cytometry.

Conclusions

- ToxTracker is suitable for the analysis of nanomaterials.
- mES cells take up nanoparticles as verified using Transmission Electron Micoscopy (TEM) as well as flow cytometry.
- Pronounced cytotoxicity and induction of markers was observed for cobalt (Co), nickel (Ni), antimony (Sb) and manganese (Mn) nanoparticles.
- Gold, platinum and several other nanoparticles were inert and showed no cytotoxicity or reporter activation.
- Metallic nanoparticles often show the same effects as their oxides, although not in all cases.
- Quantum dots are cytotoxic and activate the Srxn1 marker for oxidative stress. 1,5 nm and 8,6 nm quantum dots also activate the Ddit3 marker for protein stress.
- For quantum dots, cytotoxicity correlates with particle size, with smaller particles showing a lower LD50.