Mechanism-based genotoxicity screening of nanomaterials using the ToxTracker panel of reporter cell lines

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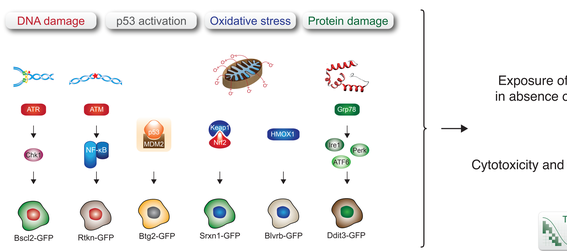
SUMMARY

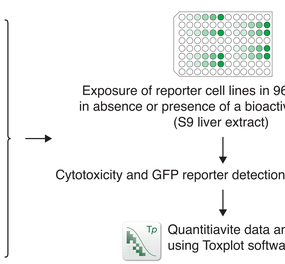
ToxTracker is a mammalian stem cell-based reporter assay that detects activation of specific cellular signalling pathways upon exposure to unknown compounds. ToxTracker contains six different GFP-tagged reporters that allow discrimination between induction of DNA damage, oxidative stress and protein damage in a single test.

We evaluated the ability of ToxTracker to identify the hazardous properties and underlying mechanisms of a panel of 24 metal and non-metallic nanoparticles (NPs). First we tested a panel of metal oxide- and Ag NPs, as well as a selection of non-metallic materials (diesel, carbon nanotubes and quartz). The reporter cells were able to take up NPs, and furthermore, exposure to CuO, NiO and ZnO NPs as well as to quartz resulted in activation of the oxidative stress reporter (Srxn1-GFP). Next, we extended the toxicity screening and tested CdTe quantum dots (QD) of various sizes (1.5-9 nm) and found clear size-dependent effects in terms of cytotoxicity and oxidative stress reporter activation. We also tested metallic NPs including nickel (Ni) and cobalt (Co). Oxidative stress appeared to be a primary mechanism. Co NPs also clearly activated the reporter associated with NF B signaling (Rtkn-GFP) and several NPs induced protein unfolding (Ddit-GFP reporter).

In conclusion, the reporter cells efficiently engulf ENMs, allow for high-throughput testing and can assess various genotoxic and non-genotoxic mechanisms of toxicity in a single assay, which makes make the ToxTracker reporter assay an attractive approach for genetic toxicology assessment of ENMs.

THE TOXTRACKER ASSAY





Biomarker gene	Mode of action	Cellular pathway
Bscl2	Mutagenic DNA lesions	ATR/Chk1 DNA damage signaling
Rtkn	DNA double-strand breaks	NF-kB signaling
Srxn1	ROS production	Nrf2 antioxidant response
Blvrb	ROS production	Nrf2-independent
Ddit3	Protein damage	Unfolded protein response
Btg2	Cytotoxicity	p53 signaling

Figure 1: The ToxTracker reporter assay provides mechanistic insight into the genotoxic properties of compounds and nanomaterials. (A) ToxTracker includes six different GFP-based reporters for detection of DNA damage, oxidative stress and protein damage. Induction of the GFP reporters is determined by high throughput flow cytometry, (B) The ToxTracker reporters indicate activation of various cellular signalling pathways that have been associated with increased cancer risk.

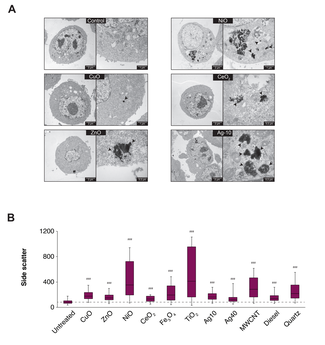


Figure 2: Nanoparticle uptake in mES cells and and genotoxic properties assessed using ToxTracker reporter cells. A) Internalization of the NPs after 24 h exposure to 20 µg/mL CuO, 30 µg/mL ZnO, 100 µg/mL NiO, 100 µg/mL CeO2 and 10 µg/mL Ag-10 NPs was determined by means of TEM. NPs were taken up by mES cells and were localized in endosomal vesicles or free in the cytoplasm (black arrow heads). B) Internalisation of the NPs by the mES cells was confirmed by increased side scatter in the flow cytometer. C) The Bscl2-GFP genotoxicity reporter for DNA replication stress, Srxn1-GFP for oxidative stress and Btg2-GFP for p53-associated cellular stress were used to provide mechanistic insight into the biological damage that is induced by the various metal-based NPs. Induction of the GFP reporters was determined by flow cytometry after 24 h exposure (Karlsson *et al*, 2014). D) For the NPs that induced activation of the ToxTracker reporters (CuO, ZnO and NiO), the accompanying metal salts were analysed in the ToxTracker assay similar as the NPs.

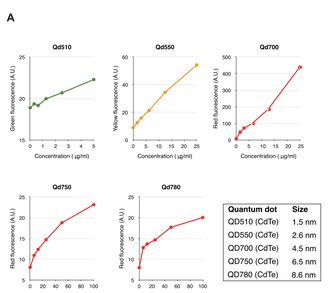


Figure 3: Genotoxic properties of quantum dots. A) Internalization of the fluorescent QDs after 24 h exposure was determined by means of flow cytometry. B) CdSe QDs in different sizes were tested using the six ToxTracker reporter cells. Clear size-dependent cytotoxic and genotoxic properties were found in the sense that the smaller QDs were toxic at much lower concentrations. All QDs tested clearly induced the oxidative stress reporter Srxn1-GFP and, at higher concentrations, also the Ddi-GFP reporter indicative of protein unfolding.

			DNA damage		Oxidative stress		p53	UPR
			BSCL2	RTKN	SRXN1	BLVRB	BTG2	DDIT3
Nanoparticle type	**Size (nm)**	**LD50* (ug/mL)**						
CuO	20-40	20						NA
ZnO	20-200	15						NA
Fe3O4	20-40	>100						NA
TiO2	20-100	>100						NA
CeO2	5-40	>100						NA
Quartz (DQ12)	-	>100						NA
MWCNTs	-	>100						NA
Diesel	-	>100						NA
Diesel+S9	-	>100						NA
NiO	2-70	20						
Ni	50-100	1.5						
Co	20-35	3						
CoO	30-50	20						
Co3O4	40-65	>100						
Ag	5	>50						
Ag	10	>50						
Ag	40	>50						
Pt	5	>50						
Au	5	>50						
QD (CdTe)	1.5	2.3						
QD (CdTe)	2.6	4.8						
QD (CdTe)	4.5	8.2						
QD (CdTe)	6.5	18.6						
QD (CdTe)	8.6	20.0						
Controls								
Cisplatin								
Diethyl maleate								
Tunicamycin								

Positive (>2-fold induction)
Weak positive (1.5 to 2-fold induction)
Negative (<1.5-fold induction)
Not analysed

Figure 4. Summary of the ToxTracker results for the tested NPs. The nanoparticle type (composition), size (nm) and LD50 (for Srxn1 reporter) is shown as well as the induction of the six different reporter cell lines. Metallic nickel and cobalt NPs as well as the smallest QDs were the most cytotoxic. The reporter cell lines could distinguish between (geno)toxic NPs and non (geno)toxic ones. Induction of oxidative stress appeared to be a main mechanism. Several NPs also induced protein folding at higher concentrations.

CONCLUSIONS

- The ToxTracker assay is compatible with analysis of various nanomaterials
- No genotoxicity by direct DNA interaction of the tested nanomaterials was observed in the ToxTracker assay
- Induction of oxidiatve stress appears to be a major cause for genotoxicity of nanomaterials
- Cytotoxicity and potential genotoxicity is strongly correlated with the particle size of nanomaterials

References:

Karlsson HL, Gliga AR, Calléja FMGR, Gonçalves CSAG, Wallinder IO, Vrieling H, Fadeel B, Hendriks G. (2014). Mechanism-based genotoxicity screening of metal oxide nanoparticles using the ToxTracker panel of reporter cell lines. Part Fibre Toxicol.

Hendriks G, Derr RS, Misovic B, Morolli B, Calléja FMGR, Vrieling H. (2016). The Extended ToxTracker Assay Discriminates Between Induction of DNA Damage, Oxidative Stress, and Protein Misfolding. Toxicol Sci.

Nelson BC, Wright CW, Ibuki Y, Moreno-Villanueva M, Karlsson HL, Hendriks G, Sims CM, Singh N, Doak SH. (2016). Emerging metrology for high-throughput nanomaterial genotoxicology. Mutagenesis.

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