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Evaluation of the ToxTracker® assay for DNA damage and pro-oxidative MoA using 10 coded chemicals

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Introduction

Unilever's Safety & Environmental Assurance Centre (SEAC) constantly seeks to bring new scientific solutions when making safety and environmental risk and impact assessments of Unilever products. Novel mechanistic information can be used in the context of making robust safety decisions about the safe use of specific ingredients in consumer products.

ToxTracker® has the potential to provide genotoxicity screening data in addition to mechanistic information on mode of action (MoA) e.g. Oxidative stress. There is potential to integrate the tool as part of a battery of Next Generation Risk Assessment (NGRA) approaches to strengthen confidence in MoA prediction.

Aim

- To evaluate the performance of ToxTracker® as a predictive tool for genotoxicity and assess the ability of the assay to provide MoA information
- Performance of ToxTracker® for a small number of compounds with existing genotoxicity datasets (DNA damage prediction).
- Use of the ToxTracker® ROS-scavenger modified assay as a method to provide MoA information.
- We tested 10 coded compounds with varying modes of genotoxic activity. All were tested using the standard (+/-S9) and ROS scavenger (N-Acetyl Cysteine (NAC)) modified (-S9) versions of the ToxTracker® assay.

ToxTracker® Standard Assay

The standard ToxTracker® assay consists of 6 GFP reporter cell lines. Developed for *in vitro* hazard screening and provides insight into mechanisms of genotoxicity.

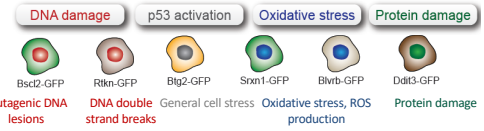

Table 1: Standard study performance evaluation

Compound	Ames	MN[a] *in vitro*	CA[b] *in vitro*	ToxTracker® DNA Damage			Concordance
				-S9	+S9	ROS	
Caffeine							+
Coumarin							+
Curcumin							+
Sulforaphane			No data				+
6-Gingerol			No data				-
Doxorubicin							+
Menadione			No data				+
Tert-butylhydroquinone (tBHQ)							-
Cumene Hydroperoxide		No data	No data				+
Sodium Benzoate							-

a) Micronucleus assay b) Chromosome Aberration test

Legend: green, negative; red, positive; blue, inconclusive data; orange, weak activation of the ToxTracker® reporter

Results and Discussion:

Standard Assay:

- Concordance of 70% between ToxTracker® and a battery of *in vitro* genotoxicity assays.
- tBHQ and Sodium benzoate generally regarded as misleading positives *in vitro*. 6-Gingerol understood to induce genotoxicity probably by oxidative stress and at high concentrations (Kirkland et al., 2008).

ROS Scavenger Modified Assay:

- When tested using the ROS modified ToxTracker® with ROS scavenger N-Acetyl Cysteine (NAC) the DNA damage and oxidative stress response for curcumin, menadione and cumene hydroperoxide was reduced or abolished, suggesting a pro-oxidant MoA leading to DNA damage for these compounds.
- ToxTracker® enabled us to distinguish between direct and indirect DNA damaging agents.

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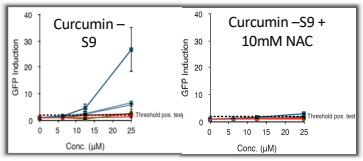
ToxTracker® ROS Modified Assay

The ROS modified ToxTracker® assay has the same principles as the standard assay but tests the compounds in the presence of ROS scavengers. The addition of ROS scavengers can differentiate between a direct and indirect DNA damaging MoA.

DNA damage	Cellular stress (p53)	Oxidative stress	Protein damage
▼ Bscl2	◆ Btg2	■ Srxn1	● Ddit3
▲ Rtkn		■ Blvrb	

Curcumin

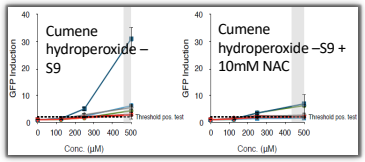


Corresponding reduction in cytotoxicity levels.

- Markers for oxidative stress strongly induced (Srxn1 and Blvrb - blue).
- DNA damage marker above threshold for positive test outcome (2.44).
- Addition of ROS scavengers (NAC) to the test system reduced DNA damage to a weak positive result (1.51).

Conclusion:
Indicative of oxidative MoA for curcumin leading to DNA damage demonstrated in the ToxTracker® assay.

Cumene hydroperoxide

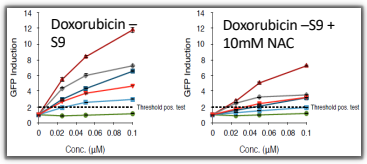


The addition of ROS scavengers did not rescue cell survival. The grey zone indicates cytotoxic concentrations.

- Activation of markers for DNA damage (2.97) accompanied by activation of oxidative stress markers.
- Addition of ROS scavengers (NAC) only marginally reduced the activation of markers for DNA damage (2.34).

Conclusion:
Indicative of Cumene hydroperoxide having DNA damage potential.
Clear evidence of oxidative stress.
Less clear whether DNA damage can be attributed to a pro-oxidant MoA.

Doxorubicin



Small improvement in cell survival observed with the addition of NAC.

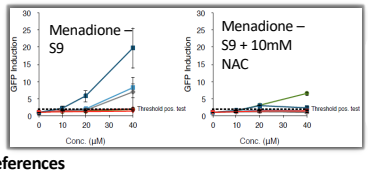
- Very high induction of DNA damage biomarkers (11.79)! Also the other markers show high activation.
- Addition of ROS scavengers (NAC) resulted in a reduction of DNA damage but overall remained highly activated.

Conclusion:
Clear evidence of DNA damaging potential.
Independent of pro-oxidant MoA.

Menadione

Standard ToxTracker® assay		
-S9	+S9	ROS
1.94	5.07	

More potent metabolites!



- Weak positive activation of biomarkers for DNA damage (1.98). Strong activation of oxidative stress biomarkers.
- Addition of ROS scavengers abolished DNA damage and oxidative stress response.

Conclusion:
DNA damaging potential of metabolites of menadione need further investigation as this is not part of the current ToxTracker® test protocol with ROS scavengers.

References

Kirkland, D., Kasper, P., Muller, L., Corvi, R., & Speit, G. (2008). Recommended lists of genotoxic and non-genotoxic chemicals for assessment of the performance of new or improved genotoxicity tests: a follow-up to an ECVAM workshop. *Mutat Res, 653*(1-2), 99-108. doi:10.1016/j.mrgentox.2008.03.008

Acknowledgements
Remco Derr and Paula van Rossum