The ToxTracker reporter assay detects indirect genotoxicity caused by high levels of oxidative stress.

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Introduction

Current tests for genotoxicity do not specifically investigate oxidative damage, eventhough this process can (indirectly) lead to genotoxicity. ToxTracker is a mammalian stem cell-based reporter assay that detects the activation of specific cellular signaling pathways upon exposure to compounds. The assay can distinguish between DNA damage, protein damage, p53 activation and oxidative damage.

ToxTracker has previously been validated extensively using various libraries of reference compounds. We have now extended the validation with 25 pesticides. For many pesticides, their precise mode of action is unknown, but oxidative stress is often suspected to play a role.

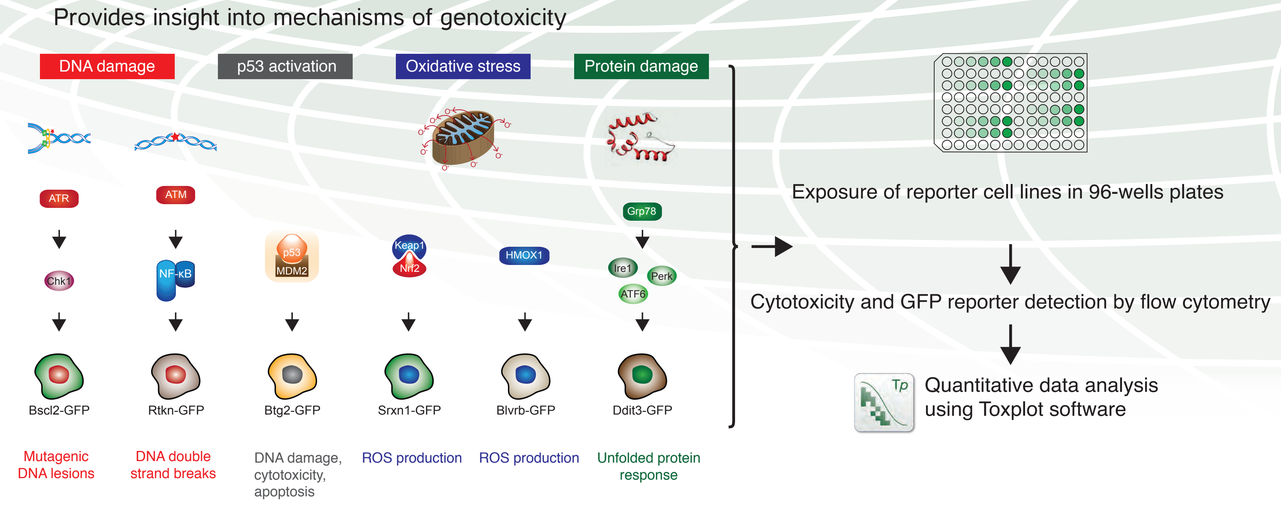
To investigate potential direct or indirect genotoxicity due to oxidative stress, several pesticides as well as a number of control compounds were tested in the presence of the ROS scavenger N-Acetyl Cysteine (NAC).

The ToxTracker reporter assay

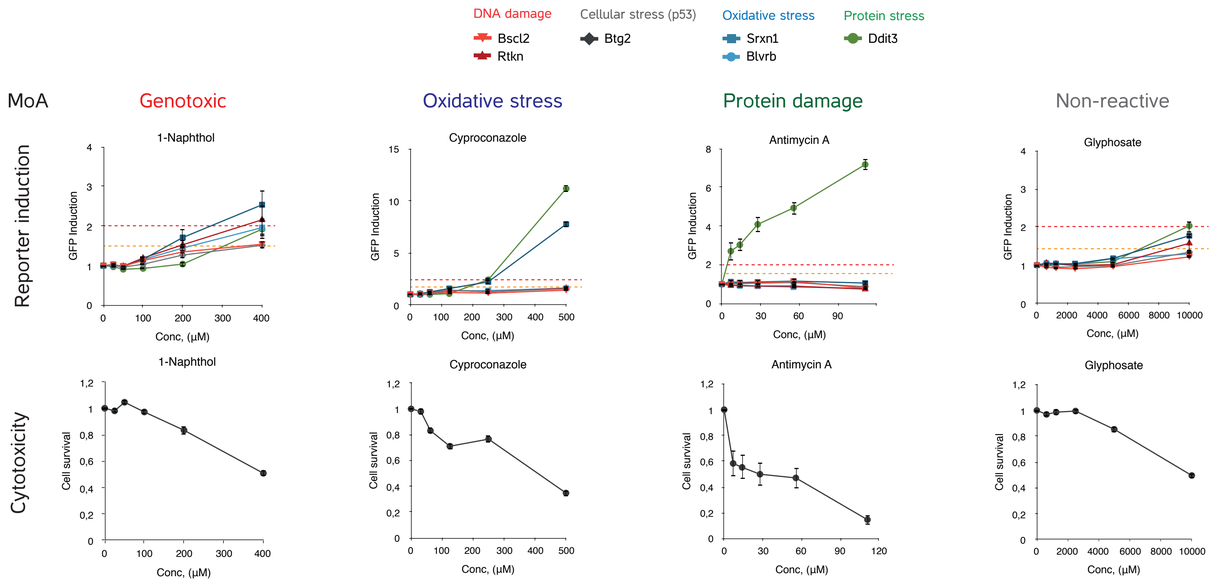
Stem cell-based reporter assay consisting of 6 GFP reporter cell lines

Developed for *in vitro* carcinogenicity hazard screening

Provides insight into mechanisms of genotoxicity

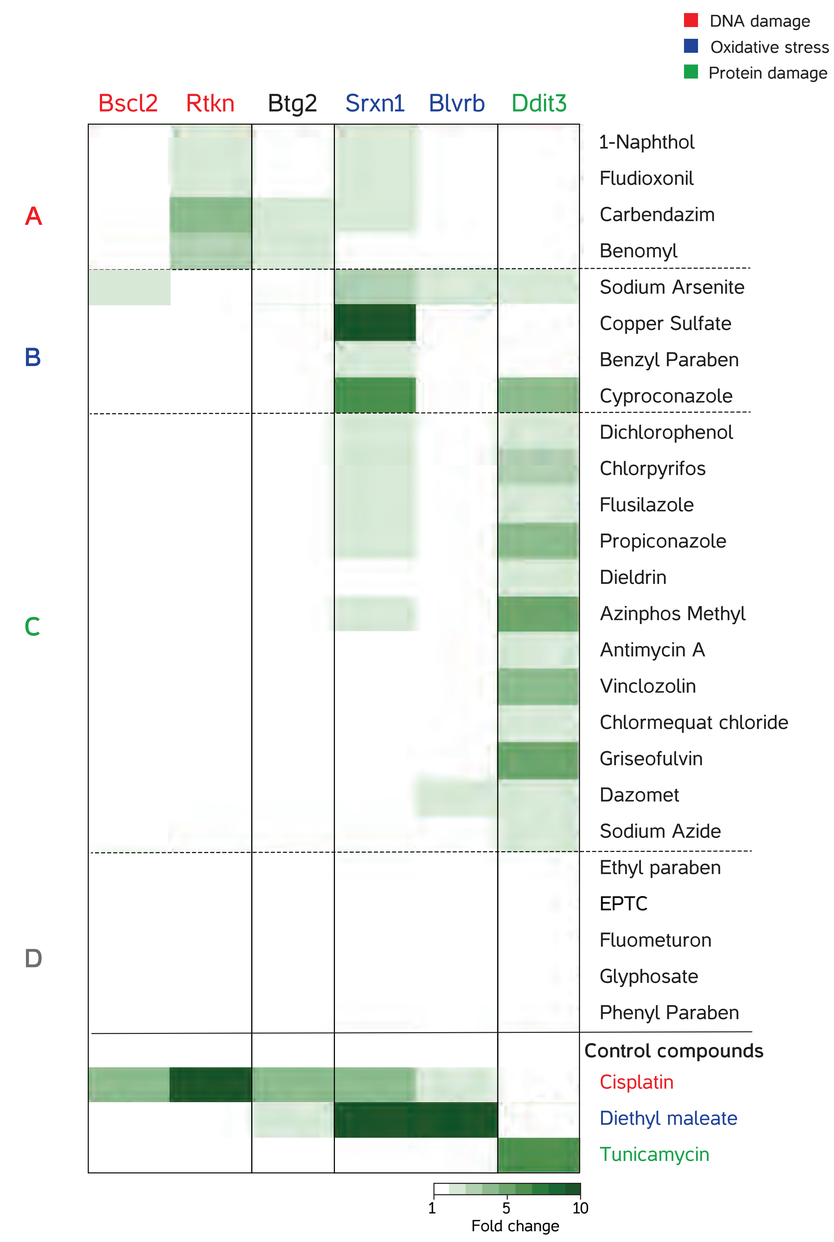


Pesticides differentially activate the ToxTracker reporters



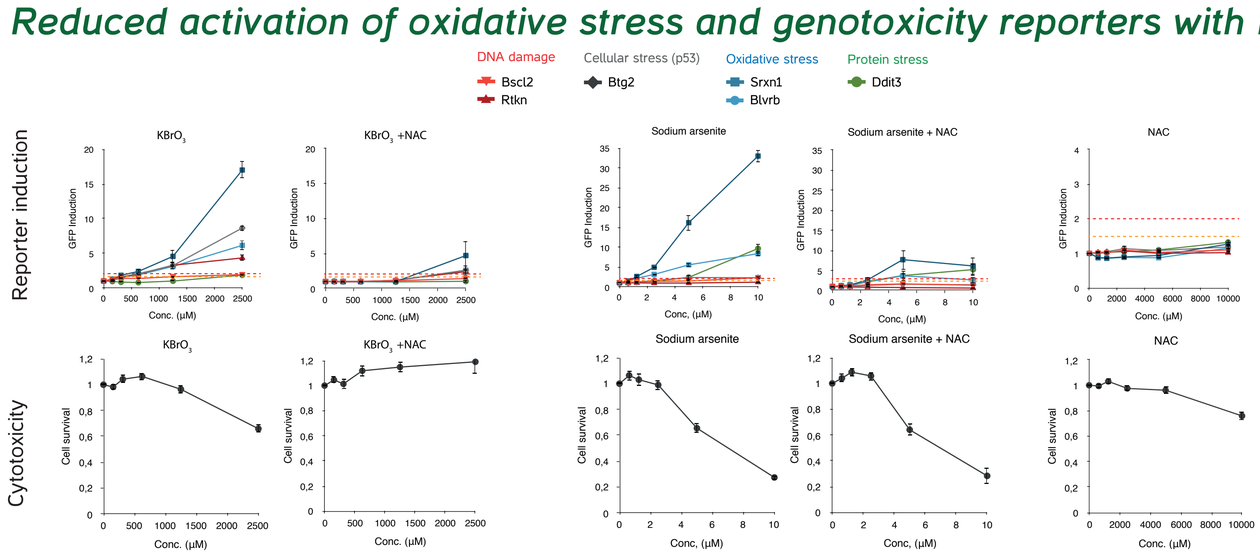
Selective activation of the ToxTracker reporter cell lines in response to exposure to a variety of pesticides. ToxTracker GFP reporter cells were exposed to increasing concentrations of a range of pesticides. GFP induction in intact cells was determined by flow cytometry at 24 h. after initiation of the exposure. The relative cell survival was determined by cell count after 24 h. exposure using flow cytometry. Cut-off for positive result (GFP induction ≥2) and weak positive (GFP induction ≥1,5) is shown, red and orange dashed line respectively.

ToxTracker reporter activation by pesticides



Selective activation of the ToxTracker reporter cell lines in response to exposure to a variety of pesticides at 50% cytotoxicity. Compounds were sorted using hierarchical clustering and four groups can be distinguished: A: genotoxic, B: oxidative stress, C: protein damage, D: not reactive. ToxTracker GFP reporter cells were exposed to increasing concentrations of a range of pesticides. GFP induction in intact cells was determined by flow cytometry at 24 h. after initiation of the exposure. Cisplatin, tunicamycin and diethyl maleate were used as control compounds for activation of the markers for DNA damage, protein damage and oxidative stress respectively. EPTC: S-Ethyl-N.N-dipropyl thopcarbamate.

Reduced activation of oxidative stress and genotoxicity reporters with NAC



Addition of the antioxidant N-Acetyl Cysteine (NAC) reduces the activation of oxidative damage reporters Srxn1 and Blvrb as well as the DNA double strand break reporter Rtkn. GFP reporter cells were exposed to one concentration of the ROS scavenger NAC (10 mM) in combination with increasing doses of the indicated agent. 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. Exposure to NAC alone does not result in activation of the ToxTracker reporters. Cut-off for positive result (GFP induction ≥2) and weak positive (GFP induction ≥1,5) is shown, red and orange dashed line respectively.

Direct versus indirect genotoxicity

		DNA damage				Oxidative stress				Protein damage		p53	
		Bscl2		Rtkn		Srxn1		Blvrb		Ddit3		Btg2	
		-	+	-	+	-	+	-	+	-	+	-	+
Pesticides	Carbendazim	2,0	1,8	5,3	3,6	2,1	1,7	1,5	1,1	1,1	1,2	4,8	3,8
	Chlorpyrifos	1,0	0,8	1,0	0,8	3,5	2,1	1,6	1,0	6,1	6,9	1,4	1,0
	$CuSO_4$	1,0	1,4	1,1	1,1	16,0	9,9	3,5	2,0	1,8	3,1	1,5	1,8
	Fludioxonil	1,0	0,9	1,7	0,5	1,8	0,9	1,0	0,8	2,8	3,3	1,8	0,8
	Flusilazole	1,2	0,8	1,2	0,9	3,0	1,4	1,3	0,8	6,6	5,3	1,6	1,0
	$NaAsO_2$	2,3	1,3	1,2	0,6	33,0	6,1	8,4	2,6	9,7	5,2	2,3	1,4
Control compounds	$KBrO_3$	1,9	1,4	4,3	2,4	17,0	4,7	6,1	2,6	1,8	1,0	8,6	2,6
	MMS	1,6	1,6	1,9	1,9	57,6	9,5	14,8	4,4	1,6	1,1	6,3	2,6
	Cisplatin	3,2	2,7	7,6	4,5	2,9	2,5	1,7	1,3	0,9	1,3	3,6	2,3
	DEM	1,3	1,0	1,1	0,9	38,6	1,4	11,3	1,2	1,8	1,2	3,8	1,0
	Tunicamycin	1,1	1,1	0,7	0,6	2,0	1,2	1,7	1,2	11,4	9,7	1,4	1,1

NAC

Addition of the ROS scavenger N-Acetyl Cysteine (NAC) reduces the activation of oxidative damage reporters Srxn1 and Blvrb and for some of the compounds also the DNA damage reporters Rtkn and/or Bscl2. Heatmap shows the average GFP induction for each reporter in the presence and absence of 10 mM N-Acetyl Cysteine.

Conclusions

- 5 out of the 25 tested pestices were genotoxic and activated the Rtkn or Bscl2 reporter for DNA damage. 13 out of 25 tested compounds caused oxidative damage.
- The 33 tested pesticides can be separated into four different groups: A) genotoxic compounds, B) compounds that primarily cause oxidative damage C) compounds that cause protein damage, D) ToxTracker negative compounds.
- Addition of the ROS scavenger N-Acetyl Cysteine reduces the activation of the oxidative damage reporters Srxn1 and Blvrb.
- For Sodium Arsenite and Fludioxonil, activation of the genotoxicity reporter, Bscl2 or Rtkn respectively, is decreased in the presence of N-Acteyl Cysteine
- Addition of a ROS scavenger helps to distinguish between direct and indirect genotoxicity due to oxidative stress in ToxTracker.