ToxTracker – A Key to Early-Stage Molecule Genetox Testing

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ABSTRACT

ToxTracker is a unique genotoxicity assay that combines multiple biomarkers through 6 reporter cell lines to evaluate the potential mode of action of genotoxic compounds. The ToxTracker assay is robust and can easily be modified for fit-for-purpose for early-stage screening of the genotoxic potential of molecules. With the extended ToxTracker ACE assay one can predict the outcomes of an AMES (BSLC2 reporter cell line) and in vitro micronucleus assay (RTKN report cell line) and determine the clastogenic or aneugenic properties of the tested molecules. Additionally, the ToxTracker ACE assay can help to identify molecules that have the potential to classify as kinase inhibitors. By modifying the assay for fit-for-purpose it saves time, and resources, by not investing in molecules that demonstrate genotoxic properties. In this poster we will present the modifications made to improve screening efficiency of the ToxTracker assay for early-stage screening of agrichemical molecules.

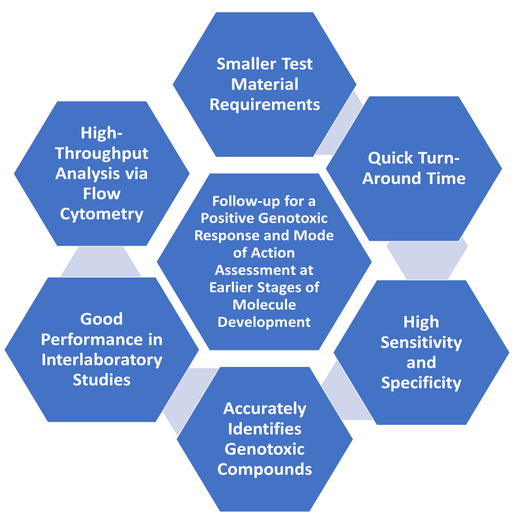
BACKGROUND

ToxTracker ACE is designed to provide a multiplexed platform that includes markers for DNA damage as predictors for the standard genotoxicity assays, and also includes markers for non-genotoxic MOA, including oxidative stress, protein misfolding and general cellular stress. All these types of cellular damage have been associated with increased cancer risk.

With the capability of conducting the ToxTracker ACE assay, testing can be performed for:

- Screening Studies
- Mode of Action for Positive Genotoxic Compounds
- Weight of Evidence for Regulatory Submissions

WHY TOXTRACKER



CHALLENGES FACED

As testing progressed there where challenges that needed to be overcome:

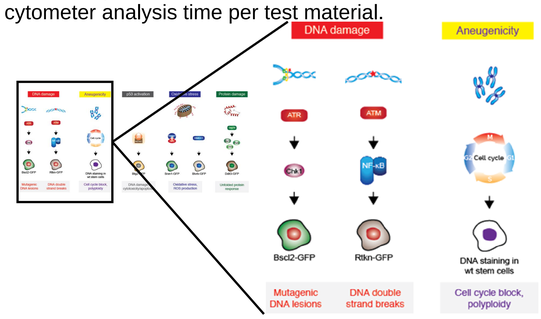
- Test material requirements
- Flow cytometer capabilities
- Cytotoxicity variations between preliminary toxicity assay and main assay
- AFB1 response does not always meet the fold-change requirement
- ACE Data analysis

MODIFICATIONS TO OVERCOME CHALLENGES

By working with Toxys, modifications were applied to the ToxTracker ACE protocol to overcome the presented challenges.

Test Material Requirements and Flow Cytometer Capabilities

1. By focusing on the genetox endpoints only, the test material volume requirements decreases, as does the flow cytometer analysis time per test material.



2. Reproducibility from run to run allowed for reduction in the number of runs from 3 to 2. If the 2 runs are not concordant, then a third run would be conducted. Reduction in the number of runs required allowed for reduced test material needs and decreased flow cytometer time.

Cell Line	Compound	Concentration	Run 1 Induction	Run 2 Induction	Run 3 Induction	Average	StDev	%CV
Bscl2		0	1.00	1.00	1.00	1.00	0.00	0.00
Bscl2		TS5	1.01	1.04	1.03	1.03	0.01	1.29
Bscl2	Compound 1	TS4	1.03	1.02	1.03	1.03	0.01	0.88
Bscl2		TS3	1.06	1.05	1.05	1.05	0.00	0.45
Bscl2		TS2	1.09	1.07	1.09	1.08	0.01	0.90
Bscl2		TS1	1.14	1.18	1.16	1.15	0.01	0.63
Srxn1		0	1.00	1.00	1.00	1.00	0.00	0.00
Srxn1		TS5	1.01	1.04	1.01	1.02	0.01	1.29
Srxn1	Compound 2	TS4	1.04	1.05	1.01	1.03	0.02	2.41
Srxn1		TS3	1.03	1.06	1.02	1.04	0.02	2.43
Srxn1		TS2	1.06	1.10	1.06	1.07	0.02	1.96
Srxn1		TS1	1.20	1.24	1.21	1.22	0.03	2.14
Rtkn		0	1.00	1.00	1.00	1.00	0.00	0.00
Rtkn		TS5	1.03	1.07	1.01	1.04	0.03	3.08
Rtkn	Compound 1	TS4	1.06	1.12	1.00	1.06	0.06	5.36
Rtkn		TS3	1.12	1.20	1.02	1.11	0.09	8.26
Rtkn		TS2	1.15	1.24	1.04	1.14	0.10	8.75
Rtkn		TS1	1.18	1.38	1.12	1.22	0.12	10.20
Rtkn		0	1.00	1.00	1.00	1.00	0.00	0.00
Rtkn		TS5	0.98	1.02	0.99	1.00	0.02	1.82
Rtkn	Compound 2	TS4	0.95	0.98	0.96	0.96	0.01	1.51
Rtkn		TS3	0.89	0.93	0.94	0.92	0.03	2.87
Rtkn		TS2	0.85	0.91	0.90	0.89	0.03	3.26
Rtkn		TS1	1.28	1.37	1.31	1.22	0.10	8.01
Srxn1		0	1.00	1.00	1.00	1.00	0.00	0.00
Srxn1		TS5	1.03	1.10	1.03	1.05	0.04	4.20
Srxn1	Compound 1	TS4	1.08	1.13	1.01	1.07	0.06	5.57
Srxn1		TS3	1.11	1.19	1.12	1.14	0.05	4.08
Srxn1		TS2	1.26	1.48	1.26	1.33	0.13	9.65
Srxn1		TS1	1.61	1.85	1.56	1.67	0.15	9.25
Srxn1		0	1.00	1.00	1.00	1.00	0.00	0.00
Srxn1		TS5	1.11	1.13	1.08	1.10	0.03	2.38
Srxn1	Compound 2	TS4	1.19	1.23	1.17	1.20	0.03	2.51
Srxn1		TS3	1.41	1.46	1.33	1.40	0.06	4.45
Srxn1		TS2	1.65	1.71	1.56	1.65	0.07	4.04
Srxn1		TS1	2.04	2.40	1.99	2.14	0.23	10.57
	negative							
	equivocal							
	positive							

Additional perk to these modifications – ability to increase the number of molecules tested at one time.

MODIFICATIONS TO OVERCOME CHALLENGES

Cytotoxicity variations between preliminary toxicity assay and main assay

1. Instead of running every-other concentration in the range finder assay, all 22 concentrations are tested. This allows for a better assessment of the toxicity profile of the test material.

Dilution plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	1	2	3	4	5	6	7	8	9	10	11
B	0	1	2	3	4	5	6	7	8	9	10	11
C	0	1	2	3	4	5	6	7	8	9	10	11
D	0	1	2	3	4	5	6	7	8	9	10	11
E	0	1	2	3	4	5	6	7	8	9	10	11
F	0	1	2	3	4	5	6	7	8	9	10	11
G	0	1	2	3	4	5	6	7	8	9	10	11
H	0	1	2	3	4	5	6	7	8	9	10	11

Dilution plate 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	12	13	14	15	16	17	18	19	20	21	22	
B	12	13	14	15	16	17	18	19	20	21	22	
C	12	13	14	15	16	17	18	19	20	21	22	
D	12	13	14	15	16	17	18	19	20	21	22	
E	12	13	14	15	16	17	18	19	20	21	22	
F	12	13	14	15	16	17	18	19	20	21	22	
G	12	13	14	15	16	17	18	19	20	21	22	
H	12	13	14	15	16	17	18	19	20	21	22	

2. In the main assay, the wild-type cells (B4418) are tested concurrently with the BSCL2 and RTKN reporter cells lines regardless of the presence or absence of autofluorescence to allow for comparison of the toxicity exhibited in the reporter cells lines to that observed in the WTs.

AFB1 response does not always meet the fold-change requirement

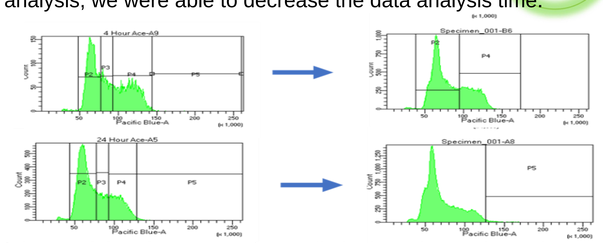
By including cyclophosphamide (CP) as an additional +S9 control, the rate of failure due to the +S9 control, Aflatoxin B1 (AFB1) was eliminated.

			Fold-change	
			Run 1	Run 2
Reporter	Treatment	Conc.	induction	induction
Bscl2	Aflatoxin B1 +S9	0	1.00	1.00
Bscl2	Aflatoxin B1 +S9	500 µM	1.37	1.28
Bscl2	Aflatoxin B1 +S9	1000 µM	1.68	1.46
Bscl2	CP +S9	0	1.00	1.00
Bscl2	CP +S9	5 mM	1.52	1.38
Bscl2	CP +S9	10 mM	2.53	2.23
Rtkn	Aflatoxin B1 +S9	0	1.00	1.00
Rtkn	Aflatoxin B1 +S9	500 µM	1.91	1.41
Rtkn	Aflatoxin B1 +S9	1000 µM	3.33	1.73
Rtkn	CP +S9	0	1.00	1.00
Rtkn	CP +S9	5 mM	1.05	2.11
Rtkn	CP +S9	10 mM	3.44	5.38

Pass
Fail

ACE Data Analysis

By elimination of the gates that are not critical to the ACE analysis, we were able to decrease the data analysis time.



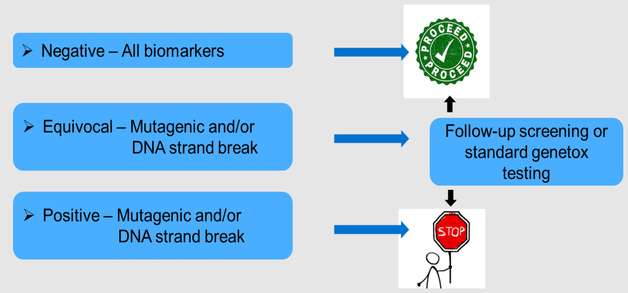
It is noted that there are software limitations that make gating challenges that need to be evaluated with the flow cytometer vendor.

IMPORTANCE OF THE MODIFICATIONS

The testing that is currently being conducted is on early-stage agricultural molecules that are being evaluated for safety based on application.

- Reduction in test material quantities needed is crucial, as the majority of the compounds being tested in ToxTracker ACE have limited supply that has been synthesized.
- It is critical that we can accurately assess the toxicity profile of these molecules so we can be confident that the results obtained meet the assay requirements as defined in the protocols supplied by Toxys.
- The same applies to have positive controls that respond as expected. Passing positive controls are required in order to consider the resulting data valid.
- Increasing the efficiency of data analysis allows for data communication to occur in a timely manner and to be readily available to help make advancement decisions.

HOW IS THE DATA USED



NEXT STEPS

- Collaborate with Toxys to automate the ToxTracker ACE assay where applicable to improve efficiency.
- Miniaturization to 384-well plates to further reduce test material needs.
- Database generation for data analysis based on modifications to increase the through-put of data analysis
- Consult with flow cytometer vendor to improve the ACE data gating to reduce the time required for evaluation.

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