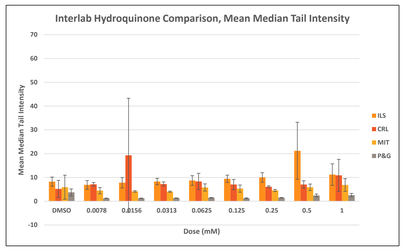
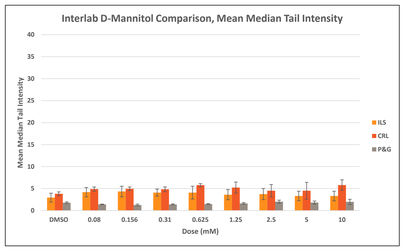
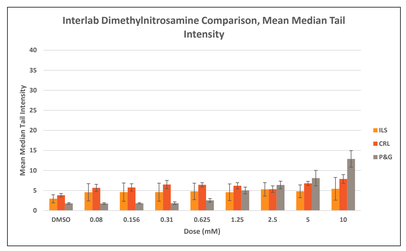
Metabolically Competent HepaRG™ Cells and CometChip®: A New Approach Methodology for a Medium Throughput Genotoxicity Assay

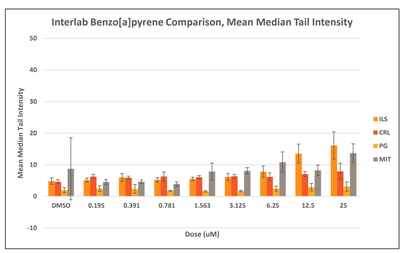
L. Martin[1], J. Fowler[1], C. Swartz[1], J. Sly[1], N. Owiti[2], S. Kaushal[2], E. Rottinger[3], B. Engelward[2], D. Roberts[4], S. Pfuhler[3], and L. Recio[5].
[1]Integrated Laboratory Systems LLC, an Inotiv Company, Research Triangle Park, NC; [2]Massachusetts Institute of Technology, Cambridge, MA; [3]The Proctor & Gamble Company, Mason, OH; [4]Charles River, Skokie, IL and [5]ScitoVation, Durham, NC.

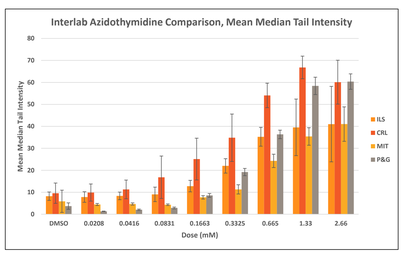
Interlaboratory Testing of HepaRG™ CometChip®

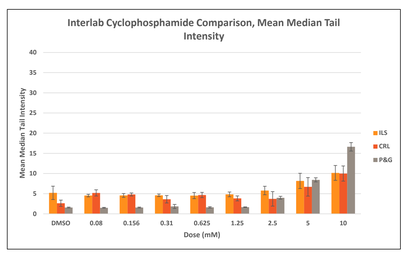


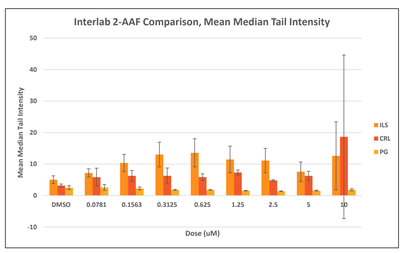


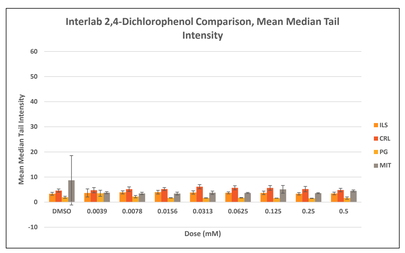


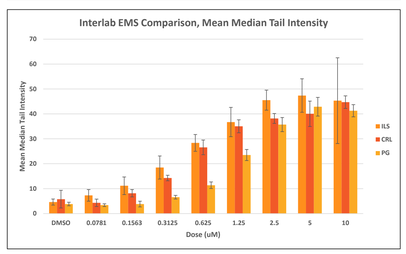




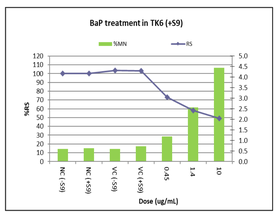




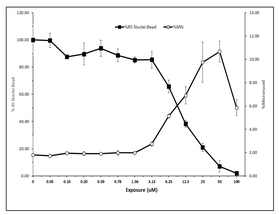




Micronucleus Assay using HepaRG™



4 hour (+S9) BaP Treatment in TK6



24 hour BaP Treatment in HepaRG™

HepaRG™ CometChip® pairs readily with the flow cytometry-based micronucleus assay, allowing for the creation of a more robust test battery using HepaRG™ Cells.

Benchmark Dose Analysis

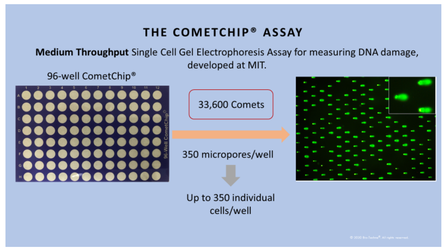
Chemical	BMD	Lower BMD	Upper BMD	Fit p-value
Amitrole (mM)	40.478	13.325	1000000.0	0.1441
Ethyl Methansulfonate (mM)	0.138	0.110	0.179	0.9383
2,4-Dichlorophenol (uM)	581.243	506.02	787.13	0.6884
Benzo[a]pyrene (uM)	5.586	4.281	7.034	0.7888
Cadmium Chloride (uM)	68.657	24.131	200000.0	0.1674
Dimethylbenzanthracene (mM)	0.016	0.010	0.023	0.8750
Di-(2-ethylhexyl) phthalate (uM)	2.814	2.543	3.906	0.5625
Aflatoxin B1 (uM)	0.101	0.091	0.156	0.8750
Eugenol (mM)	0.034	0.024	0.041	0.5625
2-Aminoacetylfluorene (mM)	0.086	0.065	0.106	0.5625
Hydroquinone (mM)	0.252	0.105	0.284	0.7969
Azidothymidine (mM)	0.225	0.160	0.294	0.9992
Phenobarbital (mM)	2.201	1.335	5.273	0.9316
Cyclophosphamide (mM)	2.630	2.024	3.310	0.6900

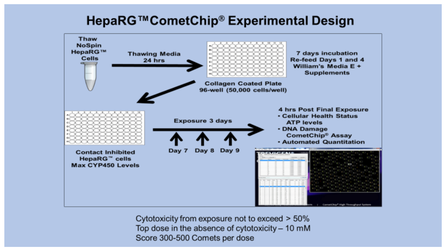
Benchmark Dose Analysis was performed using BMD Express 2 to demonstrate the possibility of using multiple analysis endpoints on a single set of data.

Introduction

The effort to reduce dependency on the use of animals in toxicology testing is an area that is receiving increased attention and resources in genetic toxicology. As part of that effort, we are combining CometChip® technology, a single cell array platform developed at the Massachusetts Institute of Technology (MIT), with metabolically competent HepaRG™ cells to develop a New Approach Methodology (NAM) as an alternative to the traditional in vivo comet assay. CometChip® utilizes an automated, unbiased image-based scoring system that replaces the traditional single-cell, slide based scoring with the rapid assessment of images in a 96-well format. With this technology, 200 or more scorable comets can be present in a single image, allowing for drastically reduced analysis times. We have developed a protocol for a 3-day repeat exposure regimen and qualified the HepaRG™ CometChip® using more than 50 known negative and positive control compounds. To further validate this method, a multi-lab trial was conducted in collaboration with MIT, Charles River Laboratories, and Proctor & Gamble. In addition, we have combined the HepaRG™ CometChip® with other endpoints such as the flow-cytometry based micronucleus assay and benchmark dose analysis to create a battery-like approach to in vitro genetic toxicology testing. By developing genotoxicity assessments in HepaRG™ and other human hepatocyte models, we can reduce our reliance on rodent-based testing models while still providing a complete genetic toxicological profile that will meet regulatory requirements for safety evaluation.
This work is funded by NIEHS SBIR 4R44ES024698-02.

Methods





Results

- **A robust method was developed for the use of metabolically competent HepaRG ™ with CometChip ® .**
- **Reproducibility of the method was demonstrated via an interlaboratory trial.**
- **BMD analysis was utilized in conjunction with standard comet analysis, providing further insight into results.**
- **Results were compared to data from Kirkland et al, 2019. This provides insight into HepaRG™ cells when compared to an *in vivo* system.**

Chemical Name	Kirkland Rat Liver 2019*	ILS CometChip	ILS CometChip + historicals
Hydroquinone	-	+	+
Ethyl Methanesulfonate (EMS)	+	+	+
Aflatoxin B1	+	-	=
Dimethylnitrosamine	+	-	=
Cyclophosphamide	+	+	+
Acrylamide	+	-	-
Acetaminophen	+	-	-
Cyproterone Acetate	+	-	-
Urethane	+	-	-
4-Aminobiphenyl	-	+	=
2-amino-3-methylimidazo[4,5-f] quinoline	+	-	-
Benzene	+	-	-
Chloroform	-	+	=
N-ethyl-N-Nitrosurea (ENU)	+	+	+
Chlorambucil	-	+	=
2,4-Diaminotoluene	+	+	=
Methyl Methanesulfonate (MMS)	+	+	+
Diethylnitrosamine (DEN)	+	+	=

*A comparison of transgenic rodent mutation and in vivo comet assay responses from 91 chemicals. David Kirkland et al, Mutation Research/Genetic Toxicology and Environmental Mutagenesis, Volume 839, March 2019
+: Positive Response -: Negative Response =: Equivocal response when compared to ILS historical CometChip data

Conclusions

- **Combining metabolically competent HepaRG™ cells and CometChip® technology provides the potential to develop a human-relevant New Approach Methodology to reduce reliance on the *in vivo* Comet Assay.**
- **The throughput of CometChip® technology enables the conduct of experiments not possible using the 30+ year old one-at-a-time manual scoring method. This is enabled through increased throughput, precision, and use of unbiased automated scoring.**
- **A possible extension of this is the use of CometChip® to score tissues collected from the *in vivo* Comet Assay.**
- **The HepaRG™ CometChip® assay may be readily combined with the Micronucleus assay to further reduce reliance on *in vivo* testing.**
- **The HepaRG™ CometChip® is highly reproducible, demonstrated by multiple laboratories and technicians.**