



New Alternative Methodologies: CometChip® in Metabolically Competent HepaRG™ Cells as a Medium Throughput Genotoxicity Assay

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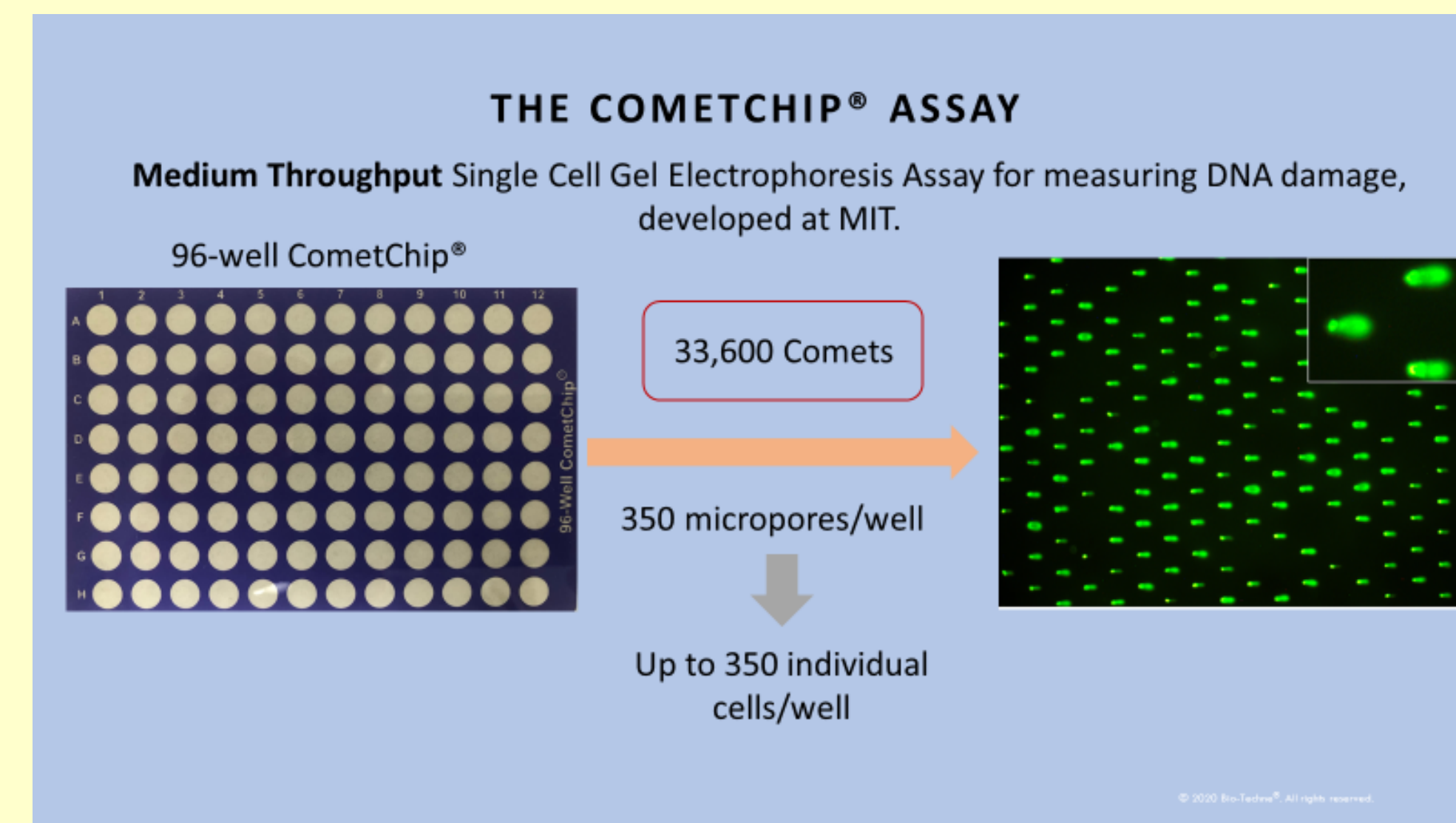


HepaRG™ CometChip® enables medium throughput Comet Assay in a metabolically competent system, and pairs readily with flow cytometry-based Micronucleus Assay

Abstract

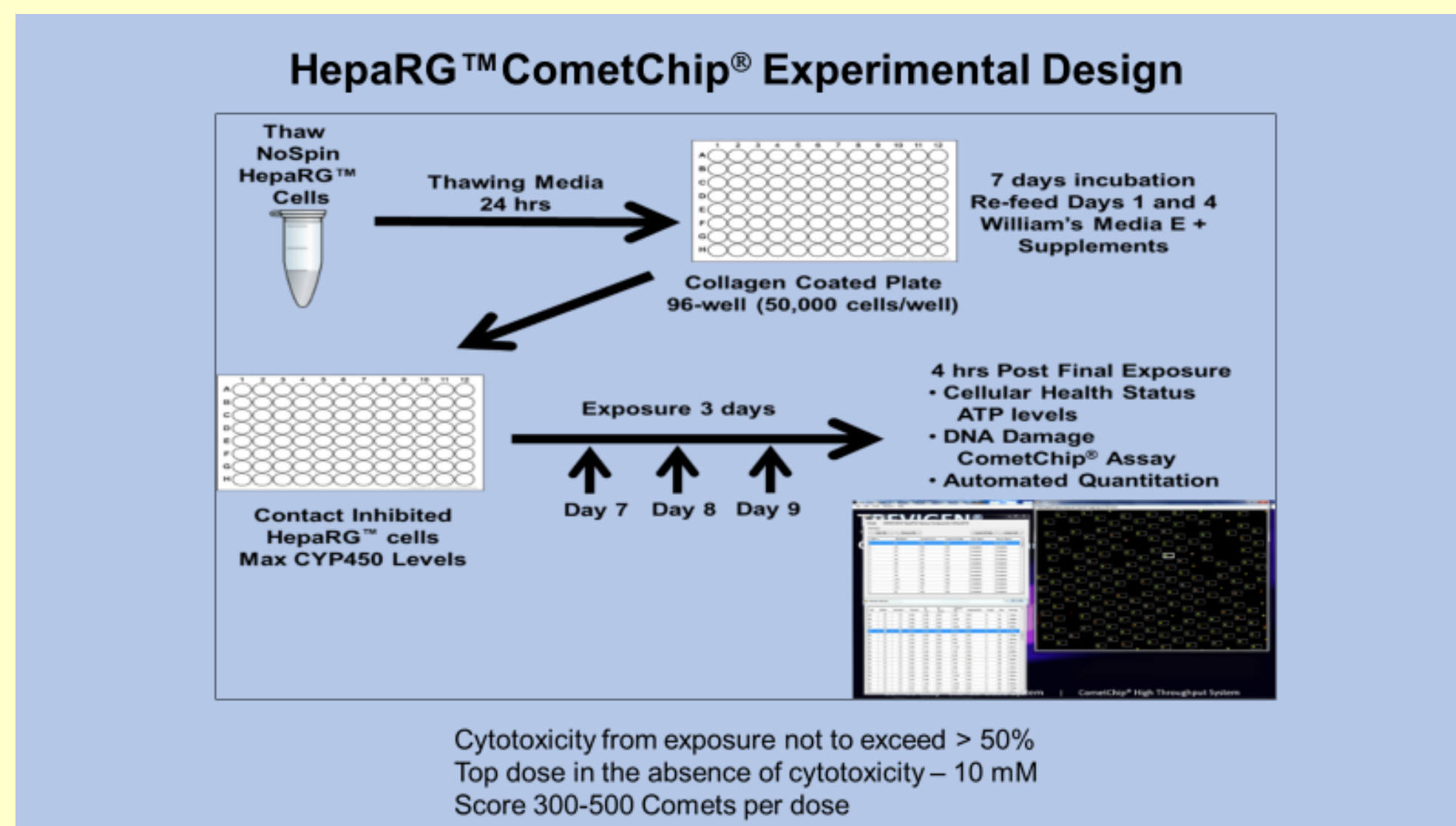
The effort to reduce dependency on the use of animals is an ongoing priority in genetic toxicology. CometChip® technology, developed at MIT, is a single cell array platform that we are combining with metabolically competent HepaRG™ cells to develop a New Alternative Methodology (NAM). CometChip® utilizes an automated, unbiased image-based scoring system that replaces the traditional one cell at a time slide based scoring with the rapid assessment of images in a 96-well format. ≥200 scorable comets can be present in a single image, with less than 45 minutes required to score an entire 96 well plate compared to multiple days via traditional scoring methods. We have developed a protocol for a 3-day repeat exposure regimen, qualified the HepaRG™ CometChip® using known negative and positive control compounds, and combined the HepaRG™ CometChip® with other endpoints such as flow-based micronucleus and benchmark dose analysis. To further qualify this method, we have collaborated with scientists at MIT, Charles River Laboratories, and Proctor and Gamble to demonstrate the interlaboratory reproducibility of the assay. By developing genotoxicity assessments in HepaRG™ and other human hepatocyte models, we can reduce our reliance on rodent based testing models. This work is funded by NIEHS SBIR 4R44ES024698-02.

CometChip® Technology



The CometChip® System uses standard 96-well format.

HepaRG™ CometChip® treatment Regime



Cytotoxicity from exposure not to exceed > 50%
Top dose in the absence of cytotoxicity – 10 mM
Score 300-500 Comets per dose

Introduction

- The *in vivo* Comet Assay, following OECD:489, is a common follow-up test to a compound testing positive in *in vitro* systems, and can use >45 animals per test article
- HepaRG™ cells contain both Phase I and Phase II metabolism enzymes
- CometChip® technology uses 96-well plate format to allow for rapid sample processing, combined with automated imaging technology. This allows for processing of an entire 96-well plate in less than 45 minutes.
- HepaRG™ cells can be readily used in the Micronucleus Assay with the addition of epidermal growth factor.
- Benchmark Dose Analysis can be used as an additional endpoint.

Study Objectives

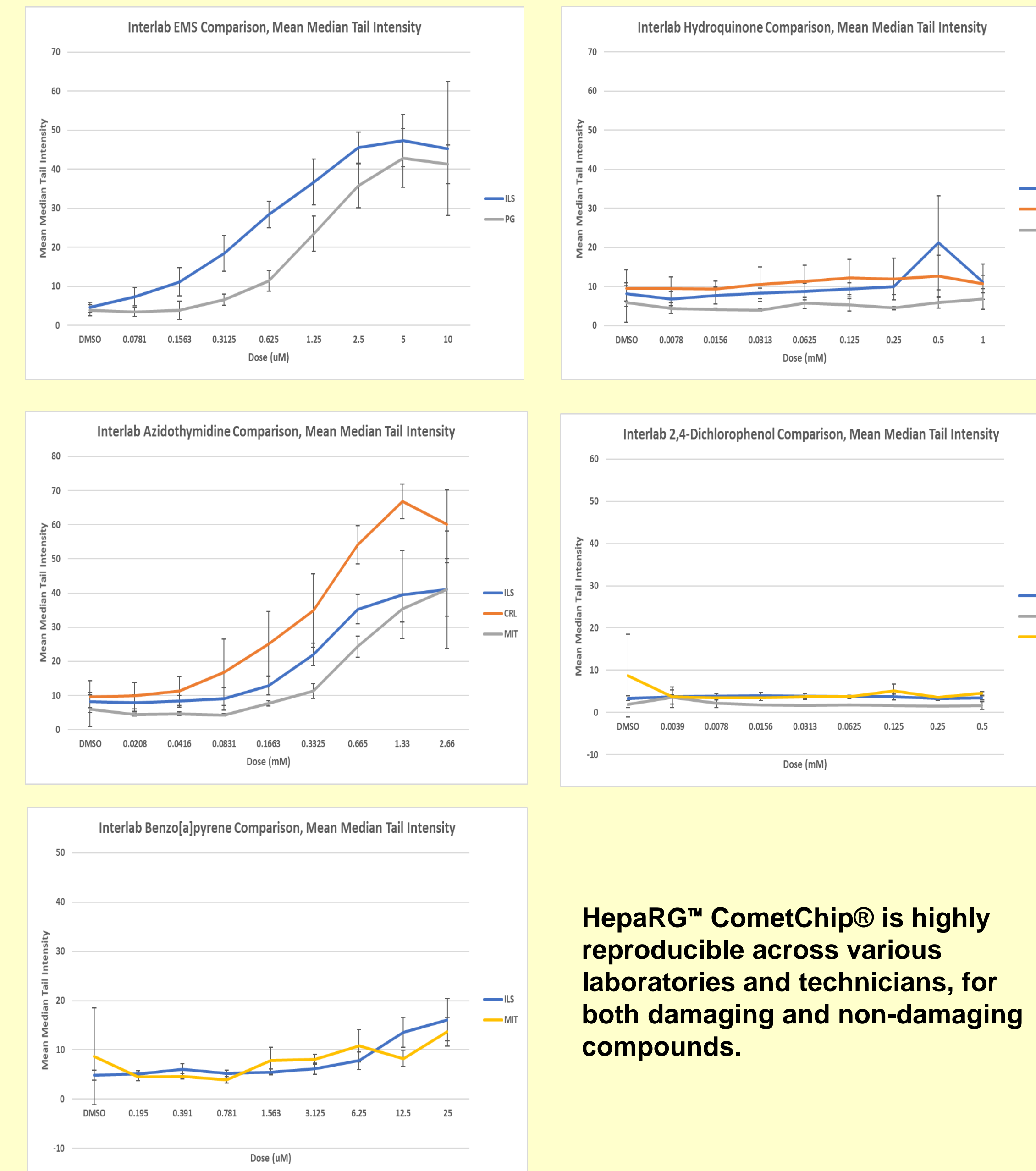
- Establish an initial protocol for HepaRG™ CometChip®.
- Conduct a “power” study to assess optimal number of comets to score per dose level.
- Test known negative and positive control test articles for use in qualifying the HepaRG™ CometChip® Assay.
- Integrate the HepaRG™ CometChip® Assay with the Micronucleus Assay.
- Demonstrate reproducibility of the HepaRG™ CometChip® through interlaboratory testing.

Power study using Ethyl methanesulfonate (EMS)

1 Chip						2 Chip					
EMS (mM)	Cells scored	NDNA in Tail	P Value	% Survivability	Cells scored	NDNA in Tail	P Value	% Survivability			
0.0	81	6.091314	1	100	317	6.392117	1	100			
0.3	399	6.412134	1	95.74	520	6.702204	1	92.57			
0.5	276	6.881189	1	89.27	516	11.611191	0.9806	75.2			
1.0	222	8.551163	0.0062	84.28	480	15.941139	0.1443	84.42			
2.0	275	18.281088	0.0167	87.53	480	27.601213	0.0127	83.89			
4.0	338	35.811039	<.0001*	95.61	430	37.301260	0.0023*	70.08			
6.0	232	42.781237	<.0001*	78.08	337	46.981089	<.0001*	76.29			
8.0	204	47.091232	<.0001*	85.56	312	50.501050	<.0001*	51.81			
10.0	105	36.091083	<.0001*	37.36	124	32.021170	0.0130*	21.11			

Reduced impact of increased comets once ~500 comets reached

Interlaboratory Testing of HepaRG™ CometChip®



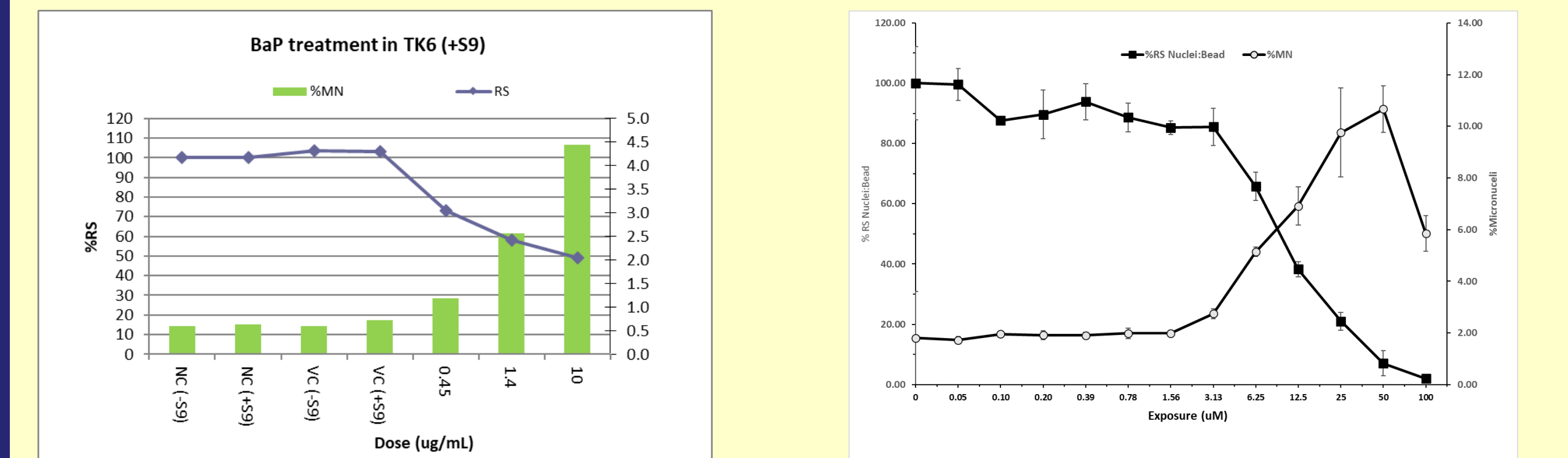
HepaRG™ CometChip® is highly reproducible across various laboratories and technicians, for both damaging and non-damaging compounds.

Benchmark Dose Analysis

Chemical	BMD	Lower BMD	Upper BMD	Fit p-value
Amitrole (mM)	40.478	13.325	1000000.0	0.1441
Ethyl Methanesulfonate (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 performed using BMD Express 2

Micronucleus Assay using HepaRG™ and Comparison to Kirkland et al Comet



4 hour (+S9) BaP Treatment in TK6

24 hour BaP Treatment in HepaRG™

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)	+	+	+

Comparison of ILS CometChip results with and without historical data to Kirkland et al, 2019 comet data

*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
= : Equivalency response when compared to ILS historical CometChip data

Summary and Conclusions

- Combining metabolically competent HepaRG™ cells and CometChip® technology provides the potential to develop a human-relevant New Alternative Methodology to reduce reliance on the *in vivo* Comet Assay.
- The throughput of CometChip® technology enables the conduct of experiment not possible using the 30+ year old one-at-a-time manual scoring. 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 *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.

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