Human Relevant Genetic Toxicology for Risk Assessments

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DNA damage assessment CometChip®

SBIR Fast Track and SBIR 2B

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Micronucleus and TGx DDI

Method Description

New Approach Methodology (NAM) is focused on key characteristics of human carcinogens: genotoxicity and mutagenicity.

Regulatory Genetic Toxicology Test Battery (OECD Guideline)

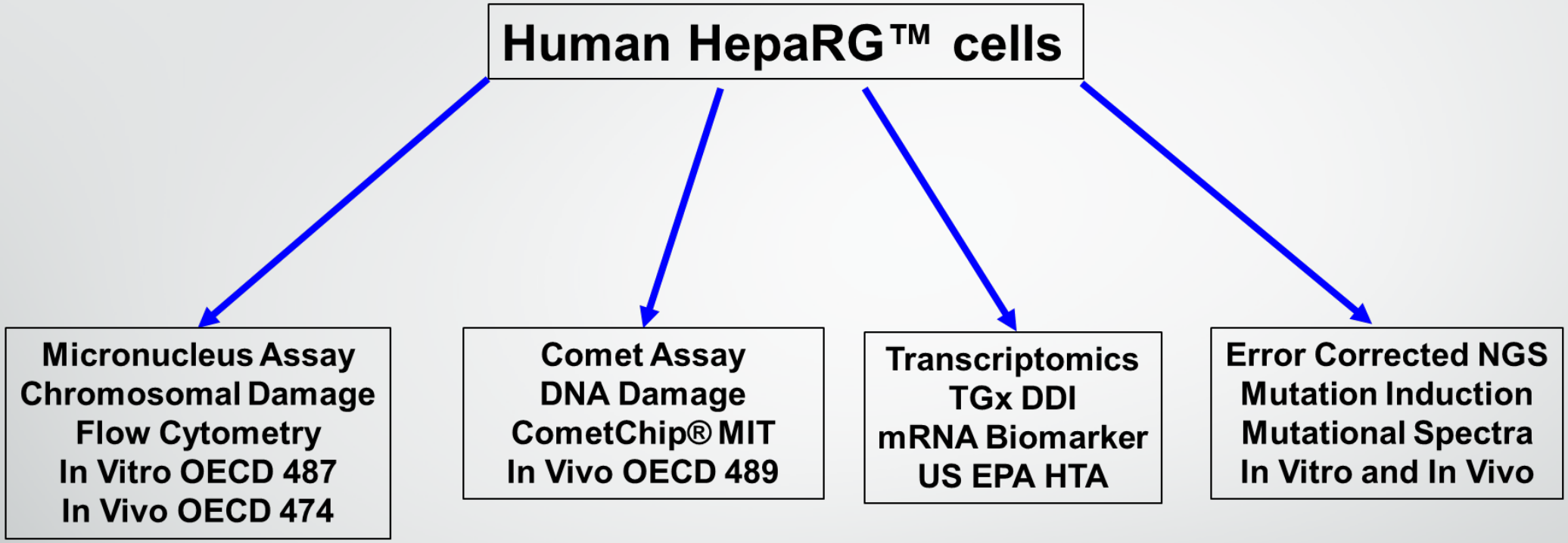
- A test for gene mutation in bacteria
- A test for chromosomal aberrations, or micronucleus assay, or an *in vitro* mouse lymphoma TK gene mutation assay
- An *in vivo* test for genotoxicity, chromosomal damage – micronucleus assay in rodent hematopoietic cells
- An in vivo test in a second tissue tissue assessment of DNA damage typically Comet assay in

Genetic Toxicology regulatory test battery is <u>hazard identification</u> focused, used to assess the potential of a test compound to cause mutation and genotoxicity (chromosomal aberrations/aneuploidy) in a 3-tiered testing approach.

DNA repair deficient bacterial cells and p53-deficient rodent cell line-based bioassays
In vitro assays require use of highly induced rat liver S9 to provide Phase I metabolism
highly induced rat liver enzymes - not human relevant
No human gene mutation assay is required
Tier 3 is animal testing for DNA damage, Chromosomal Damage and Gene Mutation

Hepatocytes based NAM has limited application to direct effects of volatile compounds – respiratory tract models are under development.

Multiple endpoint genotoxicity and mutagenicity assessments in metabolically competent human liver cell line HepaRG™: Method Description



Complimentary Genotoxicity Assessment to Cover a Broad Range of Mode-of-Action

Multiple endpoints required to interrogate the broad mode-of-action for genotoxic and nongenotoxic carcinogens.

Multiple endpoint "inputs" to benchmark dose and point of departure for *In Vitro* to *In Vivo* Extrapolation Modeling

HepaRG™ Multiple Endpoint Genetic Toxicology Testing Platform: Context of Use

Hazard identification and genotoxicity screening

Human cell alternative to the use of bacterial cells and rodent cell lines used in regulatory *in vitro* genetic toxicology testing - Chinese hamster, inbred mouse cell lines, mouse stem cells

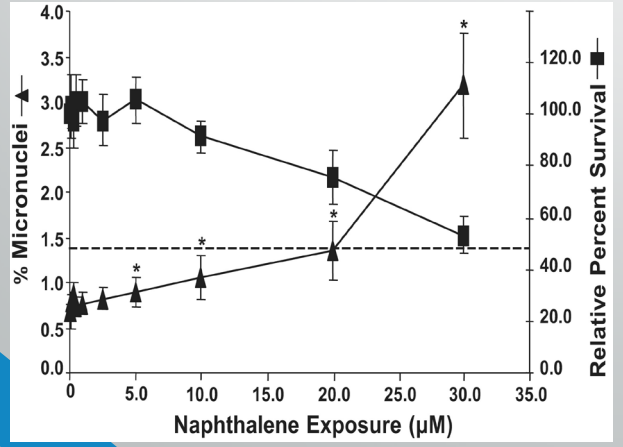
Replace reliance on rat liver S9 metabolism in genotoxicity screening assay with human based xenobiotic Phase I and Phase II metabolism

Genetic toxicology *in vitro* bioassays lack xenobiotic biotransformation enzymes and use highly induced rat liver homogenate with CYP450 cofactor

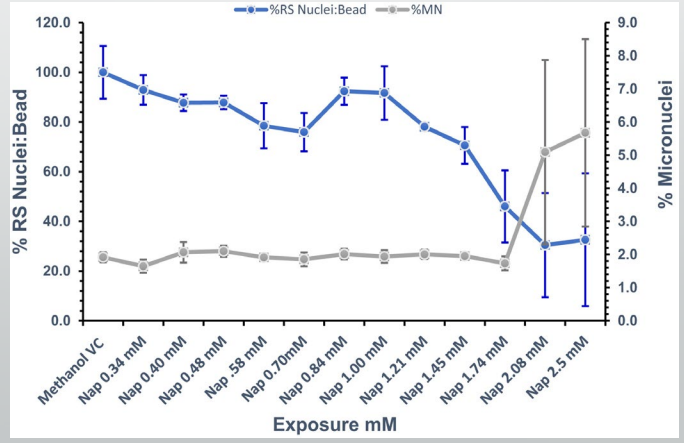
No Phase II metabolism is not relevant to *in vivo* metabolism of endogenous biochemicals and xenobiotics - incomplete/un-coupled biotransformation.

Naphthalene case study: Genotoxicity assessment in TK6 cells with S9 and in HepaRG™ cells

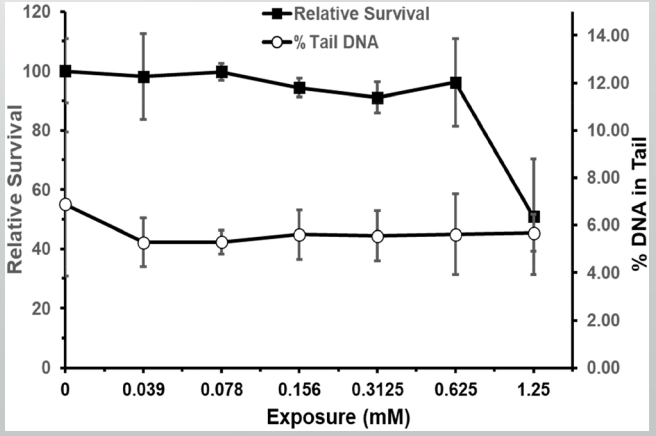
Recio L, Fowler J, Martin L, Swartz C. Genotoxicity assessment in HepaRG™ cells as a new approach methodology follow up to a positive response in the human TK6 cell micronucleus assay: Naphthalene case study. Environ Mol Mutagen. 2023 Oct-Nov;64(8-9):458-465.



TK6 cells Micronucleus with S9



HepaRG™ micronucleus Assay



HepaRG™ CometChip® Assay

HepaRG™ Multiple Endpoint Genetic Toxicology Testing Platform: Context of Use

HepaRG™ is a versatile cell line has been adapted to several cell-based design formats 2D, 3D spheroids, and organoid cultures, 96-well formats and testing strategies to examine:

cytotoxicity, enzyme induction, transcriptomics, medium throughput genotoxicity assays

Versatile applications: Hazard Identification, enzyme induction, and chemical screening to dose-response assessment for quantitative benchmark concentration modeling.

HepaRG™ is used as part of NIEHS Tox21 and US EPA TOXCAST high/medium throughput testing programs.

Stephen Ferguson

Enzyme induction to assess FDA drug-drug interaction

ECVAM validation as a replacement for primary human hepatocytes

HepaRG™ Multiple Endpoint Genetic Toxicology Testing Platform: Context of Use

HepaRG™ NAM is aimed shifting genotoxicity assessment away from rodent based assays to human based *in vitro* bioassays that captures human xenobiotic biotransformation (Phase I and II) for use in risk assessment.

- Quantification of regulatory genetic toxicology endpoints
- Human relevant alternative to *in vivo* genotoxicity assessments

In Vitro and *In Vivo* Micronucleus Assay

- Measure of chromosomal aberrations and aneuploidy
- Flow Cytometry based assays
- Requires cell division to assess chromosomal anomalies in single cell suspensions
- *In Vitro* and *In Vivo* micronucleus assay
- ScitoVation qualified human TK6 cells with and without S9 and HepaRG™ cells

In Vivo Comet Assay OECD 489

- Typically done in liver and other tissues as required by regulatory agency
- Measure of DNA damage – detects a broad range of lesions and DNA repair
- Does not require cell division – single cell suspensions
- No OECD guidance for *In Vitro* Comet Assay
- High Throughput CometChip® Assay – developed at MIT automated comet assay scoring

HepaRG™ Multiple Endpoint Genetic Toxicology Testing Platform: Context of Use

Transcriptomics based mode-of-action and dose-response in a human-relevant test system to develop and inform:

- Transcriptomics based mode-of-action used by US EPA in rodents and *in vitro* EPA/NIEHS Tox21
- Nongenotoxic Mode of Action – nuclear transcriptional activators gene signature
- Genotoxic Mode of Action – TGx DDI US EPA

HepaRG™ error corrected next generation sequencing (ecNGS) gene mutation assay

- Technological leap NGS replacement for 50 yr old clonal selection gene mutation assays
- Gene mutation assay in human cells is not a required endpoint - rodent cell lines.
- Follow up to bacterial mutation assay (Ames test) positive
- Gene mutation assay in liver requires use of transgenic rodent models with limited availability (up to 1 yr lead time and high cost >$200,000).

Quantitative dose response analysis for risk assessments

- Benchmark Dose (BMD) Modeling
- Adverse Outcome Pathways (AOP)
- Potency Evaluation by BMD
- Identify point of departure (POD) – first genome perturbation or mode-of-action
- *In Vitro* to *In Vivo* Extrapolation (IVIVE) modeling for risk assessment

HepaRG™ Multiple Endpoint Toxicology Testing Platform: Context of Use

Measures first three key characteristics of carcinogens

- Electrophile or can be metabolized to electrophiles – human-relevant metabolism
- Genotoxicity and mutagenicity – ecNGS
- Alters DNA repair and genomic instability

Directly relevant to carcinogenesis process

- Induction of chromosomal aberrations and gene mutation
- Direct contribution to development of adverse outcome pathways
  - DNA damage and mutation assessment
- Informs cancer risk assessments by quantifying BMD and POD

Reference compound assessment – significance of complimentary measurements

Buick JK, Williams A, Gagné R, Swartz CD, Recio L, Ferguson SS, Yauk CL. Flow cytometric micronucleus assay and TGx-DDI transcriptomic biomarker analysis of ten genotoxic and non-genotoxic chemicals in human HepaRG™ cells. Genes Environ. 2020 Feb 4;42:5.

Buick JK, Williams A, Meier MJ, Swartz CD, Recio L, Gagné R, Ferguson SS, Engelward BP, Yauk CL. A Modern Genotoxicity Testing Paradigm: Integration of the High-Throughput CometChip® and the TGx-DDI Transcriptomic Biomarker in Human HepaRG™ Cell Cultures. Front Public Health. 2021 Aug 18;9:694834

HepaRG™ Multiple Endpoint Toxicology Testing Platform: Context of Use

HepaRG™ multiple endpoint human-relevant genetic toxicology testing platform aimed at reducing or replacing dependence on required rodent mutagenicity genotoxicity assessments.

- Identical endpoints measured *in vivo* as required by regulatory genetic toxicology in a metabolically competent human liver cell line
  - Micronucleus and Comet Assay
- Transcriptomics based biomarkers to assess MOA and useful for IVIVE modeling based risk assessments
- ecNGS to assess gene mutation in metabolically competent human liver cell line

HepaRG™ multiple endpoint human-relevant genetic toxicology testing platform aimed as a follow up to positive findings in the hazard identification regulatory genetic toxicology test battery across multiple sectors.

- Genetic toxicology assessments are required part of safety assessment worldwide.
- pharmaceuticals, industrial chemicals, cosmetics, food additives

HepaRG™ Multiple Endpoint Genetic Toxicology Testing Platform: Biological Relevance

Human relevant metabolism

Phenobarbital/β-naphthoflavone rat liver S9 with CYP450 cofactors "gold standard" used by in vitro genetic toxicology test battery is not a valid replacement for human.

Method quantifies identical regulatory genetic toxicology endpoints as measured in rodents known to be key characteristics of carcinogens.

DNA damage
chromosomal aberrations
gene mutation

Integration of transcriptomics-based mode of action (MOA)

Non-genotoxic MOA – gene signatures for nuclear transcriptional biomarkers
Genotoxic MOA – TGx DDI
Transcriptomics based MOA, BMD and POD for risk assessments

Complimentary assessment of multiple endpoints

Traditional and genomics-based endpoints

HepaRG™ Multiple Endpoint Genetic Toxicology Testing Platform: Biological Relevance

Liver is most common tumor target tissue in NTP cancer bioassay program

Genetic toxicology hazard identification regulatory test battery is a qualitative assessment of genotoxicity not quantitative and is not designed for use in human risk assessments.

- Phenobarbital/β-naphthoflavone rat liver S9 – not human relevant metabolism
- Excision repair deficient bacteria not relevant to humans
- Rodent cell lines that are p53 deficient
- Rodent cells lines and rodents - three doses with vehicle control
- Low confidence BMD

HepaRG™ genetic toxicology testing platform allows for several doses to be tested as several replicates for quantitative assessment of dose-response curve.

- Repeat experiments
- Identify BMD – BMD50 suggested for genetic toxicology endpoints
- Determine POD for risk assessment
- *In Vitro to In Vivo* Extrapolation modeling

HepaRG™ Multiple Endpoint Genetic Toxicology Testing Platform: Technical Characterization

Significant sources of variability include:

- Source of cells – pre-differentiated and post differentiated
- 2D, 3D spheroids and organoid models
- Exposure schedule – acute to repeat dose testing
- Proprietary cells, media, and reagents from single vendor reduces variability among laboratories
- Preparation of single cell suspensions for Comet

NIEHS SBIR funding to ILS and MIT supported development of methods, qualification of assay, transfer of methods to Charles River Laboratories and Proctor and Gamble for HepaRG™ CometChip®

- Functional assessment of HepaRG™ cell – enzyme induction
- Experimental design comparing 1 to 3 day exposures
- Top dose criteria
  - Cytotoxicity assessment to set top dose – 50%
  - 10 mm to be consistent with OECD test Guidelines
- Methods paper for HepaRG™ CometChip ®

Owiti NA, Kaushal S, Martin L, Sly J, Swartz CD, Fowler J, Corrigan JJ, Recio L, Engelward BP. Using the HepaCometChip Assay for Broad-Spectrum DNA Damage Analysis. Curr Protoc. 2022 Sep;2(9):e563.

HepaRG™ Multiple Endpoint Genetic Toxicology Testing Platform: Technical Characterization

Interlaboratory reproducibility and transferability of HepaRG™ CometChip® Assay

- On-site training of collaborators from Charles River Laboratories, Proctor and Gamble
- Compound selection from rodent liver comet assay
- Provided HepaRG™ cells and CometChip®
- Data compiled and undergoing assessment/discussions
- Consistent results and reproducibility for prototypic positive controls

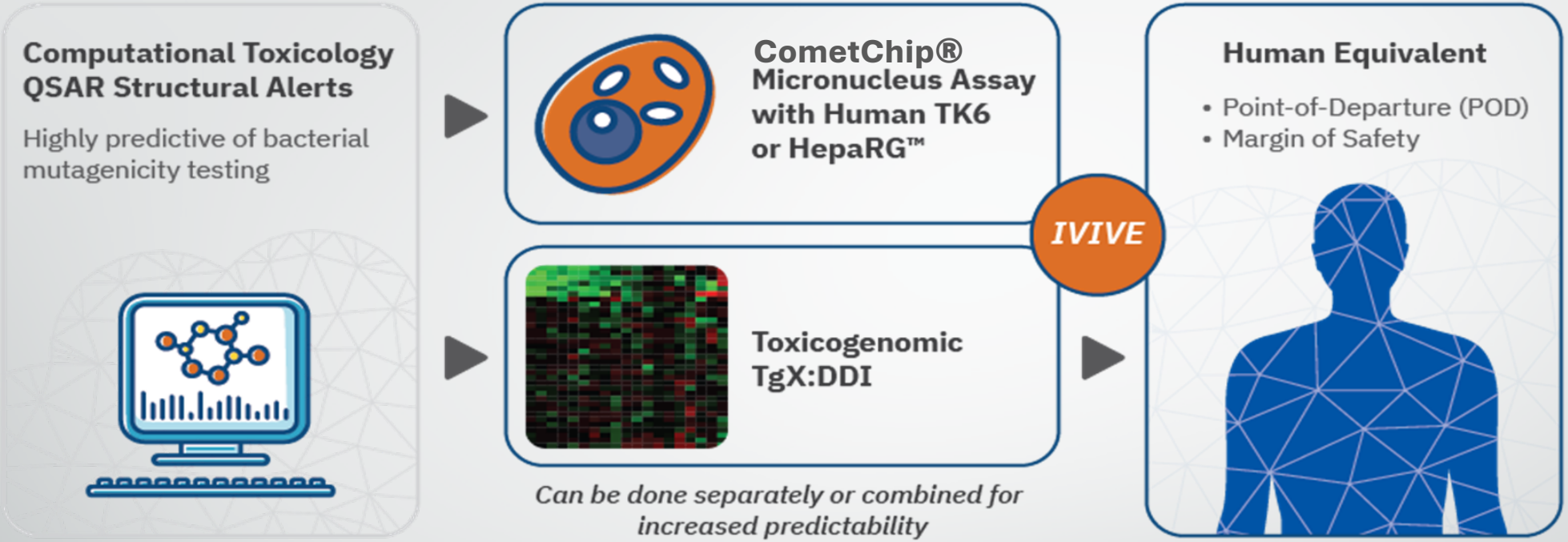
Micronucleus assay in HepaRG™ and transcriptomics-based TGx DDI biomarker

- Funded by Health Canda – Carole Yauk now at U of Ottawa
- Qualified by using 5 genotoxic Vs 5 nongenotoxic compounds
- CometChip®, Micronucleus assay, and transcriptomics can be integrated into a single exposure in 96 well plates.
- HepaRG™ Micronucleus assay and TGx DDI - Health Canada Testing Programs
- Several laboratories/publications on HepaRG™ CometChip®, Micronucleus assay, transcriptomics

Quality control metrics based on responses of known positive controls included on plate map from each study

- "universal controls" and development of historical control databases

Next Generation Genotoxicity Assessments



Thank you for your attention!

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