Genetic Toxicology Association
Translating Genetox

Keynote Address

Pfizer

I didn’t catch your NAM: advancing genotoxicity testing strategies through multi-sector collaborations

Prof. Carole Yauk, PhD
University of Ottawa

-Sponsored by Pfizer-

Genetic Toxicology Association

Translating Genetox

2023 Annual Meeting of the GTA

John M. Clayton Hall Conference Center
University of Delaware,
Newark, DE

May 3rd to 5th 2023

Genetic Toxicology Association

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MultiCASE

Exhibitor booths are open during all the breaks and poster sessions

Genetic Toxicology Association
Translating Genetox

2023 Genetic Toxicology Association Annual Meeting

Invited Speakers

Keynote Address Prof. Carole Yauk PhD, University of Ottawa

Anthony Lynch PhD, GSK Plc
Arianna Bassan PhD, Innovatune
Ashley Allemang MS, Procter & Gamble
Carol Beevers, PhD, Corteva Agriscience
Dan Roberts, MS, Toxys
David Kirkland PhD, Kirkland Consulting
Francesco Marchetti PhD, Health Canada
Giel Hendriks PhD, Toxys
Jakub Kostal, PhD, ToxFix
John Nicolette MS, Janssen
John W Wills, PhD, University of Cambridge
Kevin Cross PhD, Insteam
Krista Dobo PhD, Pfizer
Marie Vasquez MS, Helix3
Prof. Bevin P. Engelward, ScD. MIT
Prof. John P. Wise, University of Louisville
Prof. Ke Jian "Jim" Liu, Stony Brook University
Prof. Koren Mann, McGill University
Ronee Baracani MS, Eli Lilly & Company
Seda Arat, Ph.D , Pfizer
Shaofei Zhang PhD, Pfizer
Stephanie Kellum BS, Corteva Agriscience
Stephen D. Dertinger PhD, Litron
Suman Chakravarti PhD, MultiCASE
Susanne Stalford PhD, Lhasa
Tim McGovern PhD, US FDA
Vasily Dobrovolsky PhD, FDA
Xi Chen, Ph.D. NCTR, US FDA
Yax Thakur MS, New York Medical College

Session Co-Chairs

Workshop 1 Yi Yang PhD & Dan Levy PhD
Workshop 2 Maria Engel MS & Rosie Elespuru PhD
Keynote Address Sheroy Minocherhomji PhD & Penny Leavitt MS
Symposium I Dan Roberts MS & Wen Sun PhD
Symposium II Wen Sun PhD & Penny Leavitt MS
Symposium III Laura Markley PhD & Jamie Young PhD
Symposium IV David Kirkland PhD & Leon Stankowski PhD
Symposium V Melissa Masuda-Herrera MS & Kevin Cross PhD
Symposium VI Joel Bercu PhD & Jennifer Cheung BS

Genetic Toxicology Association
Translating Genetox

2023 Annual Meeting of the Genetic Toxicology Association – May 4th

8:40 – 9:45 AM	**Keynote Address**	**Auditorium 128**

Prof. Carole Yauk PhD, University of Ottawa, Canada
Co-chairs: Sheroy Minocherhomji PhD & Penny Leavitt MS, DABT

9:45 – 10:00 AM	**Coffee Break**	**Lobby A**
10:00 – 11:30 AM	**Symposium I**	**Auditorium 128**

New Technologies for Genetic Toxicology Testing
Co-chairs: Dan Roberts MS, & Wen Sun PhD

11:30 – 12:00 PM	**Awards Ceremony**	**Auditorium 128**

Excellence in Service Award
Chair: Dan Roberts MS

12:00 – 1:00 PM	**Networking Lunch**	**Room 101A**

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EMGS Environmental Mutagenesis and Genomics Society
Synergistic Interactions for a Better World

CORTEVA™ agriscience

Genetic Toxicology Association
Translating Genetox

2023 Annual Meeting of the Genetic Toxicology Association – May 4th

1:00 – 2:30 PM	**Symposium II**	**Auditorium 128**

Artificial Intelligence, Machine Learning and Modeling Advances in Genetic Toxicology Testing

Co-chairs: Wen Sun PhD, & Penny Leavitt MS, DABT

2:30 – 2:50 PM	**Coffee Break**	**Lobby A**
2:50 – 4:30 PM	**Symposium III**	**Auditorium 128**

Genetic Mechanisms in Metals Carcinogenesis

Co-chairs: Laura Markley PhD & Jamie Young PhD

Sponsors

SOT Society of Toxicology
Creating a Safer and Healthier World by Advancing the Science and Increasing the Impact of Toxicology

HESI Genetic Toxicology Technical Committee

CORTEVA™ agriscience

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Genetic Toxicology Association
Translating Genetox

2023 Annual Meeting of the Genetic Toxicology Association – May 4th

4:30 – 5:00 PM	**Poster Presentations**	**Auditorium 128**

2- to 3-minute Speed Talks – Student & Early Investigators

Co-chairs: Ashley Allemang MS & Yi Yang PhD, DABT

5:00 – 7:00 PM	**Poster Presentations & Cocktails**	**Lobby A**

5:00 – 6:00 PM Odd numbered posters. Presenters available at their posters

6:00 – 7:00 PM Even numbered posters. Presenters available at their posters

7:00 – 8:30 PM	**Dinner and Reception**	**Room 101A**

Sponsors

inotiv
analyze. answer. advance.

I didn’t catch your NAM: Advancing genotoxicity testing strategies through multi-sector collaborations

Carole Yauk

University of Ottawa

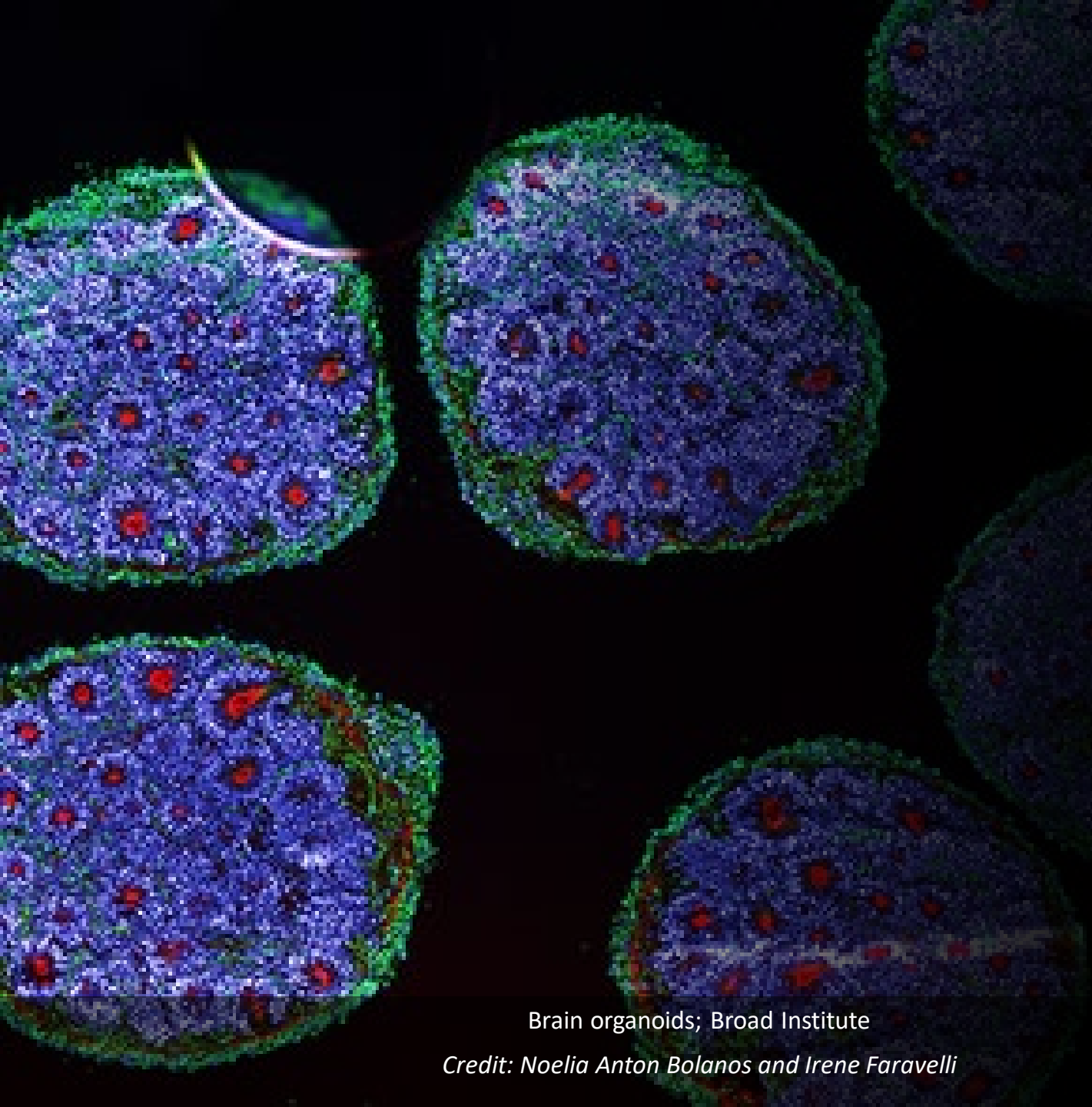
Genetic Toxicology Annual Meeting, May 2023

Outline

We have the:

1. Motivation
2. Experience
3. Know-how
4. Case examples of informative NAMs

To transform genotoxicity testing and risk assessment.



Brain organoids; Broad Institute

Credit: Noelia Anton Bolanos and Irene Faravelli

What's in a NAM*?
*New approach methodologies

Generally accepted: Non-animal-based approaches that can be used to provide information for chemical hazard and risk assessment

- In silico, in chemico, in vitro, ex vivo

Herein: Emerging tools in toxicology that inform mechanisms and reduce reliance on long-term animal tests

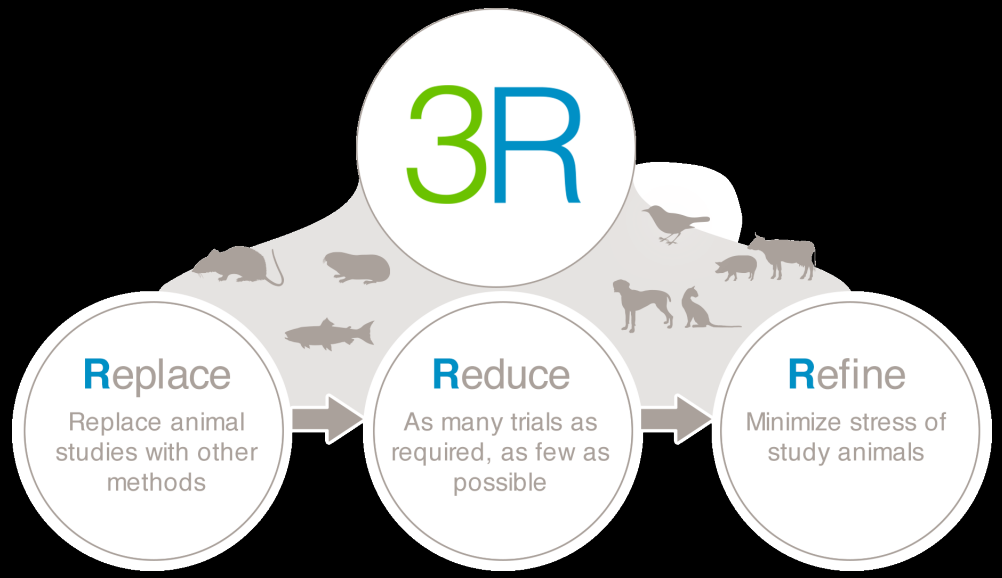
Part 1. Motivation

Shortcomings of today's genotoxicity test methods

Current tests

Human health effects

- They are
  - Slow
    - One endpoint at a time
  - Expensive
- They use too many animals
  - Not always relevant to humans
- *In vitro* methods lack specificity
  - Not sufficiently predictive of effects *in vivo*
- They generally do not tell us about mechanisms of genotoxicity
  - Necessary to predict human relevance



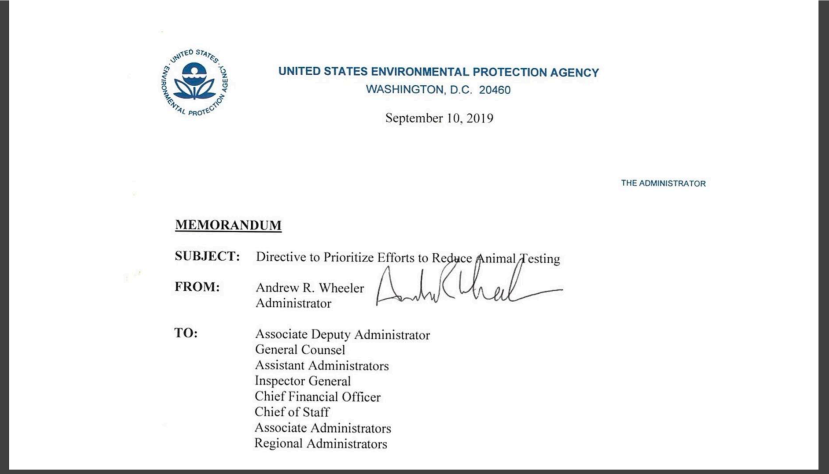
Motivation
What do we need?

Assays that are more

- Efficient
- Human-relevant
- Comprehensive
- Quantitative (not just for hazard identification)
- Predictive (mechanism-based)

And that

- Are as protective as today's assays
- Use fewer animals
- Can be integrated with other assays
- Use modern technologies (let's not ignore innovation)



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

September 10, 2019

THE ADMINISTRATOR

MEMORANDUM

SUBJECT: Directive to Prioritize Efforts to Reduce Animal Testing

FROM: Andrew R. Wheeler
Administrator

TO: Associate Deputy Administrator
General Counsel
Assistant Administrators
Inspector General
Chief Financial Officer
Chief of Staff
Associate Administrators
Regional Administrators

Government of Canada | Gouvernement du Canada

Français

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MENU

Canada.ca › Environment and Climate Change Canada

Strengthening protections for Canadians and the environment from harmful chemicals and pollutants

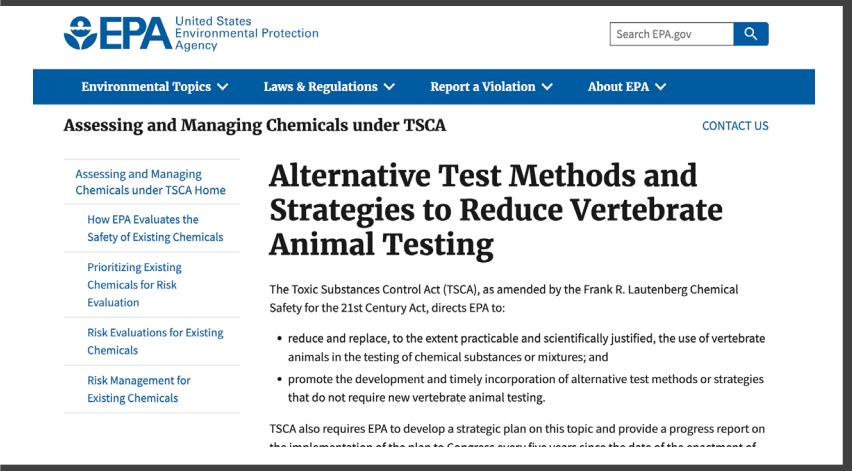
From: Environment and Climate Change Canada

News release

February 9, 2022 – Ottawa, Ontario

Canadians expect their government to protect their health and the environment from harmful chemicals and other toxic pollutants. Today, the Government of Canada took an important step forward to do just that.

The Government introduced in the Senate the bill *Strengthening Environmental Protection for a Healthier Canada Act*, which would modernize the *Canadian Environmental Protection Act, 1999* (CEPA) for the first time in twenty years and make



EPA United States Environmental Protection Agency

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Assessing and Managing Chemicals under TSCA CONTACT US

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- How EPA Evaluates the Safety of Existing Chemicals
- Prioritizing Existing Chemicals for Risk Evaluation
- Risk Evaluations for Existing Chemicals
- Risk Management for Existing Chemicals

Alternative Test Methods and Strategies to Reduce Vertebrate Animal Testing

The Toxic Substances Control Act (TSCA), as amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act, directs EPA to:

- reduce and replace, to the extent practicable and scientifically justified, the use of vertebrate animals in the testing of chemical substances or mixtures; and
- promote the development and timely incorporation of alternative test methods or strategies that do not require new vertebrate animal testing.

TSCA also requires EPA to develop a strategic plan on this topic and provide a progress report on

Office of the Prime Minister | Cabinet du Premier ministre

Ottawa, Canada K1A 0A2

Mandate letter to Minister of Health, 2021

Motivation

Legislation phasing out animal testing

FDA Modernization Act 2.0

Politicians and public pushing for NAMs implementation

"The CEPA modernization bill is in keeping with the emerging scientific discipline of **toxicogenomics** . . . the science is evolving to be able to better identify toxic impacts of substances in populations. Some groups may be at greater risk for negative impacts of substances than other groups. Combinations of substances may create toxic impacts not found in each substance separately, and cumulative effects are important to understanding toxicity."

-Senator Stan Kutcher

Senate Debates: "Strengthening Environmental Protection for a Healthier Canada" Bill

Part 2. Experience

Genetic Toxicologists are pioneers in NAMs

OECD publishing

OECD Guidelines for Testing of Chemicals Section 4: Health Effects

Test No. 471: Bacterial Reverse Mutation Test

The bacterial reverse mutation test uses amino-acid requiring at least five strains of Salmonella typhimurium and Escherichia coli to detect point mutations by base substitutions or frameshifts. The principle of this bacterial reverse mutation test is that it detects mutations which revert mutations present in the test strains and restore the functional capability of the bacteria to synthesize an essential amino acid. Suspensions of bacterial cells are exposed to the test substance (liquid or solid) in the presence and in the absence of an exogenous metabolic activation system. At least five different analysable concentrations of the test substance should be used. The recommended maximum test concentration for soluble non-cytotoxic substances is 5 mg/plate or 5 ml/plate. There are two methods: the plate incorporation method and the preincubation method. For both techniques, after two or three days of incubation at 37°C, revertant colonies are counted and compared to the number of spontaneous revertant colonies on solvent control plates.

Test No. 476: In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes

The in vitro mammalian cell gene mutation test can be used to detect gene mutations induced by chemical substances. In this test, the used genetic endpoints measure mutation at hypoxanthine-guanine phosphoribosyl transferase (HPRT), and at a transgene of xanthineguanine phosphoribosyl transferase (XPRT). The HPRT and XPRT mutation tests detect different spectra of genetic events. Cells in suspension or monolayer culture are exposed to, at least four analysable concentrations of the test substance, both with and without metabolic activation, for a suitable period of time. They are ⌄ More

Published on July 29, 2016 Also available in: French

In series: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects (view more titles)

Read online Download PDF Get citation details

Test No. 487: In Vitro Mammalian Cell Micronucleus Test

The in vitro micronucleus test is a genotoxicity test for the detection of micronuclei in the cytoplasm of interphase cells. Micronuclei may originate from acentric chromosome fragments (i.e. lacking a centromere), or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. The assay detects the activity of clastogenic and aneugenic test substances in cells that have undergone cell division during or after exposure to the test substance. This Test Guideline allows the use of protocols with and without the actin polymerisation inhibitor cytochalasin B ⌄ More

Published on July 29, 2016 Also available in: French

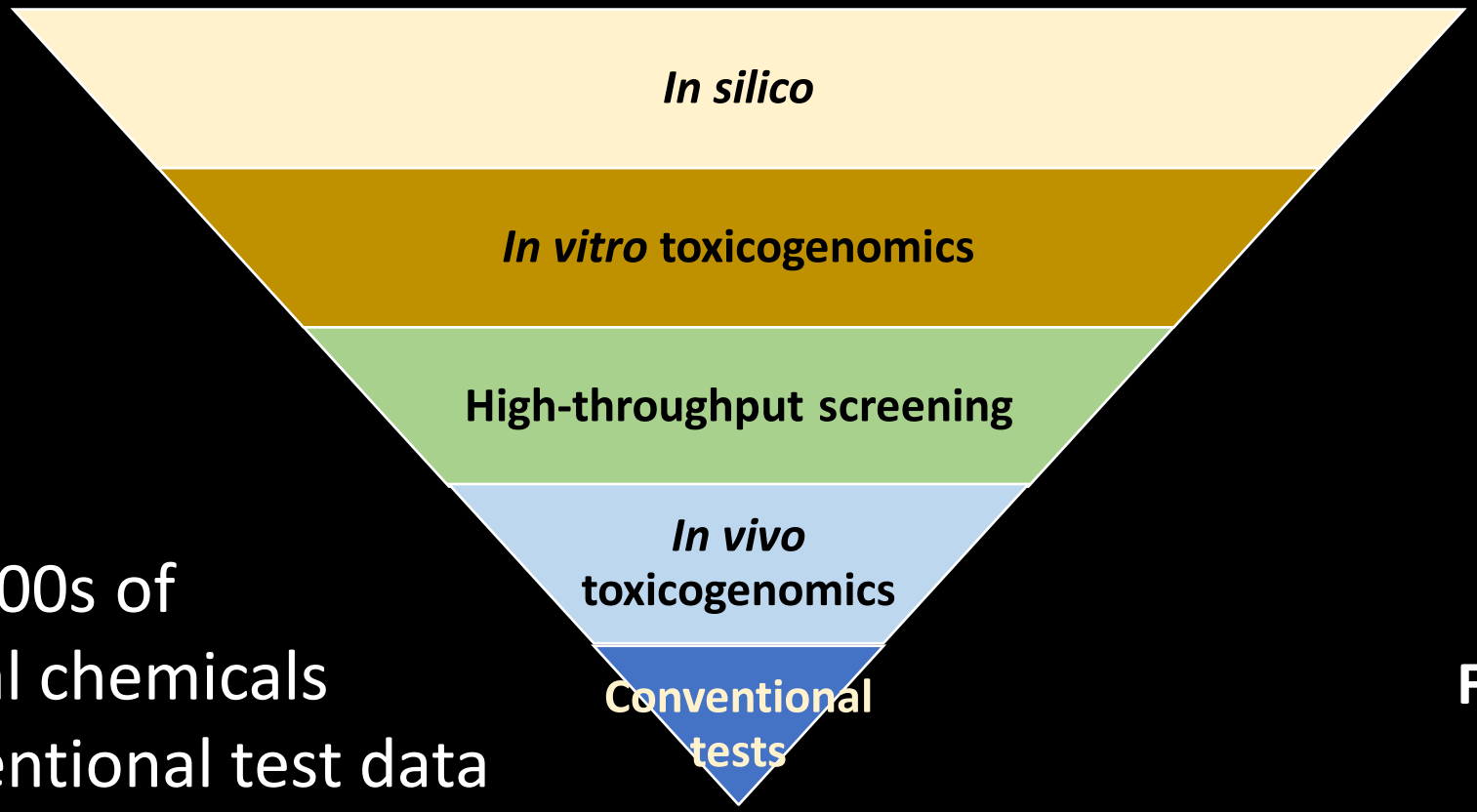
In series: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects (view more titles)

Read online Download PDF Get citation details

Calls to implement a paradigm change (> 15 years ago)

Faster
Cheaper
Mechanistic
Predictive

To address 1000s of environmental chemicals with no conventional test data

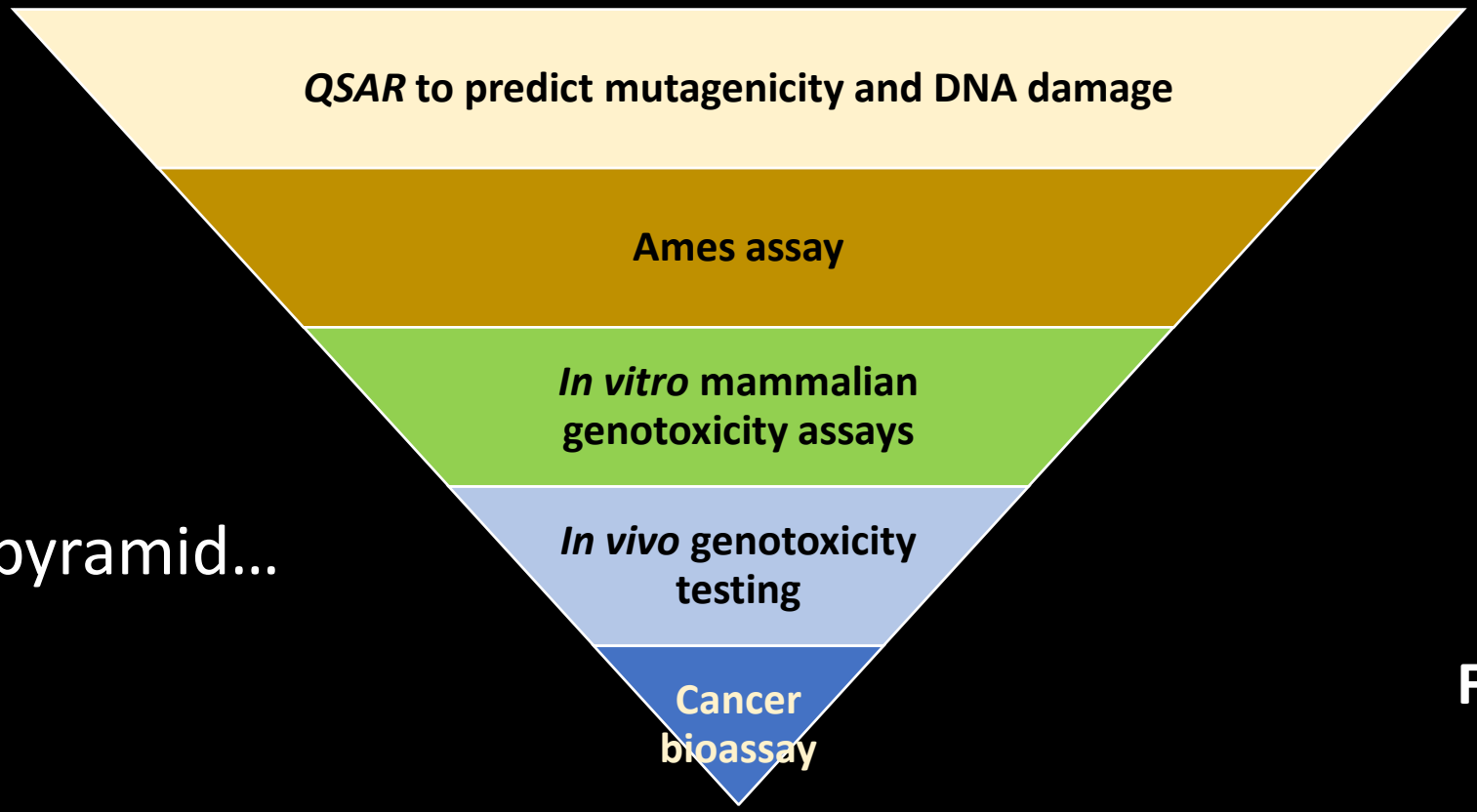


All chemicals

Few chemicals

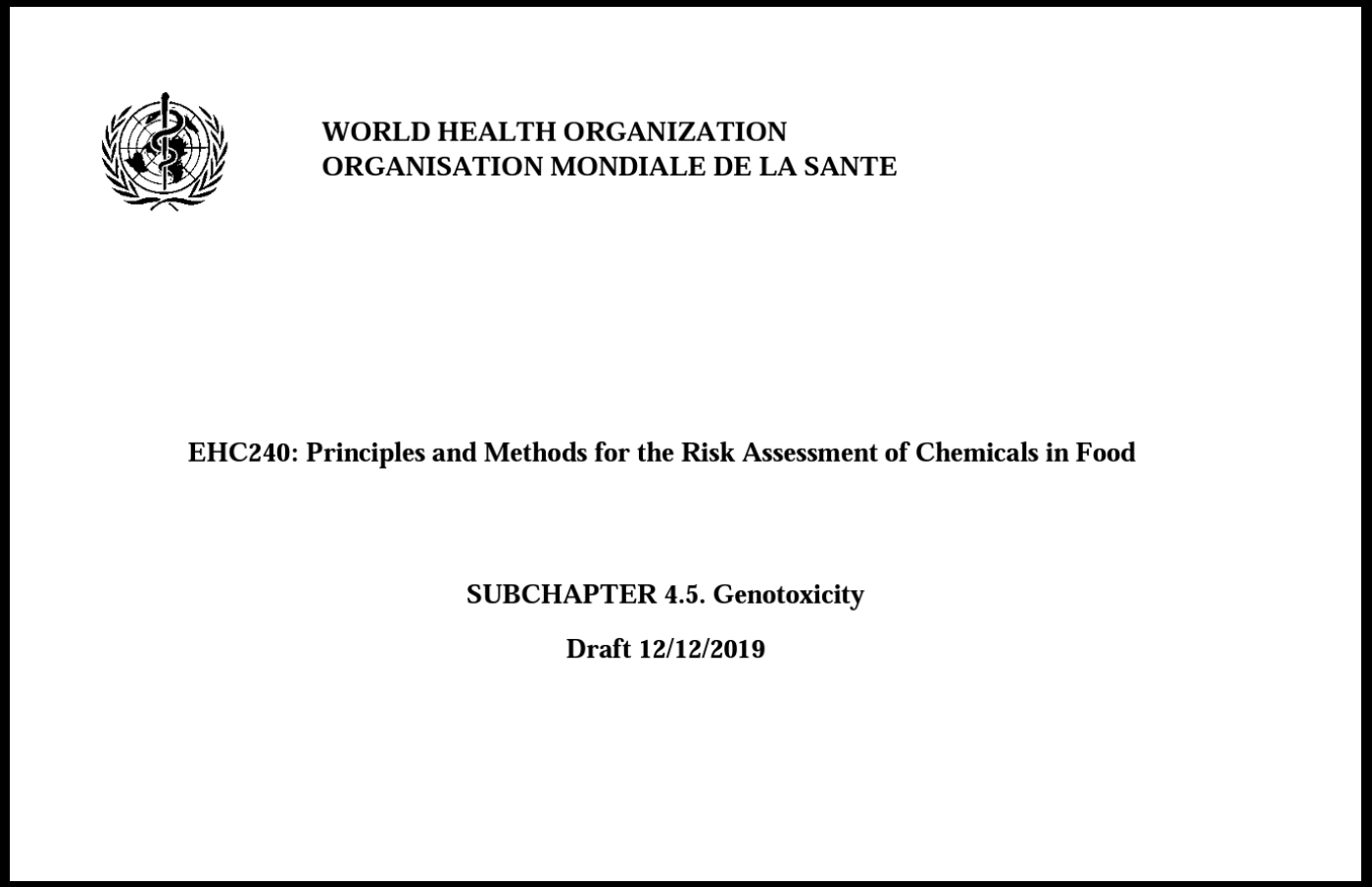
Pioneers: Genetic toxicologists have been implementing tiered testing for decades

We just
need to update
(supplement?)
the tests in this pyramid…



Part 3: Know-how
We have many NAMs

Long lists of both *in vivo* and *in vitro* NAMs



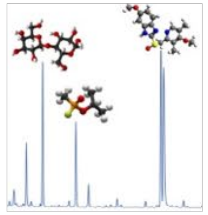
WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE

EHC240: Principles and Methods for the Risk Assessment of Chemicals in Food

SUBCHAPTER 4.5. Genotoxicity

Draft 12/12/2019

Informative NAMs: Toxicogenomics (TGx)



Genome

Transcriptome

Proteome

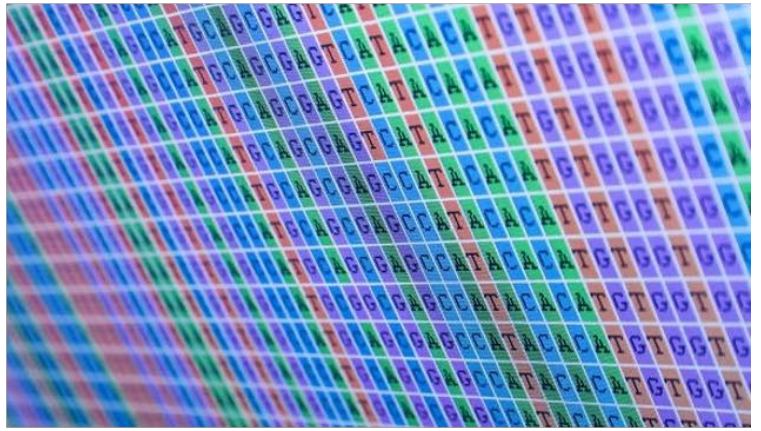
Metabolome

Exposure

Response

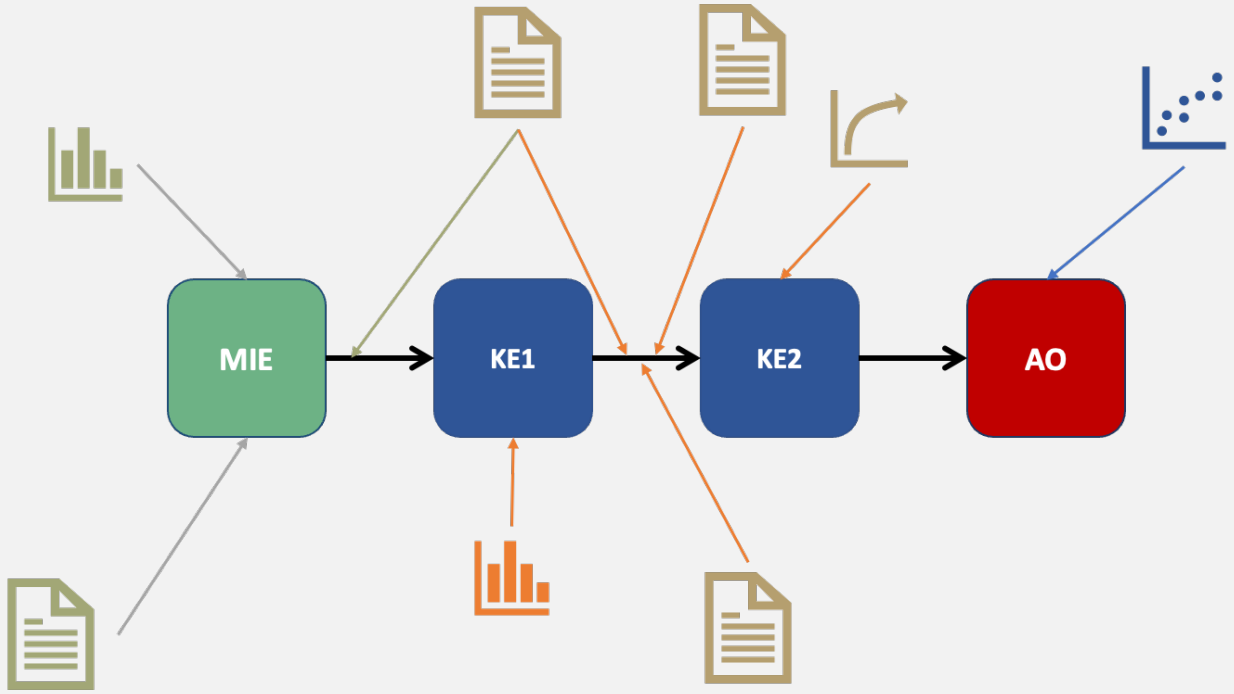
Disease

Bioinformatics



Know-how:
We have frameworks for use of NAMs in various contexts

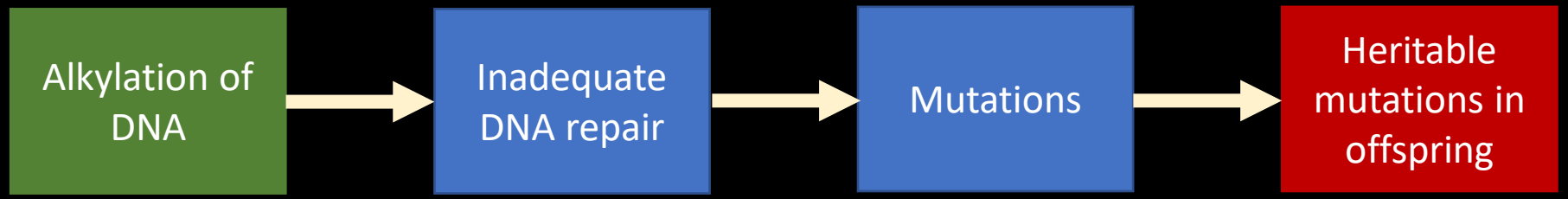
Adverse Outcome Pathways (AOPs)



A conceptual framework for organizing biological information into sequences of events

- Mapping methods to events
- Evaluating and quantifying the relationships between events

Know-how: AOP framework



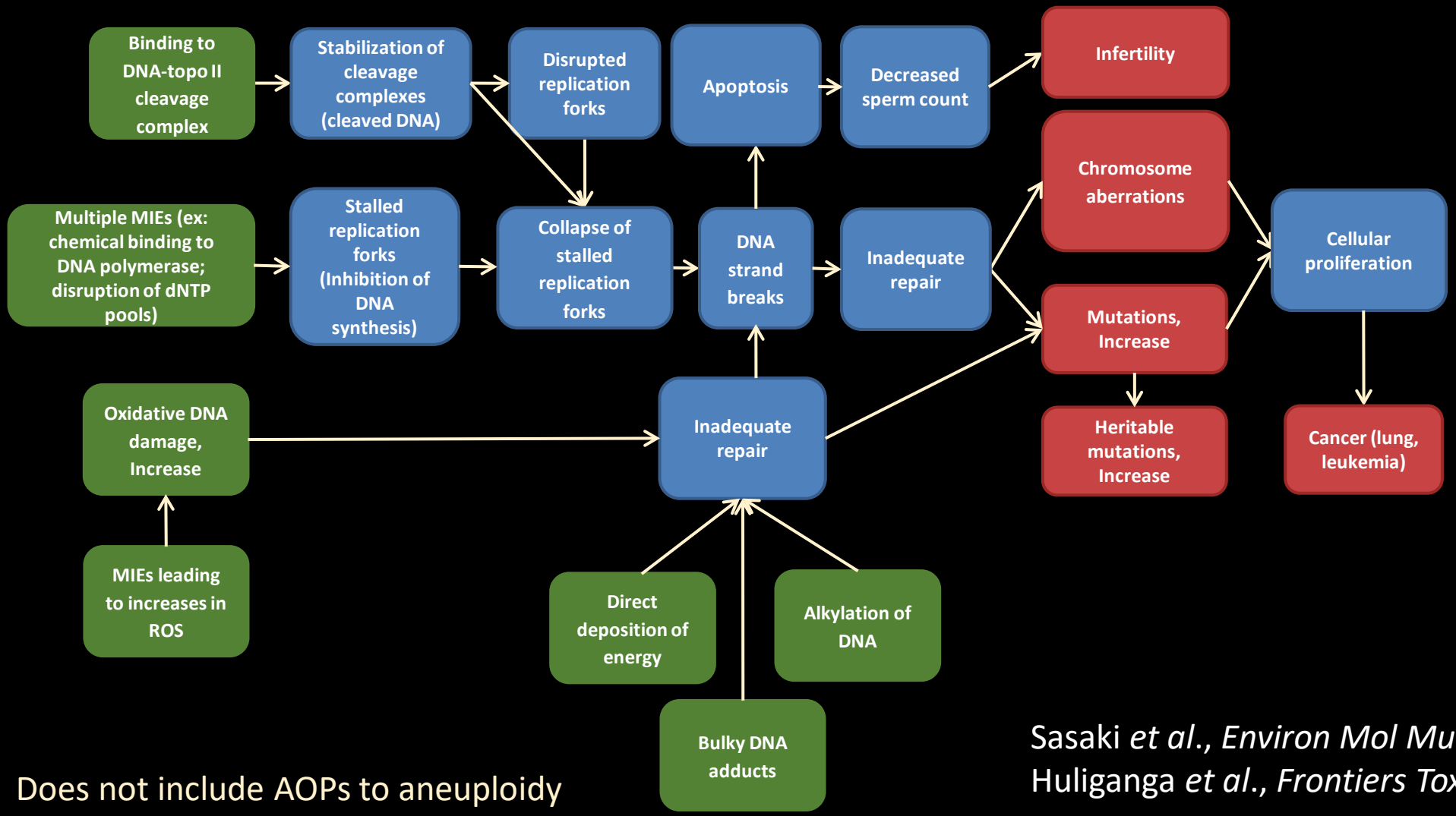
- AOP15: Alkylation of DNA leading to heritable genetic effects
- Endorsed by the OECD, https://aopwiki.org/aops/15 (Pioneers again – one of 1st five endorsed)

What purpose do they serve?

- Mode of action hypothesis
- A structure for developing test paradigms
- Predicting adverse chemical effects from mechanistic data

- Flexible and living document (update with new evidence and test methods)
- Collaborative tool
- A modern knowledge and data dissemination tool

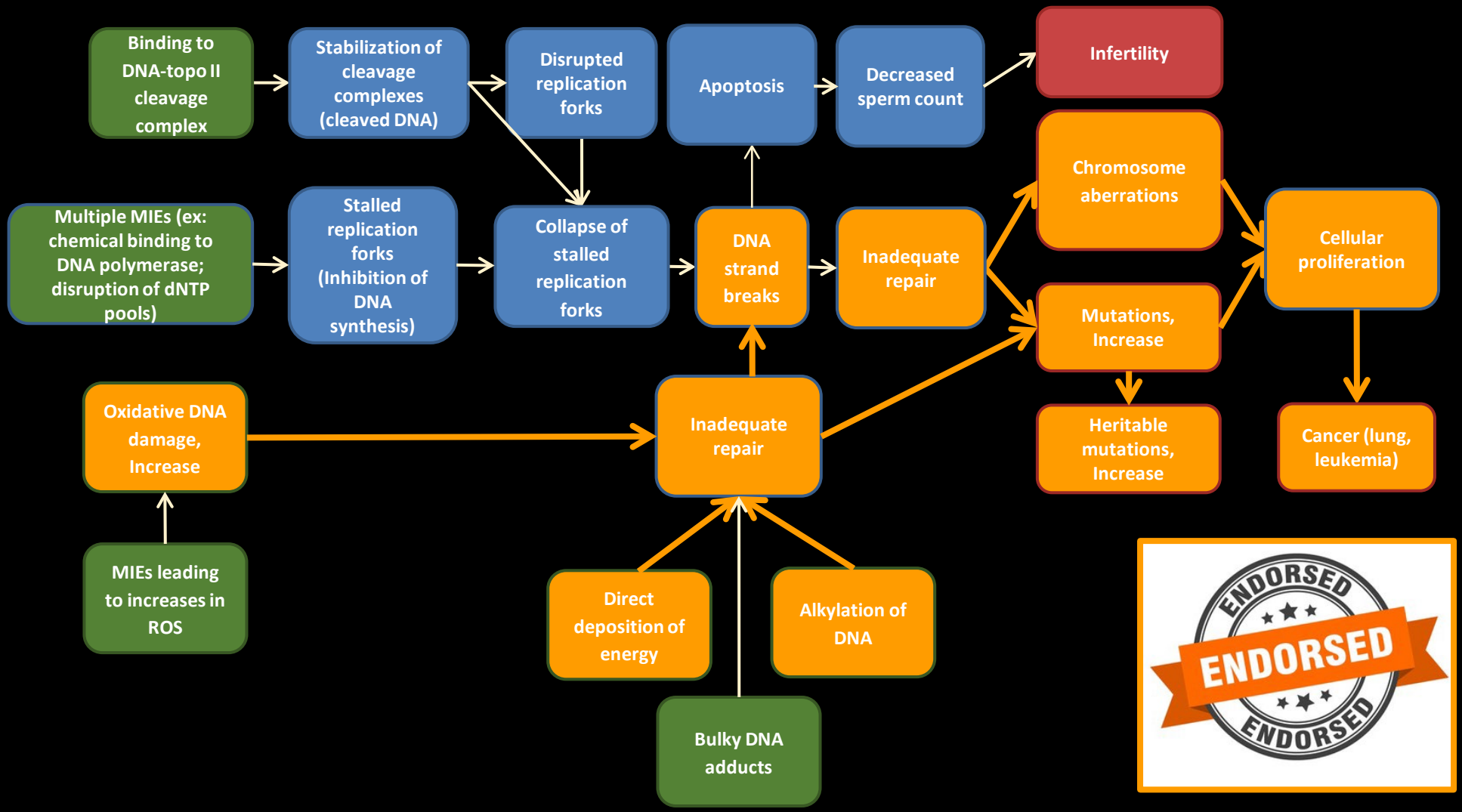
Growing genomic-damage AOP network



Does not include AOPs to aneuploidy

Sasaki *et al.*, *Environ Mol Mutagen*, 2020
Huliganga *et al.*, *Frontiers Tox*. 2022

Growing genomic-damage AOP network



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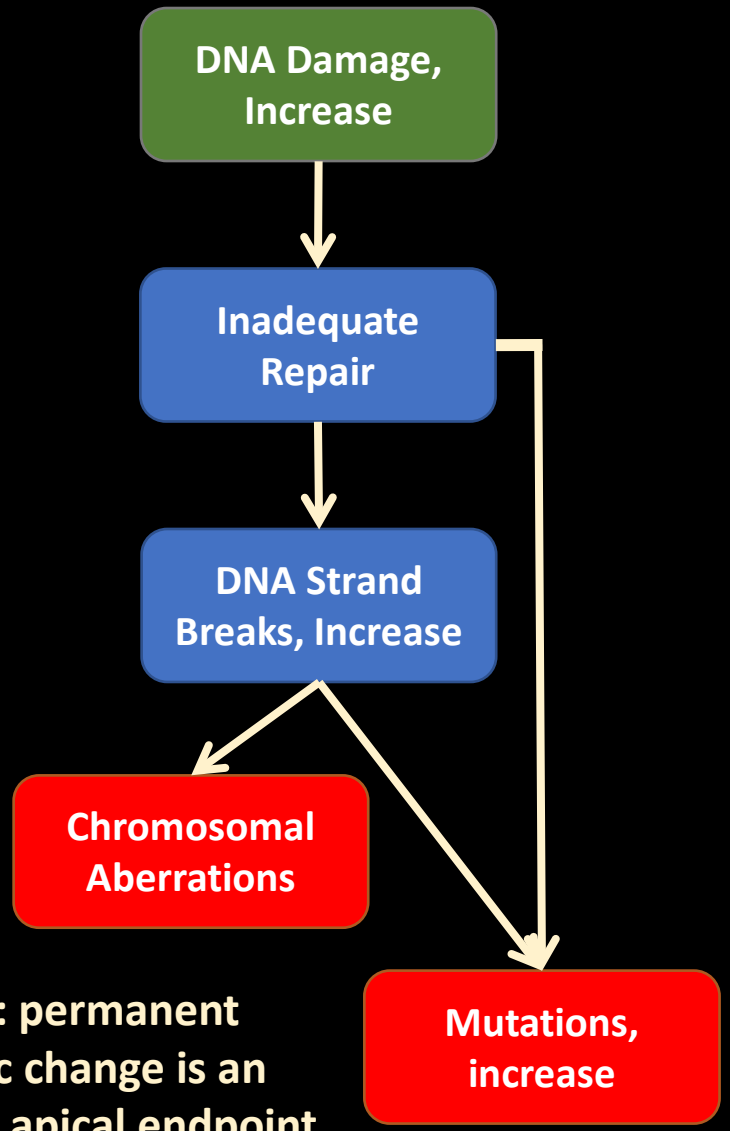
AOP-informed testing strategies



ACCEPT: permanent genomic change is an adverse apical endpoint

Key Events	Methods
DNA damage	High-throughput Comet assay TGx-DDI transcriptomic biomarker
Inadequate repair	High-throughput Comet assay DNA repair inhibitors (test essentiality)
DNA strand breaks	High-throughput comet assay MultiFlow® assay
Chromosomal aberrations	Flow cytometry micronucleus assay
Mutations	Error-corrected sequencing

Sasaki *et al.*, *Environ Mol Mutagen*, 2020

Cho *et al.*, *EMM*, 2022
OECD AOP 296 (endorsed by the OECD)

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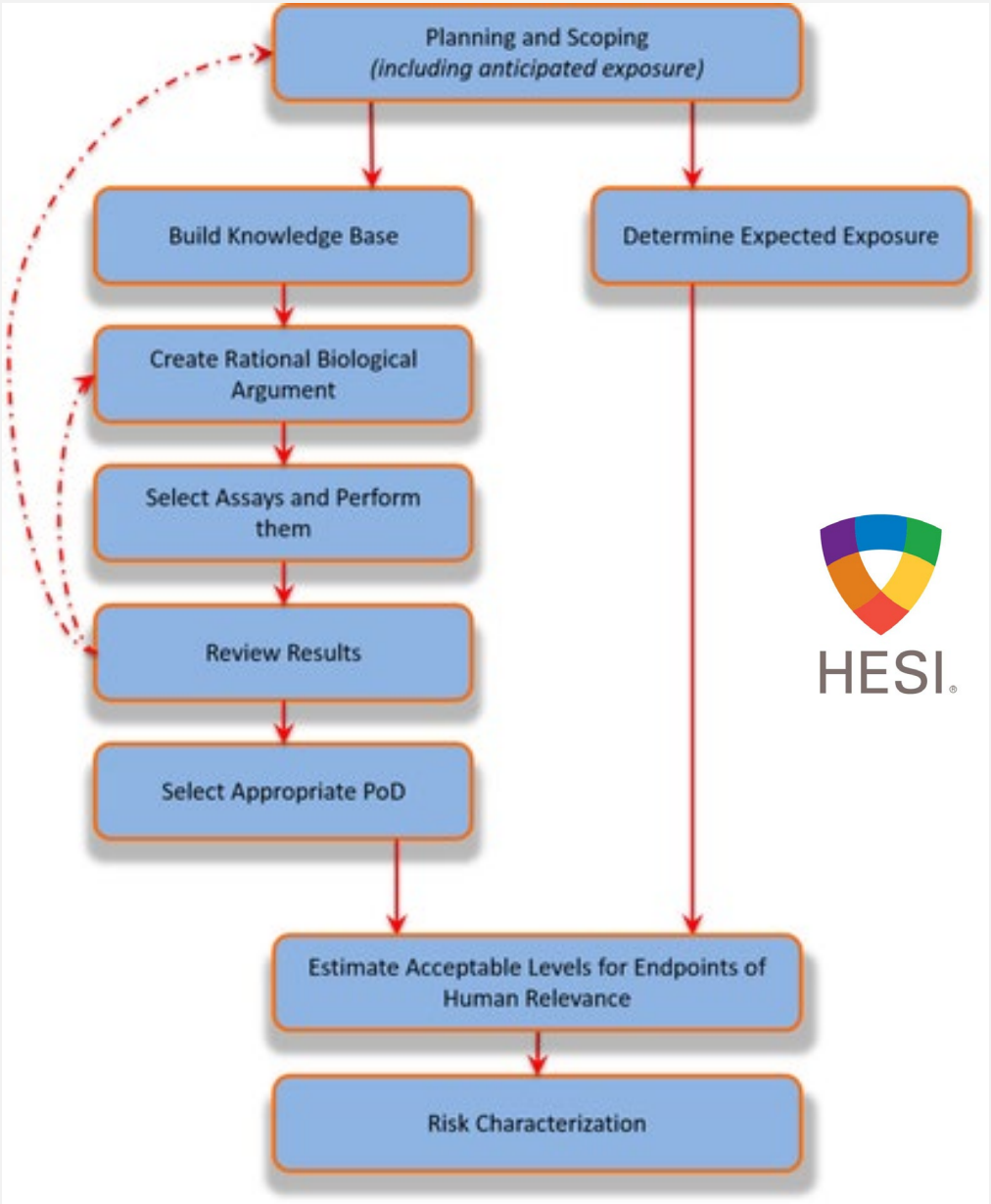
Know how: We have frameworks for implementation

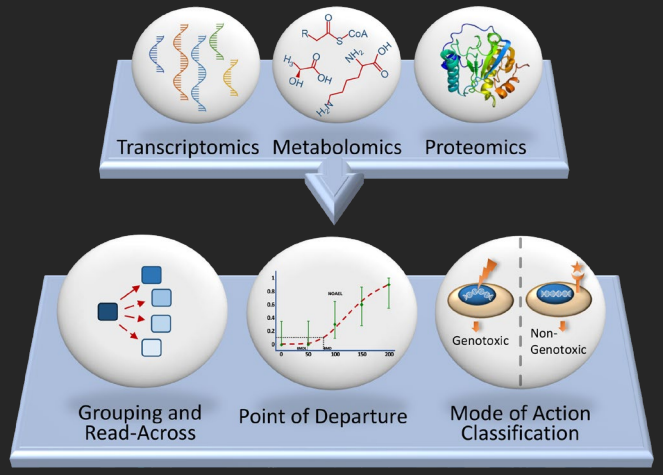
What would we do if we were starting from scratch (Clean Sheet Initiative)?

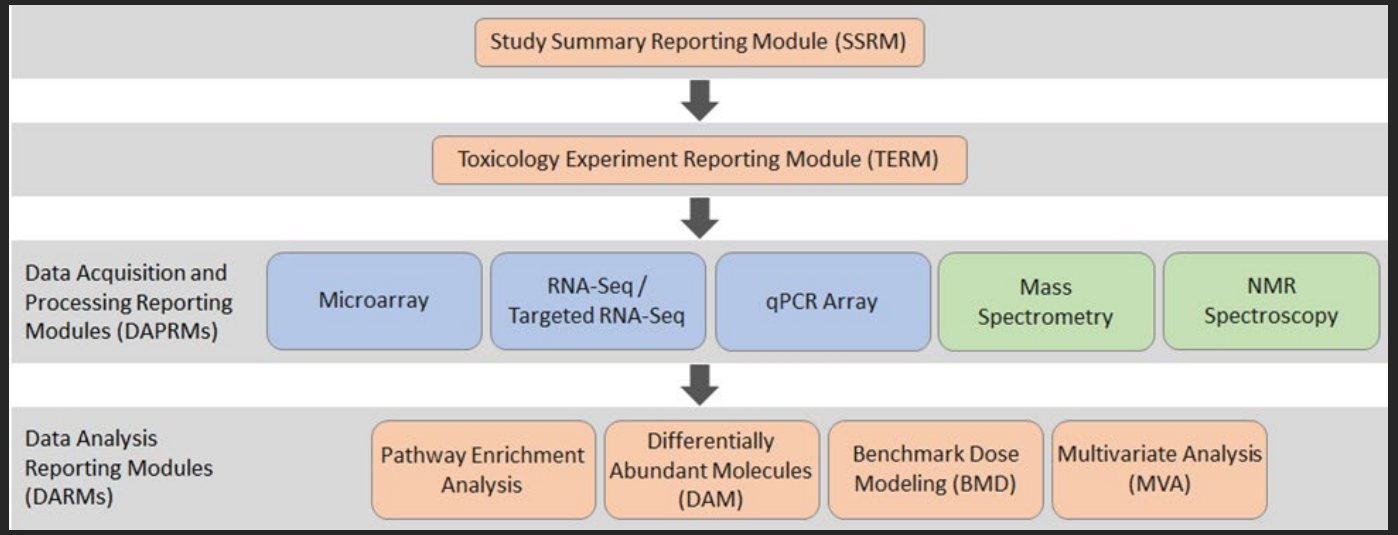
A flexible, MOA-informed framework that emphasizes human-relevant data and quantitative genetic toxicology approaches

Dearfield *et al*. *Environ Mol Mutagen*. 2017



Know-how:
OECD Omics Reporting Framework (OORF)





Supporting regulatory adoption of Omics data

Framework for the standardisation of reporting of 'omics data generation and analysis, to ensure that all of the information required to understand, evaluate the quality, interpret and reproduce an 'omics experiment and its results are available.

Modular/flexible format

https://www.oecd.org/chemicalsafety/testing/omics.htm

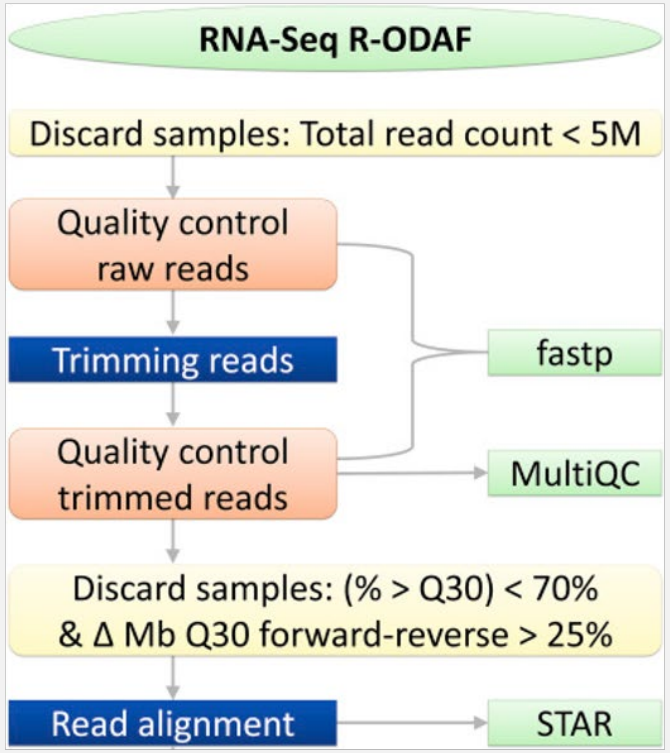
Harrill *et al.*, *Reg Tox Pharm*. 2021.

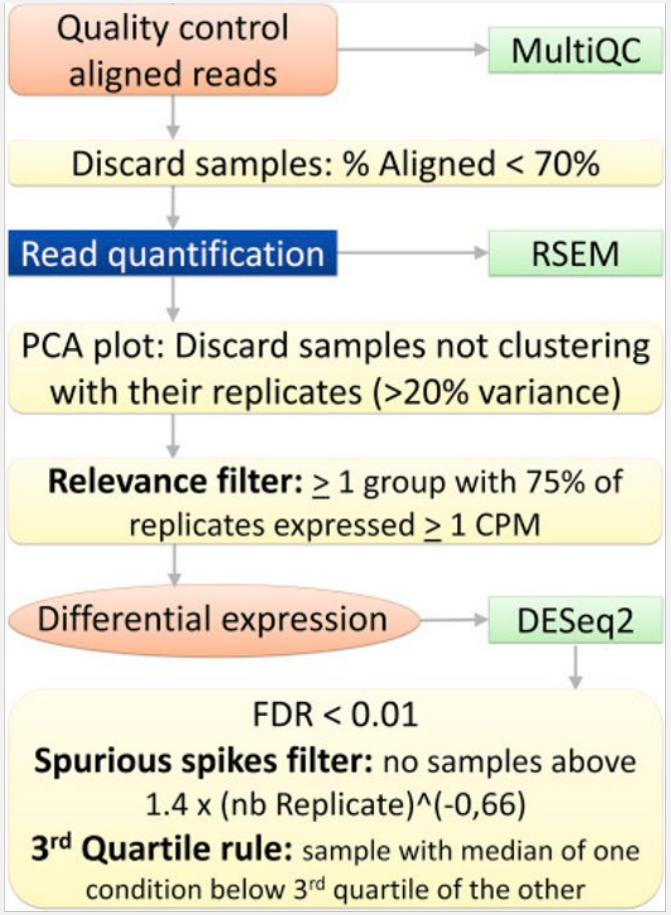
Know-how:

Regulatory - Omics Data Analysis Framework

(R-ODAF)

- Baseline analysis to encourage fair comparisons between analyses





Verheijen *et al*. *Regulatory Toxicology and Pharmacology*, June 2022, vol. 131, 105143

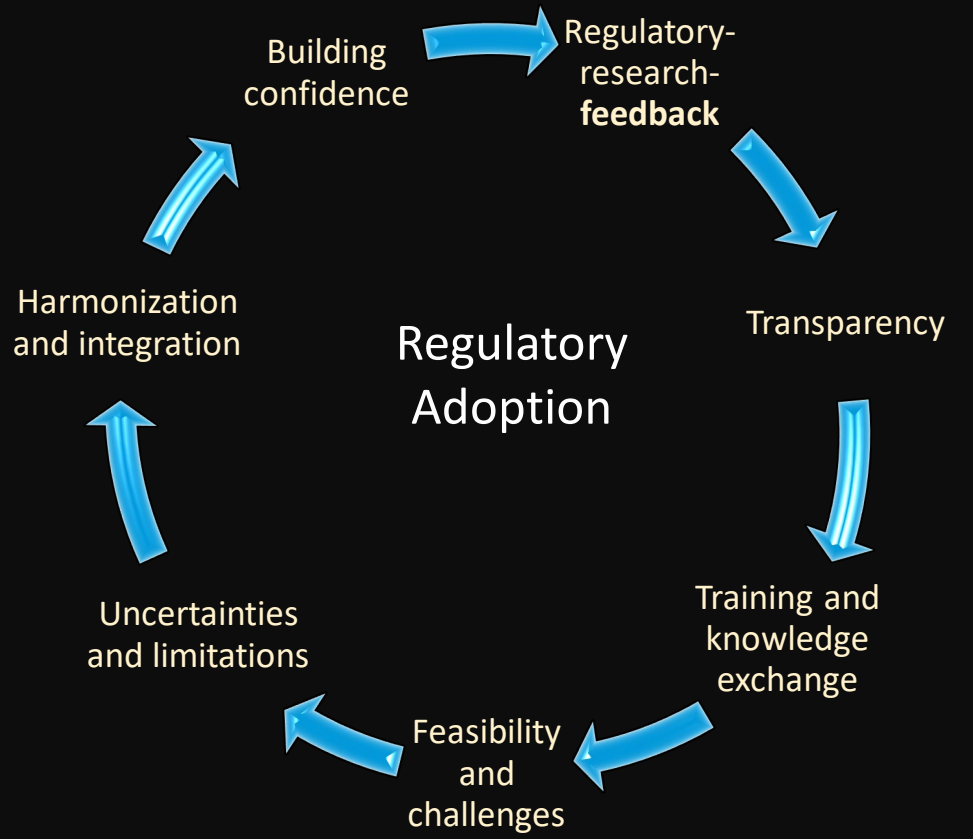
So really....

We’re not there yet…

- **Still lacking regulatory examples of adoption**
  - **Increasing pressure (motivation) for our regulatory partners**
- **Main complaint from Health Canada regulatory colleagues: We don’t receive the data!**
- **Paradigms can’t change without regulatory experience and acceptance**

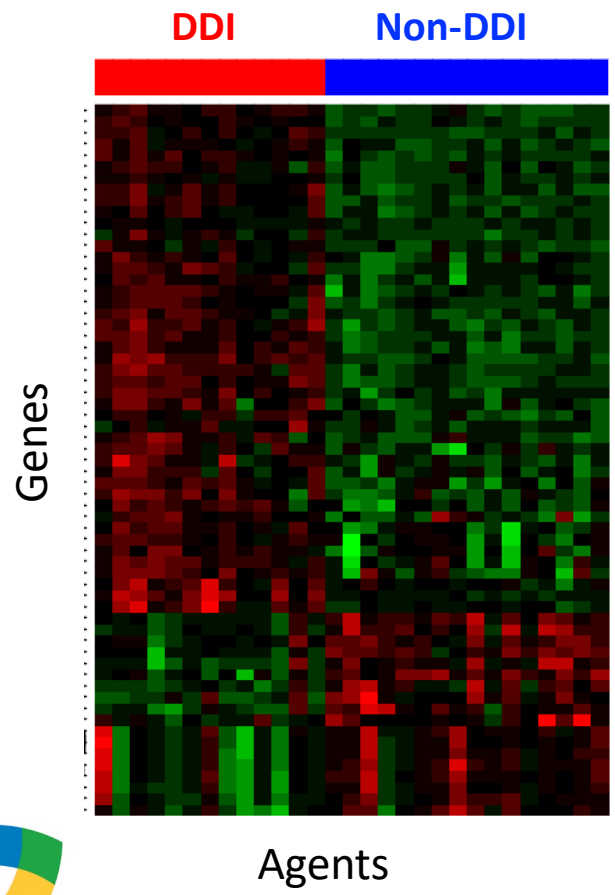
Need to push through the last mile…

How we can get there: Collaborative networks



- HESI
- IWGT
- OECD
- EMGS
- GTA

Part 4. Experience
Informative NAMs: the TGx-DDI biomarker



An *in vitro* transcriptomic biomarker to predict probability that an agent is DDI (DNA damage-inducing) or non-DDI.

- Developed using human cells in culture (TK6 cells)
- From exposure to 28 prototype DDI and non-DDI chemicals
- 64 genes identified as being predictive of DDI potential

TGx-DDI Publications for Methods Development, Validation, Application:

Biomarker development and validation

- Li, HH et al. *Environ Mol Mutagen* (2015)
- Li, HH et al. *PNAS* (2017)

Development of method for use of biomarker with metabolic activation system

- Buick, JK et al. *Environ Mol Mutagen* (2015)
- Yauk, CL et al. *Environ Mol Mutagen* (2016)

HESI

Biomarker Qualification Program

TGx-DDI ring-trial underway

TGx-DDI ring-trial underway

Objectives: To assess the cross-laboratory reproducibility of TGx-DDI classification calls involving one platform (NanoString), four sites, and 13 chemicals (plus controls)

Study design reflects years of conversation and input from FDA BQP reviewers.

		MULTI-SITE STUDY CONTRIBUTIONS				
INSTITUTION		**Study coordination** (meetings, logistics, supply procurement, shipping)	**TGx-DDI Assay** (cell culture, exposure, RNA isolation)	**NanoString** (RNA QC & Transcriptomics)	**Data Analysis, Interpretation & Reporting**	**Data Compilation Presentation and Cross Site Data Analysis**
HESI	**HESI**	X				X
Georgetown University	**Georgetown University**	X	X	X	X	
SANOFI	**Sanofi Laboratories**		X		X	
P&G Procter & Gamble	**Procter & Gamble Laboratories**		X		X	
BRT	**Burleson Research Technologies**		X	X	X	
Children's National	**Children's National Genomics Core**			X		
THE WISTAR INSTITUTE	**Wistar Institute Genomics Core**			X		

Beyond TGx-DDI: Other biomarkers work too

100% concordance in the TGx-DDI and GenoMark biomarkers in HepaRG cells

	GENOMARK	TGx-DDI
Sensitivity (%)	100	100
Specificity (%)	100	100
Accuracy (%)	100	100

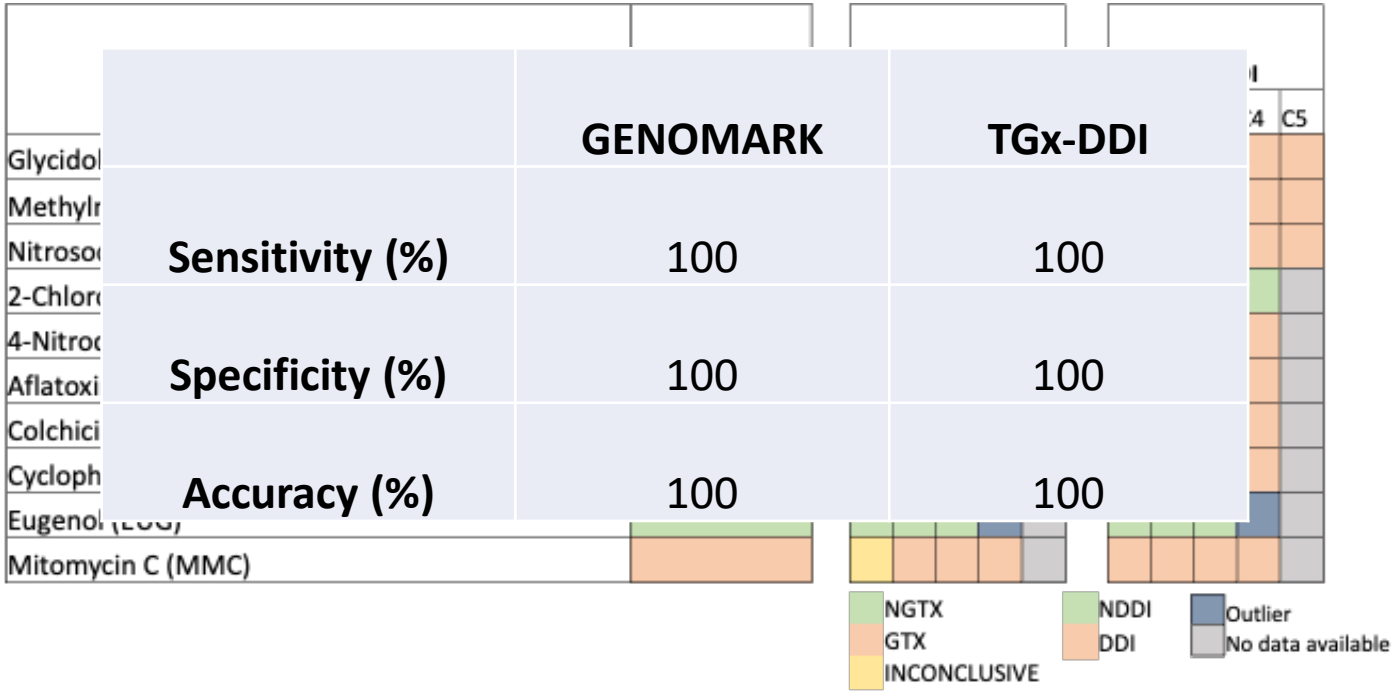
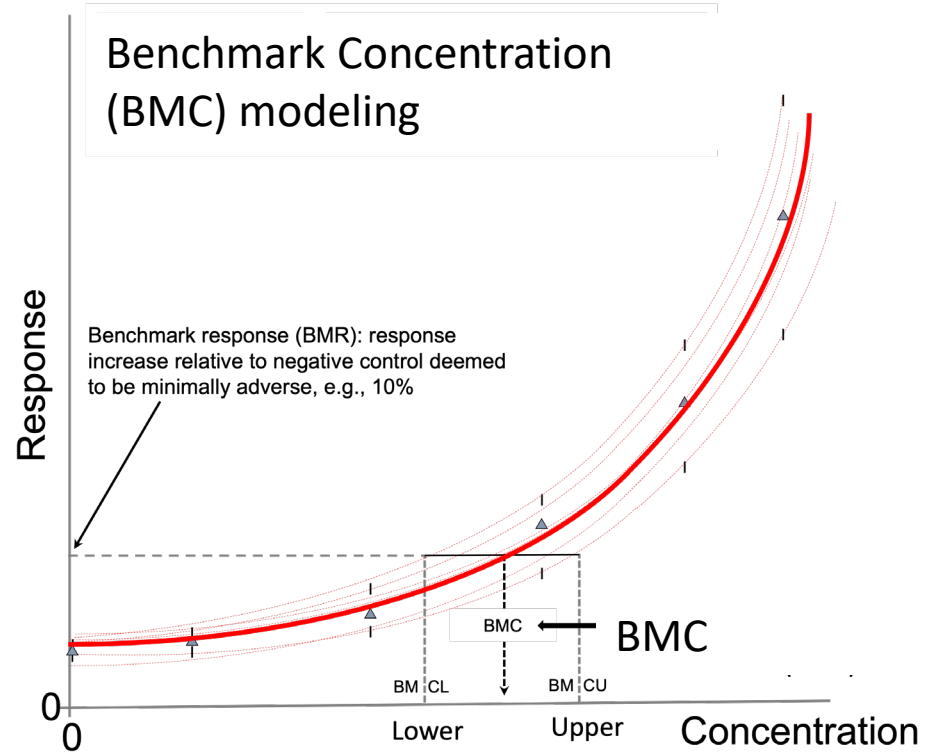


Figure created by Dr. Paul White



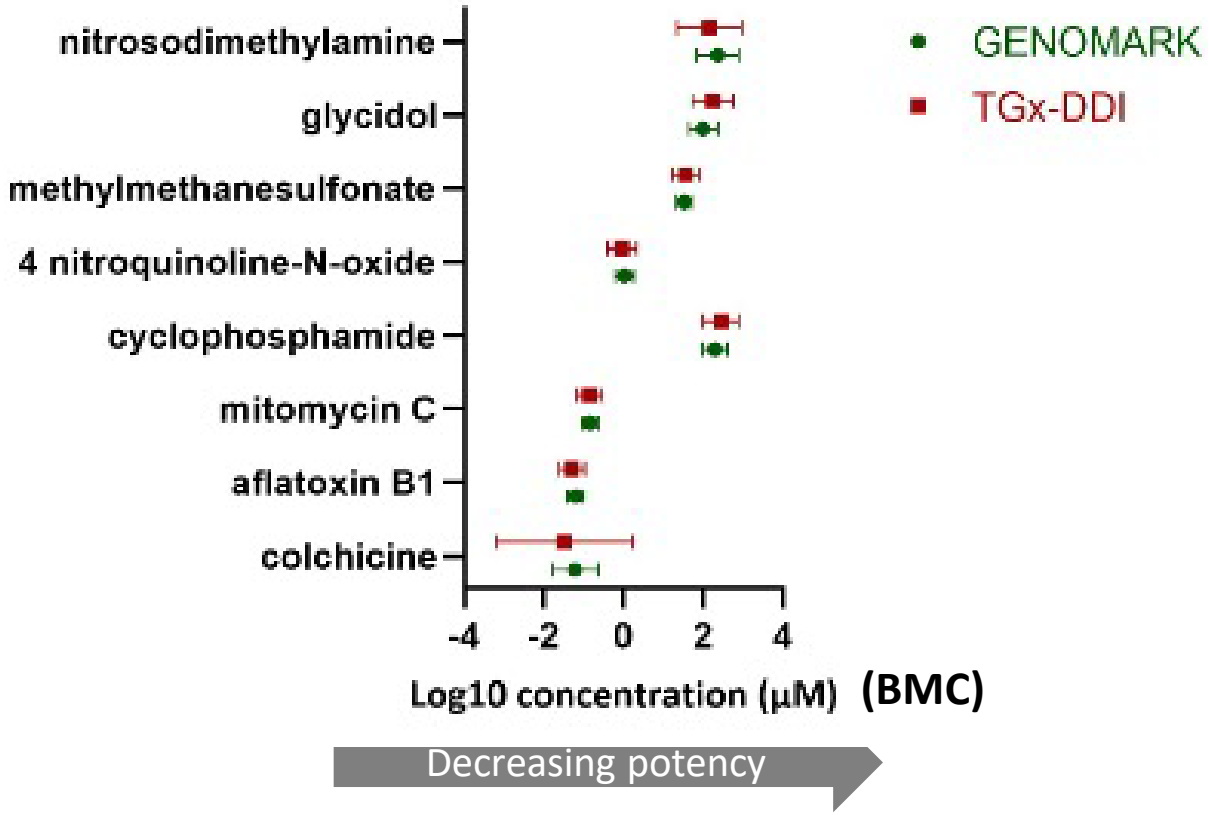
Anouck Thienpont (Vrije Universiteit Brussel)
Tamara Vanhaecke, Vera Rogiers and **Birgit Mertens (Sciensano)**

ECVAM validation study underway

VUB VRIJE UNIVERSITEIT BRUSSEL

sciensano

Beyond TGx-DDI: Other biomarkers work too
Identical potency ranking by TGx-DDI and GenoMark biomarkers in HepaRG cells using TempO-Seq



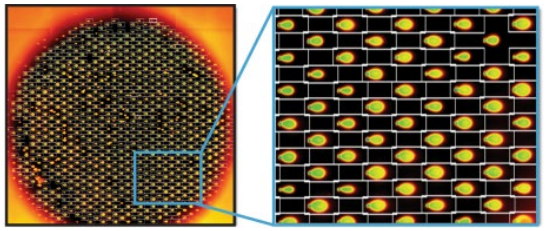
Anouck Thienpont (Vrije Universiteit Brussel)
Tamara Vanhaecke, Vera Rogiers and **Birgit Mertens (Sciensano)**

VUB VRIJE UNIVERSITEIT BRUSSEL

sciensano

Integration of TGx-DDI and the HT-CometChip in HepaRG provides an efficient next-generation genotoxicity testing strategy in HepaRG cells

8 DDI and 4 non-DDI tested in concentration-response high-throughput design



From: Sykora, P. *et al.* (2018) Nature Scientific Reports 8(1): 2771

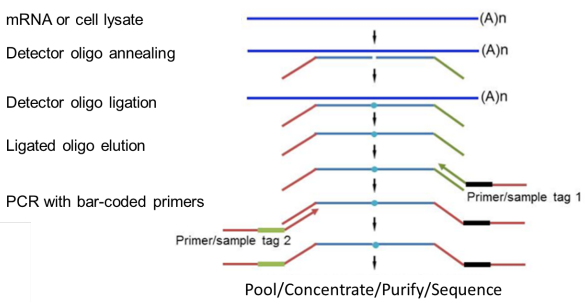
Concordant results

	TGx-DDI Classification (Gene Expression)					CometChip (DNA Damage)				
DDI Chemicals	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5
Cytosine Arabinoside	+	+	+	+	+	+	+	+	+	+
2-Deoxy-D-Glucose	-	-	-	-	-	-	-	-	-	-

Discordant results

	TGx-DDI Classification (Gene Expression)					CometChip (DNA Damage)				
DDI Chemicals	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5
Zidovudine	-	-	-	-	-	-	+	+	+	+
Aflatoxin B1	+	+	+	+	+	-	-	-	-	-

TempO-Seq

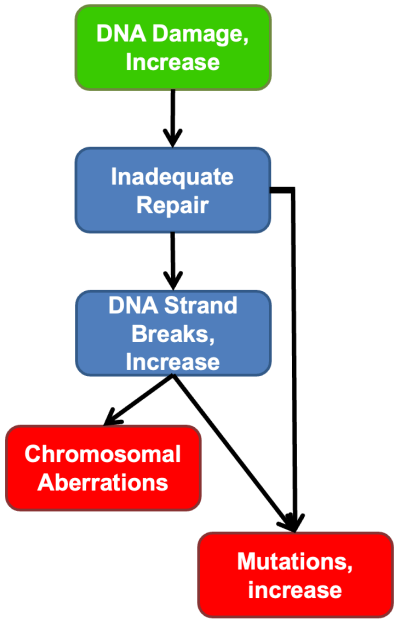


Buick et al., *Frontiers Public Health*, 2022

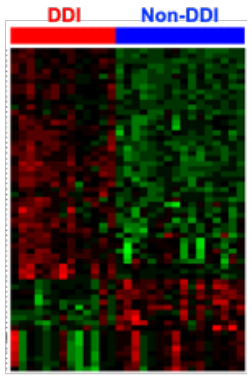
With… Bevin Engelward, Les Recio, Carol Swartz

High concordance of TGx-DDI and the HT-comet assay in both hazard identification and points of departure

AOP



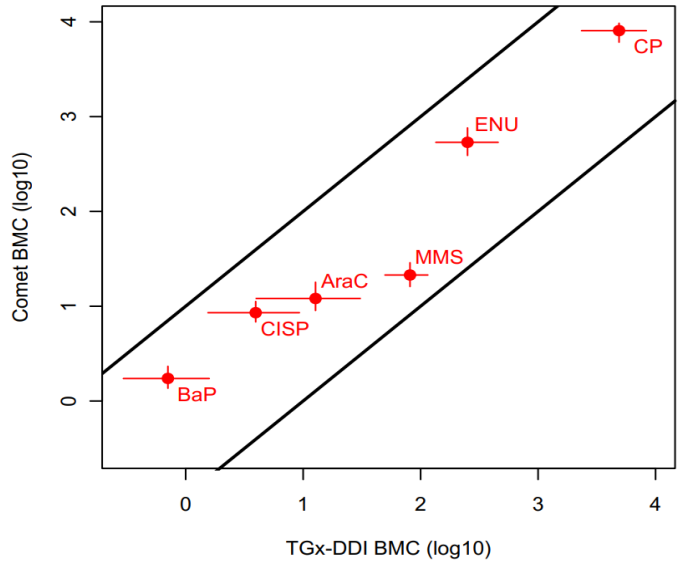
Hazard ID



Derive median gene BMC as point of departure

CometChip® DNA damage

Comparison to Comet BMC



Buick et al., *Frontiers Public Health*, 2022

Take-home messages

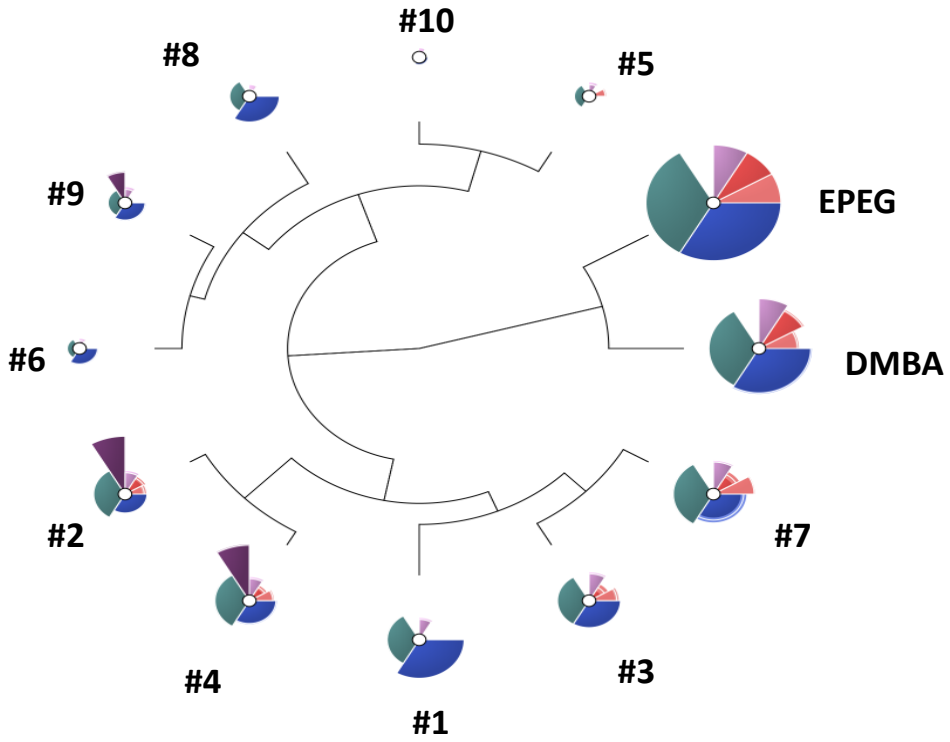
- Assay integration enables efficient identification and potency ranking of DNA damaging agents in HepaRG™ cells
- Additional mechanistic information to understand compound toxicity

Case study: integration of TGx-DDI with a battery of TK6 cell assays

Paul White, Anne-Marie Fortin

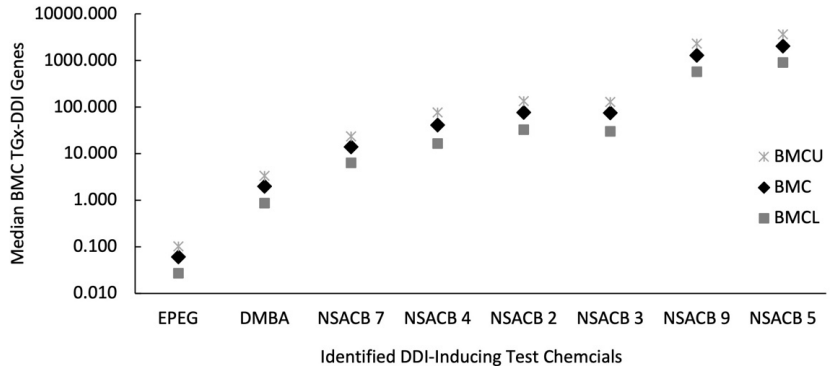
Health Canada

Objectives: Integrate TGx-DDI into the HC GeneTox21 platform to predict genotoxicity of data-poor chemicals on Canada's in-commerce list.

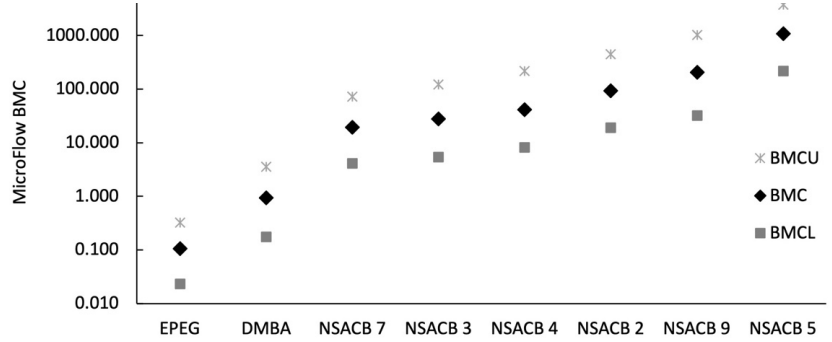


Fortin *et al.*, *Frontiers in Tox*, 2023

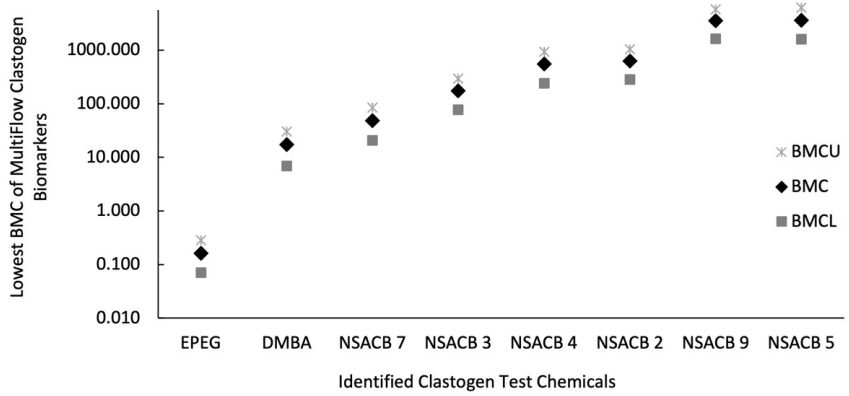
TGx-DDI



Micronucleus test (MicroFlow®)

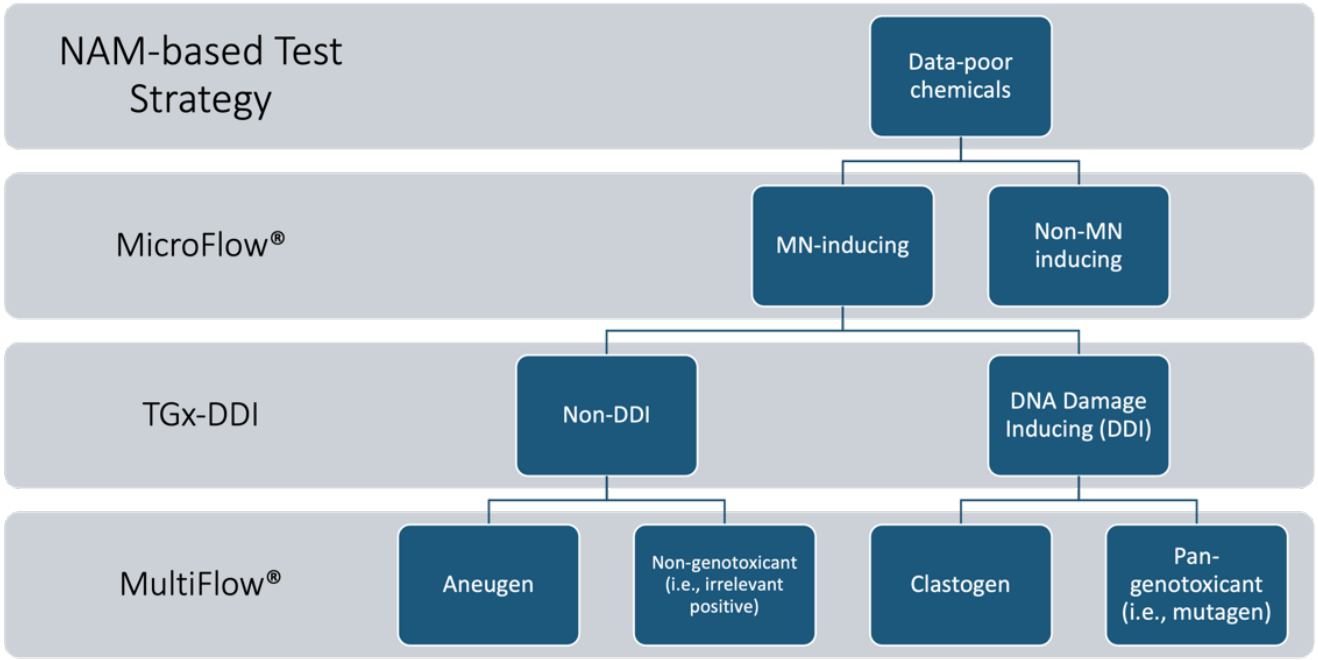


MultiFlow® assay



GeneTox21 platform data interpretation framework for modernized genotoxicity evaluation

Fortin *et al*.
Frontiers in Tox 2023



Take-home message

Integration of TGx-DDI, MicroFlow®, and MultiFlow® endpoints is an effective NAM-based strategy for genotoxicity assessment of data-poor compounds enabling:

- Hazard identification
- Mechanistic understanding
- Potency ranking
- Priority setting

Modern genotoxicity assessment requires concentration-response modeling and *in vitro-in vivo* extrapolation (IVIVE) for context and prioritization

Analysis of *in vitro* micronucleus test data for 292 chemicals (19 concentrations, with top concentration 200 μM)
From hazard identification to risk assessment application

Objectives:

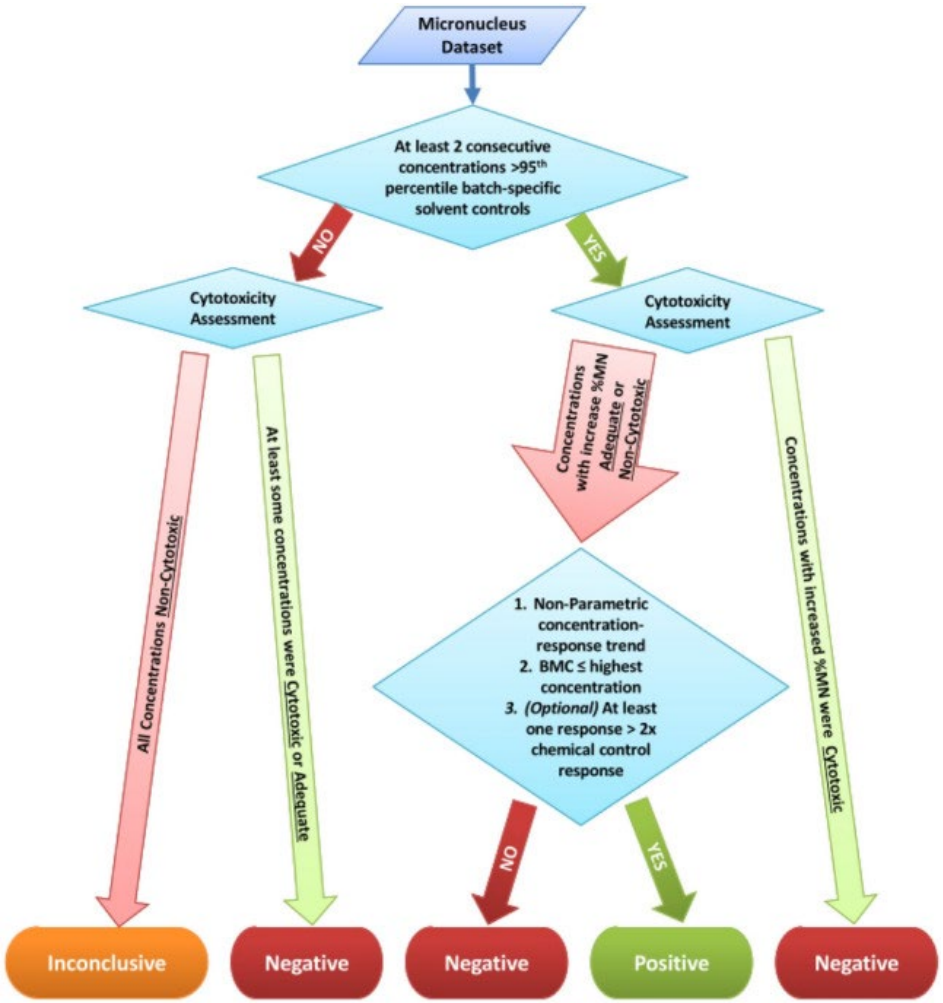
(1) Develop decision tree for hazard identification.

(2) Apply toxicokinetic modeling
- Estimate administered equivalent doses
- Determine the relationship between *in vitro* micronucleus frequency and traditional *in vivo* genetox and cancer studies
- Derive Bioactivity Exposure Ratios for prioritization.

Kuo/Beal *et al*. *Archives of Toxicology* 2022

Health Canada

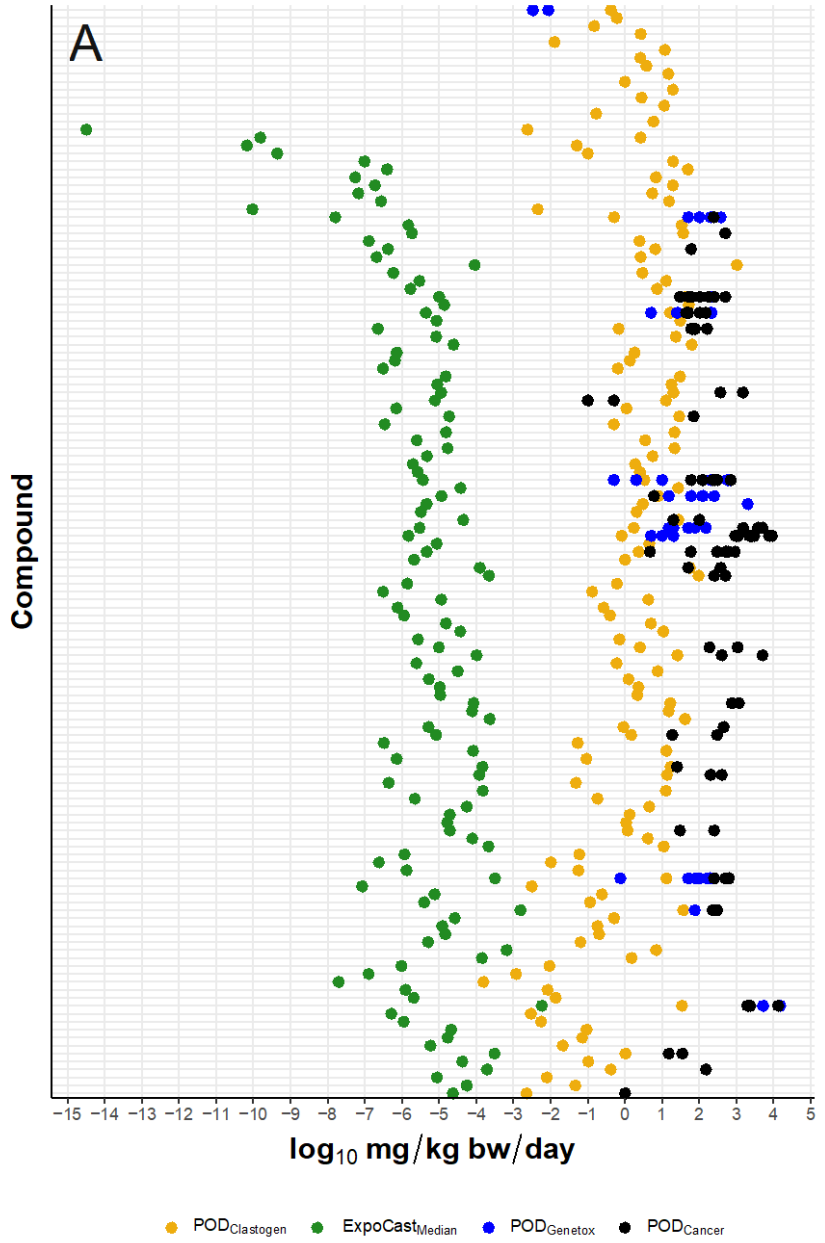
Hazard identification



IVIVE provides evidence to inform priorities for follow-up testing

TAKE HOME MESSAGES:

- *In vitro* PODs < *in vivo* PODs
  - Protective approach
- Relationship to human exposures can be established to inform priorities for further testing
- Success in thinking outside the test guideline



Kuo/Beal *et al*. *Archives of Toxicology* 2022

Expansion of approach to other assays supports this initial finding

Received: 2 October 2022 | Revised: 29 November 2022 | Accepted: 30 November 2022

DOI: 10.1002/em.22521

Environmental and Molecular Mutagenesis | Environmental Mutagenesis and Genomics Society | WILEY

RESEARCH ARTICLE

Quantitative in vitro to in vivo extrapolation of genotoxicity data provides protective estimates of in vivo dose

Marc A. Beal[1] | Marc Audebert[2] | Tara Barton-Maclaren[3] | Hannah Battaion[4] | Jeffrey C. Bemis[5] | Xuefei Cao[6] | Connie Chen[7] | Stephen D. Dertinger[5] | Roland Froetschl[8] | Xiaoqing Guo[6] | George Johnson[9] | Giel Hendriks[10] | Laure Khoury[11] | Alexandra S. Long[3] | Stefan Pfuhler[12] | Raja S. Settivari[13] | Shamika Wickramasuriya[3] | Paul White[4]

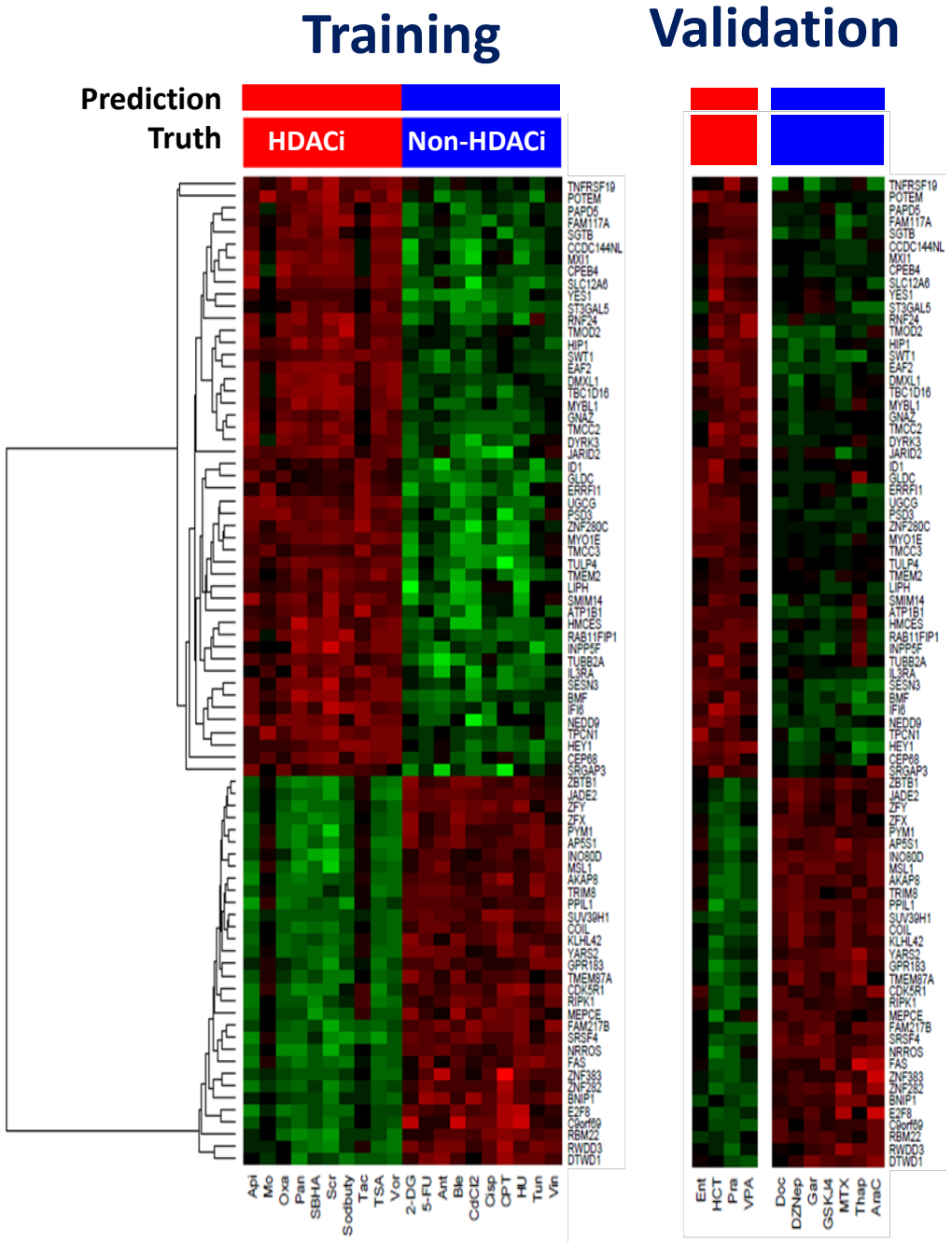
HESI Genetic Toxicology Technical Committee

The future: Multiplex!
The TGx-HDACi transcriptomic biomarker

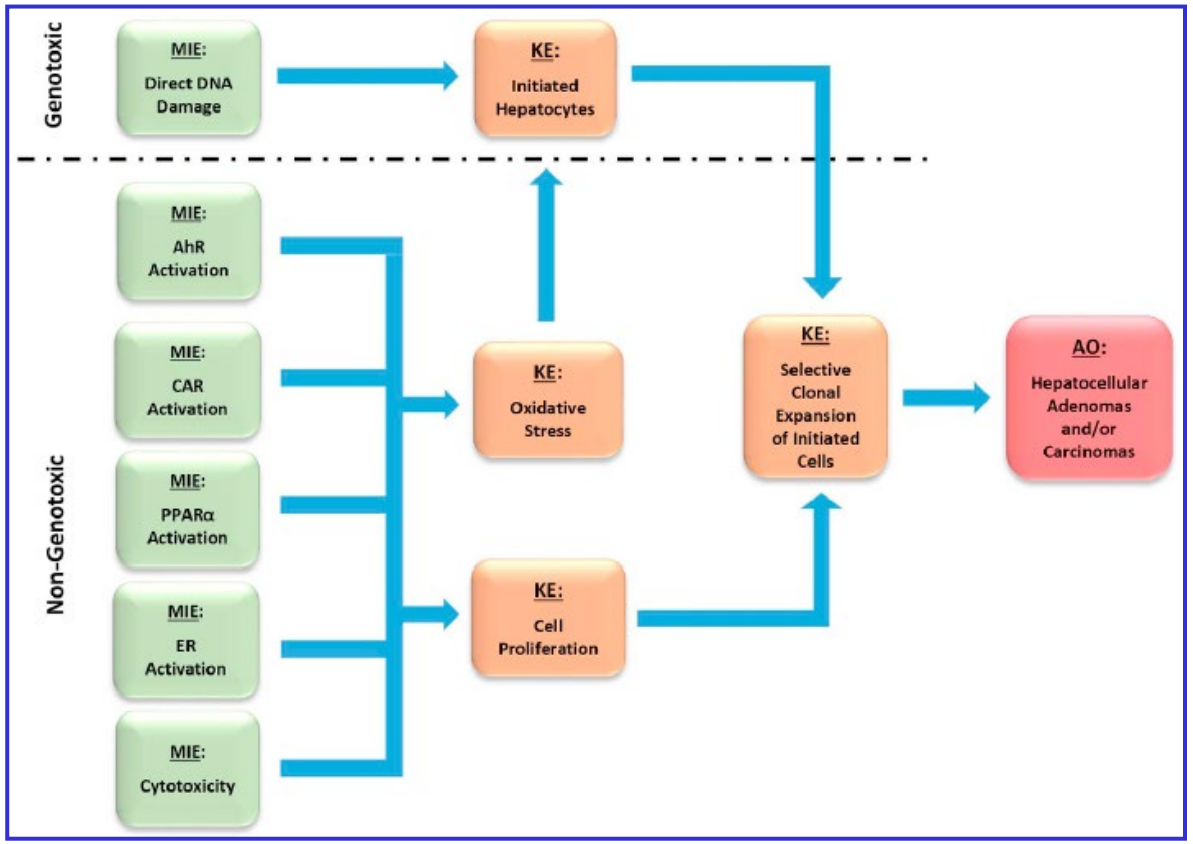
Developed in TK6 cells for integration with TGx-DDI (same time points)

- High sensitivity/specificity
- Concordant benchmark concentrations between TGx-HDACi and enzyme activity assay

Cho *et al*. *Archives of Toxicology* 2021



In vivo rodent transcriptomic biomarkers to predict hepatocarcinogenicity



Rooney *et al.* (2018) *TAAP.* **356**, p99-113

Hypothesis: Measuring MIEs and downstream KEs in short-term rodent assays identifies chemicals and doses that cause tumors in the liver in two-year bioassays.

Biomarker accuracy ranged from 91% to 98%

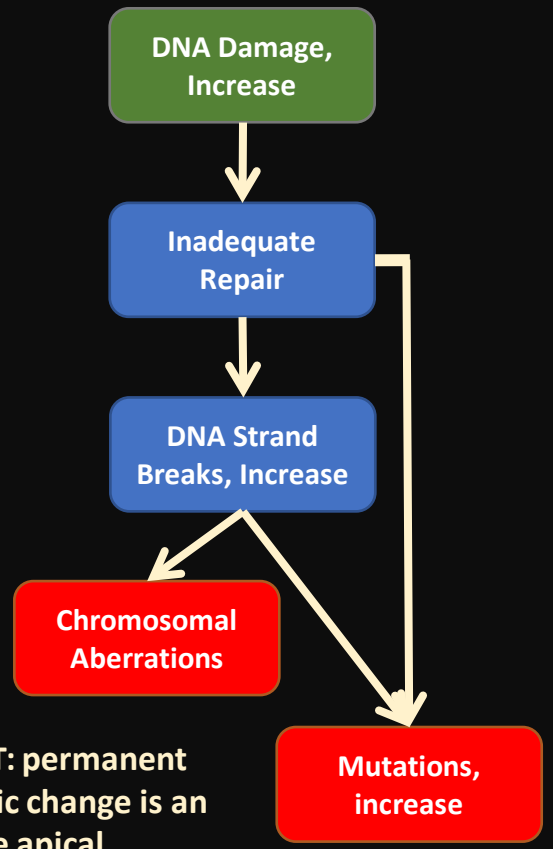
HESI eSTAR Carcinogenomics Projects

Goal: Provide biomarkers that can be used in ICHS1b Revision, Special Studies and Endpoints, as rationale (weight of evidence) to waive the need for the 2-year cancer bioassay

Leads: Keith Tanis and Chris Corton

HESI

AOP-informed testing strategies



ACCEPT: permanent genomic change is an adverse apical endpoint

Key Events	Methods
DNA damage	High-throughput Comet assay TGx-DDI transcriptomic biomarker
Inadequate repair	High-throughput Comet assay DNA repair inhibitors (test essentiality)
DNA strand breaks	High-throughput comet assay MultiFlow® assay
Chromosomal aberrations	Flow cytometry micronucleus assay
Mutations	Error-corrected sequencing

Sasaki et al., *Environ Mol Mutagen*, 2020.

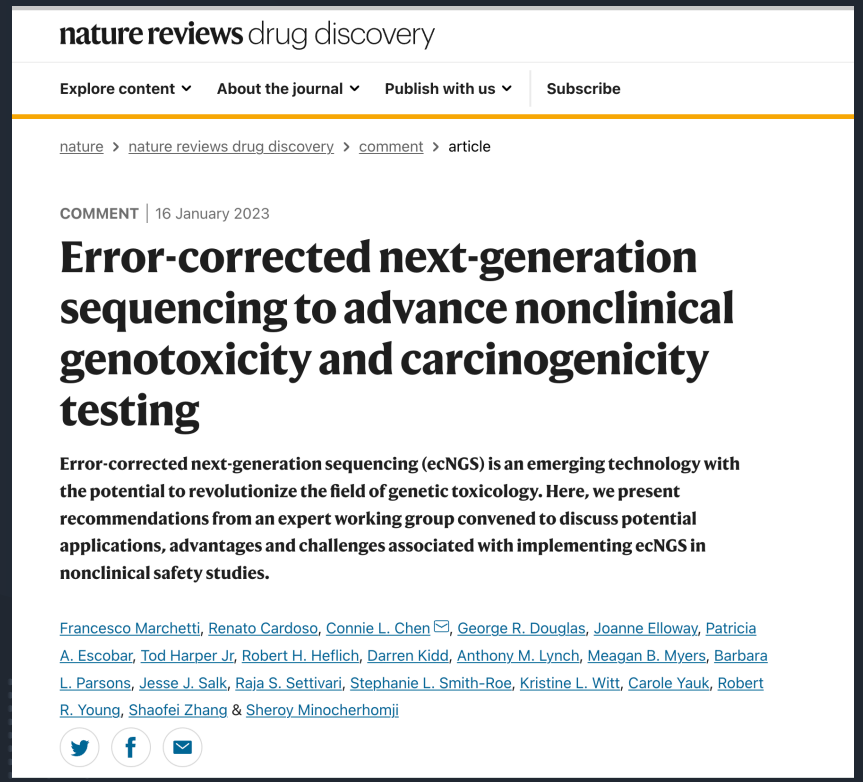
Cho et al., *EMM*, 2022

OECD AOP 296 (endorsed by the OECD)

Are you fed up with working on bacterial genes, one locus at a time, or in stand-alone mutagenicity tests?

Error-corrected Next-Generation Sequencing

- Detection of mutations and spectral changes in endogenous loci
- Genome-wide or locus-specific
- Any tissue or species (integration with other tests)
- Identify clonally expanded mutations in cancer driver genes to predict cancer outcomes



nature reviews drug discovery

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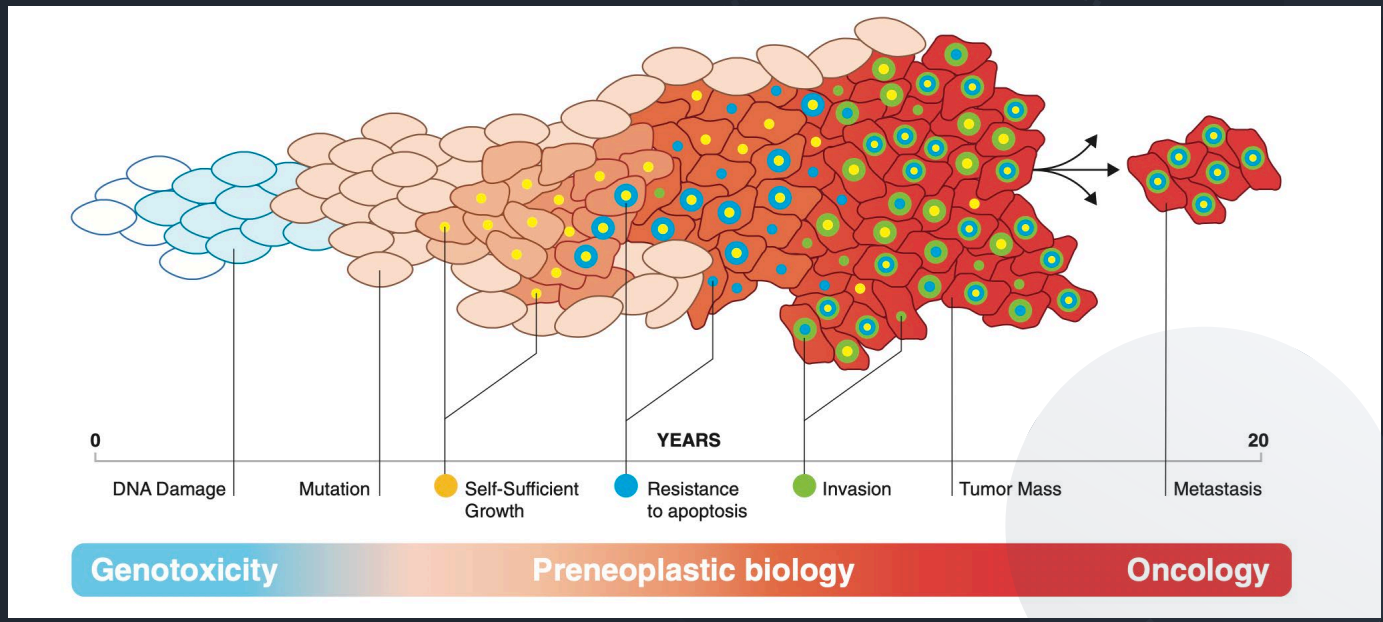
COMMENT | 16 January 2023

Error-corrected next-generation sequencing to advance nonclinical genotoxicity and carcinogenicity testing

Error-corrected next-generation sequencing (ecNGS) is an emerging technology with the potential to revolutionize the field of genetic toxicology. Here, we present recommendations from an expert working group convened to discuss potential applications, advantages and challenges associated with implementing ecNGS in nonclinical safety studies.

Francesco Marchetti, Renato Cardoso, Connie L. Chen, George R. Douglas, Joanne Elloway, Patricia A. Escobar, Tod Harper Jr, Robert H. Heflich, Darren Kidd, Anthony M. Lynch, Meagan B. Myers, Barbara L. Parsons, Jesse J. Salk, Raja S. Settivari, Stephanie L. Smith-Roe, Kristine L. Witt, Carole Yauk, Robert R. Young, Shaofei Zhang & Sheroy Minocherhomji

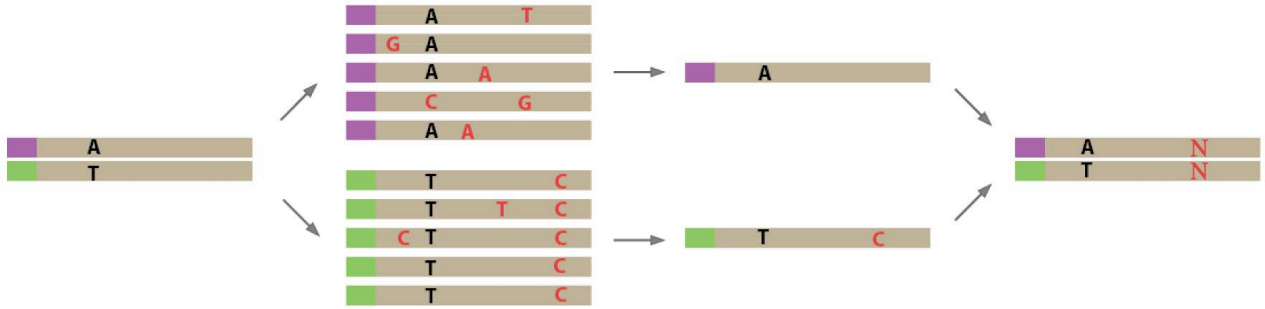


Salk and Kennedy, 2020

HESI

Error-corrected next-generation sequencing

- Unique tags on target DNA
- Sequence and group by tags
- Develop duplex consensus call on every nucleotide in the sequence



- reduces sequencing errors from
  - 1 in 1000 (regular NGS)
  - 1 in 10 million (Duplex Sequencing)

TWINSTRAND™ BIOSCIENCES

Investigating mutagenic responses using Duplex Sequencing (DS)

Mouse Mutagenesis Panel (*in vivo* studies)

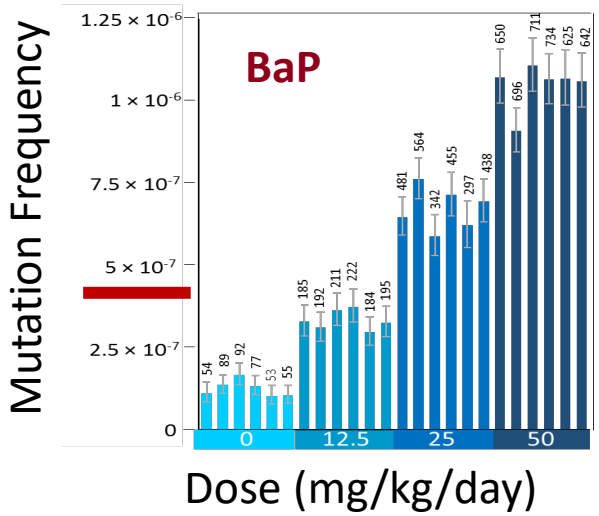
Human Mutagenesis Panel (*in vitro and in vivo*)

- Proof of concept in different models
- Experimental design
- Exploring what mechanisms can be detected
- Concordance with conventional assays
- Cross-laboratory concordance

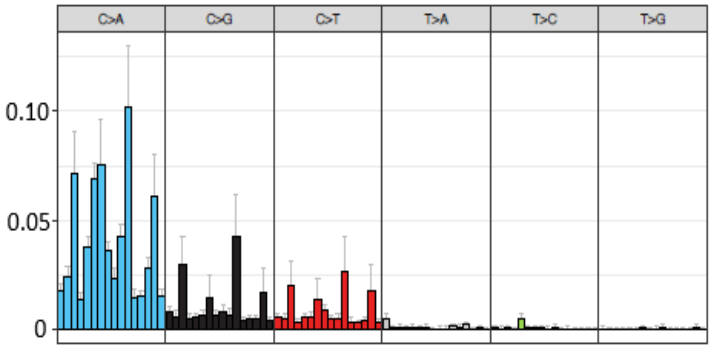
TWINSTRAND™ BIOSCIENCES

Potent vs weak mutagens: We see the expected response

Potent mutagen: benzo[a]pyrene



28 day exposure
MutaMouse
Bone Marrow



COSMIC signatures:

SBS4 (lung cancer)

SBS24

SBS29

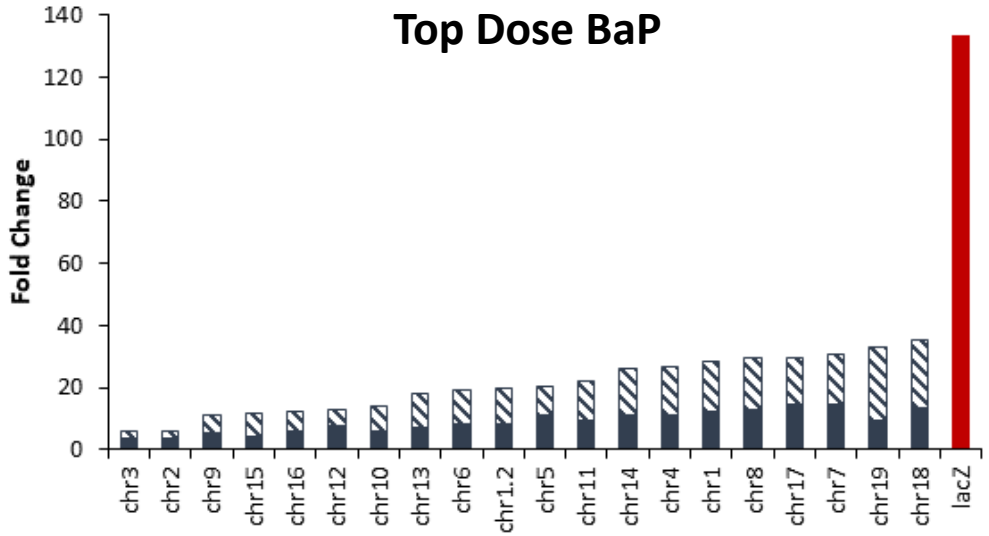
LeBlanc et al., *BMC Genomics*, 2022

How does it compare to the old assays?

Potent mutagen: benzo[a]pyrene

Correlation with Transgenic Rodent (TGR) mutation assay

$R^2 = 0.94$



Unique

All mutations (including clonally expanded ones)

Weak mutagen: procarbazine

Correlation with TGR assay

$R^2 = 0.73$

Development, application, evaluation: Team work!

TECHNICAL METHOD DEVELOPMENT: wet lab protocol, sequencing depth, regions of DNA, bioinformatics approach

STUDY DESIGN: what time points, what tissues, top dose selection

PERFOMANCE EVALUATION: accuracy (concordance with conventional test and across labs), sensitivity, different genotoxic mechanisms, value of mechanistic information

CASE STUDIES: applications in real-life

BURROUGHS WELLCOME FUND

INNOVATION IN REGULATORY SCIENCE AWARDS

Health Canada Santé Canada

HESI

FDA charles river Pfizer Roche TWINSTRAND BIOSCIENCES NIEHS National Institute of Environmental Health Sciences

Collaborative time-series, dose-response study: informing concordance, study design, uncertainty factors

Exposure duration

28 days | 60 days | 90 days | 120 days | 180 days

- MutaMouse males
- Daily exposures to **benzo[b]fluoranthene**
- 5 doses + vehicle controls
- N = 8 per dose group (4 for DS)

At each time point

Conventional endpoints

<u>Established protocols</u>
LacZ mutation (liver, bone marrow, germ cells)
Pig-a mutation (blood)
Micronucleus frequency (blood)
Histopathology

Mutation frequency
Potency analysis

Genomics endpoints

TwinStrand DS MMP + *lacZ* + *Pig-a* + CDM panel (?)
Carc-seq
PacBio HiFi

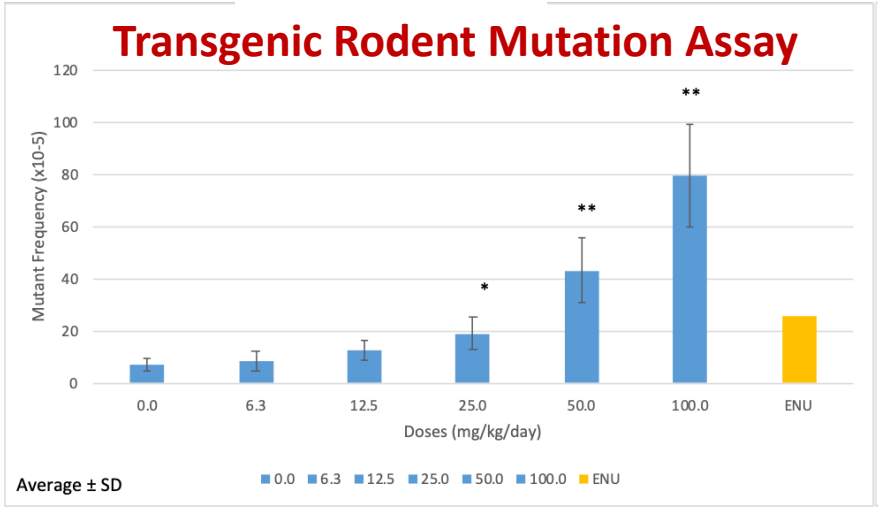
Mutation frequency (genome and locus-specific)
Potency analysis

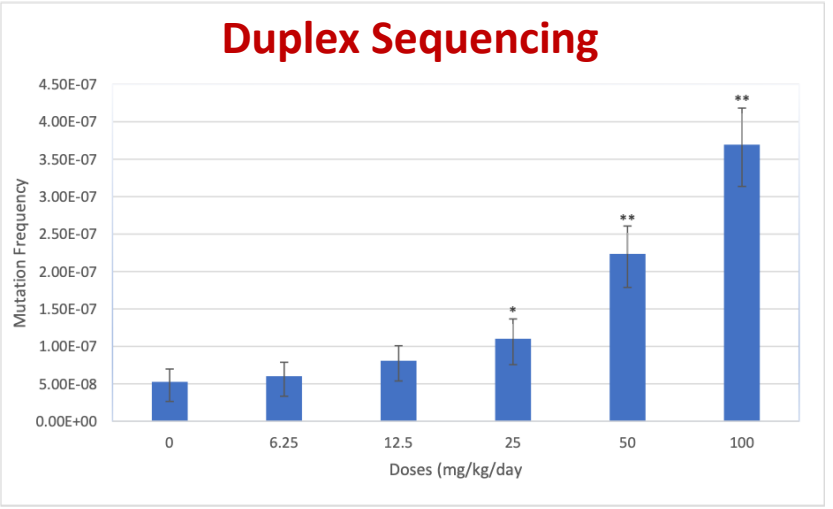
Mutation spectrum analysis
Clonal expansion over time

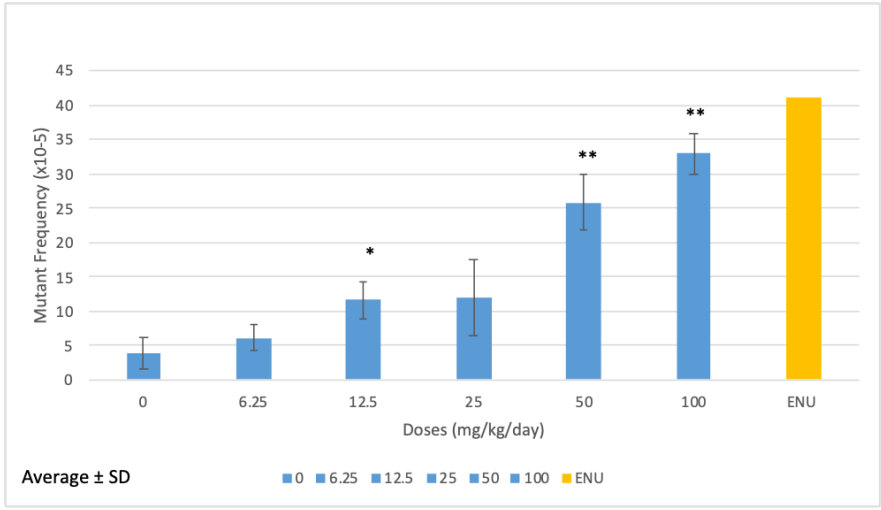
Robust mutagenic responses in all assays at 28 days

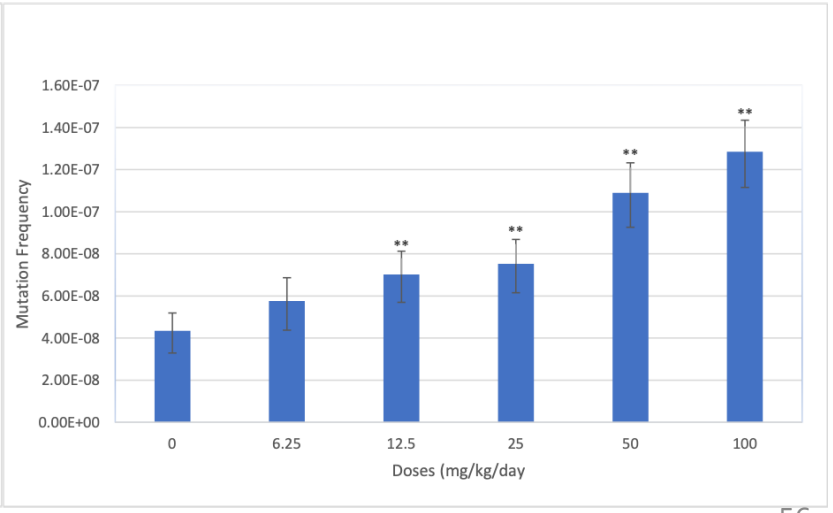
Liver

Bone Marrow





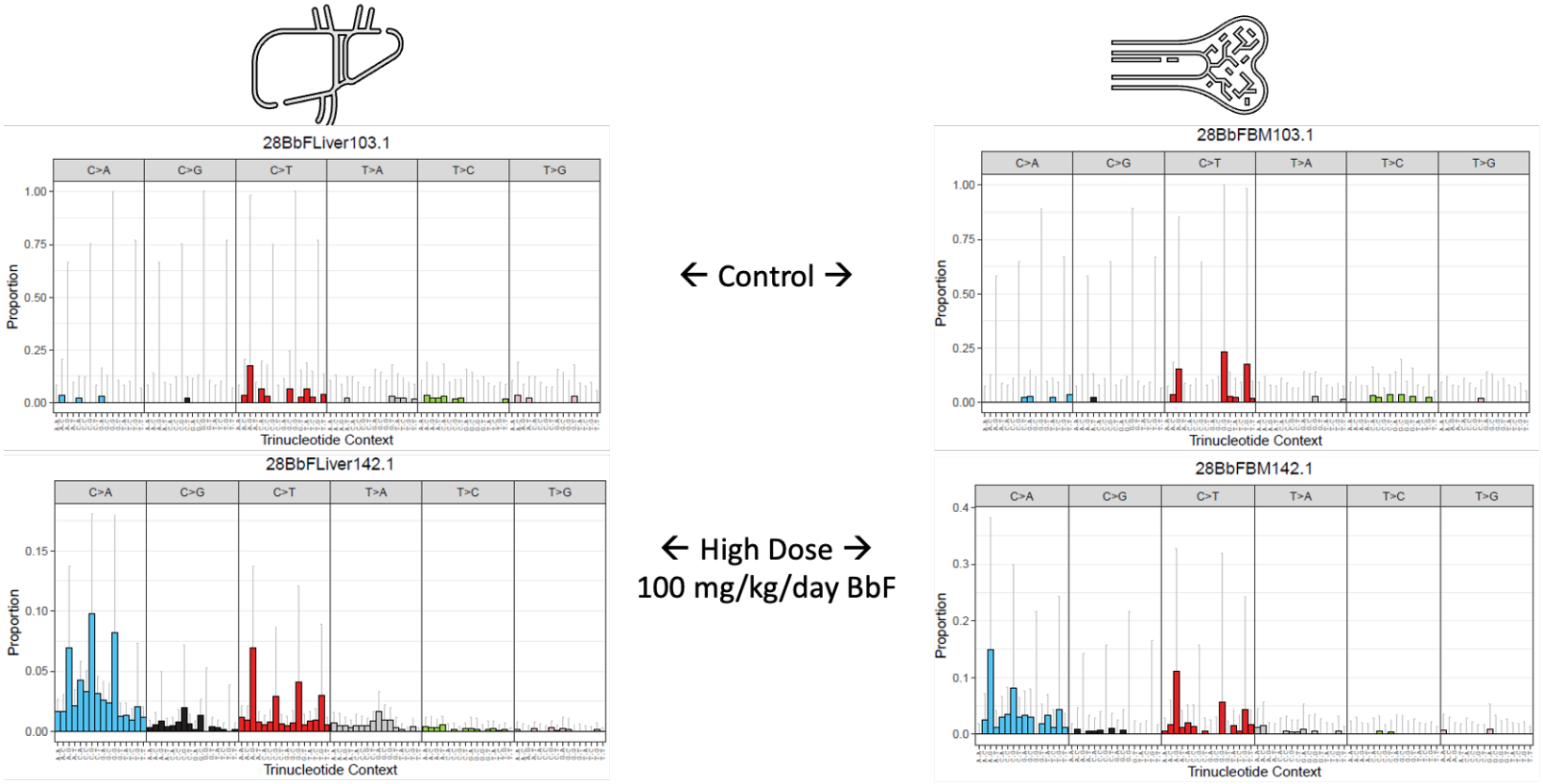


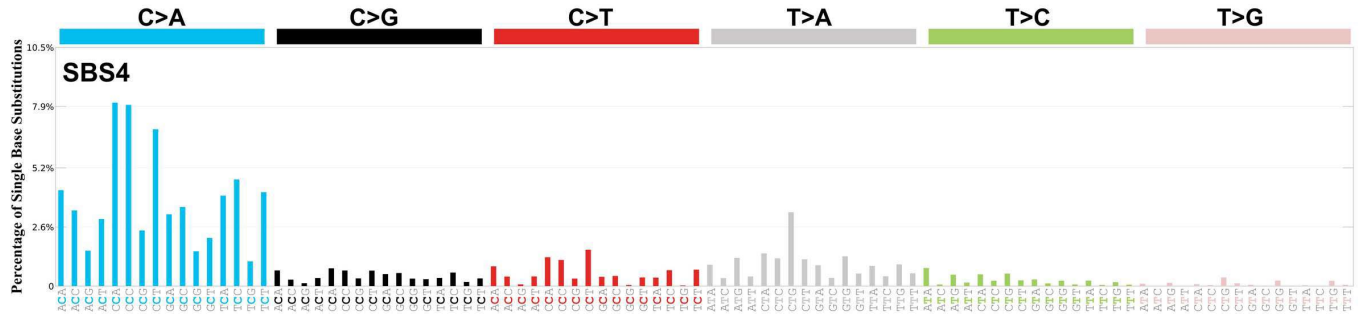


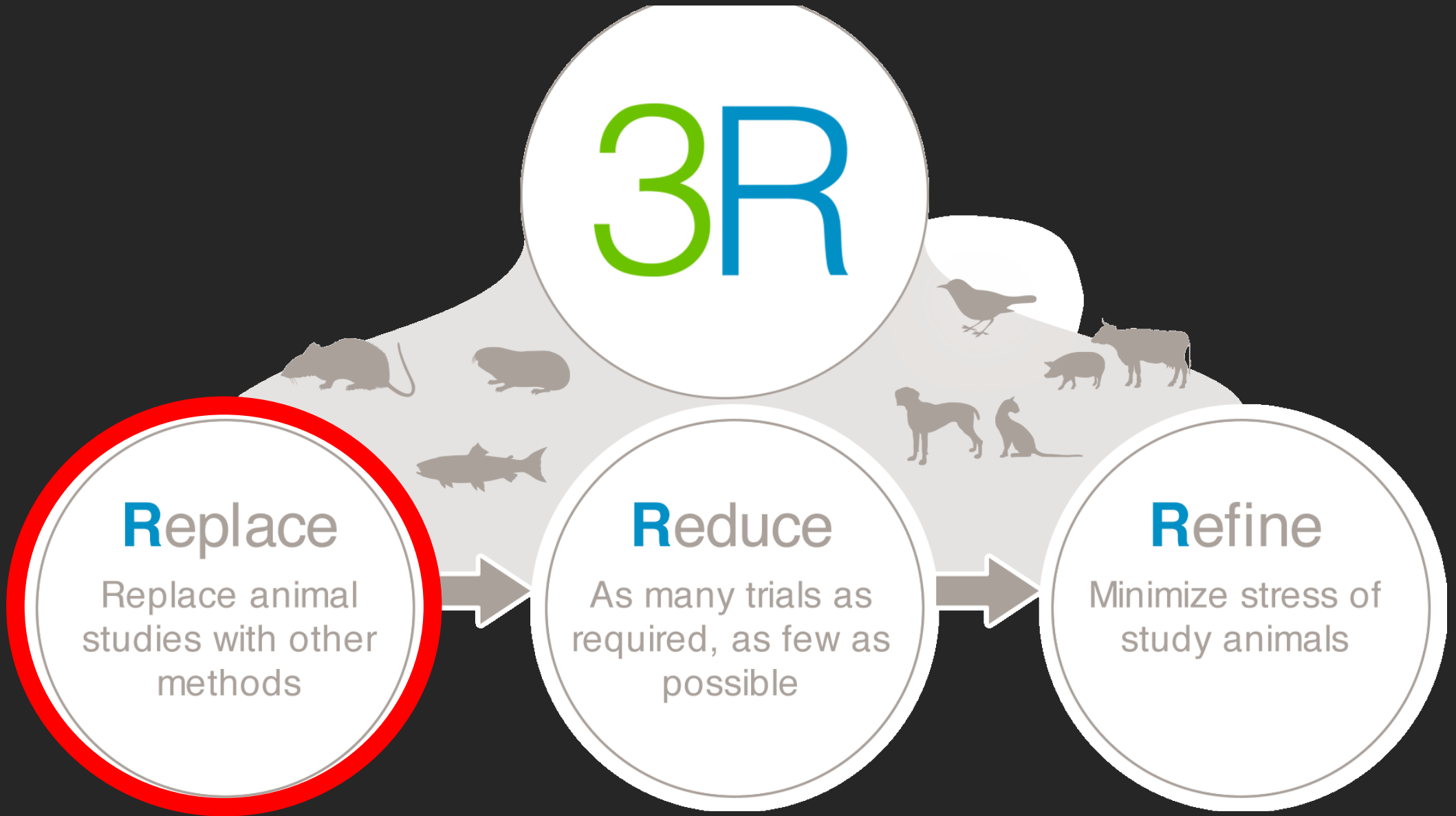
David Schuster, Health Canada crew!

p<0.05; **adj p<0.01

Enrichment of SBS4 , found in lung cancers of smokers





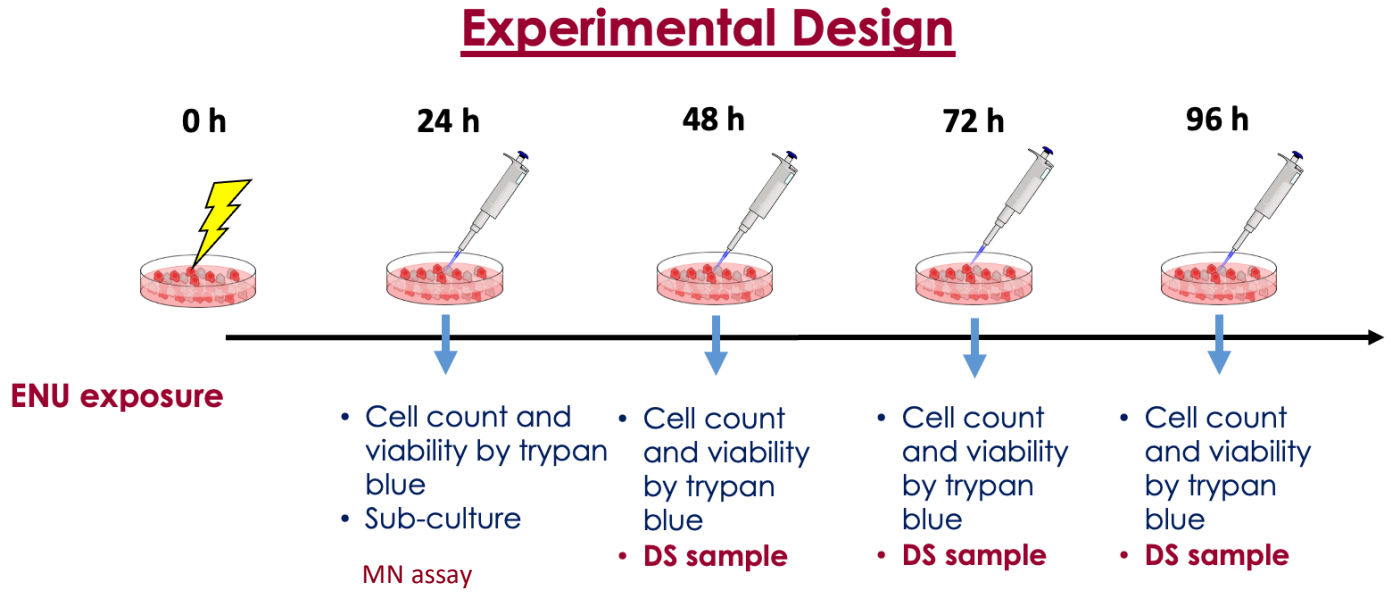

3R
Replace
Replace animal studies with other methods
Reduce
As many trials as required, as few as possible
Refine
Minimize stress of study animals

Study design for *in vitro* analyses: DS analyses in TK6 cells

- Identify appropriate experimental design parameters for mutation analysis in TK6 cells
- Explore utility of the mechanistic information acquired through application of DS
- Evaluate inter-laboratory reproducibility

Health Canada Concentrations:

0 (μM)
25
50
100
150

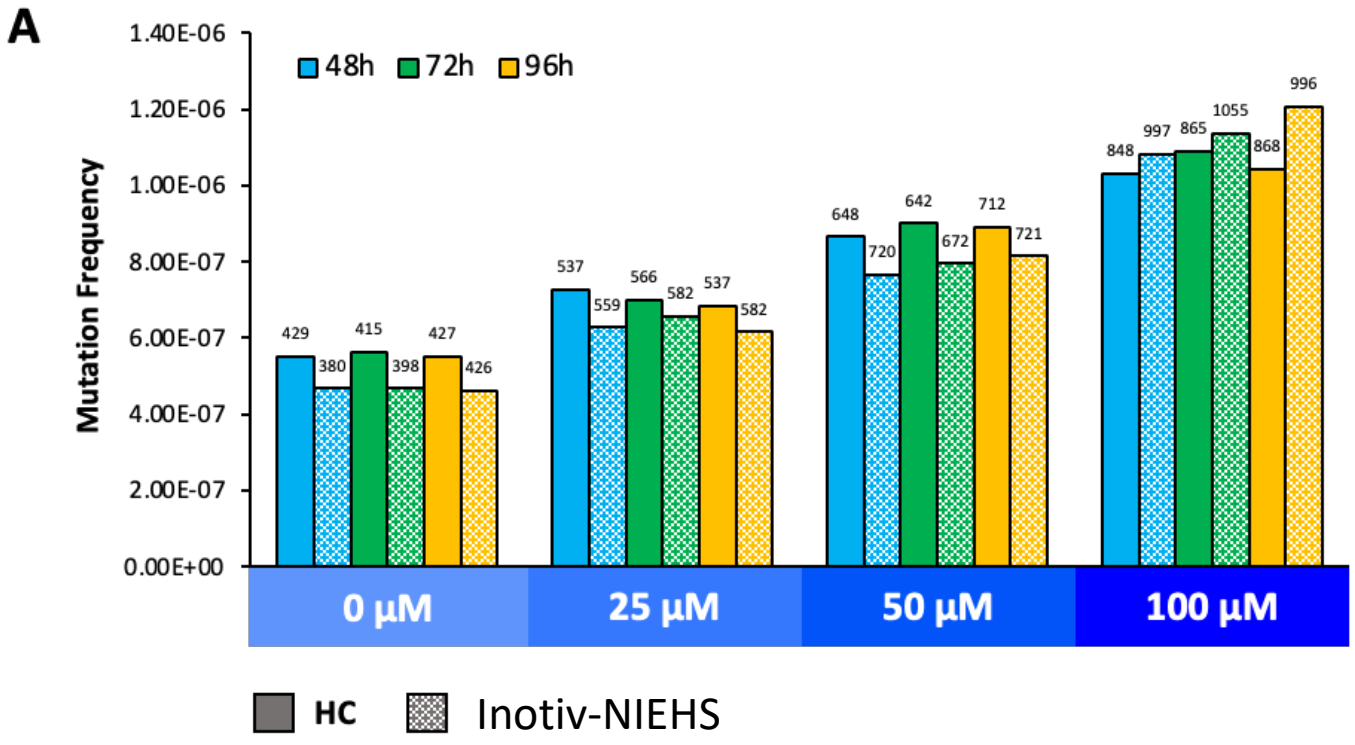


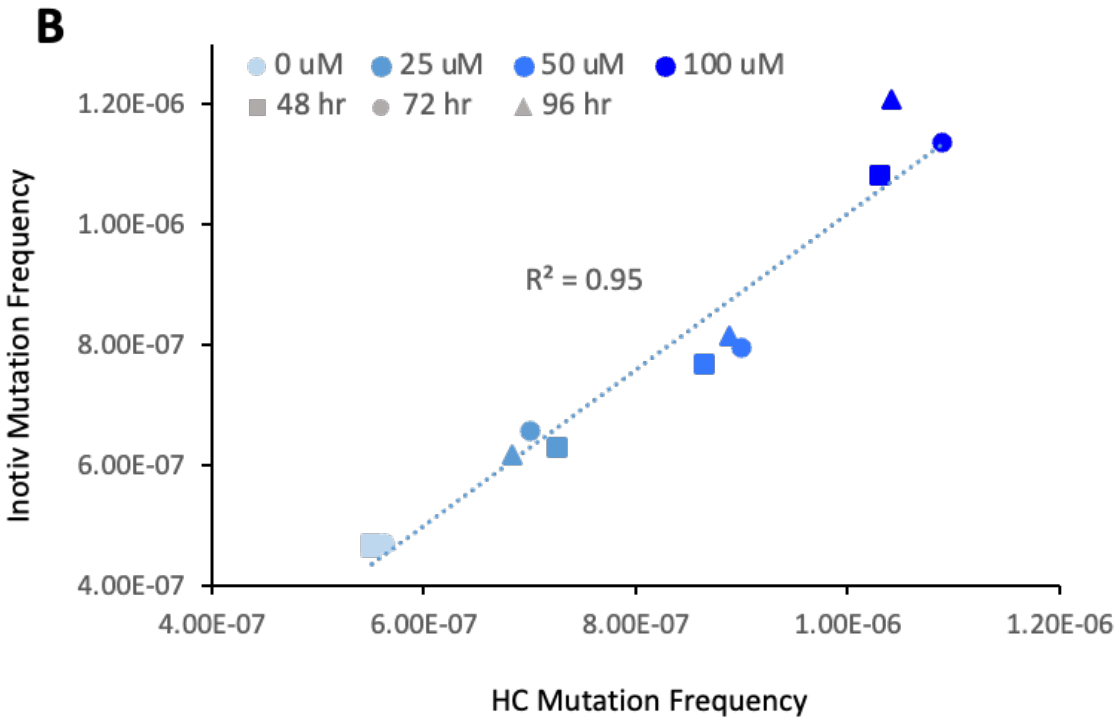
Inotiv/NIEHS Concentrations:

0 (μM)
25
50
100
150
200

Eunnara Cho, Carol Swartz, Stephanie Smith-Roe, Kristine Witt, Recio, Rivas, Health Canada and TS

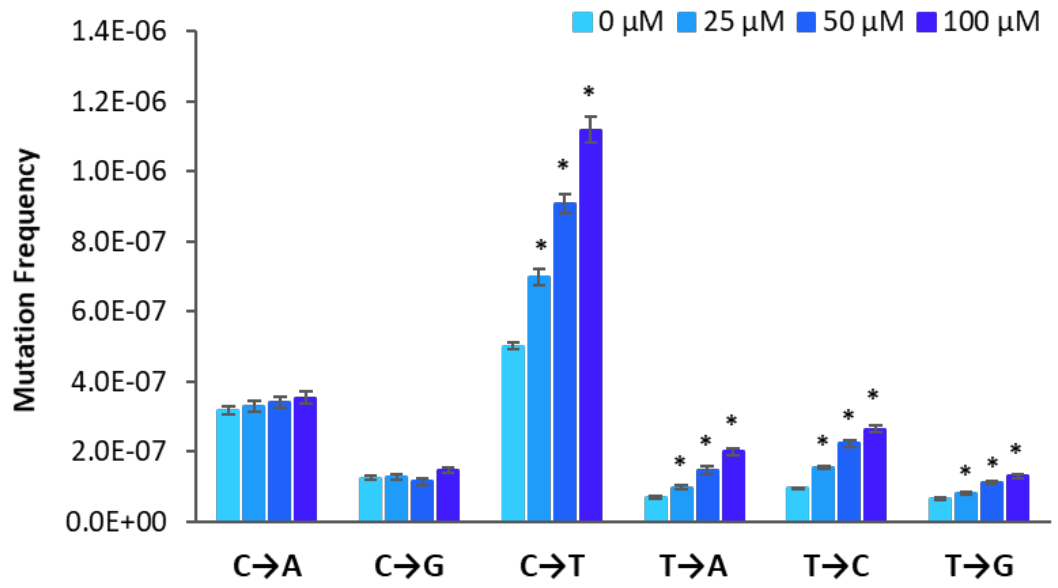
Remarkable consistency in mutation frequency by DS across time and between laboratories



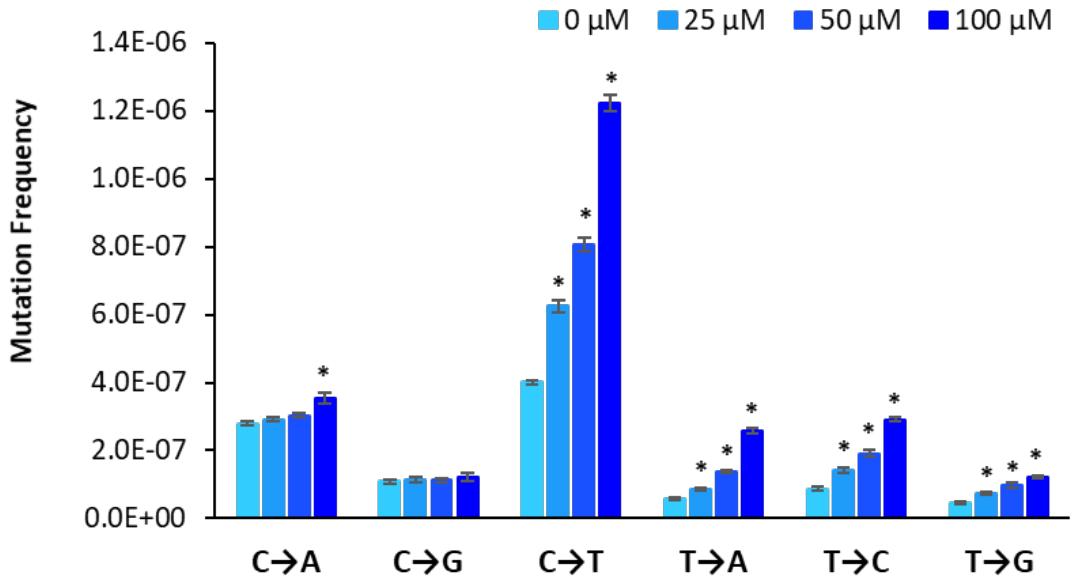


Nearly identical mutation spectrum between the two labs

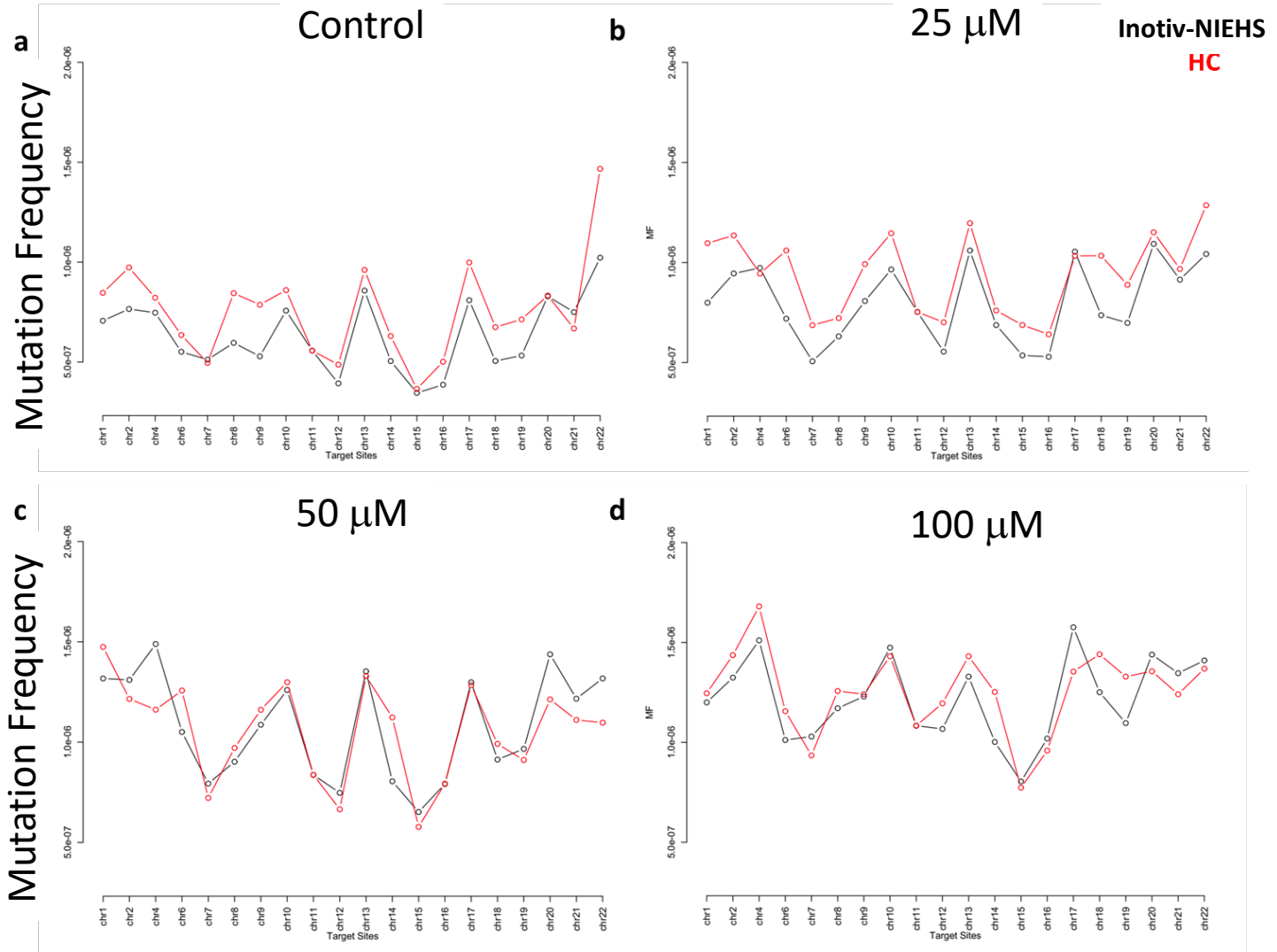
Health Canada



Inotiv-NIEHS

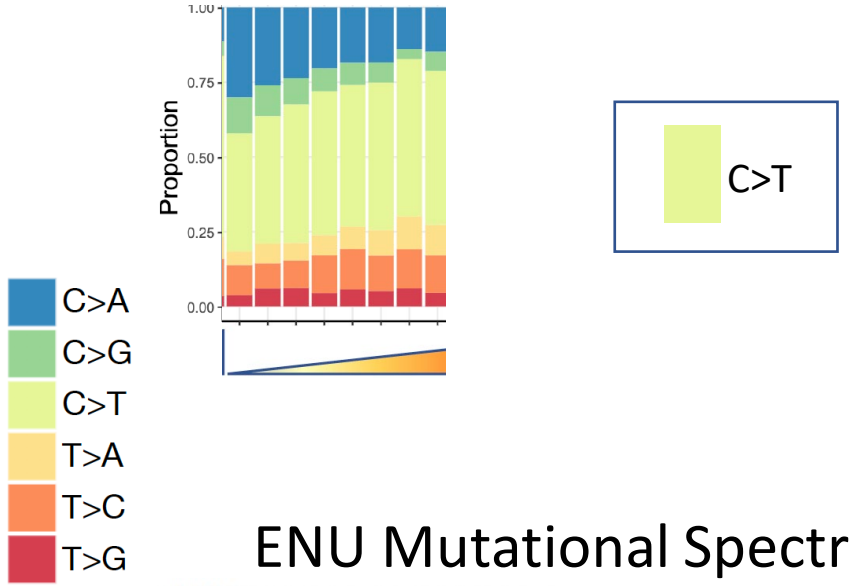


Nearly identical locus-specific responses between the two labs



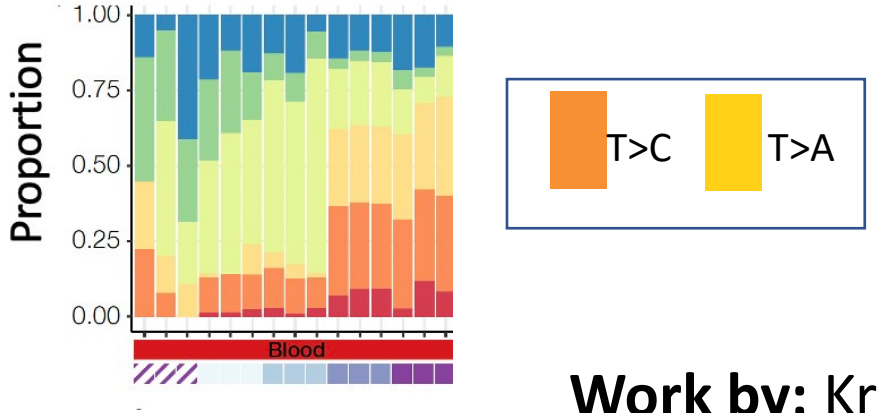
Differences in ENU mutational signatures in TK6 cells vs *in vivo*

ENU Mutational Spectrum *In Vitro*



- *In vitro* – consistent spectrum across time
  - C>T substitutions

ENU Mutational Spectrum *In Vivo*



- *In vivo* – Characteristic mutational spectrum for ENU established by 7 d post-exposure in vivo
  - T>C and T>A substitutions

- Differences due to lack of the AGT enzyme necessary for repairing O^6-alkylguanine residues in TK6 cells (Bronstein et al., 1991)

Work by: Kristine Witt, Les Recio, Carol Swartz, Cheryl Hobbs, Miriam Rivas, Stephanie Smith-Roe, TwinStrand (in prep.)

New and improved cell culture models: Many out there show promise

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RESEARCH ARTICLE

Genetic toxicity testing using human in vitro organotypic airway cultures: Assessing DNA damage with the CometChip and mutagenesis by Duplex Sequencing

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Abstract

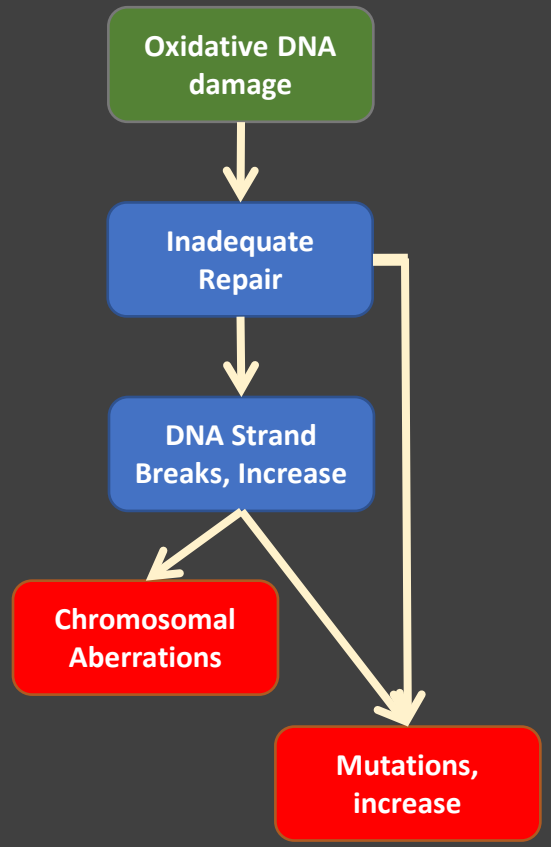
The organotypic human air-liquid-interface (ALI) airway tissue model has been used as an in vitro cell culture system for evaluating the toxicity of inhaled substances. ALI airway cultures are highly differentiated, which has made it challenging to evaluate genetic toxicology endpoints. In the current study, we assayed DNA damage with the high-throughput CometChip assay and quantified mutagenesis with Duplex Sequencing, an error-corrected next-generation sequencing method capable of detecting a single mutation per 10^7 base pairs. Fully differentiated human ALI airway cultures were treated from the basolateral side with 6.25 to 100 μg/mL ethyl methanesulfonate (EMS) over a period of 28 days. CometChip assays were conducted after 3 and 28 days of treatment, and Duplex Sequencing after 28 days of treatment. Treating the airway cultures with EMS resulted in time- and concentration-dependent increases in DNA damage and a concentration-dependent increase in mutant frequency. The mutations observed in the EMS-treated cultures were predominantly C → T transitions and exhibited a unique trinucleotide signature relative to the negative control. Measurement of physiological endpoints indicated that the EMS treatments had no effect on anti-p63-positive basal cell frequency, but produced concentration-responsive increases in cytotoxicity and perturbations in cell morphology, along with concentration-responsive decreases in culture viability, goblet cell and anti-Ki67-positive proliferating cell frequency, cilia beating frequency, and mucin secretion. The results indicate that a unified 28-day study can be used to measure several important safety endpoints in physiologically relevant human in vitro ALI airway cultures, including DNA damage, mutagenicity, and tissue-specific general toxicity.

HESI Genetic Toxicology Technical Committee

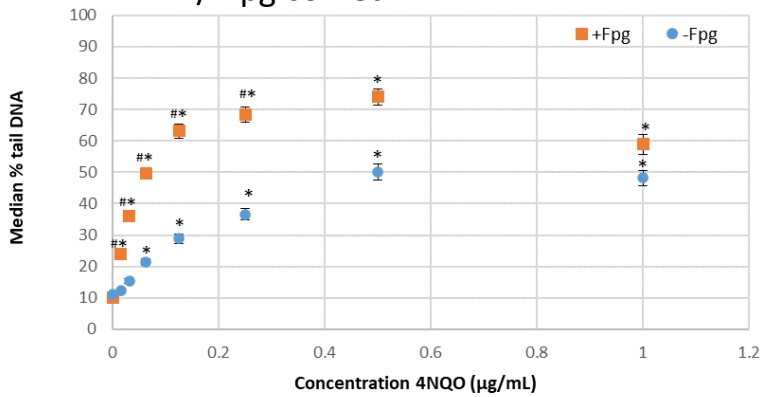
In vitro error-corrected Next-Generation Sequencing Working Group

Chair, Leslie Recio, ScitoVation

AOP-informed study design and quantitative analyses: Case study on 4-Nitroquinoline 1-oxide (4NQO)



2 hr +/-Fpg comet



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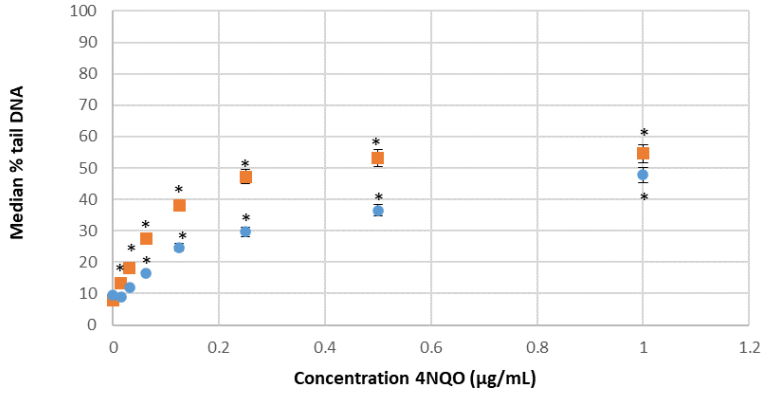

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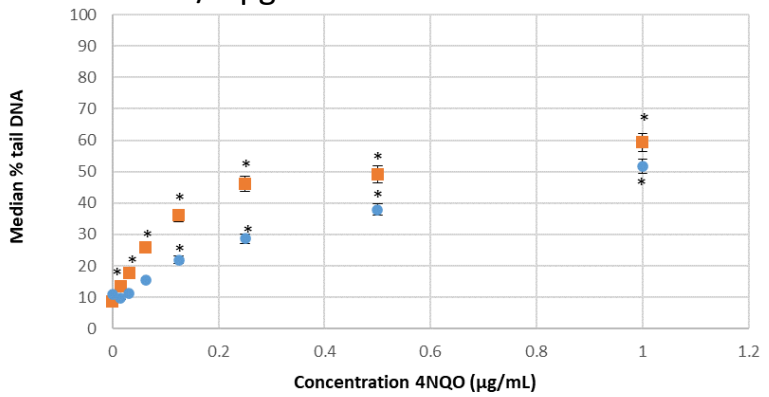
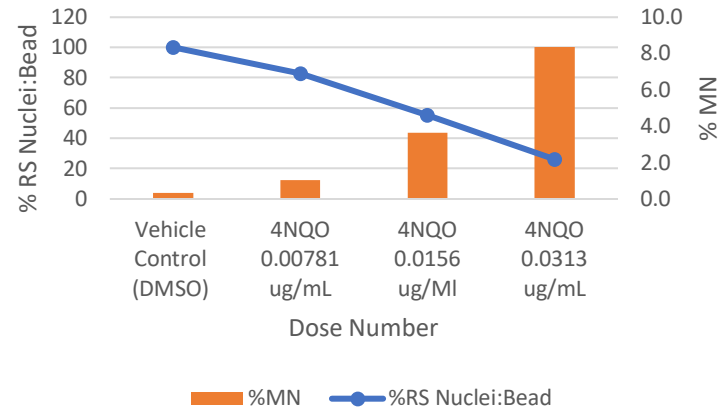
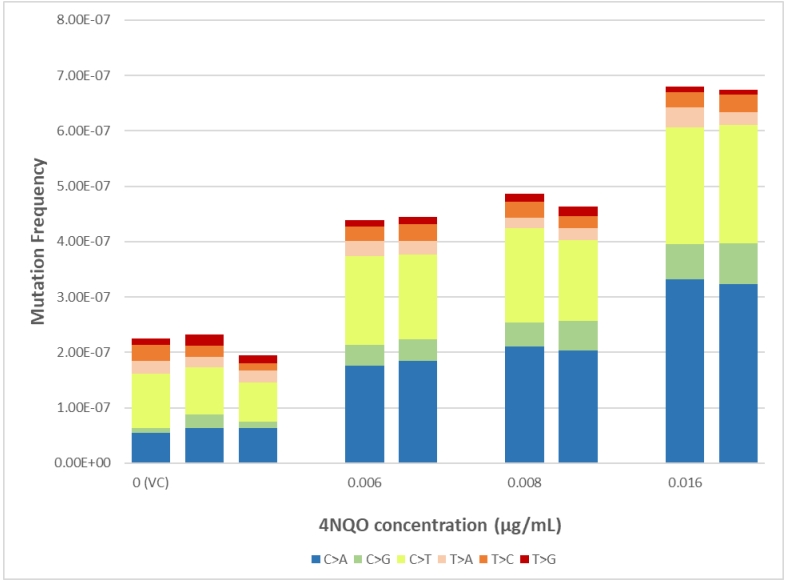


Micronucleus frequency and relative survival



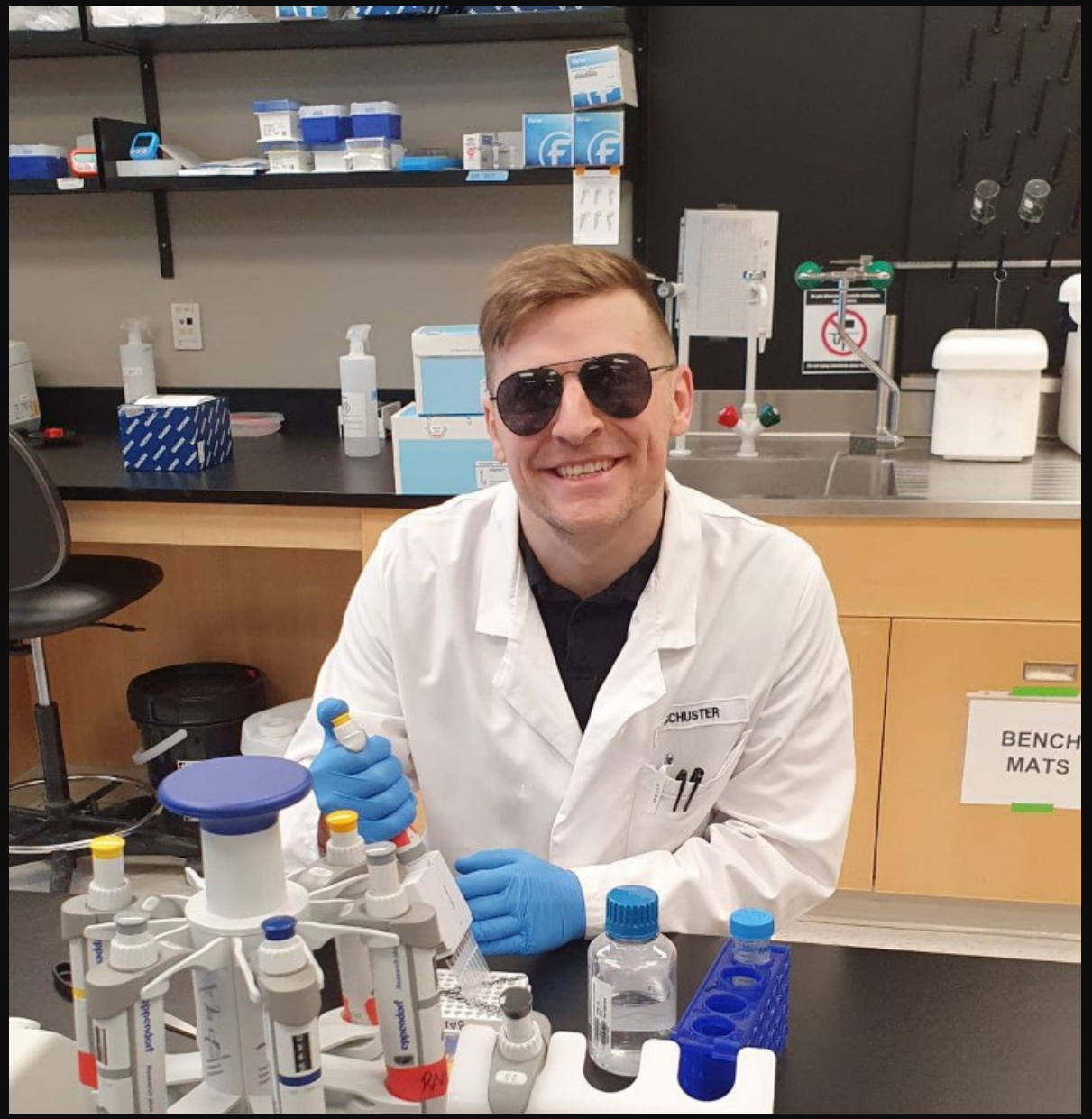
Duplex Sequencing



Huliganga, manuscript in prep.

Future’s so bright

Gotta wear shades?



David Schuster, PhD candidate

Critical feedback from excellent reviewers

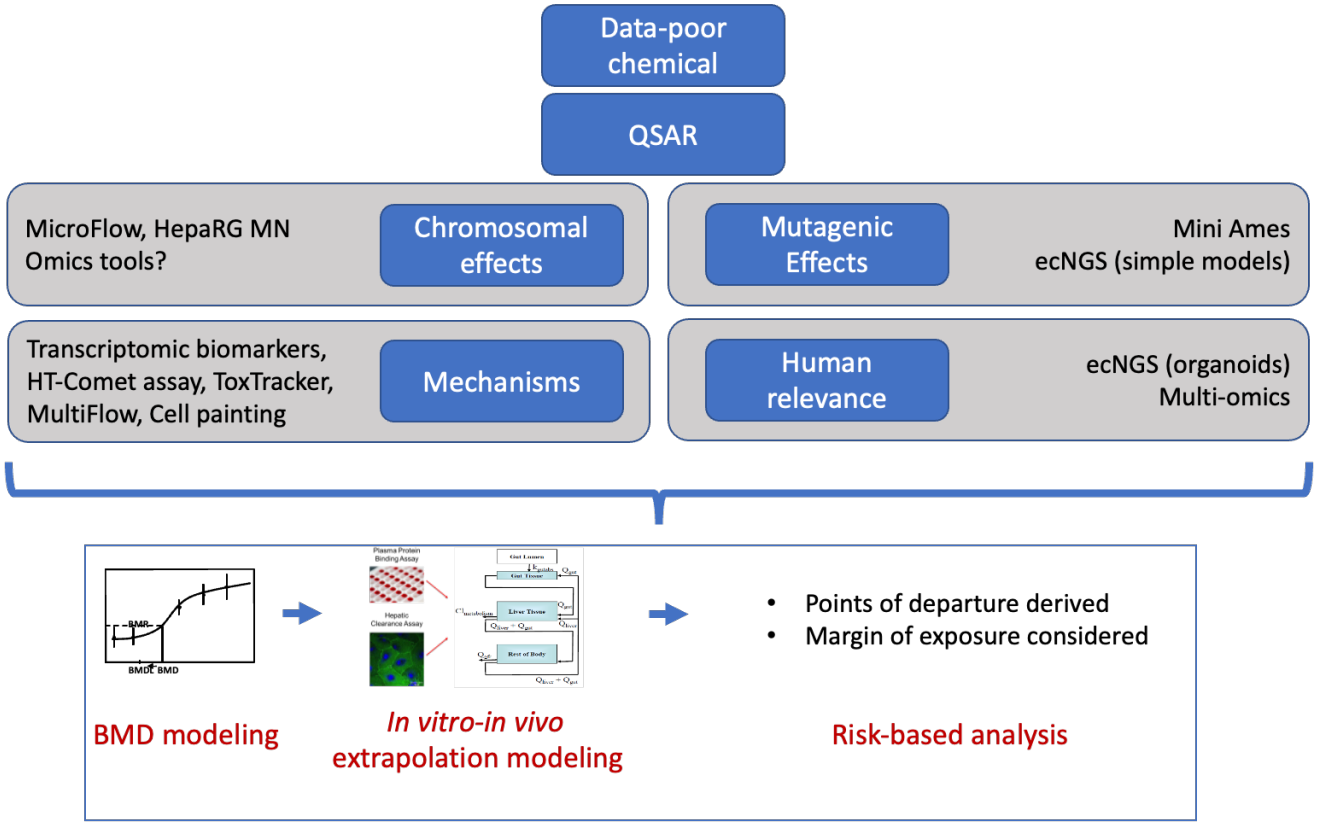
Problem with Duplex Sequencing

- Provides information on small sequence changes only
  - Ames already picks these up
  - Major obstacles to overcome to meet the speed, convenience, cost, and regulatory acceptance of Ames
- Does not capture the endpoint of interest for which *in vitro* mammalian cell mutagenesis assays are used
  - i.e., large events (cytogenetic) or both large/small events (MLA or TK mutation assay)
  - DS, as used in this study, does not provide these types of data

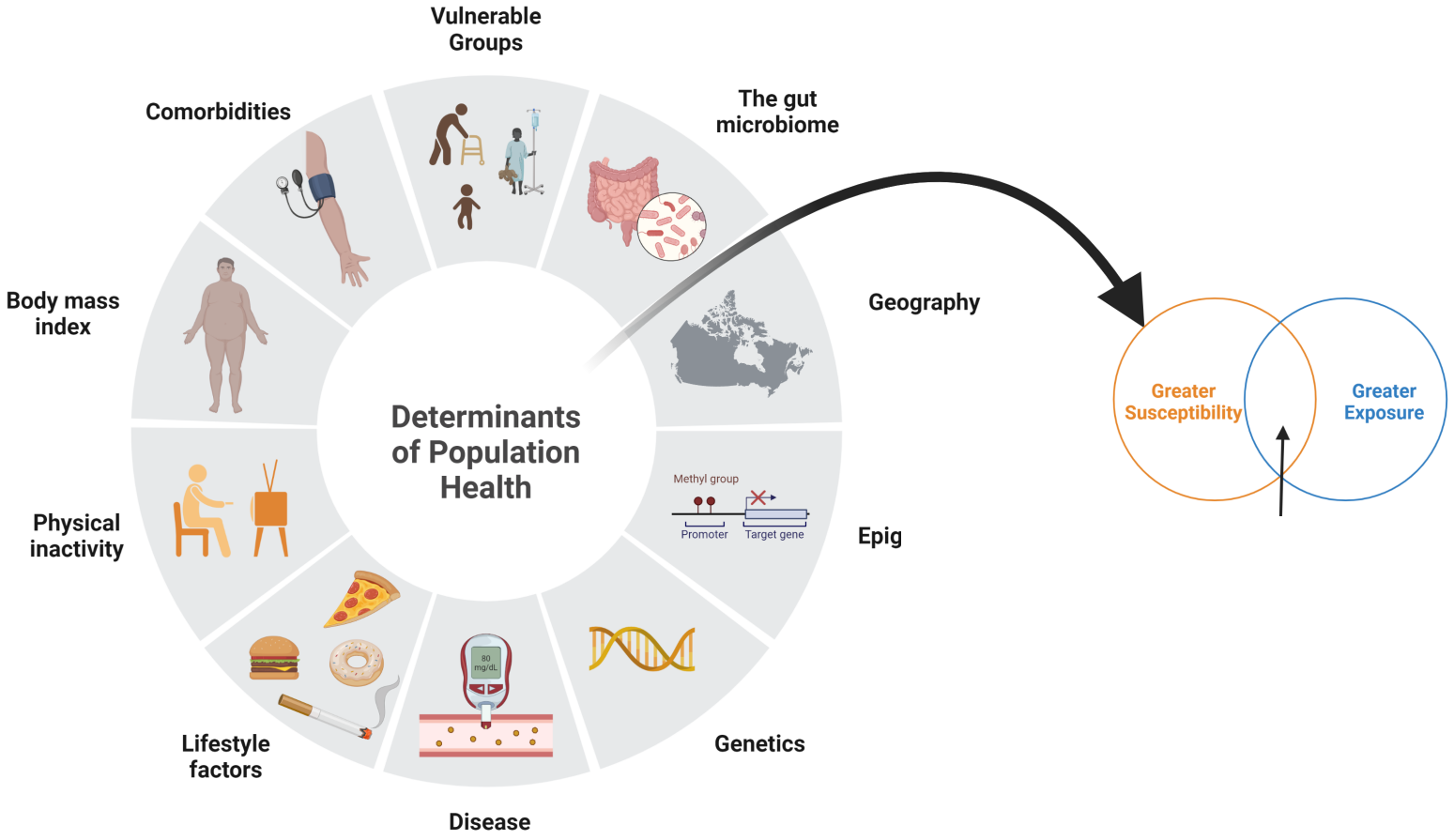
"Thus, suspect role in regulatory science… will be small."

- We need to stop comparing our new tests to our old tests.
  - Our old tests aren't necessarily the best
    - e.g., can't do IVIVE with Ames data
  - We can pick up the major types of mutations found in genetic diseases and cancer using error-corrected sequencing
- We need to stop benchmarking against animal outcomes, particularly cancer
- We need to protect human health and use a flexible, integrated approach that relies on endpoints relevant to humans
- To industry partners – please submit the data!
- To regulators – please participate in these collaborations!
- We have to be creative – can't wait for an OECD TG for everything.
  - Use the Clean Sheet, use IATAs paired with AOPs.
- If using Omics - fill in the OORF!

INSERT TODAY'S TESTING PARADIGM HERE

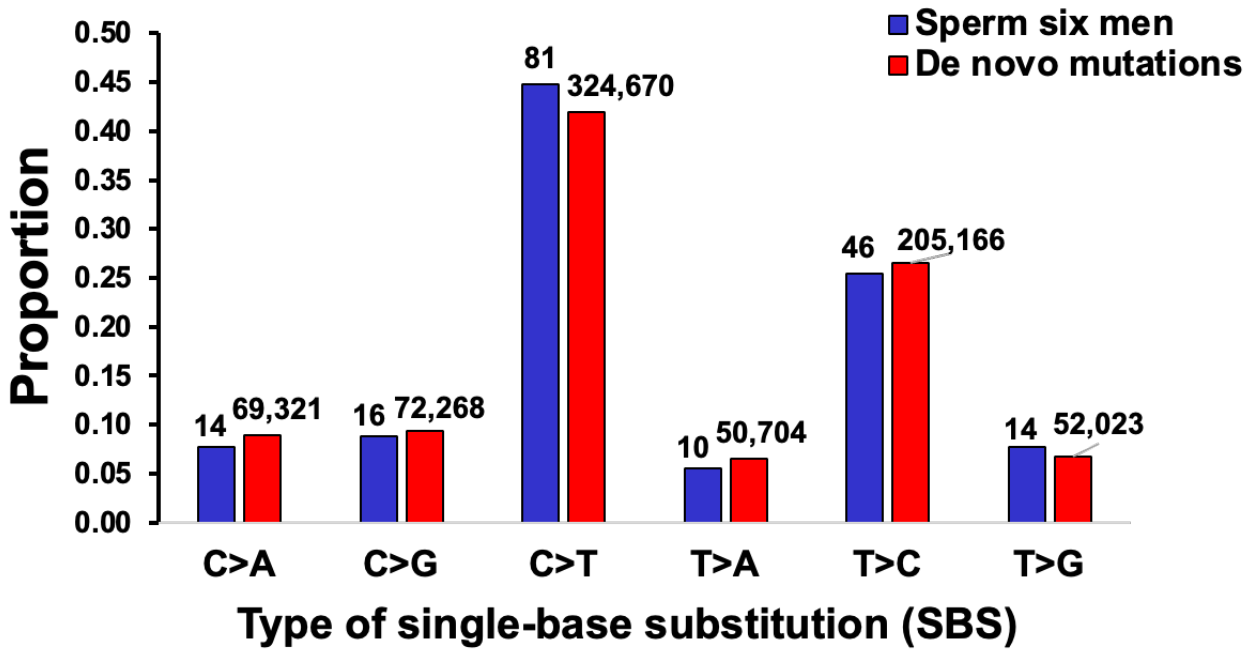


The Future: multi-omics and building in determinants of population health



Duplex Sequencing in human sperm and blood: A pilot study

- **Blood:**
  - MF: **1.2×10^{-7} bp**
- **Sperm:**
  - MF: **2.8×10^{-8} bp**



Jonatan Axelsson, Habib Shojaei, manuscript in preparation.

The extended team of incredible people involved

Health Canada, uOttawa

PIs: Francesco Marchetti, Paul White, Matthew Meier, Marc Beal

Regulatory partners: Tara Barton-Maclaren, Ivy Moffat, Alexandra Long

Incredible people at the bench: Eunnara Cho, Julie Buick, Danielle LeBlanc, Annette Dodge, Anne-Marie Fortin, David Schuster, Elizabeth Huliganga, Jonatan Axelsson, Habib Shojaei

Awesome people on the pipelines: Andrew Williams, Matthew Meier, Byron Kuo

HESI teams!!! Thank you to all our HESI partners!

TwinStrand BioSciences

Integrated Laboratory Systems, Inc./Inotiv: Leslie Recio (ScitoVation), Carol Swartz, Cheryl Hobbs

NIEHS National Institute of Environmental Health Sciences

United States Environmental Protection Agency

Massachusetts Institute of Technology

Health Canada Santé Canada

VUB Vrije Universiteit Brussel

sciensano

HESI

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- Canada Research Chairs program
- Natural Sciences and Engineering Research Program of Canada
- Burroughs Wellcome Fund

Health Canada Santé Canada

Health Canada
www.hc-sc.gc.ca

PULLED SLIDES

PULLED SLIDES

Data-poor chemical

QSAR

MicroFlow, HepaRG MN
Omics tools?

Chromosomal effects

Mutagenic Effects

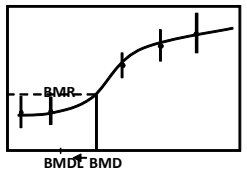
Mini Ames
ecNGS (simple models)

Transcriptomic biomarkers, HT-Comet assay, ToxTracker, MultiFlow, Cell painting

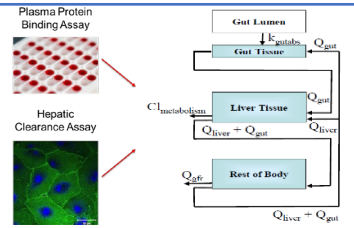
Mechanisms

Human relevance

ecNGS (organoids)
Multi-omics



BMD modeling



In vitro-in vivo extrapolation modeling

- Points of departure derived
- Margin of exposure considered

Risk-based analysis