A Decade of Progress with NAMs & NGRA

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Safety, Environmental & Regulatory Science Unilever, UK





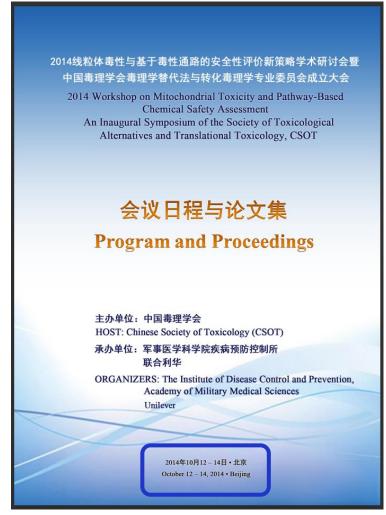








A Decade of TATT







2011 to 2025 - From TT21C to NAMs/NGRA











A Decade of:

Next Generation Risk Assessment (NGRA) New Approach (non-animal) Methodologies (NAMs)

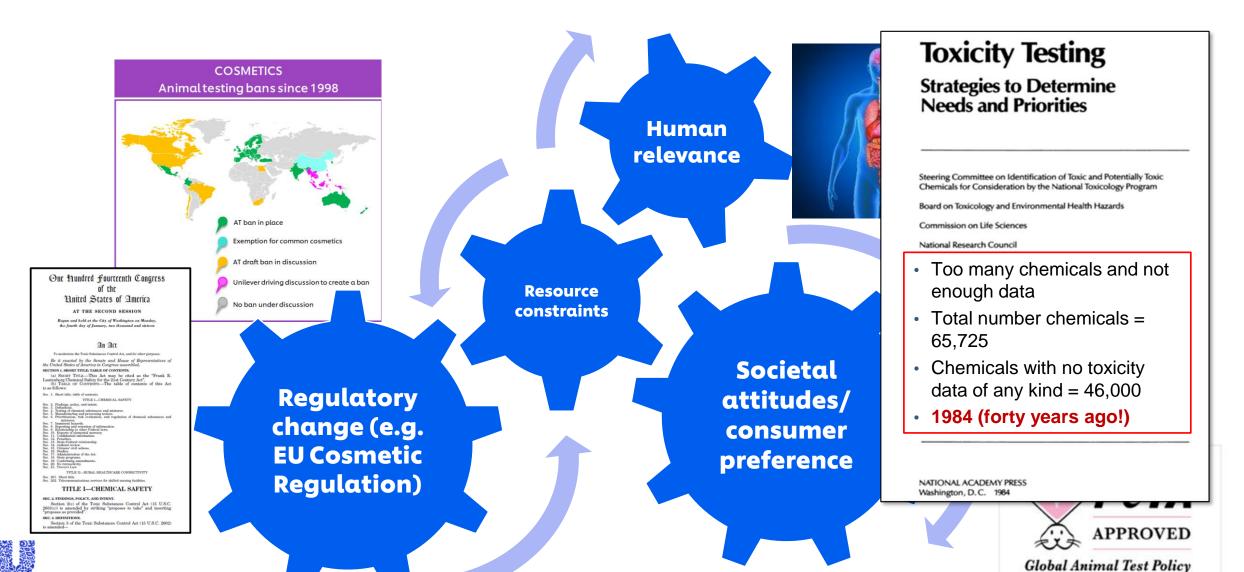
NGRA is an exposure-led, risk assessment approach that integrates New Approach Methodologies (NAMs) to assure (human & environmental) safety without the use of animal testing

NAMs are any *in vitro* or computational (*in silico*) method that enables mechanistically based chemical safety assessment and contributes to the replacement of animals



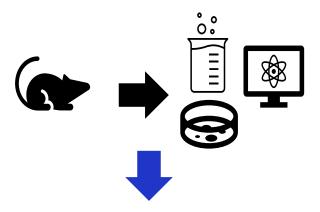
讨论争议点:下/新一代风险评估; Next/New?

The Need for Implementation of NAM-Based Safety Assessments



Non-Animal Frameworks for Safety Decisions:

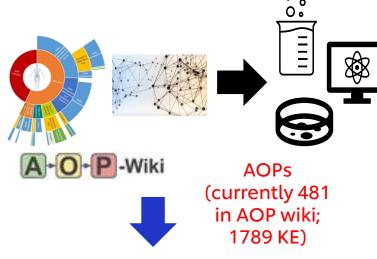
Non-animal NAMs strategies for 1-2-1 replacement prediction of animal outcome



Prediction of an animal test is **not** necessarily relevant to assess human safety

Rodent studies have been used in a protective manner with the use of uncertainty factors rather than in a predictive way

Development of battery of assays aligned to AOPs



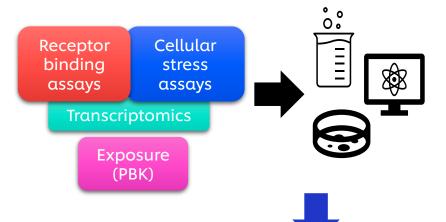
~ Multiple 1000s of assays need to be if multiple AOPs are covered

How to identify the relevant AOP?

Not feasible for initial safety testing

Value in bespoke safety assessment when differentiation between bioactivity & adversity is needed (higher tier)

Development of high-throughput & broad coverage set of non-animal NAMs



Protection Hypothesis:

If biological activity measured using a broad suite of humanrelevant test systems is above the predicted exposure in humans, then systemic adverse effects are highly unlikely



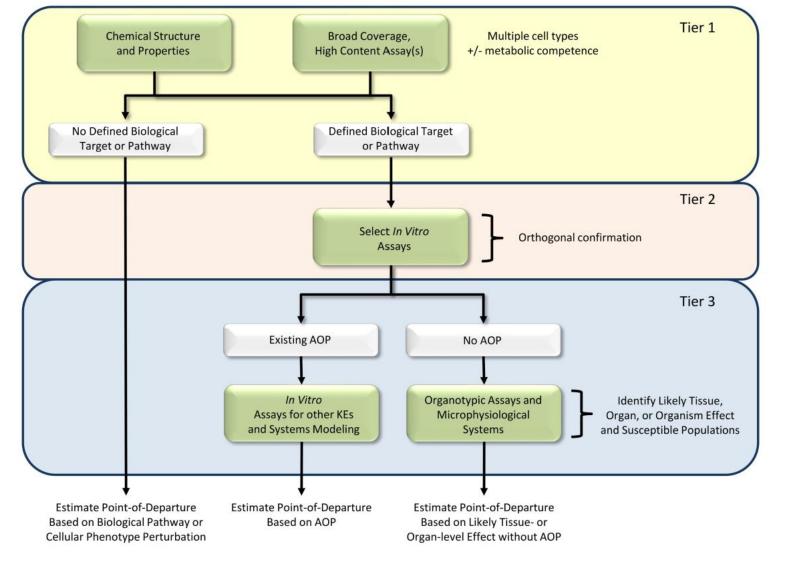
Why Tier NAMs in α Protective NGRA?

- Can then implement the available and valid tools in a measured and logical way
- Maintain a core dynamic system relevant to the risk assessment/safety question
 - Be ever prepared to improve and update
- Can lead from early-tier rapid computational tools to potentially more complex testing systems in later tiers
 - But only as necessary
- Helps give greater confidence to Regulators?
 - Improving
- Early Tiers come at a 'utility' cost the loss of otherwise useful chemicals?
 - That's what we have encountered but we must build confidence





The EPA Blueprint: A 'Tiered' Approach





TOXICOLOGICAL SCIENCES, 169(2), 2019, 317-332 Advance Access Publication Date: March 5, 2019

The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency

Russell S. Thomas,*,1 Tina Bahadori,† Timothy J. Buckley,‡ John Cowden,* Chad Deisenroth,* Kathie L. Dionisio, Fiffrey B. Frithsen, Christopher M.





NAMs are Generally *More* Protective than Animal Tests

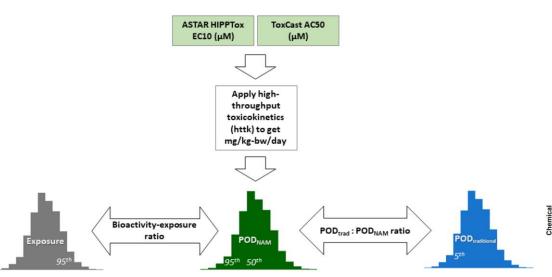
Accelerating the Pace of Chemical Risk Assessment (APCRA)





Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization

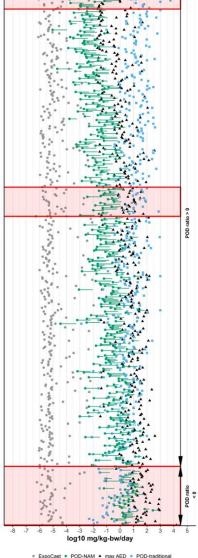
Katie Paul Friedman (1), *,1 Matthew Gagne, † Lit-Hsin Loo, ‡ Panagiotis Karamertzanis, § Tatiana Netzeva, § Tomasz Sobanski, § Jill A. Franzosa, ¶ Ann M. Richard,* Ryan R. Lougee,*,|| Andrea Gissi,§ Jia-Ying Joey Lee,‡ Michelle Angrish, || Jean Lou Dome, || Stiven Foster, Kathleen Raffaele, Tina Bahadori, Maureen R. Gwinn, Jason Lambert, Maurice Whelan, Mike Rasenberg,§ Tara Barton-Maclaren,† and Russell S. Thomas 60 *



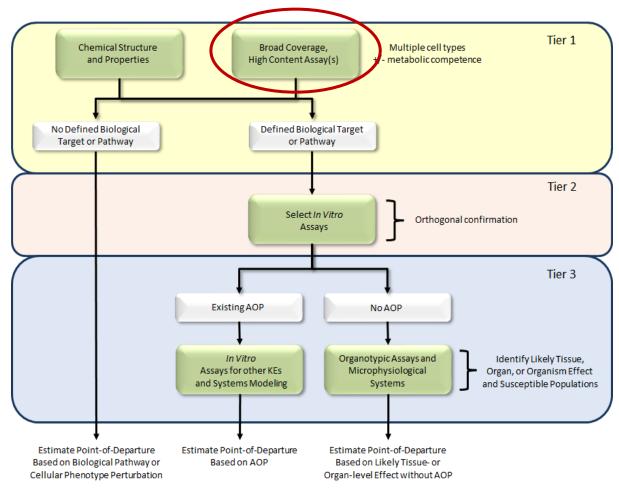
Of the 448 substances, ~90% had a $POD_{NAM,95}$ that was less than the traditional POD (POD_{traditional}) value

Bioactivity: exposure ratios (BERs), useful for identification of priority substances, demonstrated that high-throughput exposure predictions were greater than the $POD_{NAM,95}$ for 11 substances





EPA Transition from ToxCast to Broad Coverage NAM 'Product'



High throughput profiling (HTP) assays are proposed as the first tier in a NAMs-based hazard evaluation approach

HTP Assay Criteria:

- 1. Yield bioactivity profiles that can be used for potency estimation, mechanistic prediction and evaluation of chemical similarity
- 2. Compatible with multiple human-derived culture models
- 3. Concentration-response screening mode
- 4. Potential to detect specific and non-specific bioactivity

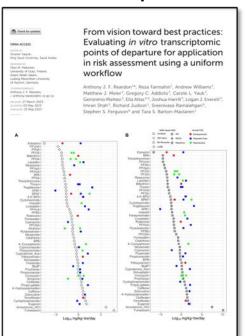
To date, EPA has identified and implemented two HTP assays that meet this criteria:

- High-Throughput Transcriptomics [HTTr]
- High-Throughput Phenotypic Profiling [HTPP]

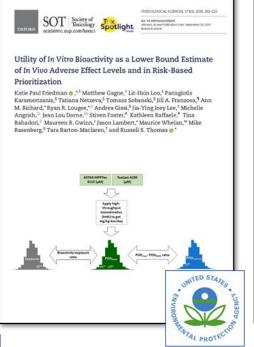


Building NAMs/NGRA Confidence: End-to-End Case Studies

≈40 compounds



448 compounds

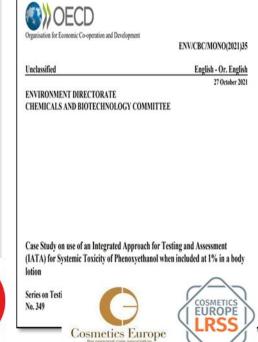


46 compounds



https://www.canada.ca/en/environment-climate-change/services/evaluatingexisting-substances/science-approach-document-bioactivity-exposure-ratioapplication-priority-setting-risk-assessment.html

30 compounds



>70 compounds

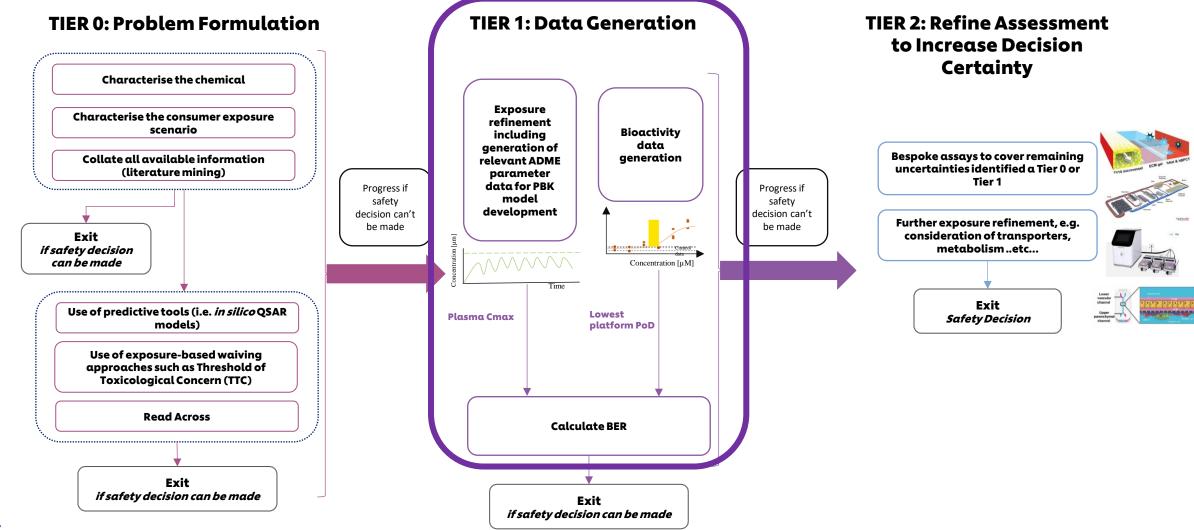






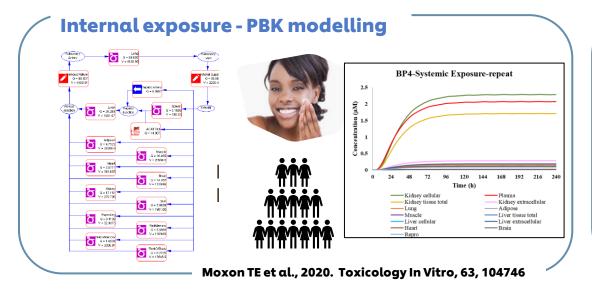


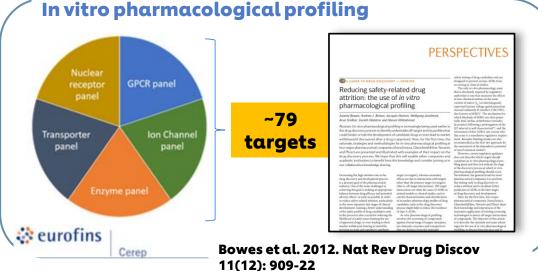
Unilever: A NAMs/NGRA Tiered Framework Approach: The overall goal is a human safety risk assessment





Unilever: Our Key NAMs

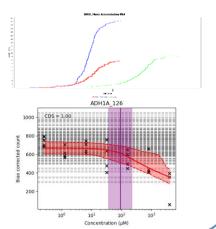




High-Throughput transcriptomics (HTTr)

- TempO-seq technology full gene panel
- 24hr exposure
- 7 concentrations
- · Various cell models (e.g. HepG2, MCF7, HepaRG)
- Dose-response analysis using **BMDExpress2** and BIFROST model

Reynolds et al. 2020. Comp Tox 16: 100138 Baltazar et al. 2020. Toxicol Sci 176(1): 236-252



Cell stress panel (CSP)

- 36 biomarkers covering 10 cell stress pathways
- HepG2
- 24hr exposure
- 8 concentrations
- Dose-response analysis using BIFROST model

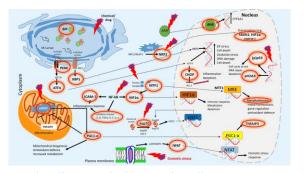


Image kindly provided by Paul Walker (Cyprotex)

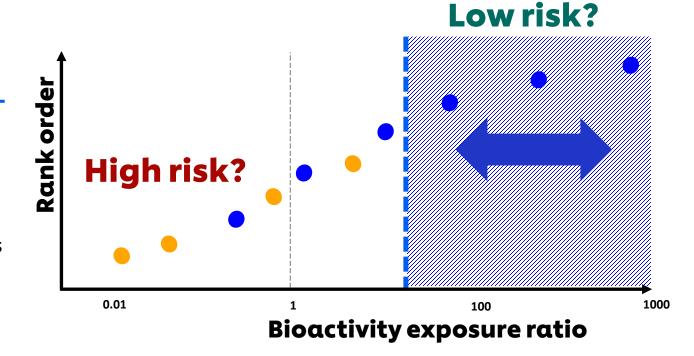
Hatherell et al. 2020. Toxicol Sci 176(1): 11-33



Unilever: Testing the Performance of NAMs in an NGRA

Benchmarking using chemicalexposure scenarios

- Chemicals with well-defined human exposures
- Traditional safety assessment available
- High certainty in the risk classification for each chemical-exposure scenario from a consumer goods perspective
- Risk class is relative to consumer health (N.B. drugs = high-risk)





'Low' risk for consumers from systemic perspective



'High' risk for consumers from systemic perspective

Protectiveness

How many of the high-risk exposure scenarios are identified as uncertain/high risk?

(i.e. BER < threshold)

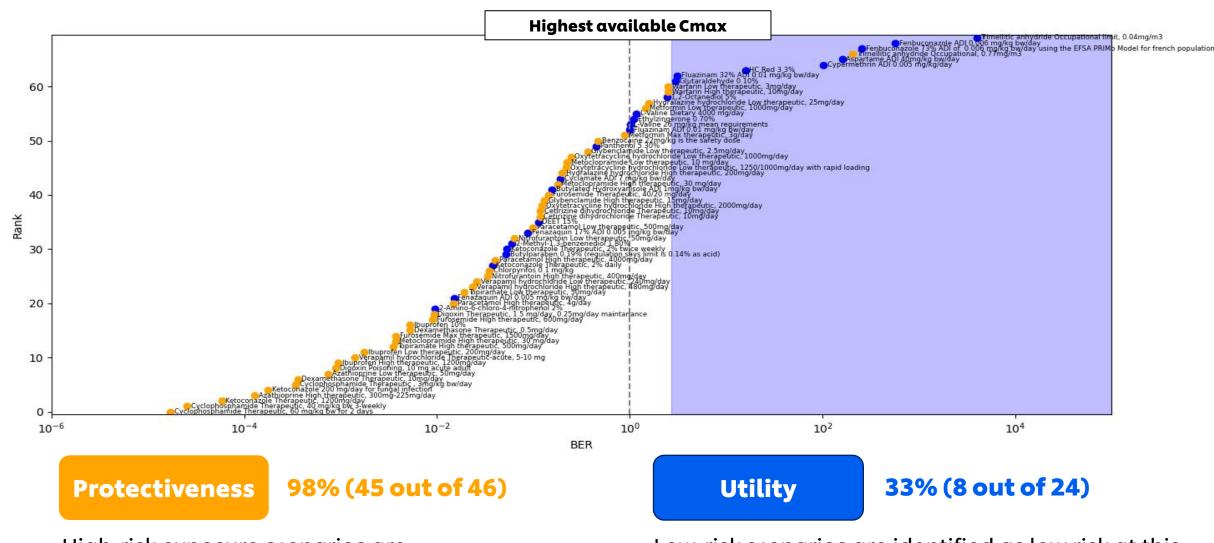
Utility

How many of the low-risk scenarios are identified as low-risk at this early tier stage in a risk assessment framework?

(i.e. BER > threshold)



Results for 38 Test Chemicals and 70 Exposure Scenarios





High-risk exposure scenarios are identified as uncertain/high risk (i.e. BER < threshold)

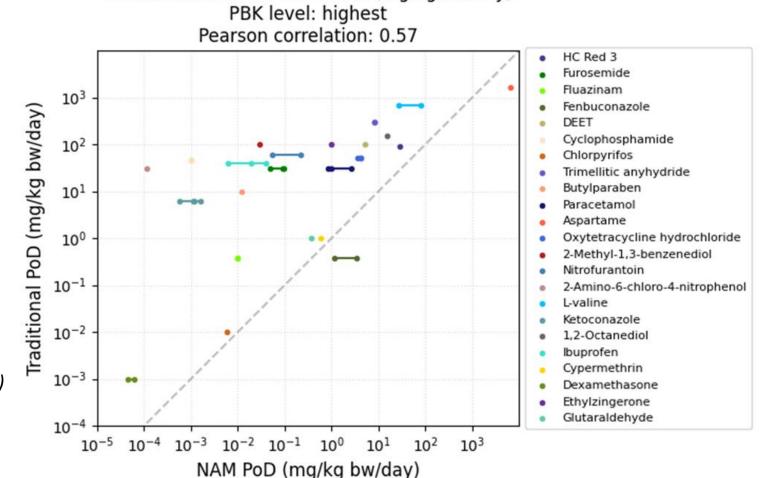
Low-risk scenarios are identified as low risk at this early tier stage in a risk assessment framework (i.e. BER > threshold)

Comparison of NAM-based Tier 1 Toolbox with Decisions Made Using *in vivo* Data – Protective not Predictive

What if we took the same approach with *in vivo* data?

- Repeat dose in vivo data identified for 27 chemicals of the 38 tested.
- In most cases NAM PoDs are more conservative than traditional PoDs

In agreement with Paul-Friedman et al. (2020)



Traditional PoDs vs. NAM PoDs (mg/kg bw/day)







Toxicological Sciences, 2025, 204(1), 79-95

https://doi.org/10.1093/toxsci/kfae159 Advance Access Publication Date: December 18, 2024 Research article

Advancing systemic toxicity risk assessment: Evaluation of a NAM-based toolbox approach

Sophie Cable*, Maria Teresa Baltazar, Fazila Bunglawala, Paul L. Carmichael, Leonardo Contreas, Matthew Philip Dent, Jade Houghton, Predrag Kukic, Sophie Malcomber, Beate Nicol, Katarzyna R. Przybylak, Ans Punt, Georgia Reynolds, Joe Reynolds, Sharon Scott, Dawei Tang, Alistair M. Middleton (1)

Safety and Environmental Assurance Centre (SEAC), Unilever, Colworth Science Park, Sharnbrook MK44 1lQ, United Kingdom





A Decade of Progress for TATT with NAMs/NGRA/TT21C





And Yet, Today, Nearly All Regulatory Safety Testing is Still Based on:







Despite the current toxicity testing paradigm not sufficiently serving our needs... woefully limited data, high testing costs, extremely long timelines, lack of human relevance, etc.

What does the USA FDA think?

TDA U.S. FOOD & DRUG

ADMINISTRATION

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/ Considerations for the Use of Artificial Intelligence To Support Regulatory Decision-Making for Drug and Biological Products

GUIDANCE DOCUMENT

Considerations for the Use of Artificial Intelligence To Support Regulatory Decision-Making for Drug and Biological Products

Draft Guidance for Industry and Other Interested Parties

JANUARY 2025

THE AMERICAN JOURNAL

of MEDICINE.

Official Journal of the Alliance for Academic Internal Medicine

The FDA Modernization Act 2.0: Drug Testing in Animals is Rendered Optional

Eli Y. Adashi, MD, MS A a A Daniel P. O'Mahony, MSLS b · I. Glenn Cohen, JD c

Affiliations & Notes ✓ Article Info ✓

The FDA Modernization Act 2.0 (5.5002), a bipartisan bill co-authored by Senators Cory Booker (D-NJ) and Rand Paul (R-KY), was passed by the Senate on September 29, 2022, by unanimous consent and without amendments. Later ratified by the US House of Representatives, the FDA Modernization Act 2.0, now part of the Consolidated Appropriations Act, 2023, was enacted into law on December 29, 2022. The new law an the Federal Food, Drug, and Cosmetic Act by authorizing sponsors of novel drugs to make use of "certain alternatives to animal testing, incl cell-based assays and computer models, to obtain an exemption from the Food and Drug Administration to investigate the safety and effect of a drug." The new law also "removes a requirement to use animal studies as part of the process to obtain a license for a biological product biosimilar or interchangeable with another biological product." In this Commentary we review the history of legislative efforts to curtail the mandated use of animals in the testing of drugs for safety and efficacy, discuss the emergence of nonanimal drug testing technology, and refuture prospects thereof.

Congressional expressions of concern over the reliance of the US Food and Drug Administration (FDA) on animals for safety and efficacy test have been a matter of record since 1998. It was in the course of hearings of the Senate Committee on Appropriations that the Doris Day Anii League lamented the discontinuation of "all assessments of in vitro, or non-animal test methods, to substantiate the safety of products." Congressional consideration of the authorization of an Interagency Coordinating Committee on the Validation of Alternative Methods followe before too long. However, it was not until 2019 that a flurry of Congressional bills were introduced with an eye toward limiting the use of an the testing of new drugs on humane grounds. Examples include, but are not limited to, the Reducing Animal Testing Act (S.4288), a bill introd Senator Ben Ray Lujan (D-NM) on May 19, 2022. Perhaps the most compelling illustration of the leanings of the Senate is illustrated by the "Senate on Animal Testing" the central missive of which was that "animal testing should not be used for the purposes of safety testing on



Roadmap to Reducing Animal Testing in Preclinical Safety Studies

Executive Summary

This roadmap outlines a strategic, stepwise approach for FDA to reduce animal testing in preclinical safety studies with scientifically validated new approach methodologies (NAMs), such as organ-on-a-chip systems, computational modeling, and advanced *in vitro* assays. By partnering with federal agencies like NIH and VA through ICCVAM, FDA can accelerate the validation and adoption of these human-relevant methods, improving predictive accuracy while reducing animal use. This transition will enhance public health by streamlining drug development and ensuring safer therapies reach patients faster, while positioning FDA as a global leader in modern regulatory science and innovation.

FDA NEWS RELEASE

FDA Announces Plan to Phase Out Animal Testing Requirement for Monoclonal Antibodies and Other Drugs

numan health and sceive FDA approval we been particularly heimer's (3) and mans, such as aspirin, peared safe in animal differences between

puncement Immediate Release: April 10, 2025

Today, the U.S. Food and Drug Administration is taking a groundbreaking step to advance public health by replacing animal testing in the development of monoclonal antibody therapies and other drugs with more effective, human-relevant methods. The new approach is designed to improve drug safety and accelerate the evaluation process, while reducing animal experimentation, lowering research and development (R&D) costs, and ultimately, drug prices.

The FDA's animal testing requirement will be reduced, refined, or potentially replaced using a range of approaches, including Al-based computational models of toxicity and cell lines and organoid toxicity testing in a laboratory setting (so-called New Approach Methodologies or NAMs data).



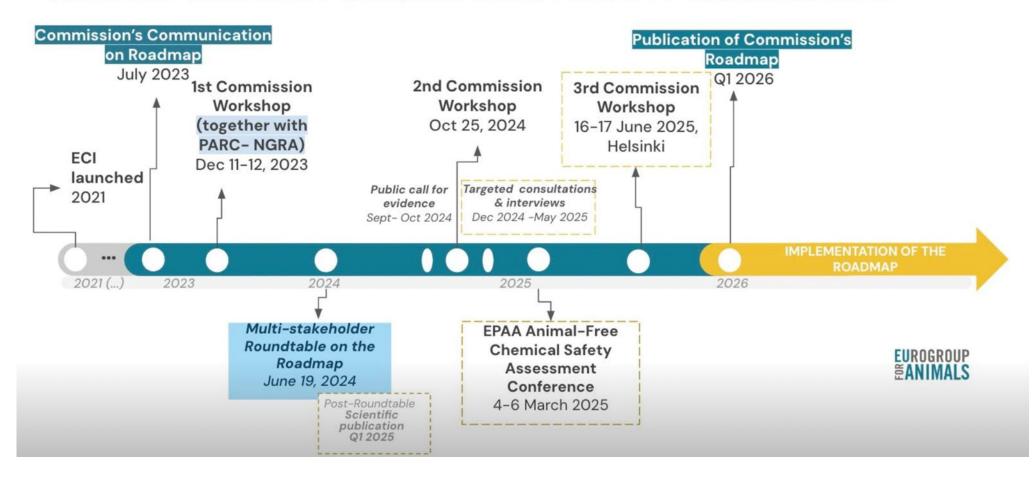
Reflected in Funding at USA National Institutes of Health?





In Europe, A Roadmap? Talk or Action? Lots to Decide On

COMMISSION ROADMAP TOWARDS PHASING OUT ANIMAL TESTING FOR CHEMICAL SAFETY ASSESSMENT





In Our Own Work: Selection of Cells?

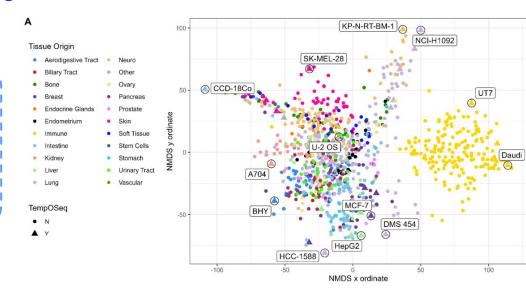
- Initially generated HTTr data in **MCF-7**, **HepG2** and **HepaRG** cell lines for all chemicals, and the Cell Stress Panel is conducted in HepG2 cells.
- Now focussed on using hTERT cell lines in place of cancer cell lines for new data generation
- Factors that have been considered:
 - Biological diversity. Using content maximisation methods and baseline gene expression data to rank cells
 - Metabolic capacity. Defining HepaRG 3D as the most competent and using the baseline gene expression data to identify the most and least competent cell lines
 - Presence of DART related genes. Making use of the baseline gene expression data and published lists of DART genes
 - Tissue of origin. Consideration of including most likely target organs or barrier tissues

Already tested:

CHON-001 → Bone HBEC3-KT → Lung HepG2 → Liver hNP1 → Brain TeloHAEC → Vascular U-2-0S → Bone

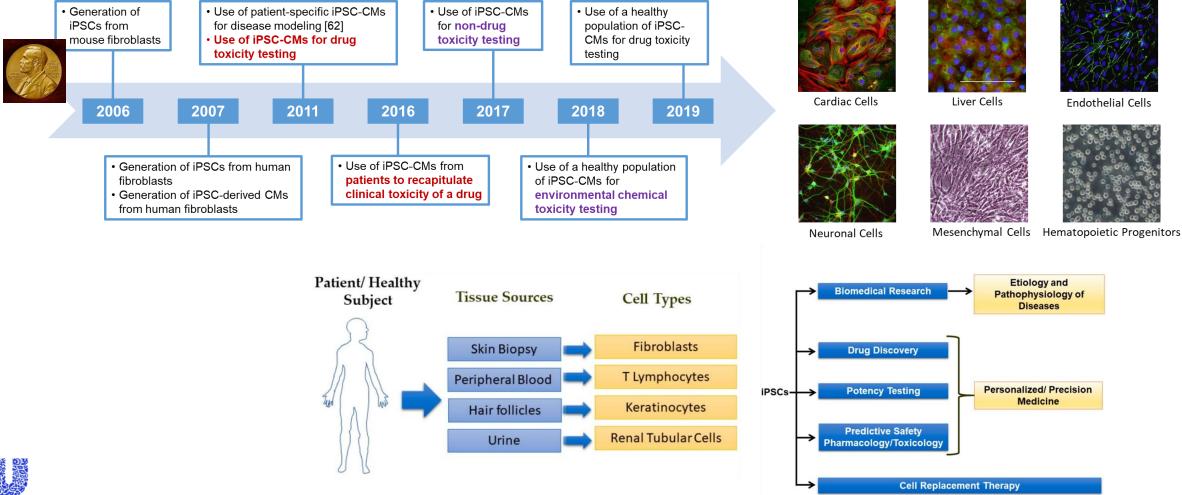
In progress:

RPTEC → Kidney
Ker-CT → Skin
CCD-18Co → Fibroblast
ASC52telo → Mesenchymal stem cell
Two zebrafish cell lines





Are hTERT Cells Likely to be Superseded? YES! By Human iPSCs





And They Are Being Used in Decision Making:

Grouping of UVCBs to waive (or minimize) animal testing requirements

House et al 2021

House et al 2022

Grouping of UVCB Substances with New Approach Methodologies (NAMs) Data

Research Article

Grouping of UVCB Substances

with Dose-Response Transcriptomics Data from Human Cell-Based Assays John S. House 1-8, Fabian A. Grimm², William D. Klaren^{2,9}, Abigail Dalze Tsai et al 2023

 $^{\rm mol\ J}$ A tiered testing strategy based on in vitro phenotypic and $^{\rm AdMI}$ transcriptomic data for selecting representative

petroleum UVCBs for toxicity evaluation in vivo Han-Hsuan Doris Tsai 🔘 1,2 John S. House 📵 3 Fred A. Wright, 1,45 Weihsueh A. Chiu 📵 1,2 Ivan Rusyn 1,2,4

To whom correspondence should be addressed. E-mail: irusyn@cvm.tamu.edu.

Abstract "Hazard evaluation of substances of "unknown or variable composition, complex reaction products and biological materials" (IV/Cls) remains a single reliability of the products of th

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approach in mechanistic and predictive toxicology (National Research Council, 2007s; Nuwaysir et al., 1999) and in risk assess-ment (Buesen et al., 2017; Kavlock et al., 2018; National Toxicology Frogram, 2018). Because gene expression is reflective of the considered to be both sensitive and early indicators of chemical-induced perturbations, they also may inform mode of action by providing gene- and pathway-level data (Chen et al., 2012; Cui of Paules, 2010). Over the last 2 decades, technologies used to pery gene expression and associated data analysis methods query gene expression and associated data analysis methods have evolved from microarrays to next generation sequescing-based approaches (Kinaret et al., 2020). More recent toxicology studies have used high-throughput transcriptomics methods allowing for rapid evaluation of the effects of large numbers of chemicals in both time-course and concentration-response study Sesigns (Harrill et al., 2019; House et al., 2017; Lamb et al., 2006; Seakley et al., 2017). The data from high-throughput

exposure, often referred to as transcriptomics, is now a common

anistic underpinnings of the effects of chemicals on biological systems but also to derive quantitative estimates of chemical potency (ie, hazard) more broadly, without a narrow focus on the meaning of perturbed pathways or genes (Harrill et al., 2021).

methods, the use of these data in toxicology is rapidly evolving changes and the "apical" toxicity phenotypes (Geter et al., 2014; Locemoner et al., 2004; Rouquie et al., 2007; Zanii et al., 2010; More recent work that include a larger number of chemicals confirmed that "apical" advense effect-based PCDs derived from sub-chronic (months) or chronic (years) studies in rodents are highly correlated with gene expression-based PCDs derived from short-term (days) in vivo studies in the same species (Shat et al.

Deriving "protective" points of departure and bioactivity-exposure ratios



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Chen et al 2020

Rapid Hazard Characterization of Environmental Chemicals Using a Compendium of Human Cell Lines from Different Organs

Zunwei Chen 1-3, Yizhong Liu1-3, Fred A. Wright 3-4, Weihsueh A. Chiu1-3 and Ivan Rusyn 1-2

Jang et al 2023

chemicals tests classes of cher

Abstract Article
The lack of ac Cumulative Risk Meets Inter-Individual Variability: of new appro rapidly evalua Probabilistic Concentration Addition of Complex Mixture using human i Exposures in a Population-Based Human In Vitro Model imoging. Con
used os surroy

Suji Jang 1,2, Lucie C. Ford 1,2, Ivan Rusyn 1,2 and Weihsueh A. Chiu 1,2,x 1

- - Interdisciplinary Faculty of Tostoology College of Vetertrany Medicine and Biomedical Sciences,
 Tean A&M University, College Station, 10, 77843, USA
 Department of Veteritaring Physiology and Pharmacology, College of Vetertrany Medicine and Biomedical
 Sciences, Tean A&M University, College Station, 17, 77843, USA
 Correspondence vehiclence Transacting Lett 1-19(79)/48-4166

ment, it has been extremely challenging to investigate the resulting cumulative risks and impacts. Recent studies proposed the use of "new approach methods," in particular in vitro assays, for hazard and dose-response evaluation of mixtures. We previously found, using five human cell-based assays that concentration addition (CA), the usual default approach to calculate cumulative risk, is mostly accurate to within an order of magnitude. Here, we extend these findings to further investigate how cell-based data can be used to quantify inter-individual variability in CA. Utilizing data from testing 42 Superfund priority chemicals separately and in 8 defined mixtures in a human cell-base population-wide in vitro model, we applied CA to predict effective concentrations for cytotoxicity for each individual, for "typical" (median) and "sensitive" (first percentile) members of the population, and for the median-to-sensitive individual ratio (defined as the toxicodynamic variability factor TDVF). We quantified the accuracy of CA with the Loewe Additivity Index (LAI). We found that LAI

varies more between different mixtures than between different individuals, and that predictions of pulation median are generally more accurate than predictions for the "sensitive" individual or the TDVE Moreover, LAI values were generally <1, indicating that the mixtures were more potent than predicted by CA. Together with our previous studies, we posit that new approach methods data from human cell-based in vitro assays, including multiple phenotypes in diverse cell types and studies in a population-wide model, can fill critical data gaps in cumulative risk assessment, but more sophisticated models of in vitro mixture additivity and bioavailability may be needed. In the meantime, because simple CA models may underestimate potency by an order of magnitude or more, either whole-mixture testing in vitro or, alternatively, more stringent benchmarks of cumulative risk indices (e.g., lower hazard index) may be needed to ensure public health protection.

Keywords: cumulative risk; dose addition; concentration addition; inter-individual variabilit toxicodynamics; chemical mixtures; defined mixtures; human health risk assessment; uncertaint factors; new approach methods



environment (NRC, pean Union, recent of Complex Mixture Exposures in a Population-Based Human In Vitro of Commodity and Model. Truit's 2022, 10, 549. https://

Received February 29, 20 Epub June 8, 2020; © The Allicia Palmi and Peter P. Egraphy

ALTEX 37(0, 623-636. dt Received: 20 July 2022 Correspondence: Iran Ru Accepted: 16 September 2022 Department of Veterinary Charles And Conference 2022 Festal ASM University, Co. Published: 20 September 2022

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Humans are continuously exposed to complex chemical mixtures in the convincement, but the same MOVE, fast, Swortest.

The artists are upon some attail. Swortest and support the same and

Testing validity of dose reconstruction assumptions for chemical mixtures

Hsieh et al 2021

Risk Characterization and Probabilistic Concentration-Response Modeling of Complex Environmental Mixtures Using New Approach Methodologies (NAMs) Data from Organotypic in Vitro Human Stem Cell Assays

Nan-Hung Hsieh,14 Zunwei Chen,14 Ivan Rusyn,1 and Weihsuch A. Chiu!

Z.: Zhou, Y.-H.: Gallins, P.L. Wright

health effec EA; Chiu, WA; Rusyn, LA oping appr Population-Based Human in Vitro

*These auth Tintis 2022, 10, 441. https:// Address of doi.org/10.3390/toxics100800

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Accepted: 29 July 2022

interests. Publisher: 1 August 2022.
Received 3
7020: Publish
Publisher's Note: MDPI stays neutral

framework: Approach to Quantify

Research

-Ford et al 2023

OBJECTIVES A Population-Based Human In Vitro Approach to Quantify Inter-Individual Variability in Responses to Chemical Mixtures

Lucie C. Ford ^{1,2}, Suji Jang ^{1,2}, Zunwei Chen ^{1,2}, Yi-Hui Zhou ^{3,4}, Paul J. Gallins ⁴, Fred A. Wright ^{3,4}, Weihsueh A. Chiu ^{1,2} and Ivan Rusyn ^{1,2}

constituents in a mixture for cumulative assessment

- Inter line planes provide of Encyclege, College of Vertrany Medicine and Biomedical Sciences, Tean AdM University, College Stellars, 77, 2831, USA; Intelline Versum and Int. CF2, singility on Issue and USA; 17, 2000, College Stellars, 77, 2831, USA; Intelline Versum and Int. CF2, singility on Issue and USA; 17, 2000, College Stellars, 18, 2000, College Stellars, 18, 2000, College Stellars, 18, 2000, College Stellars, 17, 27, 284, USA. Department of Wellongia Sciences and Stellars, 18, 2010, College Stellars, 18, 2010, College
- yihui_zhou@ncsu.edu (Y-H.Z.); fred_wright@ncsu.edu (F.A.W.)
 Bioinformatics Research Center. North Carolina State University. Raleigh. NC 27695. USA; pigalli2@ncsu.edu Correspondence: irus/n@tamu.edu; Tel.: +979-458-9866

Abstract: Human cell-based population-wide in vitro models have been proposed as a strategy to derive chemical-specific estimates of inter-individual variability; however, the utility of this approach has not yet been tested for cumulative exposures in mixtures. This study aimed to test defined mixtures and their individual components and determine whether adverse effects of the mixtures were likely to be more variable in a population than those of the individual chemicals. The in vitro model comprised 146 human lymphoblastoid cell lines from four diverse subpopulations of European classes of environmental pollutants: in addition, eight defined mixtures were prepared from these chemicals using several exposure- or hazard-based scenarios. Points of departure for cytotoxicity were derived using Bayesian concentration-response modeling and population variability was quantified in the form of a toxicodynamic variability factor (TDVF). We found that 28 chemicals and all mixtures exhibited concentration-response cytotoxicity, enabling calculation of the TDVF. The median TDVF across test substances, for both individual chemicals or defined mixtures, ranged from a default assumption $(10^{1/2})$ of toxicodynamic variability in human population to >10. The data also provide a proof of principle for single-variant genome-wide association mapping for toxicity of the chemicals current sample size. This study demonstrates the feasibility of using a set of human lymphoblastoid cell lines as an in vitro model to quantify the extent of inter-individual variability in hazardous properties of both individual chemicals and mixtures. The data show that population variability of the mixtures is unlikely to exceed that of the most variable component, and that similarity in genomewide associations among components may be used to accrue additional evidence for grouping of

Keywords: population-wide; inter-individual variability; toxicodynamics; chemical mixtures; defined ixtures; human health risk assessment; uncertainty factors; genome-wide association study

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Humans are exposed to a wide variety of chemicals from both dietary and non-dietary arms arms is an open access arms:

distributed under the terms and sources; therefore, developing approaches for the risk assessment of combined exposures to multiple agents is the pressing challenge in regulatory science [1]. Evaluation of mixtures

Rapid hazard screening after disaster events that may involve re-distribution of chemicals



Chen et al 2021

Risk Characterization of Environmental Samples Using In Vitro Bioactivity and Polycyclic Aromatic Hydrocarbon Concentrations Data

Zunwei Chan and Dillon I loud \$5 vi. Hui Thou \$5 Wai Chen et al 2021

Potential Human Health Hazard of Post-Hurricane Harvey Interdisciplis Sediments in Galveston Bay and Houston Ship Channel: A Veterinary M Case Study of Using In Vitro Bioactivity Data to Inform Risk University, R Management Decisions

Zunwei Chen ¹0, Suji Jang ¹, James M. Kaihatu ², Yi-Hui Zhou ³0, Fred A. Wright ³, Weihsueh A. Chiu ¹0

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Data to Inform Risk Management
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sessment; risk Hadib 2021, 15, 13378. https:// doi.org/10.3390/ijerph18241337

plex mixtures c man health and cynthia V. Rider O The Author(s) 20 Reserved. 20 October 2021 All rights reserved. Accepted: 16 December 2021

Published: 19 December 2021

This article is an open access article Attribution (CC BY) liornse (https:// (GB/HSC) areas after hurricane Harvey, a disaster event that led to broad redistribution of chemically contaminated sediments, including deposition of the sediment on shore due to flooding. Samples were extracted with cyclohexane and dimethyl sulfoxide and screened in a compendium of humaprimary or induced pluripotent stem cell (iPSC)-derived cell lines from different tissues (hepatocytes neuronal, cardiomyocytes, and endothelial) to test for concentration-dependent effects on various functional and cytotoxicity phenotypes (n = 34). Bioactivity data were used to map areas of potentia concern and the results compared to the data on concentrations of polycyclic aromatic hydrocarbon (PAHs) in the same samples. We found that setting remediation goals based on reducing bioactivit is protective of both "known" risks associated with PAHs and "unknown" risks associated with bioactivity, but the converse was not true for remediation based on PAH risks alone. Overall, we an example of a new approach method (NAM) to inform risk management decisions on site cleanua

contaminants in the environment; however, current methods for assessing hazards and risks of

complex mixtures are not suitable for disaster response. This study investigated the suitability of

in vitro toxicity testing methods as a rapid means of identifying areas of potential human health

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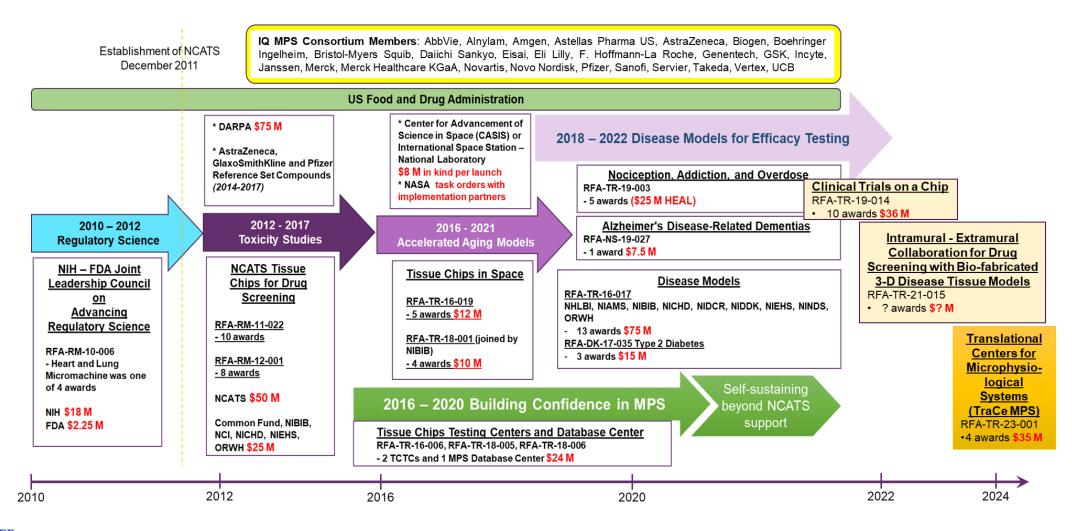
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Keywords: new approach methods (NAMs); environmental mixtures; disaster research

Natural disasters such as floods and hurricanes can lead to severe damage to urban ized estuarine environments and pose potential environmental and public health risks due to the re-distribution of chemical contaminants [1]. These challenges are especially acute in areas with known legacy contaminations whereby natural and anthropogenic disasters may alter the contamination patterns and change potential hazards in unpredictable ways One recent example of such an event is Hurricane Harvey (2017), which resulted in extrem flooding in the Houston/Galveston Bay region, a heavily industrialized area on the shore of the Galveston Bay and the Gulf of Mexico. The sediments in the Galveston Bay are known to be contaminated by various types of hazardous chemicals including polycyclic aromatic hydrocarbons (PAHs), polycyclic biphenyls (PCBs), pesticides, and heavy metals [2]. Indeed, recent studies indicated that post-Harvey, pollutants such as PAHs were

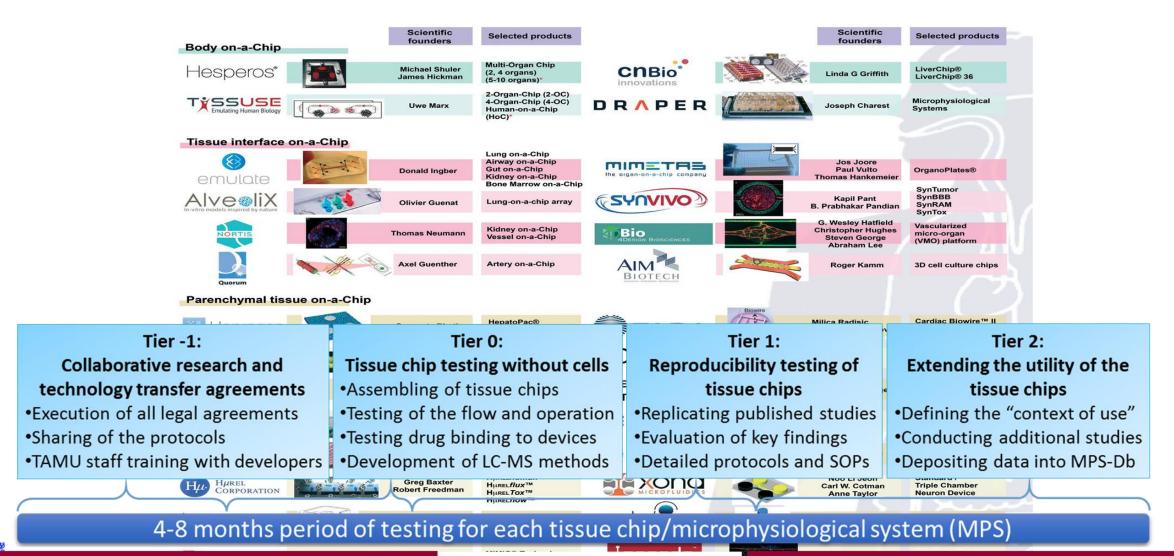


And That is Without Factoring the MPS/Organ-on-Chip Possibilities!





Why Not Spin-Off Your Own Organ-on-Chip Company (NIH Supported):



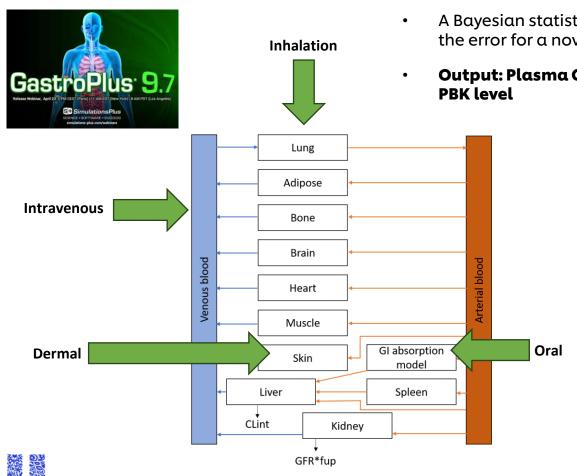
TEX-VAL





PBK Modelling in NGRA – 'Black Box' or Fully Understood?

Physiologically based kinetic (PBK) models are used to simulate the behaviour of a chemical in the body for a given exposure scenario



 A Bayesian statistical model to quantifies the error for a novel chemical

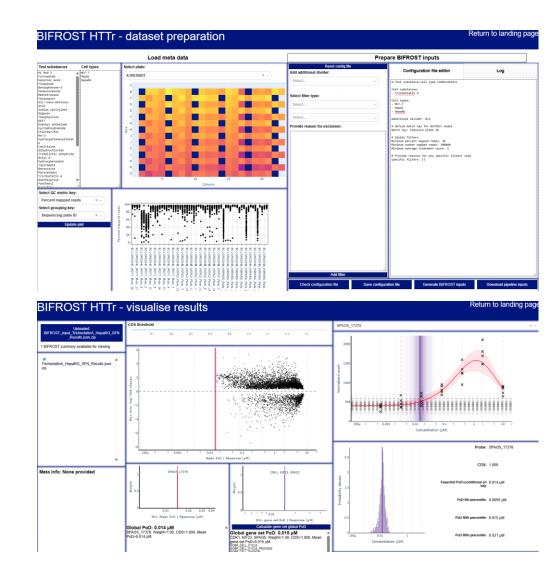
Output: Plasma C_{max} distribution at each PBK level

- PBK models are composed of multiple coupled ordinary differential equations.
- The model have various parameters that need to be determined.
- Example equation for determining the concentration of chemical in the liver:

$$V_{\text{Liver}} \frac{dC_{\text{Liver}}}{dt} = Q_{\text{Liver}} \left(C_{\text{Arterial}} - \frac{C_{\text{Liver}}}{P_{\text{Liver}}} \right) - CLint \left(\frac{C_{\text{Liver}}}{P_{\text{Liver}}} * Fup \right)$$
[Concentration of chemical in the arterial blood] [Concentration of chemical in the liver] [Concentration of chemical in the liver]

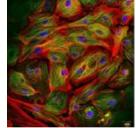
Defined Approaches for Complex Data Integration: POD in HTTr

- High throughput transcriptomic (HTTr) studies provide high dimensional datasets for the bioactivity of a chemical
- Downstream results are highly sensitive to a plethora of decisions around experimental design, data normalisation, modelling choices, summary statistics and more
- For robust inferences, it is critical that the consequence of each decision, as well as the consequence of having made an alternative choice, is understood

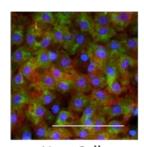




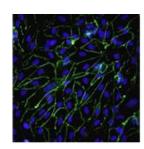
Put Together iPSC of Multiple Lineages, into MPS/OoC, Treat with 'Toxins', Determine POD from HTTr (and other NAMs) by Bayesian Statisics and Compare to Exposure Estimated by Computational PBK with Population Variability... Oh, and VALIDATE THAT!







Liver Cells



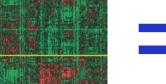
Endothelial Cells

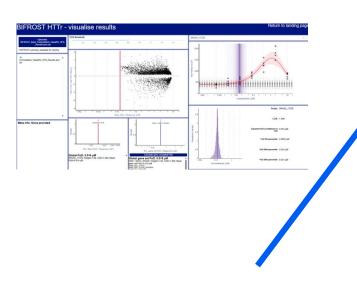


Adipose

GI absorption







And let Al make the decision on: Safe or Not-Safe



Reproducible? The Regulators Replication Validation Dilemma

News in focus

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be surprised i iles are already

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But the idea of

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NEWS FEATURE 05 December 2023

nature > news feature > article

Is Al leading to a reproducibility crisis in science?

Scientists worry that ill-informed use of artificial intelligence is driving a deluge of unreliable or useless research.

Drug design

Last year, for instance, Gitter's team releases protein targets³. The designs haven't been lab-tested, but computational 'docking' tools widely used in drug discovery suggested that

Scientists are also using bio-Als to 'talk' to cells. Efforts to sequence all the RNA molecules in individual cells have become a bedrock technique in cell biology, revealing unappreciated diversity. But making sense of (Al) tools, researcher's have warned.

In a study published in PLoS Biology to statistical one tween biologists and biometicines collaboration between biologists and biometicines collaboration biometicines collaboration biometicines collaboration between biologists and biometicines collaboration biometicines collaboration between biologists and biometicines collaboration between biometicines collaboration between biologists and biometicines collaboration between biometicines collaboration biometicines collaboration biometicines collaboration between biometicines collaboration between biometicines collaboration biometicines collaboration between biometicines collaboration biometicines collaboration between biometicines collaboration biometicines collaboration between biometicines collaboration biometici

bot called CellWhisperer*. It can take plain English instructions – 'describe these cells in English instructions – 'describe the English instructions – '

AI LINKED TO EXPLOSION IN LOW-QUALITY **BIOMEDICAL PAPERS**

a model that designs small molecules in response to text prompts, and showed that it could design drug-like inhibitors of known it could design drug-like inhibitors of known

he scientific literature is at risk of rates [of papers] that reextremely formulaic to process using artificial intelligence of Surrey in Guildford, UK.

data scientists, says Christoph Bock, a com- 300 papers that used data from the US seemed to have cherry-picked data. tion Survey (NHANES), an open data set of health records (T. Suchak et al. PLoS Biol. 23, questions as you want. You see which ones you

be coming flooded with papers that
that could easily have been generated by large
make misleading health claims based
on openly available data that are easy
Spick, a biomedical scientist at the University



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News Feature | Published: 25 May 2016

1,500 scientists lift the lid on reproducibility

Monya Baker

Nature 533, 452–454 (2016) Cite this article

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'Publish or perish' culture blamed for reproducibility crisis

Survey of more than 1,600 biomedical researchers also flagged small sample sizes and cherry-picking of data as leading causes of reproducibility problems.

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'Doing good science is hard': retraction of high-profile reproducibility study prompts soul-searching

A paper by some of the biggest names in scientific integrity is retracted for issues including misstatements about the research plan.



Are We Even Scratching the Surface of the Wealth of Data in Transcriptomics?





Toxicological Sciences, 2025, 205(2), 310-325

https://doi.org/10.1093/toxsci/kfaf036 Advance Access Publication Date: March 20, 2025 Research article



A workflow for human health hazard evaluation using transcriptomic data and Key Characteristics-based gene sets

Han-Hsuan D. Tsai (1,2 , King D. Oware (3 , Fred A. Wright^{1,4,5}, Weihsueh A. Chiu (1,2 , Ivan Rusyn^{1,2,*}

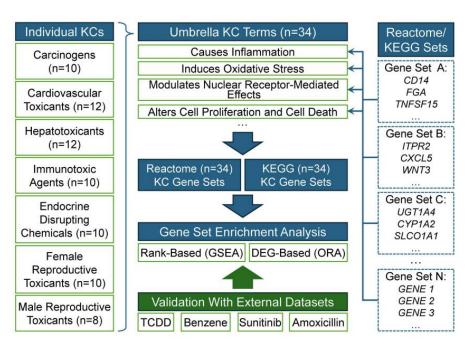




Fig. 1. Overview of the study workflow. Abbreviations: KC, key characteristic; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, gene set enrichment analysis; DEG, differential expressed gene; ORA, over-representation analysis; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

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S AND CHROA

New Collaboration Between Unilever and CMU for 2026+





Jingbo Pl



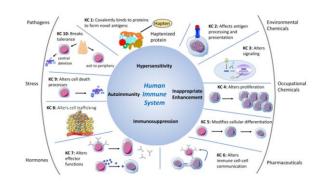
THE KEY CHARACTERISTICS OF HUMAN CARCINOGENS 10. Alters Cell Proliferation, Cell Death, or Nutrient Supply 改变细胞增殖。死亡或营养需求 3 发布死化 9. Causes Immortalization Human Carcinogens 次变DNA修复或遗传稳定性 3. Alters DNA Repair or Genomic Instability Proposition of Genomic Instability 1. Is Immunosuppressive 1 发表观遗传改变 4. Induces Epigenetic Alterations 1 发生化应激 5 Induces Oxidative Stress 6. Induces Oxidative Stress

Guyton KZ, et al. Chem Res Toxicol. 2018; 31: 1290-1292.

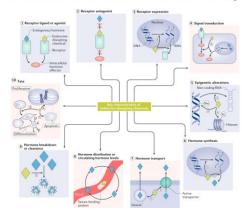
Outline

- CMU-Unilever Workshop on NAMs-based NGRA
- Understanding from IARC Monographs
- ➤ Key characteristics (KCs) vs. AOPs
- > A vision for future: KCs-structured NAMs-based NGRA?

KCs associated with immunotoxicants



KCs associated with endocrine disruptors



lature Reviews Endocrinology (Nat Rev Endocrinol) ISSN 1759-







A Decade of Progress for TATT with NAMs/NGRA/TT21C





