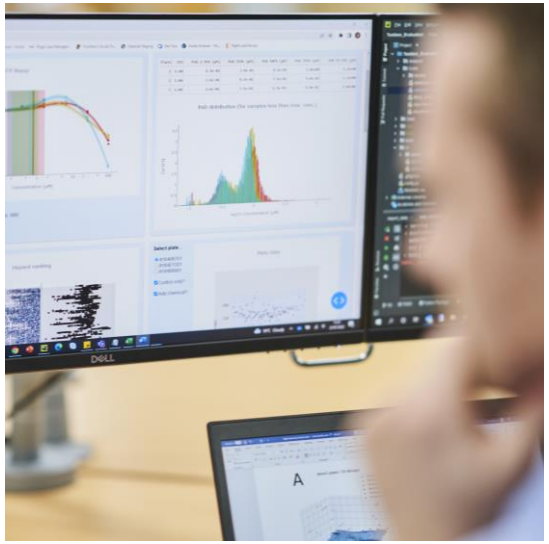
Practical Application of New Approach Methods in Developmental and Reproductive Toxicity Testing

Dr Predrag Kukic
Unilever Safety and Environmental Assurance Centre

Theme: NAMs as Problem-Solvers
23th October 2023



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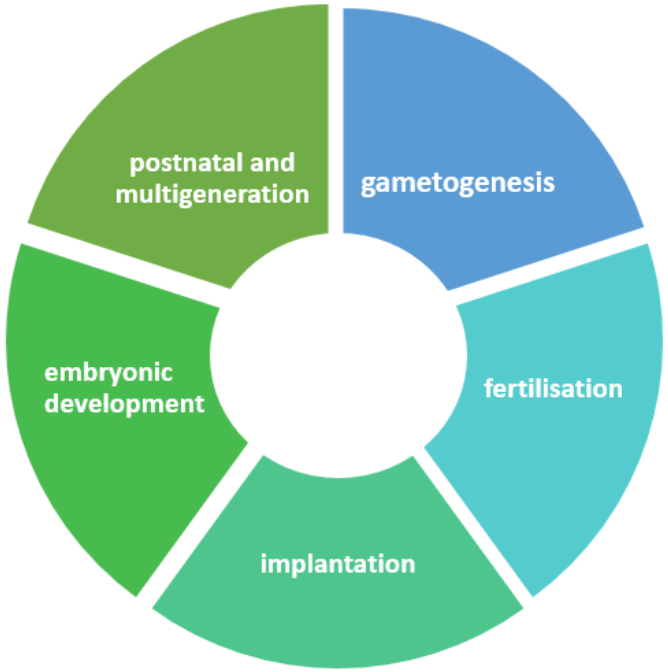
Outline

- **Overview of Unilever's NGRA Framework for DART testing**
- **Biological relevance of the NGRA Framework for DART testin**
- **Case studies / fit for purpose validation, next steps**



DART endpoint

Unilever Policy & Approach

Safe & Sustainable Products without Animal Testing

What we believe

- **Every Unilever product must be safe for people and our environment**
- Non-animal testing to assess ingredient & product safety – there are a wide range of non-animal alternatives grounded in modern science and new technology

How we do it

40+ years of developing non-animal safety science

70+ collaborations

600+ publications

seac.unilever.com



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A paradigm shift is underway as use of non-animal safety science increases & safety assessment frameworks evolve to embed NAMs

- Non-animal safety science is increasingly being used to make decisions on **consumer safety**, **safety of workers**, and safety of **people and non-human species** in the **environment**.

Regulatory Animal Testing of Chemicals is increasingly seen as unjustifiable / unethical by the majority of society

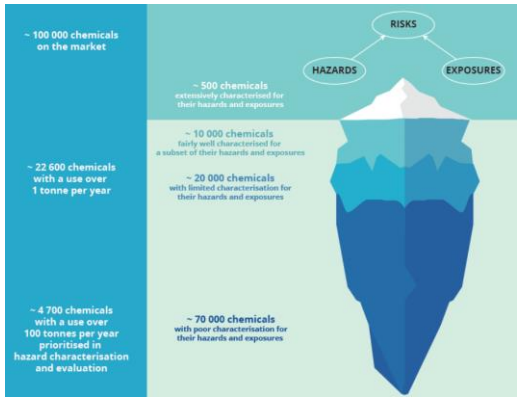
Aug 2021 – Aug 2022:
1.4M+ signatures

European Union
EUROPEAN CITIZENS' INITIATIVE

Save Cruelty Free Cosmetics

NAMs to fully replace the need for chemical regulatory animal testing

High throughput – more testing before the chemical is put on the market, data reuse, etc.



Potential to address information requirements for all substances in the market

Move to more sustainable sources of chemicals (e.g. bio-based) is transforming chemical innovation & use

Potential to ensure new chemicals are Safe & Sustainable by Design

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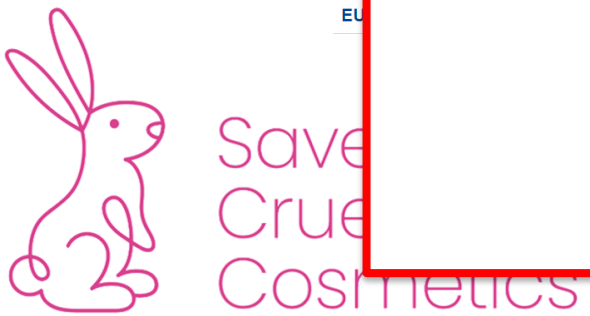

NAMs to fully replace the need for chemical regulatory animal testing

High throughput – more testing before the chemical is put on the market, data

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Move to more sustainable sources of chemicals (e.g. bio-based) is

ming chemical innovation & use

Potential to ensure new chemicals are Safe & Sustainable by Design

Human-relevant

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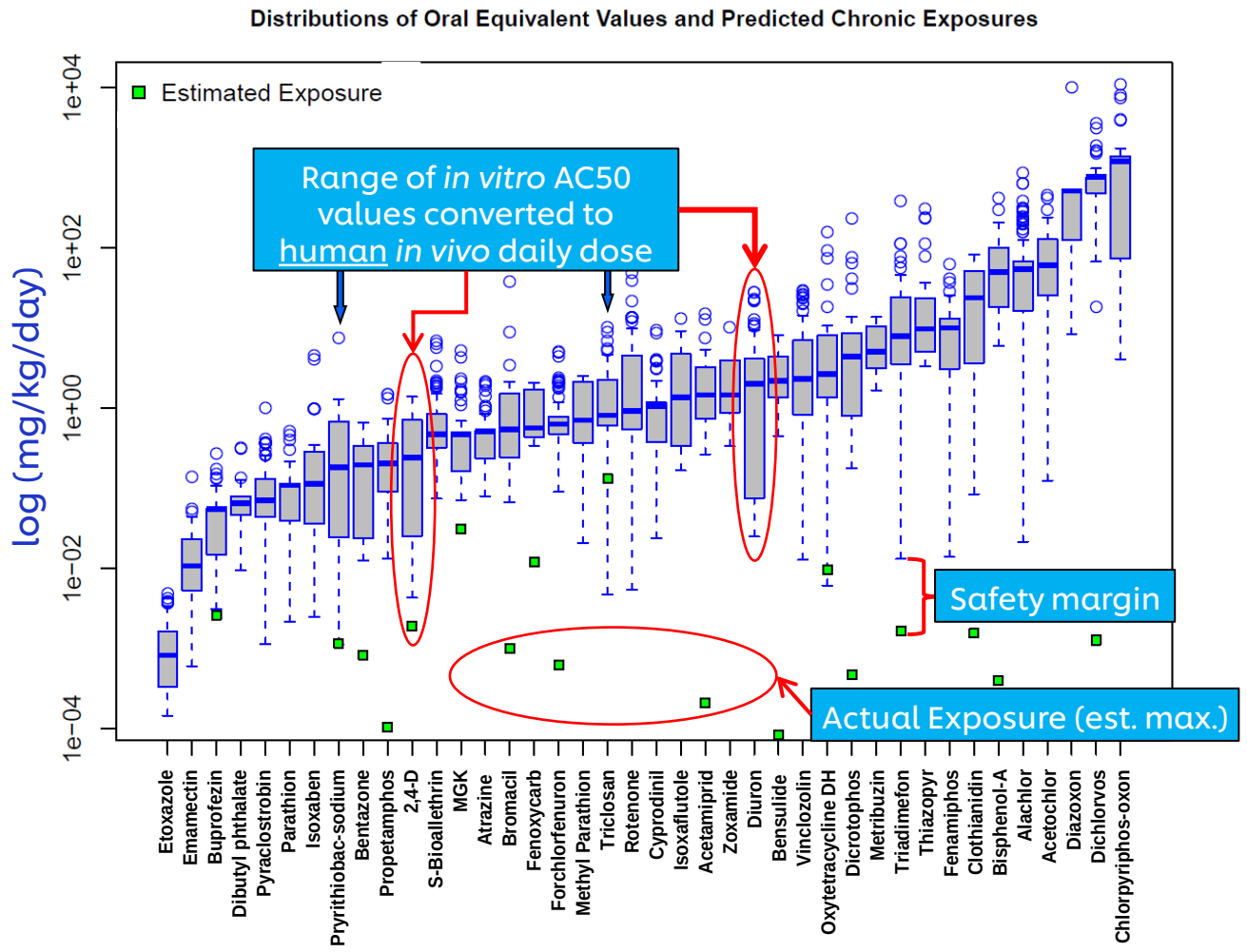
US EPA Next Generation Blueprint Tiered Testing Framework



Graph from Rusty Thomas EPA, with thanks. Rotroff *et al* (2010) Toxicological Sciences , **117**, 348-358

- NGRA is defined as **an exposure-led**, **hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure safety without the use of animal testing
- If there is **no** bioactivity observed at consumer-relevant concentrations, there can be **no adverse health effects.**
- If there **is** bioactivity observed at consumer-relevant concentrations, **follow up testing** is required to establish if that could result in an adverse effect
- At no point does NGRA attempt to predict the results of high dose toxicology studies in animals.

US EPA Next Generation Blueprint Tiered Testing Framework

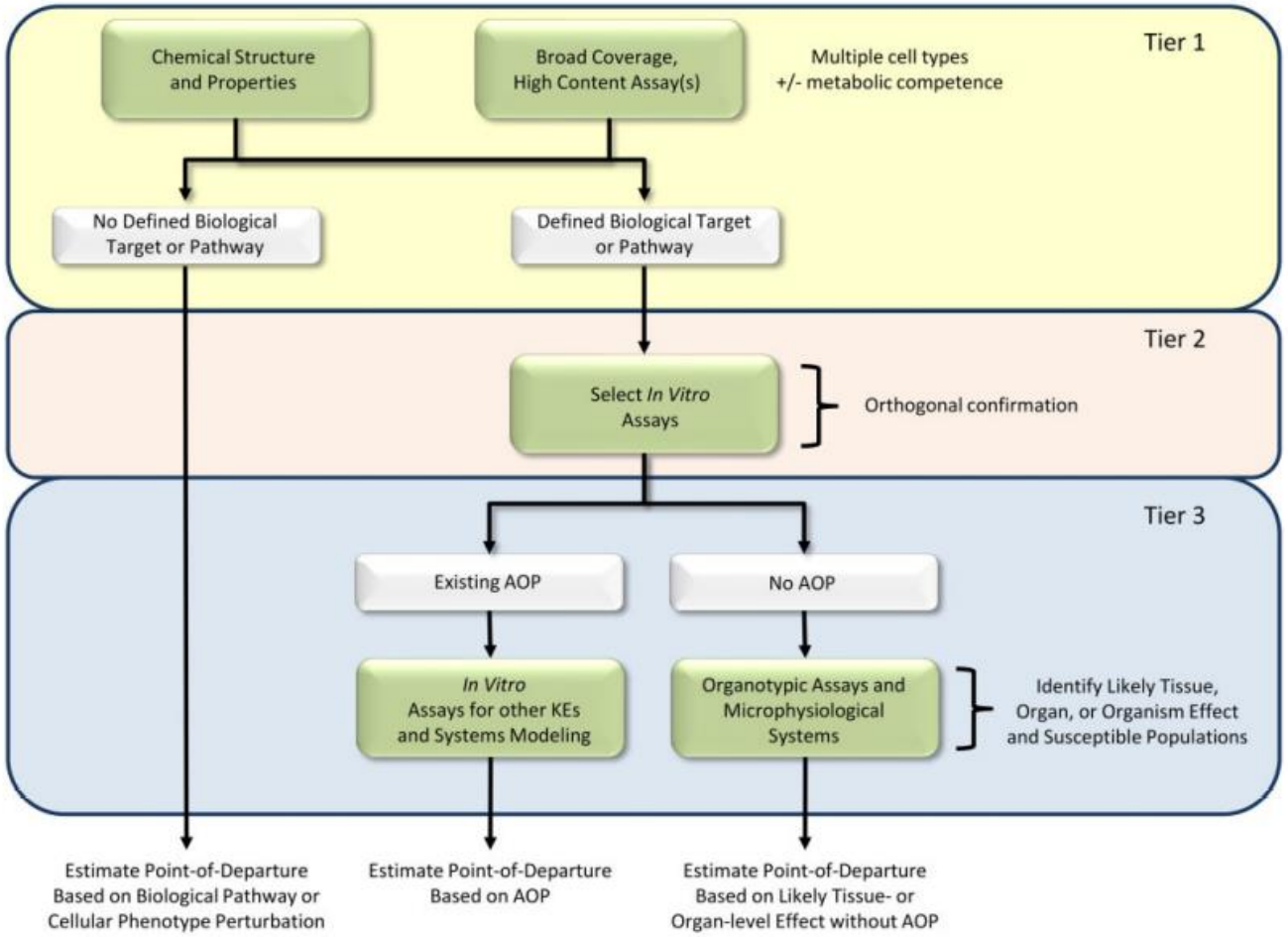


Figure 2. Tiered testing framework for hazard characterization. Tier 1 uses both chemical structure and broad coverage, high content assays across multiple cell types for comprehensively evaluating the potential effects of chemicals and grouping them based on similarity in potential hazards. For chemicals from Tier 1 without a defined biological target / pathway, a quantitative point-of-departure for hazard is estimated based on the absence of biological pathway or cellular phenotype perturbation. Chemicals from Tier 1 with a predicted biological target or pathway are evaluated Tier 2 using targeted follow-up assays. In Tier 3, the likely tissue, organ, or organism-level effects are considered based on either existing adverse outcome pathways (AOP) or more complex culture systems. Quantitative points-of-departure for hazard are estimated based on the AOP or responses in the complex culture system.

EPA United States Environmental Protection Agency

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 169(2), 2019, 317–332
doi: 10.1093/toxsci/kfz058
Advance Access Publication Date: March 5, 2019
Forum

FORUM

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,‡ Jeffrey B. Frithsen,§ Christopher M. Grulke,* Maureen R. Gwinn,* Joshua A. Harrill,* Mark Higuchi,¶ Keith A. Houck,* Michael F. Hughes,¶ E. Sidney Hunter, III,¶ Kristin K. Isaacs,‡ Richard S. Judson,* Thomas B. Knudsen,* Jason C. Lambert,‖ Monica Linnenbrink,* Todd M. Martin,‖ Seth R. Newton,‡ Stephanie Padilla,¶ Grace Patlewicz,* Katie Paul-Friedman,* Katherine A. Phillips,‡ Ann M. Richard,* Reeder Sams,* Timothy J. Shafer,¶ R. Woodrow Setzer,* Imran Shah,* Jane E. Simmons,¶ Steven O. Simmons,* Amar Singh,* Jon R. Sobus,‡ Mark Strynar,‡ Adam Swank,‡ Rogelio Tornero-Valez,‡ Elin M. Ulrich,‡ Daniel L. Villeneuve,‖ John F. Wambaugh,* Barbara A. Wetmore,‡ and Antony J. Williams*

*National Center for Computational Toxicology, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, †National Center for Environmental Assessment, U.S. Environmental Protection Agnecy, Washington, D.C. 20004, ‡National Exposure Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, §Chemical Safety for Sustainability National Research Program, U.S. Environmental Protection Agency, Washington, D.C. 20004, ¶National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, ‖National Center for Environmental Assessment, U.S. Environmental Protection Agency, Cincinnati, OH 45220,

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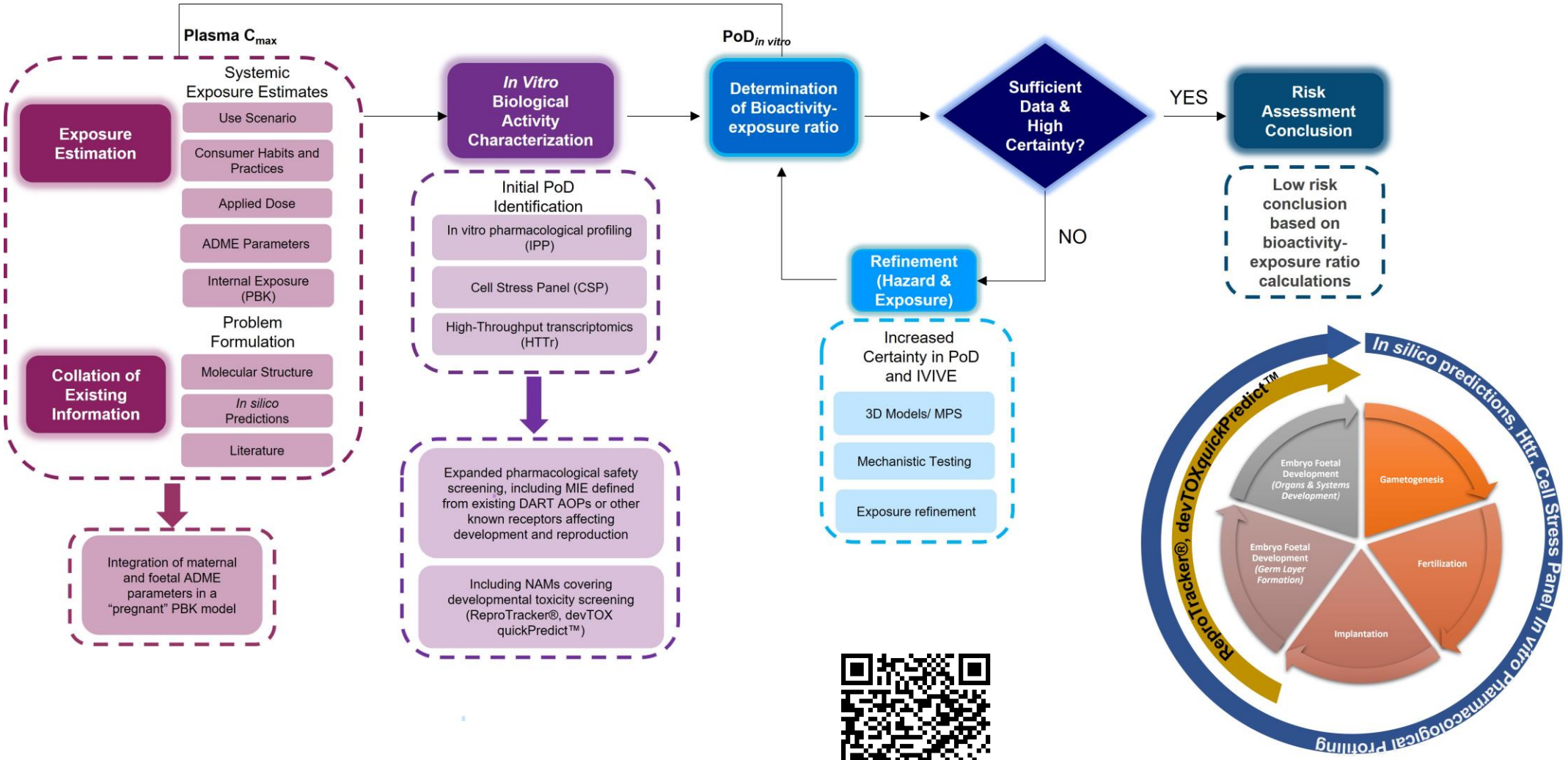
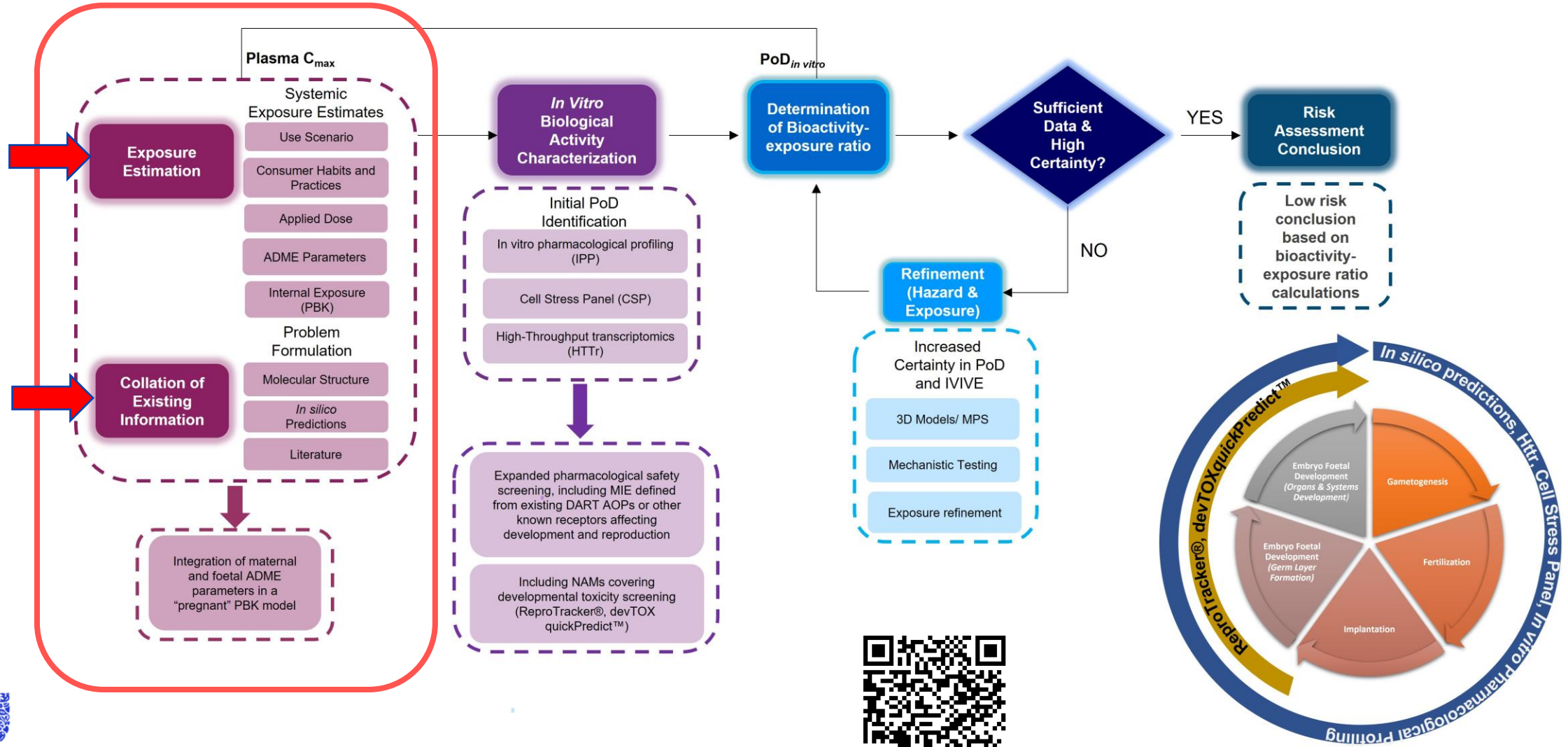
NGRA Framework for DART – tiered approach



Rajagopal et al., Front. Toxicol., 07 March 2022 https://doi.org/10.3389/ftox.2022.838466

NGRA Framework for DART – exposure module



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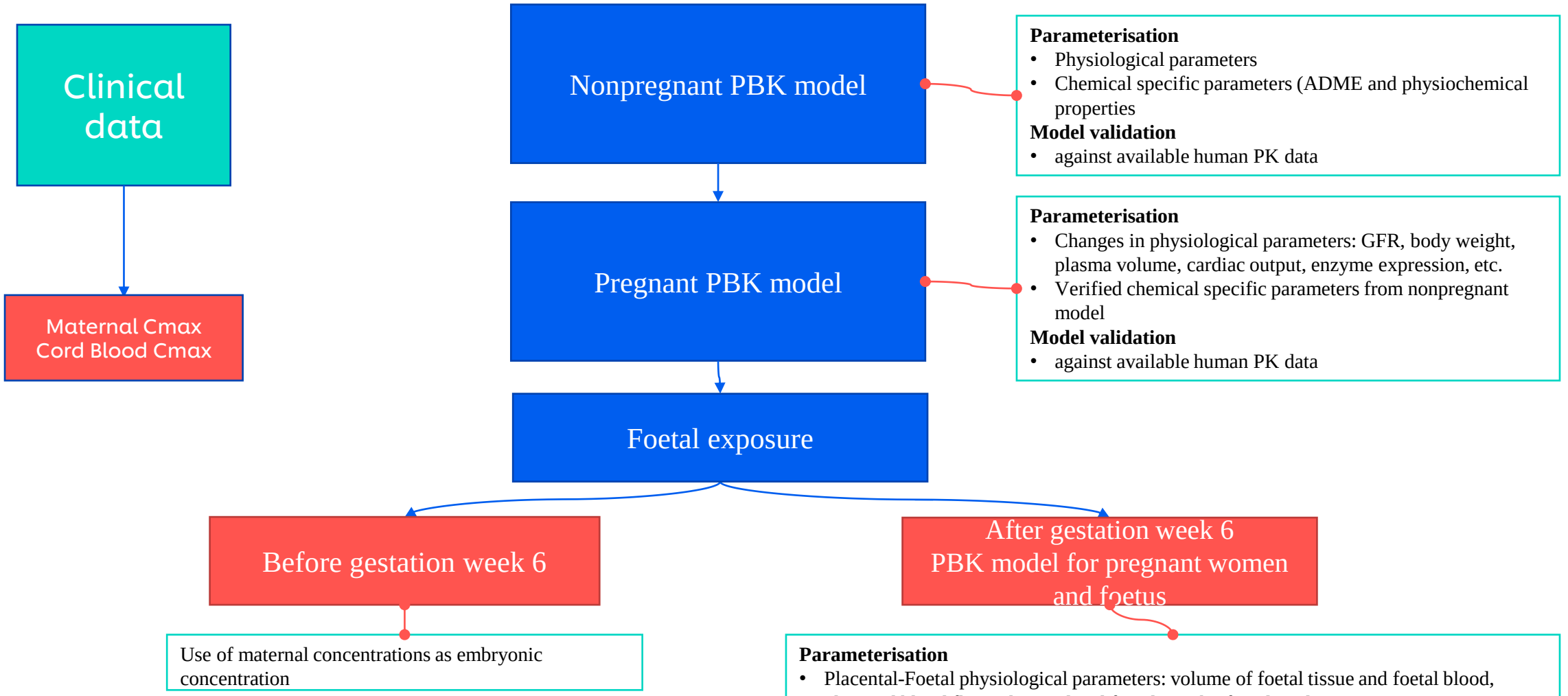
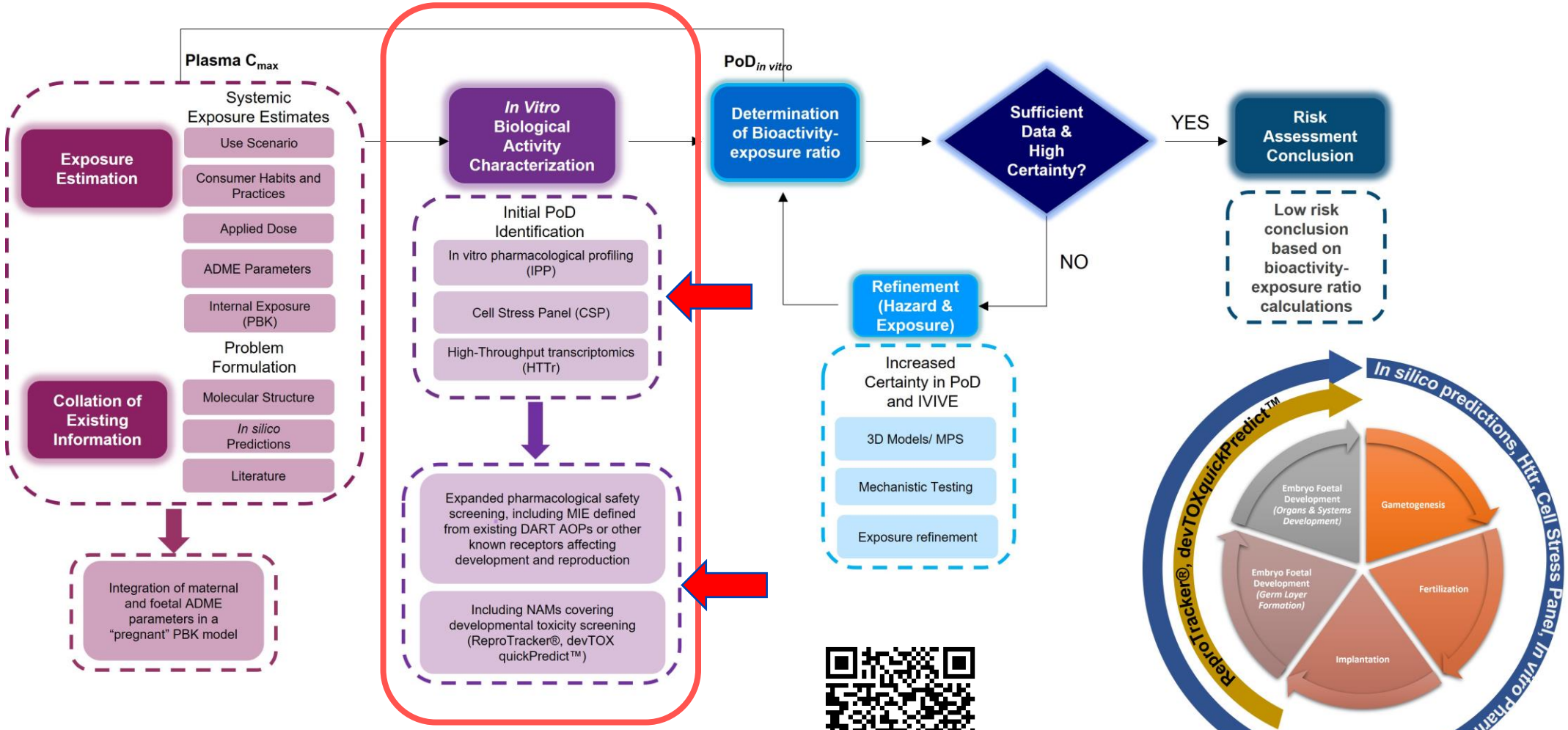
NGRA Framework for DART – exposure module



Rajagopal et al., Front. Toxicol., 07 March 2022
https://doi.org/10.3389/ftox.2022.838466

NGRA Framework for DART – bioactivity module



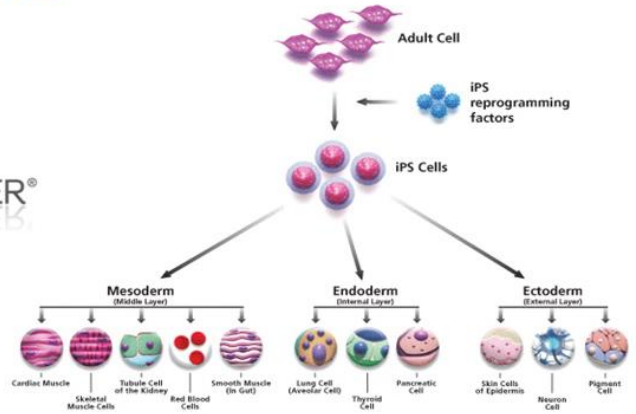
Rajagopal et al., Front. Toxicol., 07 March 2022 https://doi.org/10.3389/ftox.2022.838466

NGRA Framework for DART – bioactivity module

iPSC based tools

devTOX qP quickPREDICT

REPROTRACKER®

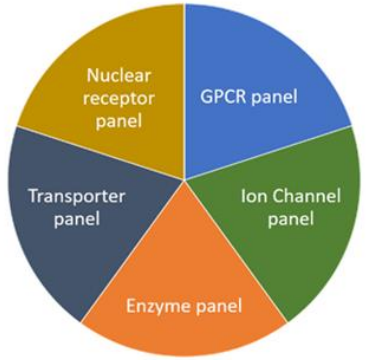


Toxicology in Vitro (2020), **63**, 104746

In vitro Pharmacological Profiling (IPP)


PERSPECTIVES

Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

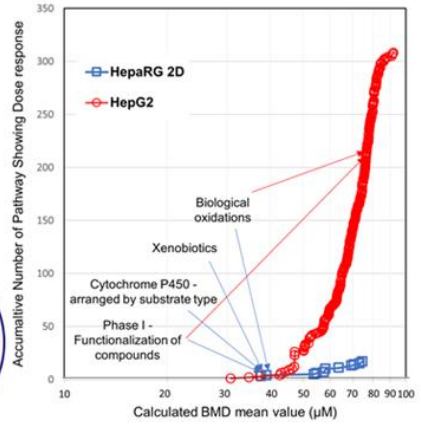


eurofins | Cerep

High-throughput Transcriptomics (HTTr)

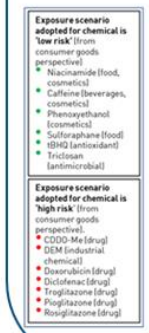
- Use of full human gene panel ~ 21k
- 24 hrs exposure
- 7 concentrations
- 3 cell lines HepG2/ HepaRG/ MCF7
- 3D HepaRG spheroid

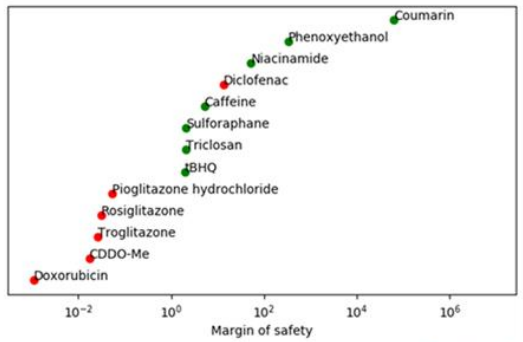
BMDExpress 2



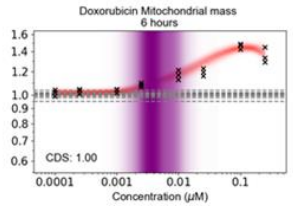
Cell Stress Panel (CSP)

36 Biomarkers, 3 Timepoints, 8 Concentrations – 10 Stress Pathways





cyprotex AN EVOTEC COMPANY



Toxicol Sci (2020), **176**, 11-33

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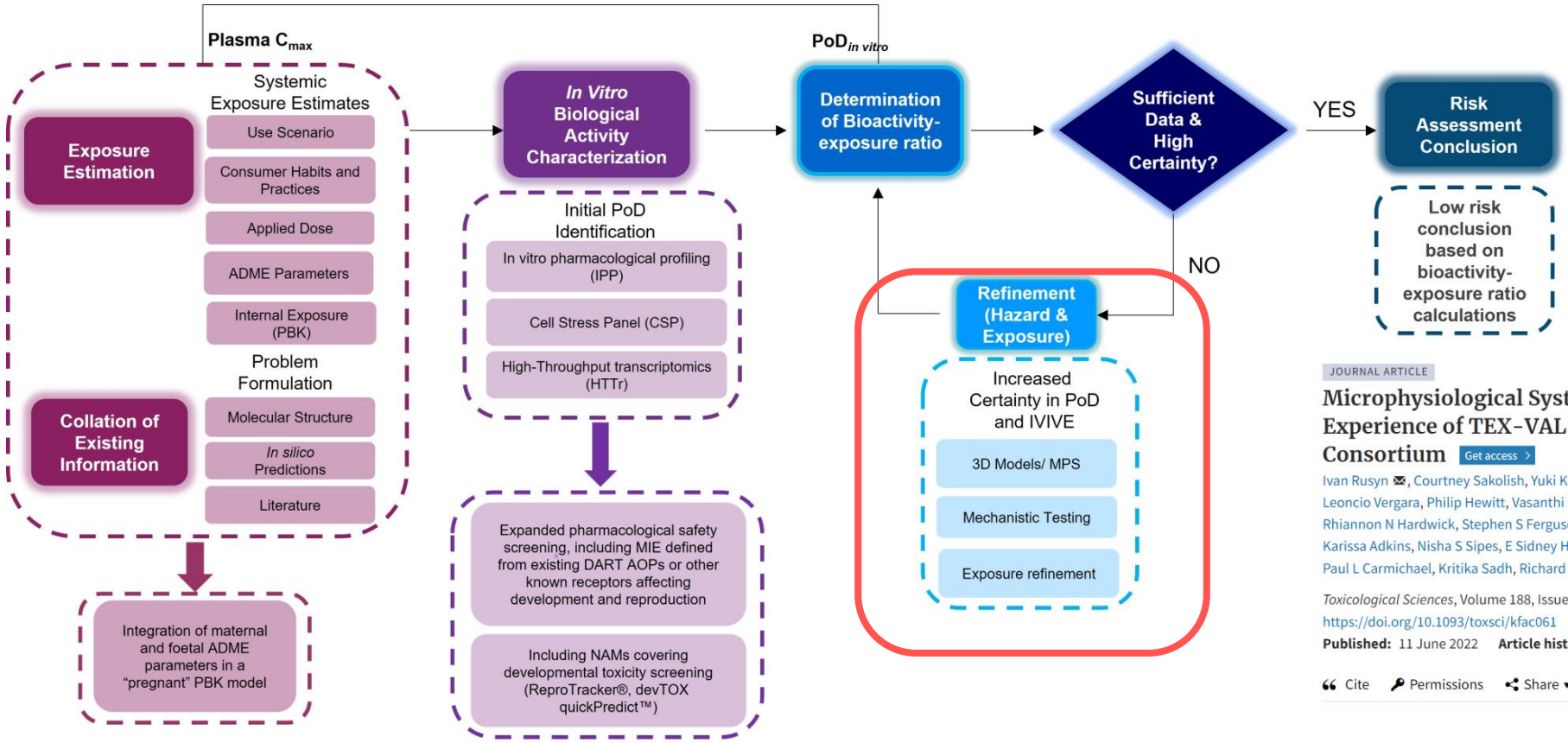
Refinement of Biological Activity and Exposure



- **Tex-Val: public-private collaboration established for testing of diverse microphysiological system**
- **Use of metabolically competent models (cell lines, alginate immobilization, etc)**

JOURNAL ARTICLE

Microphysiological Systems Evaluation: Experience of TEX-VAL Tissue Chip Testing Consortium Get access

Ivan Rusyn, Courtney Sakolish, Yuki Kato, Clifford Stephan, Leoncio Vergara, Philip Hewitt, Vasanthi Bhaskaran, Myrtle Davis, Rhiannon N Hardwick, Stephen S Ferguson, Jason P Stanko, Piyush Bajaj, Karissa Adkins, Nisha S Sipes, E Sidney Hunter, 3rd, Maria T Baltazar, Paul L Carmichael, Kritika Sadh, Richard A Becker

Toxicological Sciences, Volume 188, Issue 2, August 2022, Pages 143–152, https://doi.org/10.1093/toxsci/kfac061

Published: 11 June 2022 **Article history**

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JOURNAL ARTICLE FEATURED

The Alginate Immobilization of Metabolic Enzymes Platform Retrofits an Estrogen Receptor Transactivation Assay With Metabolic Competence FREE

Chad Deisenroth, Danica E DeGroot, Todd Zurlinden, Andrew Eicher, James McCord, Mi-Young Lee, Paul Carmichael, Russell S Thomas

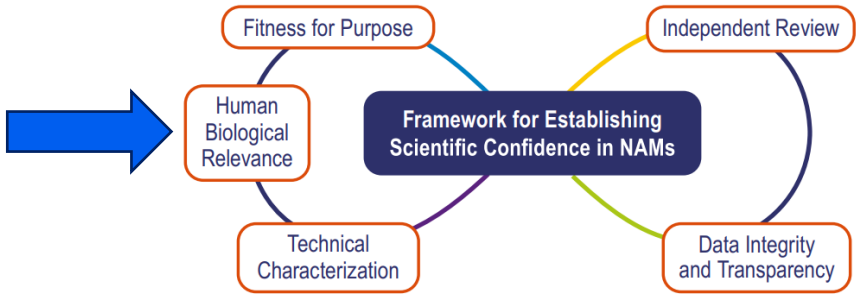
Toxicological Sciences, Volume 178, Issue 2, December 2020, Pages 281–301, https://doi.org/10.1093/toxsci/kfaa147

Published: 29 September 2020

NGRA Framework for DART – Scientific and Technical challenges

- Metabolic capacity of the framework (cell models, MPS, alginate technology, etc.)
- Spatio-temporal complexity of developmental and reproductive processes
- Short duration exposures and extrapolation to chronic effects
- Ability to generate reliable and consistent reproducible results (HTTr, cell line variability, cell stress, IPP, reprotracker)
- Complex data interpretation and uncertainty analysis
- Coverage of important cellular and intercellular processes – biological relevance
- Chemical domain of applicability / case studies – need for a flexible and fit for purpose validation

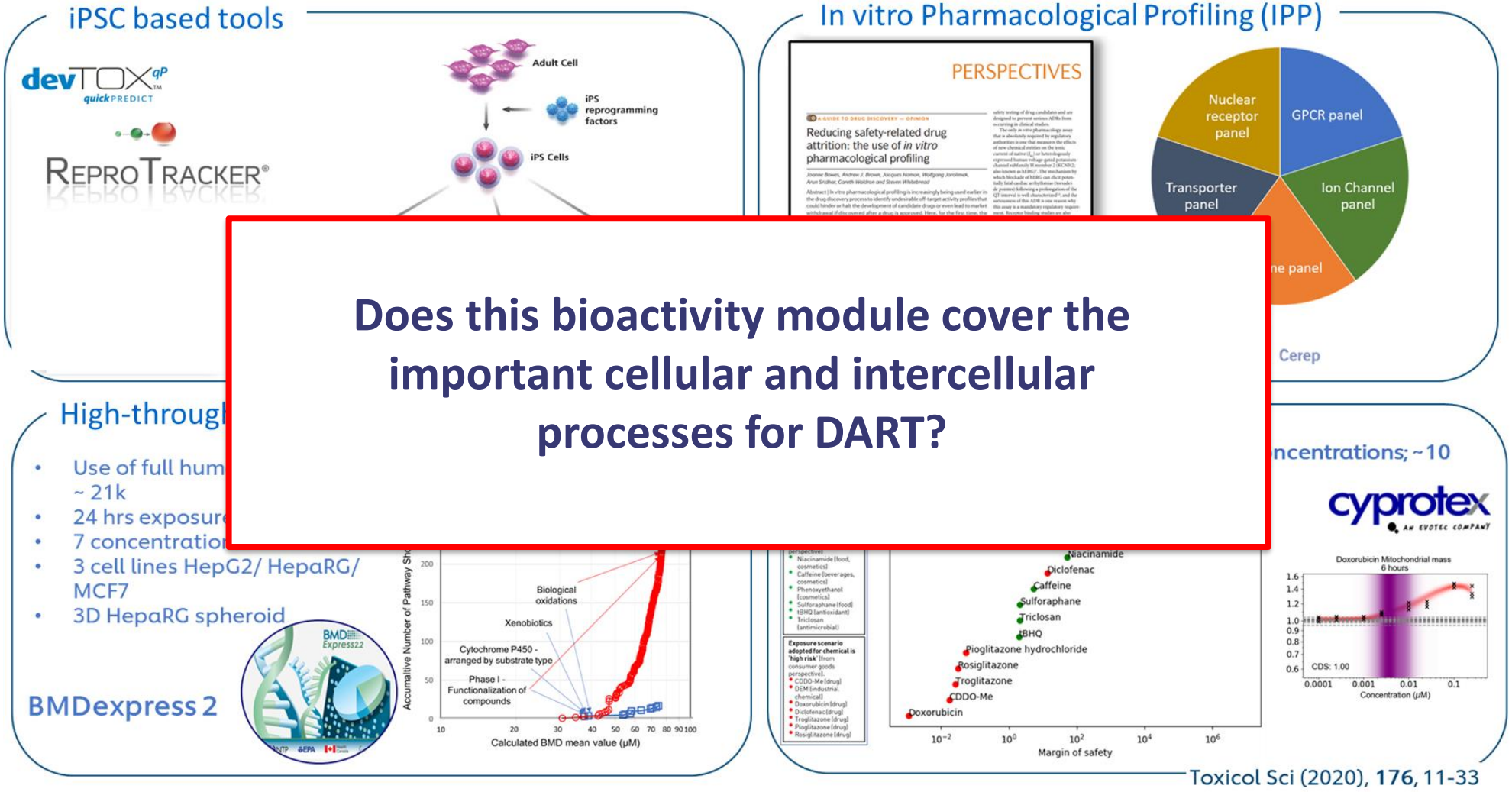
Biological relevance of the NGRA Framework for DART



van der Zalm et al. Archives of Toxicology (2022) 96:2865–2879

Coverage of important cellular and intercellular processes for DART

Does this bioactivity module cover the important cellular and intercellular processes for DART?



Toxicol Sci (2020), **176**, 11-33

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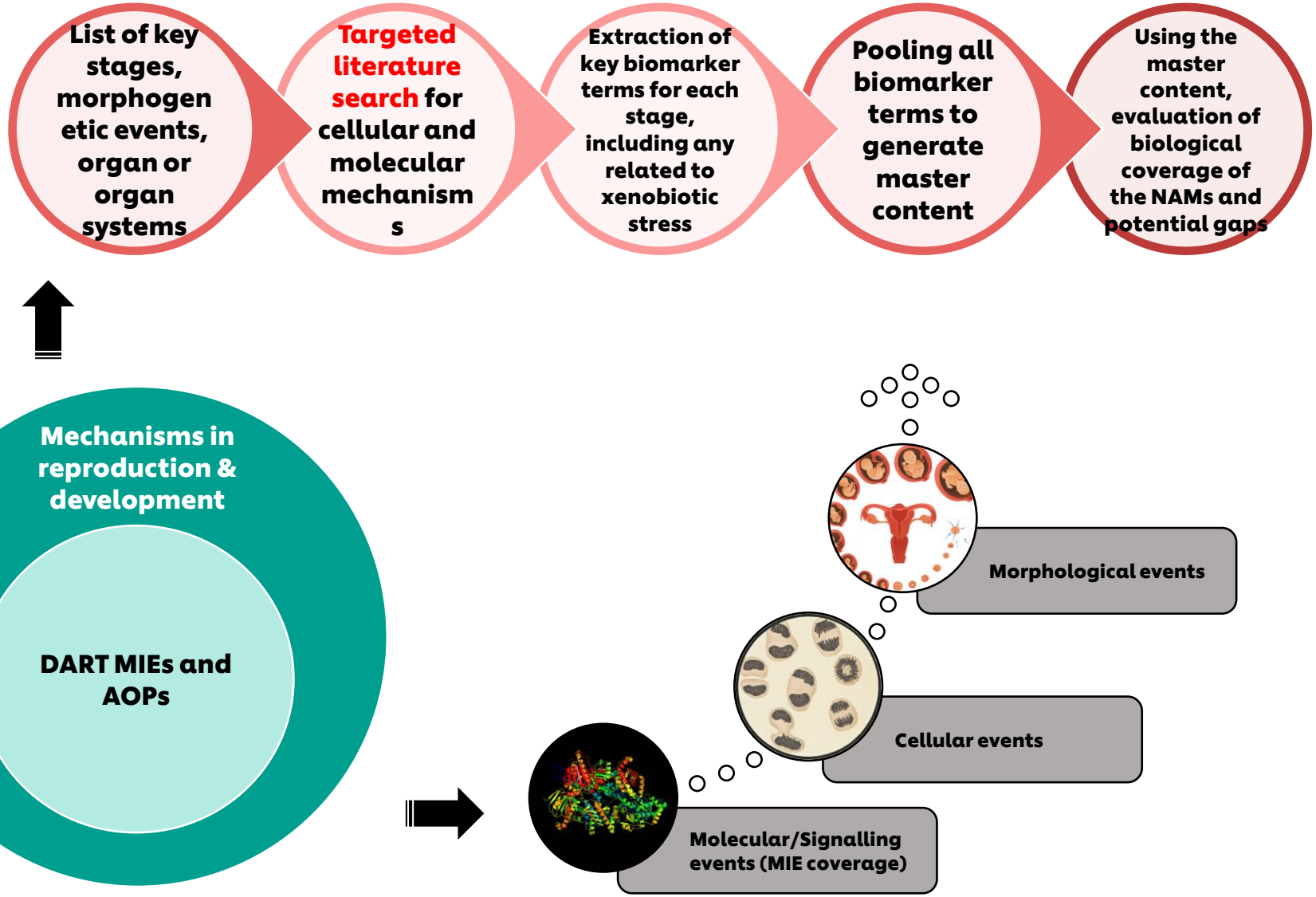
Key Biomarkers for DART - Systematic literature search

AOPs based approach

- 11 DART-related Adverse Outcome Pathways (AOPs)
- At present, a decision framework based only on AOPs is not feasible. **However, AOPs can used as a knowledge base for enhancing a testing strategy**



Key Stages, Morphogenetic Events and Derivatives Organs & Systems in Human Reproduction and Development

Sex determination

Gametogenesis

Fertilization

Zygote formation

Implantation

Blastulation

Gastrulation

Placenta formation

Neurulation

Ectoderm formation and its derivatives

- Central nervous system
- Peripheral nervous system
- Autonomous nervous system
- Integumentary system

Mesoderm formation and its derivatives

- Somitogenesis
- Hematopoiesis
- Heart and circulatory system
- Immune system
- Spleen
- Urinary system and urethra
- Reproductive system – testis
- Reproductive system – ovary
- Skeletal system
- Limbs

Endoderm formation and its derivatives

- Digestive system
- Respiratory system
- Thymus
- Parathyroid
- Thyroid

Structures developing from mesenchyme or multiple germ layers

- Adrenal glands
- Eyes
- Ears
- Face and neck

Intrauterine growth

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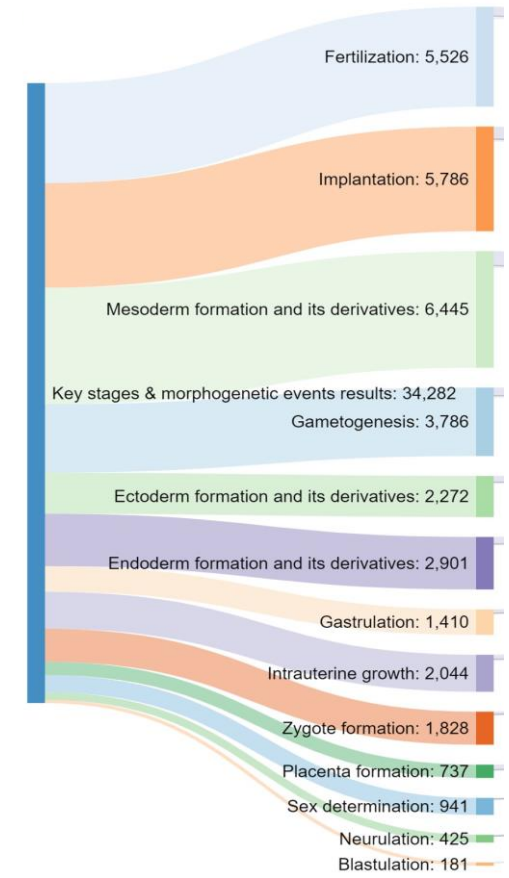
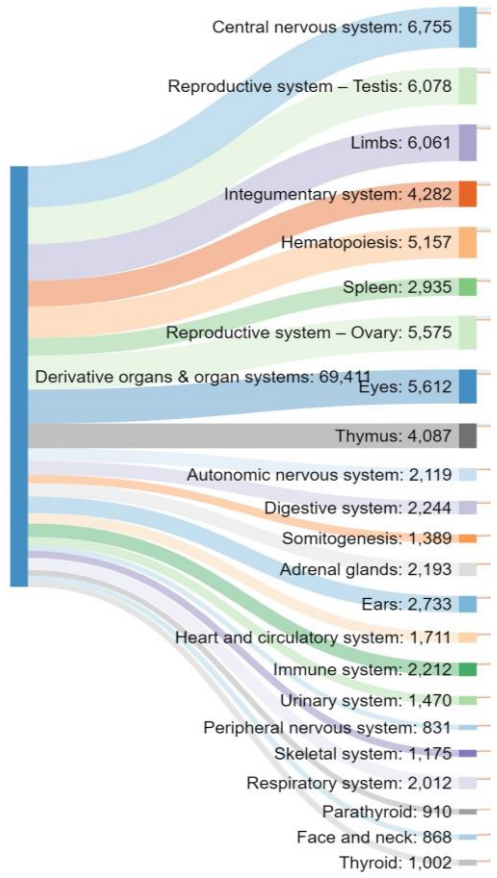
Overview of Literature Search and Extraction of Key Markers Information

Literature search
MeSH Ontology
37 million Articles

Validation and quality check of results; finalising the articles





Query run: ("CNS") AND (embryonic development OR fetal development) AND (cell physiology OR nervous system physiology) OR (signalling OR pathway OR gene OR protein) AND (human OR mammalian) NOT (infections)

34,308 articles on key stages and morphogenetic events

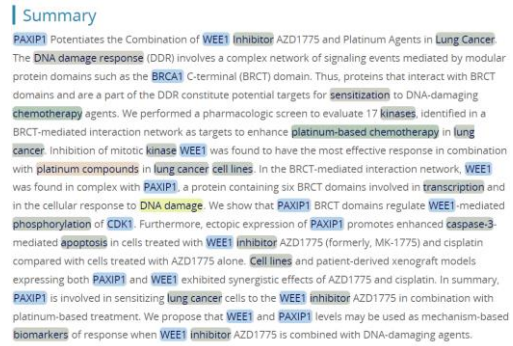
69,299 articles on organs and organ systems development

103,607 total articles

Abstracts extracted and collated

Semantic enrichment using HGNC, miRNA and biological processes ontologies

Pooling extractions, Thresholding of hit counts


Summary

PAXIP1 Potentiates the Combination of WEE1 Inhibitor AZD1775 and Platinum Agents in Lung Cancer
The DNA damage response (DDR) involves a complex network of signaling events mediated by modular protein domains such as the BRCA1 C-terminal (BRCT) domain. Thus, proteins that interact with BRCT domains and are a part of the DDR constitute potential targets for sensitization to DNA-damaging chemotherapy agents. We performed a pharmacologic screen to evaluate 17 kinases, identified in a BRCT-mediated interaction network as targets to enhance platinum-based chemotherapy in lung cancer. Inhibition of mitotic kinase WEE1 was found to have the most effective response in combination with platinum compounds in lung cancer cell lines. In the BRCT-mediated interaction network, WEE1 was found in complex with PAXIP1, a protein containing six BRCT domains involved in transcription and in the cellular response to DNA damage. We show that PAXIP1 BRCT domains regulate WEE1-mediated phosphorylation of CDK1. Furthermore, ectopic expression of PAXIP1 promotes enhanced caspase-3-mediated apoptosis in cells treated with WEE1 inhibitor AZD1775 (formerly, MK-1775) and cisplatin compared with cells treated with AZD1775 alone. Cell lines and patient-derived xenograft models expressing both PAXIP1 and WEE1 exhibited synergistic effects of AZD1775 and cisplatin. In summary, PAXIP1 is involved in sensitizing lung cancer cells to the WEE1 inhibitor AZD1775 in combination with platinum-based treatment. We propose that WEE1 and PAXIP1 levels may be used as mechanism-based biomarkers of response when WEE1 inhibitor AZD1775 is combined with DNA-damaging agents.

Pooled List of DARS biomarkers

3551 DARS Genes

	A	B	C
1	Gene symbol	Name	HitCount
2	CGA	glycoprotein hormones, alpha polypeptide	11924
3	SHH	sonic hedgehog	6622
4	WNT1	Wnt family member 1	6428
5	TGFB1	transforming growth factor beta 1	6056
6	IGF1	insulin like growth factor 1	4556
7	INS	insulin	4395
8	GNRH1	gonadotropin releasing hormone 1	3943
9	CTNNB1	catenin beta 1	3912
10	VEGFA	vascular endothelial growth factor A	3777
11	SRY	sex determining region Y	3479
12	POMC	proopiomelanocortin	3454
13	EGF	epidermal growth factor	3396
14	KIT	KIT proto-oncogene receptor tyrosine kinase	3380
15	POU5F1	POU class 5 homeobox 1	3307
16	CD4	CD4 molecule	3152
17	PAX6	paired box 6	3124
18	LIF	LIF, interleukin 6 family cytokine	3070
19	BMP4	bone morphogenetic protein 4	3027
20	CD34	CD34 molecule	3027
21	ESR1	estrogen receptor 1	2946
22	SOX9	SRY-box 9	2649
23	TNF	tumor necrosis factor	2620
24	TP53	tumor protein p53	2520
25	PTHLH	parathyroid hormone like hormone	2436
26	AMH	anti-Mullerian hormone	2431
27	NR5A1	nuclear receptor subfamily 5 group A member 1	2341
28	IGF2	insulin like growth factor 2	2290
29	LEP	leptin	2058
30	AKT1	AKT serine/threonine kinase 1	1977
31	FGF2	fibroblast growth factor 2	1912

474 DARS Biological Processes

	A	B	C
1	HitID	Name	HitCount
2	GO_0023052	signaling	21733
3	GO_0007049	cell cycle	3228
4	GO_0008219	cell death	2514
5	GO_0006306	DNA methylation	2440
6	GO_0001837	epithelial to mesenchymal transition	2422
7	GO_0016310	phosphorylation	2372
8	GO_0030154	cell differentiation	2262
9	GO_0048468	cell development	2248
10	GO_0001556	oocyte maturation	1973
11	GO_0022008	neurogenesis	1567
12	GO_0006412	translation	1541
13	NCIT_C17741	Oxidative Stress	1449
14	GO_0048477	oogenesis	1243
15	GO_0001171	reverse transcription	1235
16	GO_0016477	cell migration	1209
17	GO_0007165	signal transduction	1146
18	GO_0030218	erythrocyte differentiation	1134
19	GO_0016049	cell growth	1041
20	GO_0006914	autophagy	1021

338 DARS miRNA

	A	B
1	HitID	HitCount
2	LET7	155
3	MIR-21	127
4	MIR-145	85
5	MIR-125B	73
6	MIR-17	73
7	MIR-17-92	65
8	MIR-1	64
9	MIR-302	62
10	MIR-124	56
11	MIR-29B	55
12	MIR-34C	52
13	MIR-34A	51
14	MIR-130B	51
15	MIR-375	49
16	MIR-200C	46
17	MIR-24	45
18	MIR-29A	44
19	MIR-429	41
20	MIR-223	41

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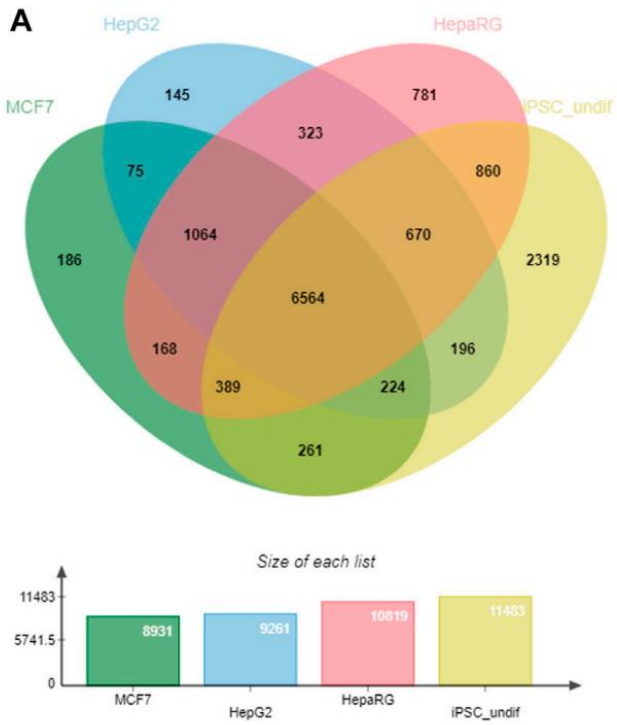
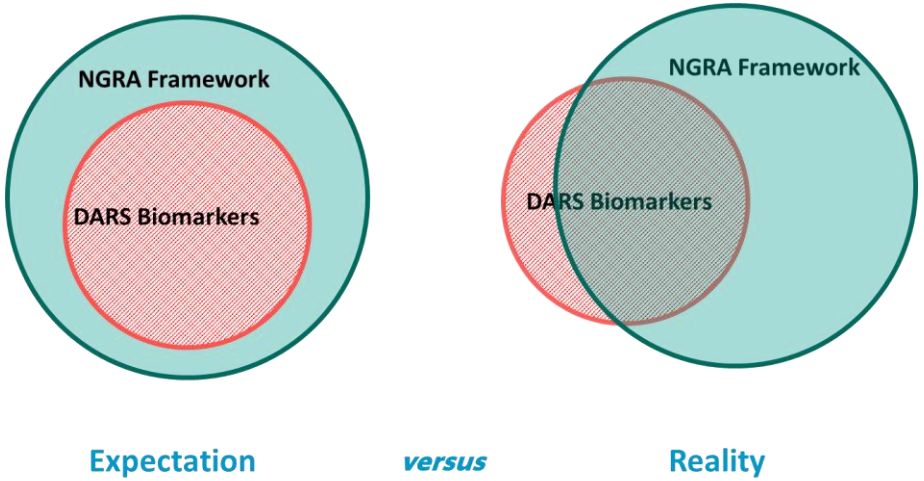
Coverage of important DART biomarkers using Literature Search

- HepG2, MCF-7, HepaRG, hiPSCs

14,225 genes in total





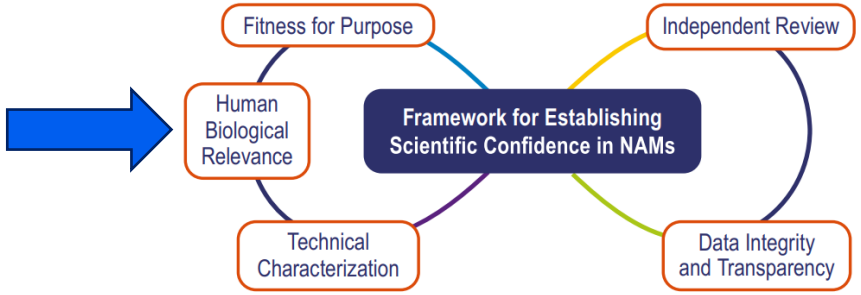
Differentiated hiPSCs not included in this study but in scope for future work

Gaps

- 41 **GPCRs** (6 present in IPP)
- 60 HTH transcription factors (mainly **homeobox transcription factors**)
- **Intercellular** signal molecules (chemokines, cytokines, growth factors, neurotropic factors, peptide hormones)

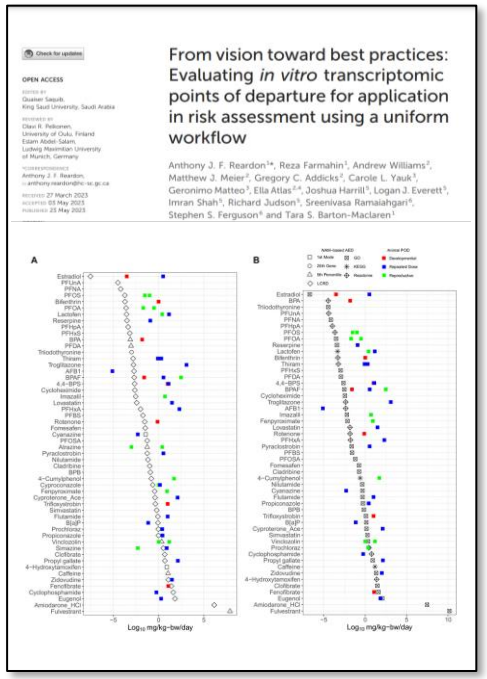
➢ **Filling the gaps – work in progress: placenta transfer measurements, DNT, DIT, studying epigenetics in germline development, advanced cell models for refinement.**

Case studies / fit for purpose validation, next steps

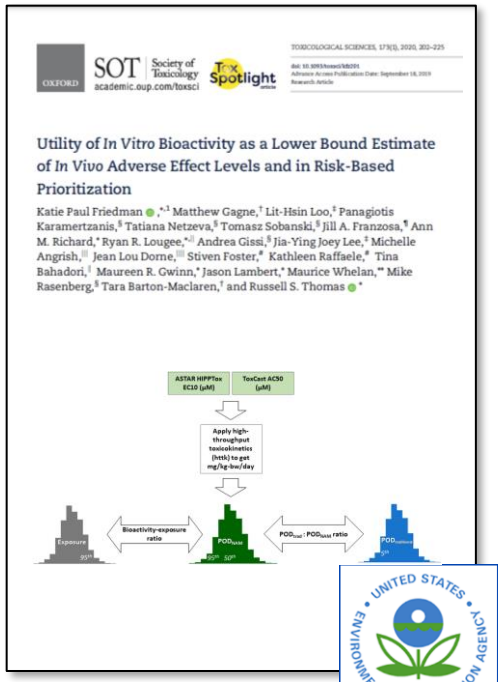


van der Zalm et al. Archives of Toxicology (2022) 96:2865–2879

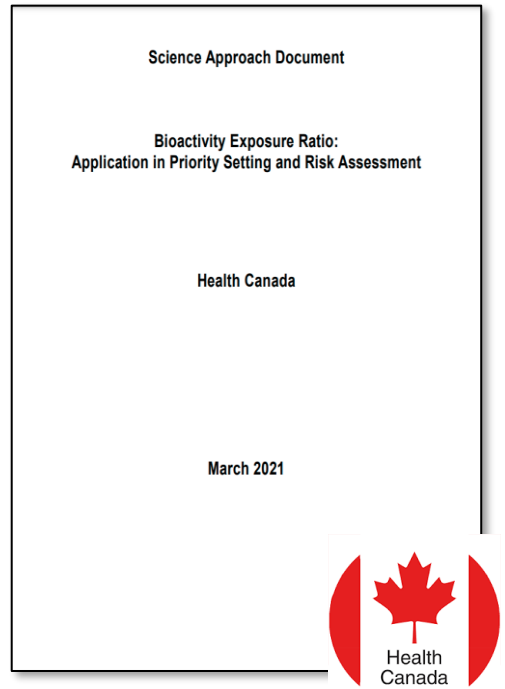
Examples of ongoing or completed case studies for NAM/NGRA BER based risk assessment or prioritisation


From vision toward best practices: Evaluating *in vitro* transcriptomic points of departure for application in risk assessment using a uniform workflow

Anthony J. F. Reardon[1]*, Reza Farmahin[1], Andrew Williams[2], Matthew J. Meier[2], Gregory C. Addicks[2], Carole L. Yauk[3], Geronimo Matteo[3], Ella Atlas[2,4], Joshua Harrill[5], Logan J. Everett[5], Imran Shah[5], Richard Judson[5], Sreenivasa Ramaiahgari[6], Stephen S. Ferguson[6] and Tara S. Barton-Maclaren[1]


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

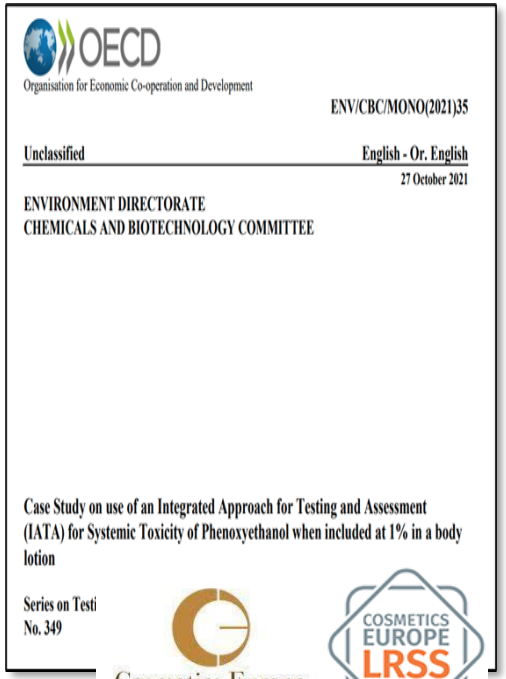
Katie Paul Friedman,*,[1] Matthew Gagne,[†] Lit-Hsin Loo,[‡] Panagiotis Karamertzanis,[§] Tatiana Netzeva,[§] Tomasz Sobanski,[§] Jill A. Franzosa,[¶] Ann M. Richard,* Ryan R. Lougee,*,[||] Andrea Gissi,[§] Jia-Ying Joey Lee,[‡] Michelle Angrish,[||] Jean Lou Dorne,[||] Stiven Foster,[#] Kathleen Raffaele,[#]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


Science Approach Document

Bioactivity Exposure Ratio:
Application in Priority Setting and Risk Assessment

Health Canada

March 2021


OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)35

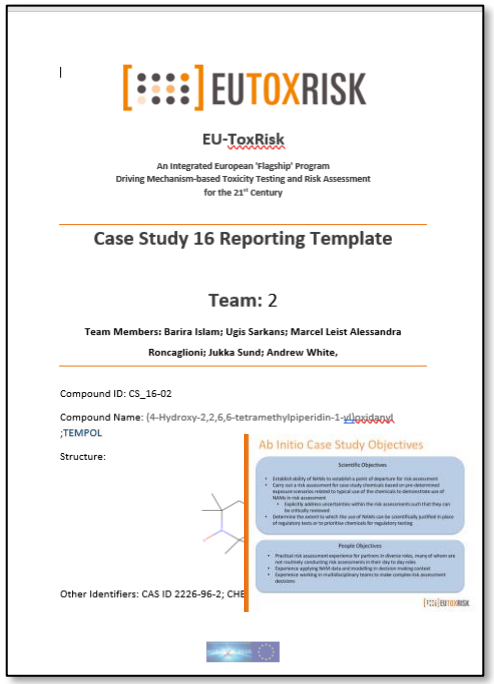
Unclassified — English - Or. English

27 October 2021

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Case Study on use of an Integrated Approach for Testing and Assessment (IATA) for Systemic Toxicity of Phenoxyethanol when included at 1% in a body lotion

Series on Testi
No. 349


EUTOXRISK

EU-ToxRisk

An Integrated European 'Flagship' Program
Driving Mechanism-based Toxicity Testing and Risk Assessment
for the 21st Century

Case Study 16 Reporting Template

Team: 2

Team Members: Barira Islam; Ugis Sarkans; Marcel Leist Alessandra Roncaglioni; Jukka Sund; Andrew White,

Compound ID: CS_16-02

Compound Name: (4-Hydroxy-2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl ;TEMPOL

Structure:

Ab Initio Case Study Objectives

Other Identifiers: CAS ID 2226-96-2; CHE

Health Canada

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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

Cosmetics Europe
the personal care association

COSMETICS EUROPE LRSS

RISK HUNT3R

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Is the NGRA Framework protective – fit for purpose validation

- Aim: evaluate protectiveness of the NGRA Framework for DART for a given chemical-exposure scenario
- Each chemical-exposure scenario is classified as “high” or “low” risk for pregnancy
- For each chemical-exposure scenario we generate NAM data using NGRA Framework

> Toxicol Sci. 2022 Aug 25;189(1):124-147. doi: 10.1093/toxsci/kfac068.

Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow

Alistair M Middleton [1], Joe Reynolds [1], Sophie Cable [1], Maria Teresa Baltazar [1], Hequn Li [1], Samantha Bevan [2], Paul L Carmichael [1], Matthew Philip Dent [1], Sarah Hatherell [1], Jade Houghton [1], Predrag Kukic [1], Mark Liddell [1], Sophie Malcomber [1], Beate Nicol [1], Benjamin Park [2], Hiral Patel [3], Sharon Scott [1], Chris Sparham [1], Paul Walker [2], Andrew White [1]

Is the NGRA Framework protective – fit for purpose validation

Exposure Scenario: Oral 0.5 mg tablet daily during pregnancy = risk for pregnancy

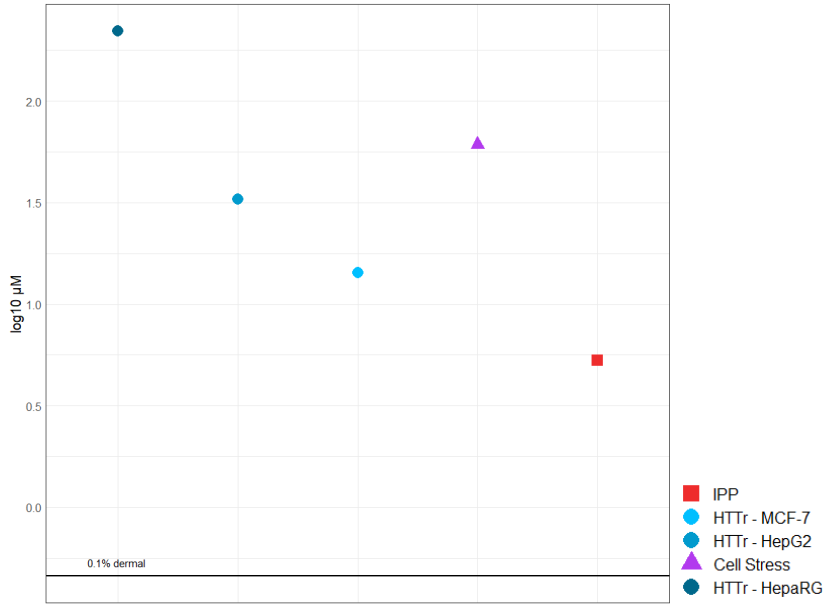
Diethylstilbesterol

Outcome: Bioactivity detected at or below the plasma Cmax = <u>risk for pregnancy</u>

The lowest PoD is coming from HTTR data from MCF7 cells expressing the Estrogen receptor, and from IPP (ER binding)

Exposure Scenario: Daily dermal application of 0.1% caffeine in a body lotion = low risk for pregnancy

Caffeine



Outcome: Bioactivity across the DART toolbox occurring at much higher concentrations than the plasma C_{max} = <u>low risk for pregnancy</u>

The lowest PoD coming from IPP ADORA2A

Is the NGRA Framework protective – fit for purpose validation

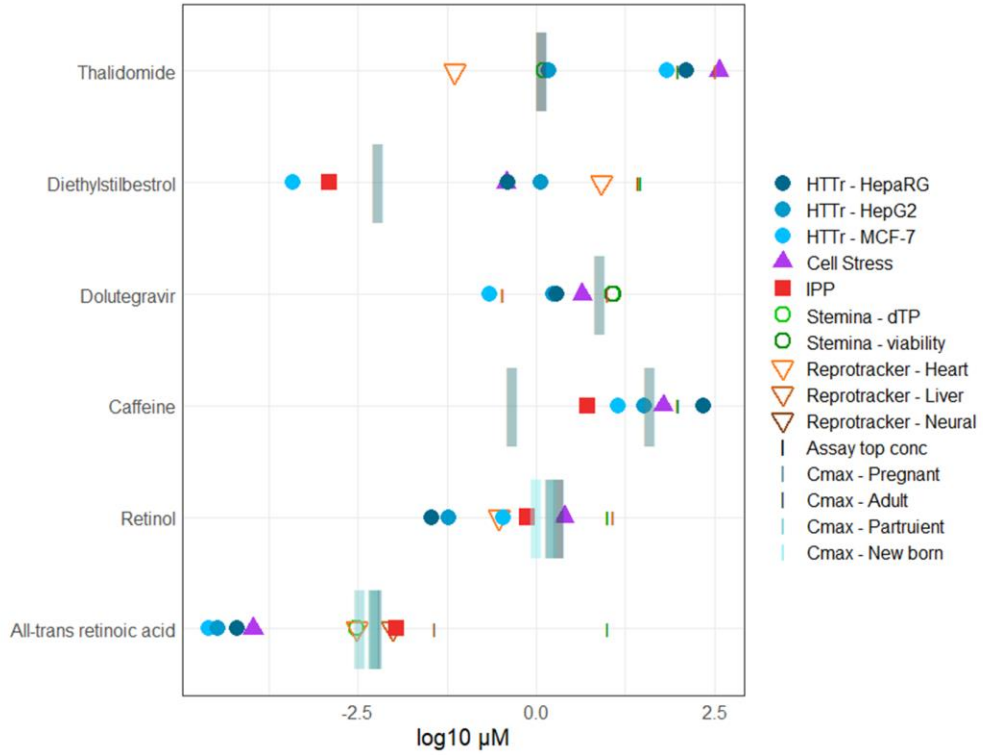
50mg oral application of Thalidomide, high risk, causing dev. toxicity.

5mg oral application of DES, high risk, causing estrogen activity/ED

50mg oral application of Dolutegravir, high risk, causing dev. toxicity

Dermal application of 0.1% caffeine in body lotion (lower Cmax), or oral uptake at recommended TDI of 200mg per days (higher Cmax) of caffeine, both low risk risk.

Uptake of vitamin A/retinol or retinol equivalents in normal diet, low risk. Cmax concentration of retinol and all-trans retinoic acid (metabolite of retinol) were measured in blood of adult, pregnant and parturient woman as well as in newborns[3].



Lowest PoD for Thalidomide is below Cmax value, the toolbox has correctly identified Thalidomide as high risk with lowest PoD coming from ReproTracker® assay.

Lowest PoD for DES is below Cmax value, the toolbox has correctly identified DES as high risk, lowest POD coming from MCF7 HTTr and estrogen receptor binding (IPP).

Lowest PoD for Dolutegravir is below Cmax value of exposure scenario, the toolbox has correctly identified it as high risk. Refinement for hazard classification as dev. Toxicant would be needed, if requested, as there are indications on dev. tox. but above Cmax values. Cell models like gastroloid systems can detect effects at relevant conc.[4].

Cmax for dermal application of caffeine is below lowest PoD, the toolbox has correctly identified it as low risk. For oral uptake of caffeine, the lowest PoD is below Cmax values indicating risk. Refinement for risk assessment would be needed.

Lowest PoD for retinol as well as all-trans retinoic acid is below Cmax values indicating high risk. Further tools would be needed to refine between bioactivity versus adversity of the compound .

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Is the NGRA Framework protective – fit for purpose validation

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5mg oral application of DES, high risk, causing estrogen activity/ED

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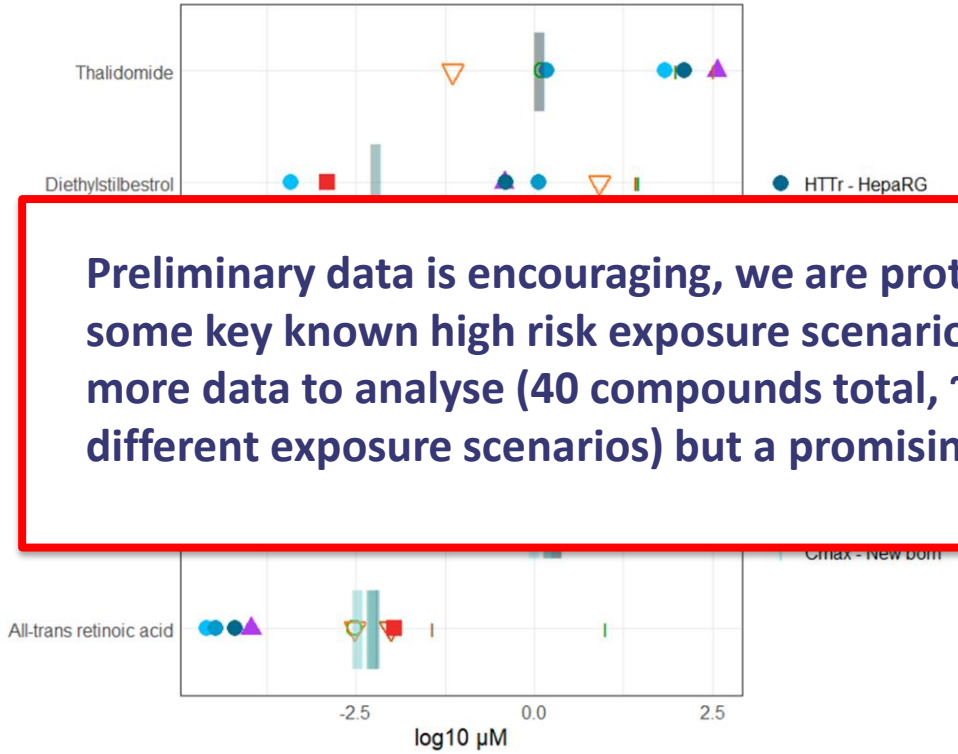

Preliminary data is encouraging, we are protective for some key known high risk exposure scenarios. Lots more data to analyse (40 compounds total, ~60+ different exposure scenarios) but a promising start!

Lowest PoD for Thalidomide is below Cmax value, the toolbox has correctly identified Thalidomide as high risk with lowest PoD coming from ReproTracker® assay.

Lowest PoD for DES is below Cmax value, the toolbox has correctly identified DES as high risk with lowest PoD coming from MCF7 HTTr and estrogen receptor binding (IPP).

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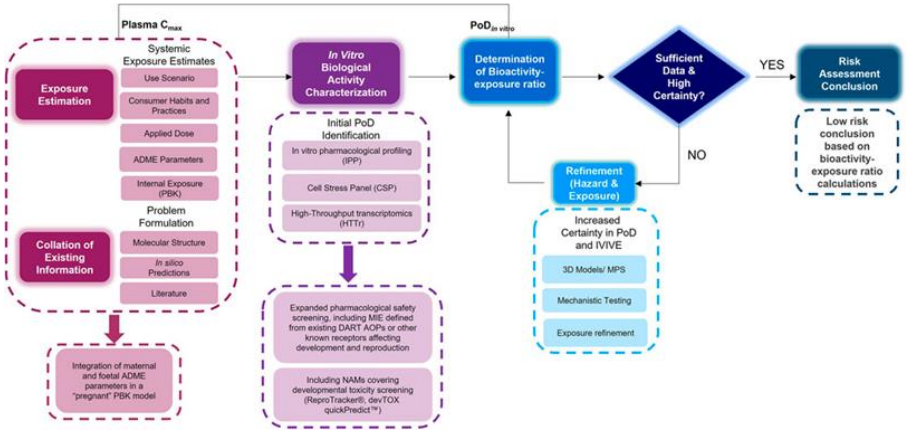
Lowest PoD for retinol as well as all-trans retinoic acid is below Cmax values indicating high risk. Further tools would be needed to refine between bioactivity versus adversity of the compound .

Next Steps

- Evaluation of DART NGRA across many chemistries
- ReproTracker assay
  - Development and evaluation of an osteoblast differentiation protocol



Rajagopal et al., Front. Toxicol., 2022

- Identification and filling of existing gaps (placenta transfer measurements, DNT, DIT, endocrine disruptors, multigenerational effects, studying epigenetics in germline development, advanced cell models for refinement)
- CLP/GHS hazard classification with NAMs

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Acknowledgments

DART NGRA Team – Paul Carmichael, Matt Dent, Jade Houghton, Predrag Kukic, Hequn Li, Alistair Middleton, Iris Muller, Beate Nicol, Ramya Rajagopal, Sandrine Spriggs, Gopal Pawar, Katy Wilson, Kathryn Wolton

40+ years of developing non-animal safety science

70+ collaborations

600+ publications

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