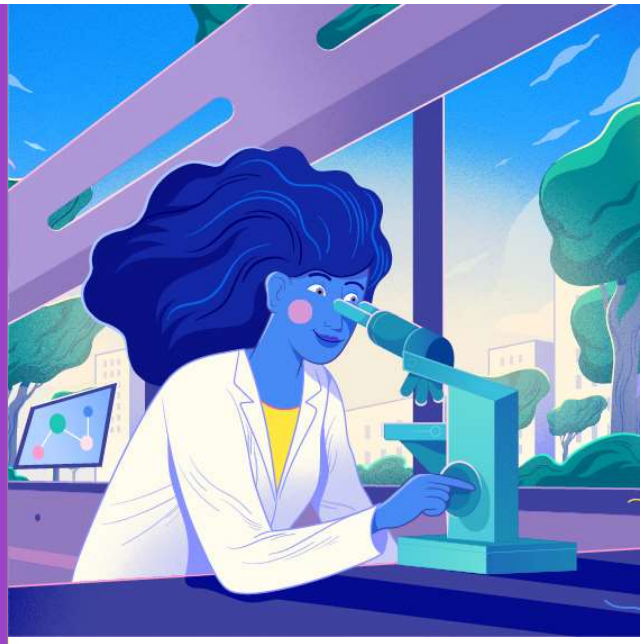
New Approach Methodologies (NAMs) and Next Generation Risk Assessment (NGRA) for DART



Dr Predrag Kukic
Science Lead
Unilever Safety and Environmental Assurance Centre (SEAC)

ETS, 28/09/2022

Outline

- Unilever's science-based safety approach
- Overview of *in vitro* methods and NGRA Framework for DART
- Biological coverage of the NGRA Framework for DART
- Case studies / fit for purpose validation

Unilever Policy & Approach
Safe & Sustainable Products without Animal Testing

We say use science.
Not animals.

What we believe

- **Every Unilever product must be safe for people and our environment**
- **Animal testing is not needed 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

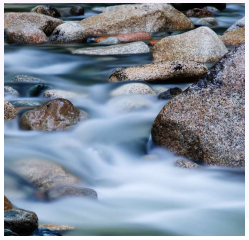
40+ years of developing non-animal safety science

70+ collaborations

600+ publications

https://tt21c.org



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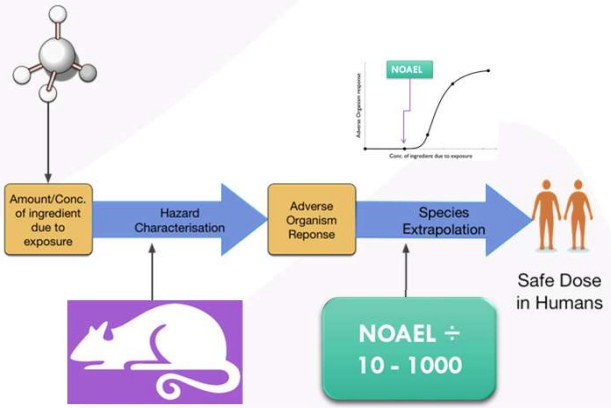
A paradigm shift is underway as use of non-animal safety science increases & safety assessment frameworks evolve to embed NGRA

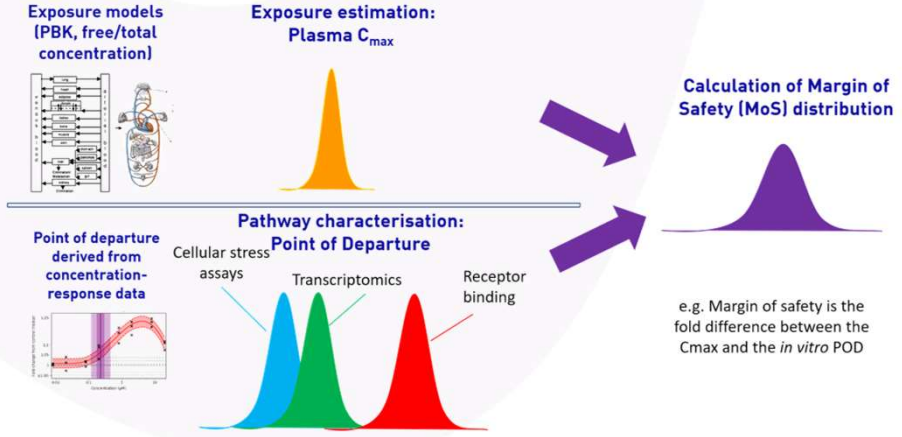
Non-animal safety science is increasingly being used to make decisions on:

1. safety of **consumers** exposed to chemicals in **products**
2. safety of **workers** exposed to chemicals during product **manufacture**
3. safety of **non-human species** if exposed to chemicals in the **environment**

‘Traditional’ Risk Assessment

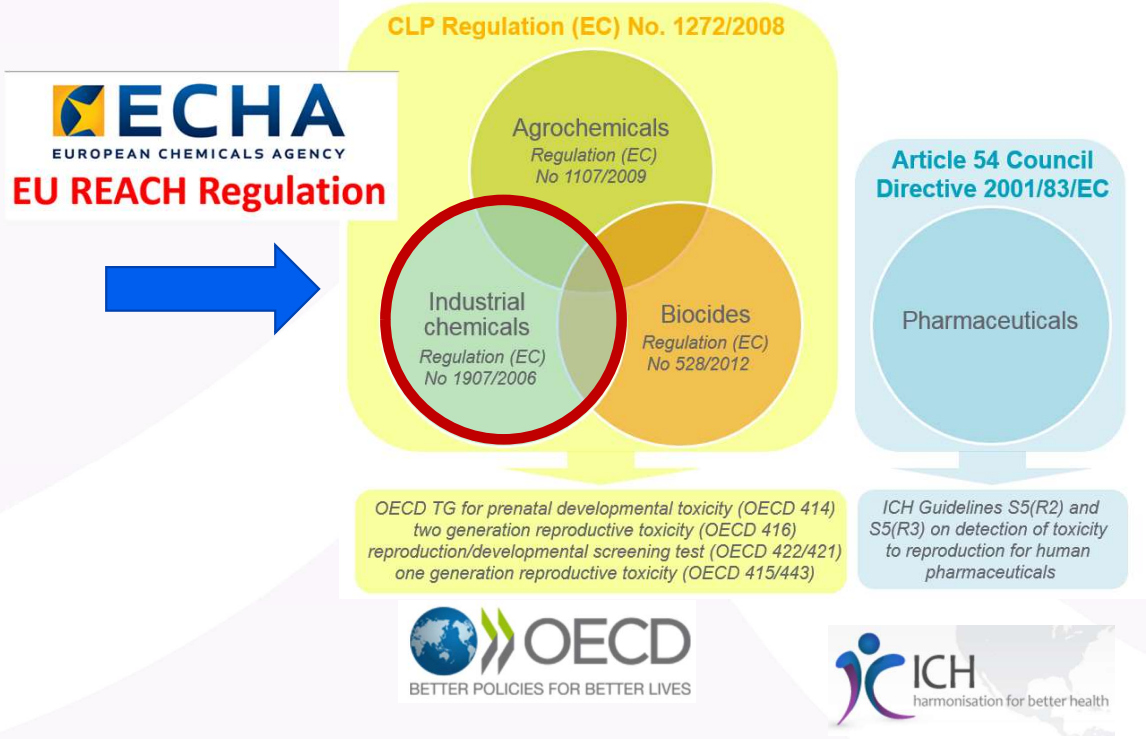


‘Next Generation’ Risk Assessment



… animal testing for DART endpoints under REACH

- **In the European Union, selling cosmetic products tested on animals is prohibited. The ban applies to both the final formulation and the ingredients of the product (Cosmetics Regulation No 1223/2009)**
- **Those same chemical ingredients may, however, also need to be registered under REACH or their dossiers updated, which may involve animal testing.**



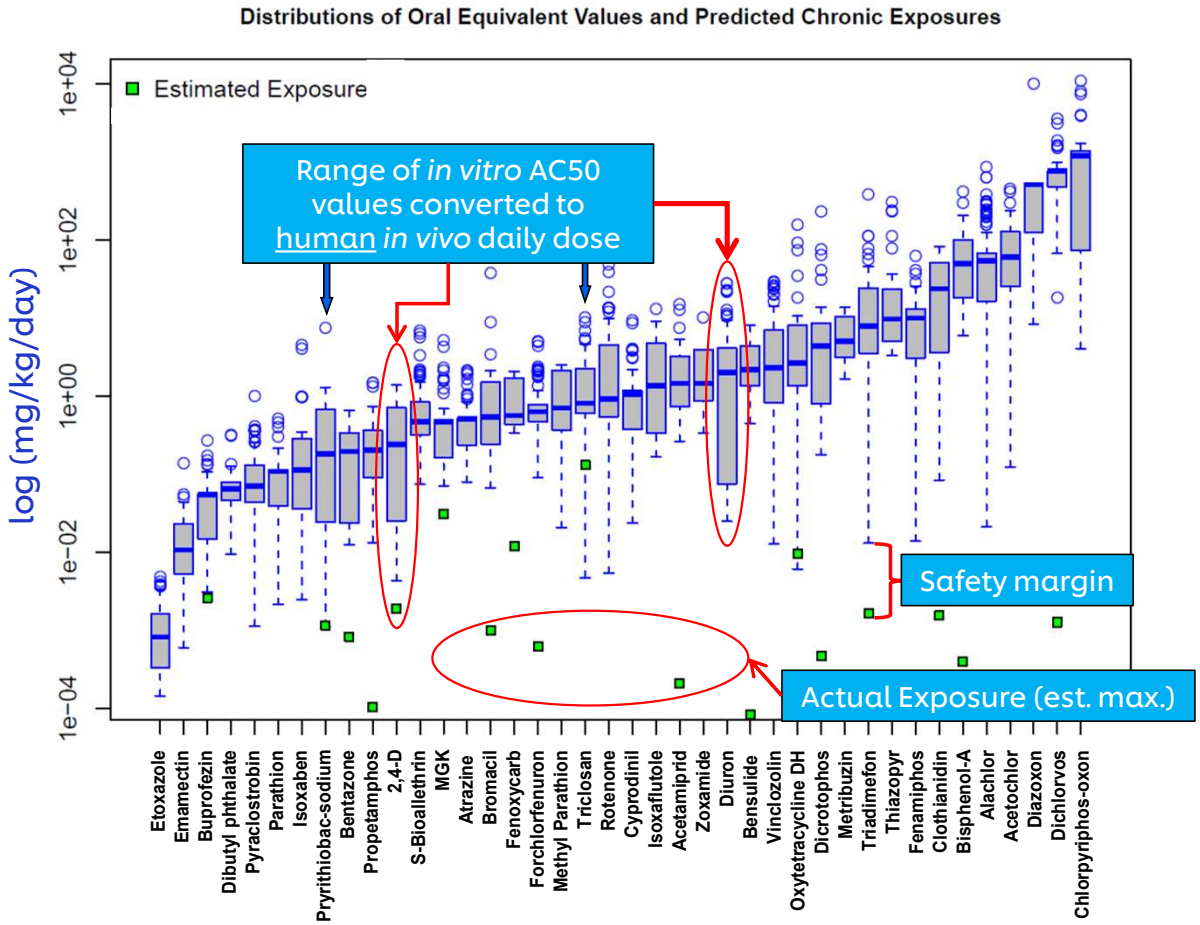
Study	Annex VII	Annex VIII 10-100 tpa	Annex IX 100-1000 tpa	Annex X 1000+ tpa
Screening test for reproductive /developmental toxicity (OECD TG 421 or 422)		Required	Strongly recommended if no higher tier fertility study (such as OECD 443) is/will be available	
Prenatal developmental toxicity study (EU B.31, OECD TG 414)		May be proposed in case of (serious) concern[1] for prenatal developmental toxicity. However, it is strongly recommended to consider conducting a screening study in addition to the prenatal developmental toxicity[2] study	Required in one species; second species may be triggered[2]	Required in two species
Extended one-generation reproductive toxicity study (EU B.56, OECD TG 443)[3]		Recommended instead of the screening study in case of serious concern[1] for fertility	Required if triggered[4]	Required

Draft Guidance document (europa.eu)

Unilever's approach: science-based safety

- Plans to address information requirements for REACH using science-based safety approach that is not based on the generation of new animal data:
  - Strengthen the existing read-across submissions
  - Exposure-led safety assessment that also includes worker exposure assessment from all facilities
  - Generation of new in vitro data, including NAMs for DART using the Next Generation Risk Assessment (NGRA) Framework

Using 21st century science to assure safety – NGRA



The hypothesis underpinning NGRA is that **if no bioactivity is observed at consumer-relevant concentrations, there can be no adverse health effects.**

At no point does NGRA attempt to predict the results of high dose toxicology studies in animals.

NGRA **uses new exposure science and understanding of human biology.**

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

US EPA Next Generation Blueprint Tiered Testing Framework

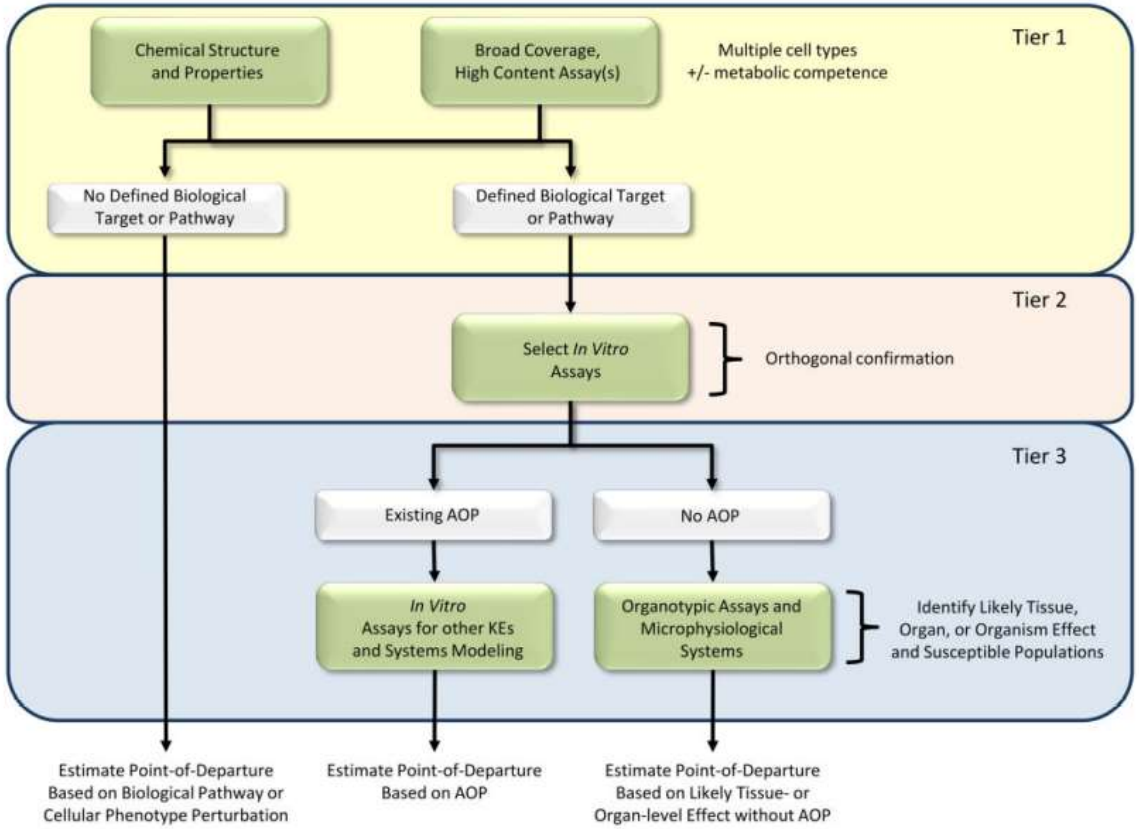


Figure 2. Tiered testing framework for hazard characterization. Tier 1 uses both chemical structure and broad coverage, high content assays across multiple cell types for comprehensively evaluating the potential effects of chemicals and grouping them based on similarity in potential hazards. For chemicals 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

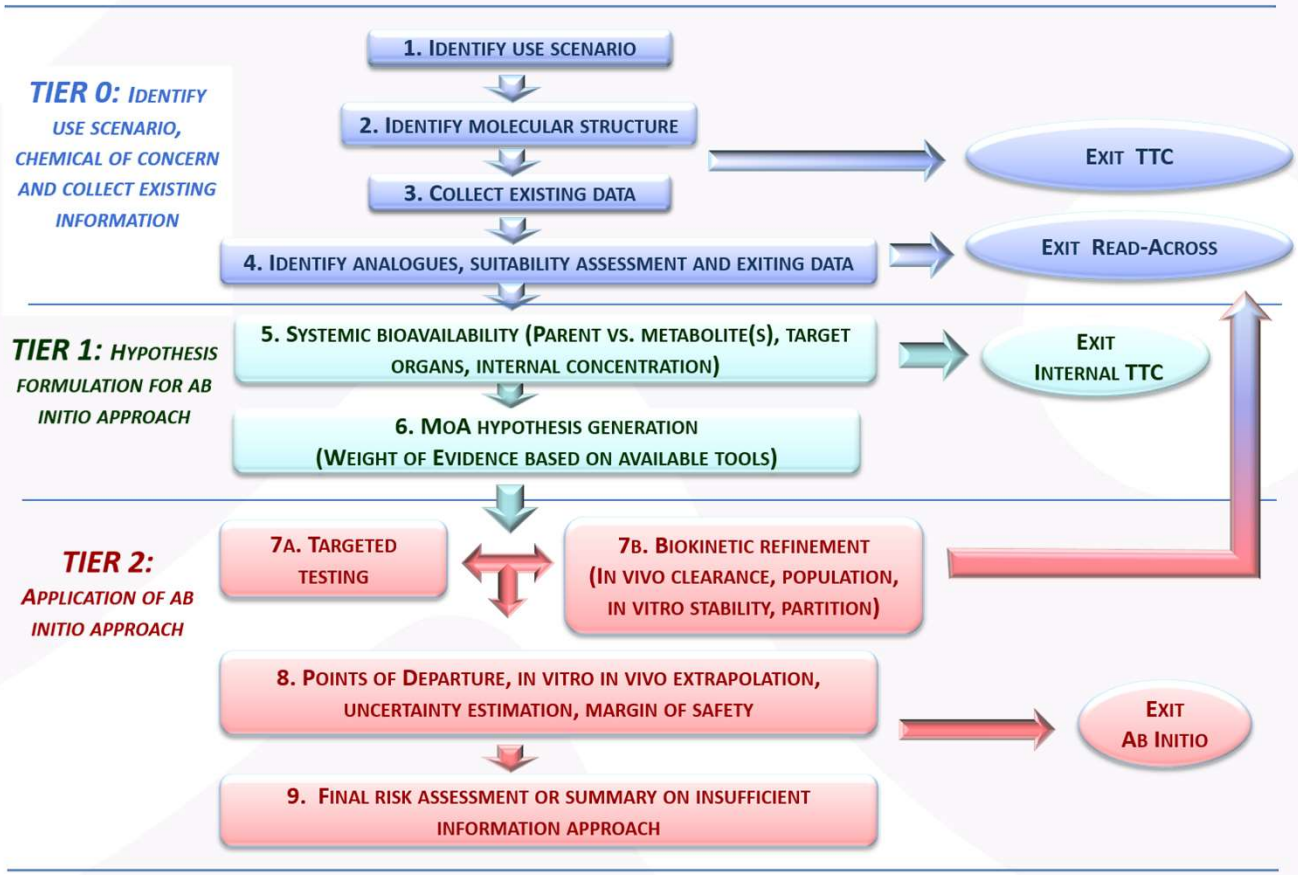
The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency

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

Unilever

FP7 (€200 mil) - Ab initio chemical safety assessment: Tiered testing to support human health safety assessment



Read across
Exposure-based waiving
In silico tools

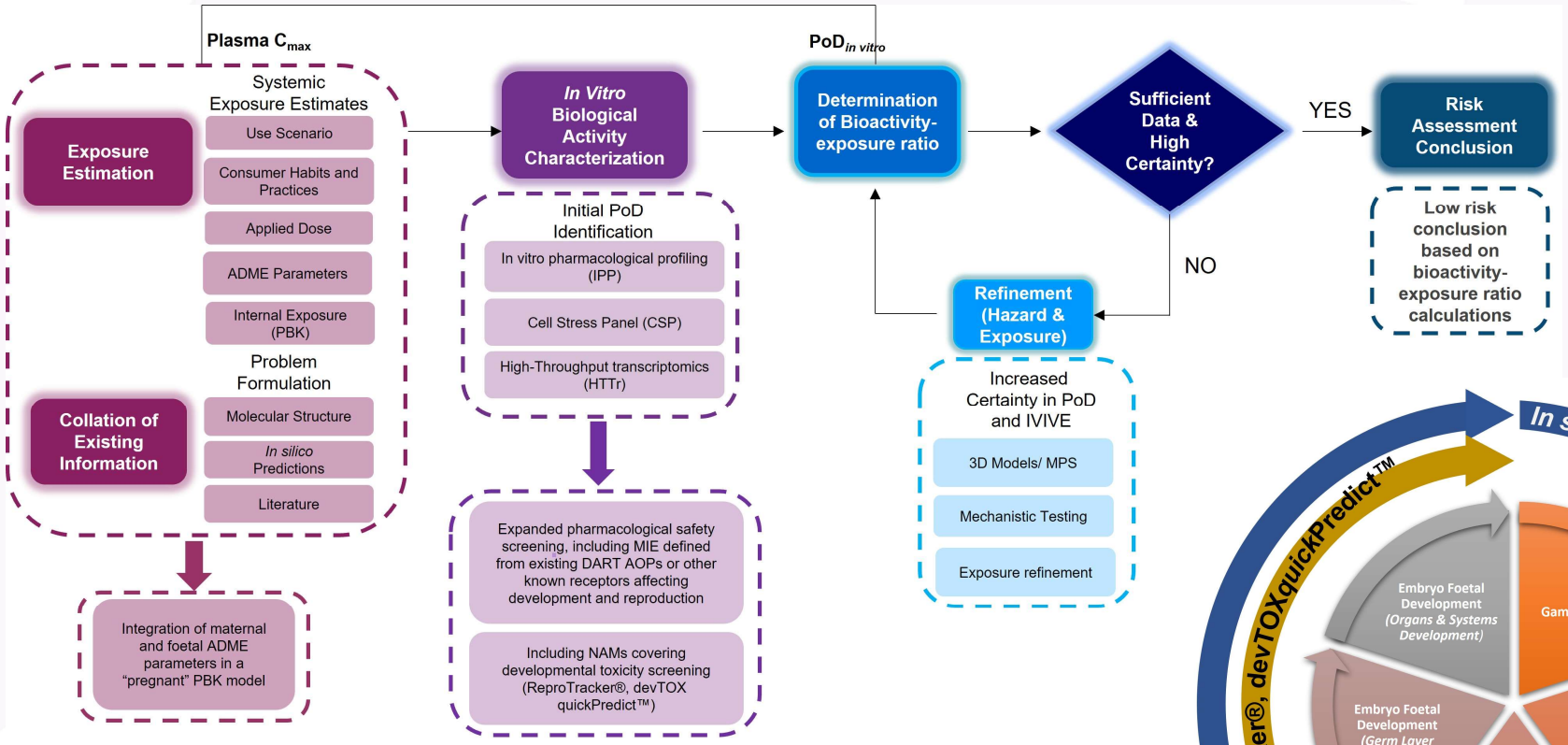
Metabolism and metabolite identification
Physiologically-based kinetic modelling
In chemico assays
'Omics
Reporter gene assays
In vitro pharmacological profiling

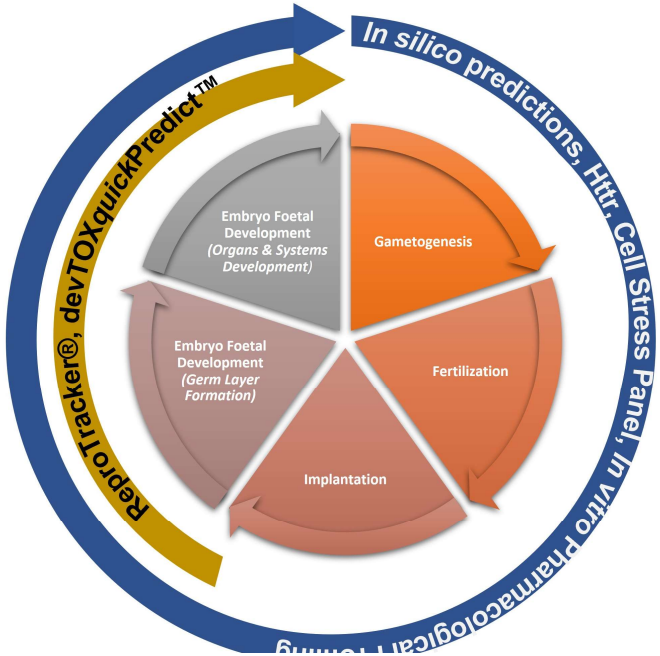
3D culture systems
Organ-on-chip
Zebrafish larva assays
Pathways modelling
Human studies

Berggren *et al.*, (2017) *Computational Toxicology* 4: 31-44

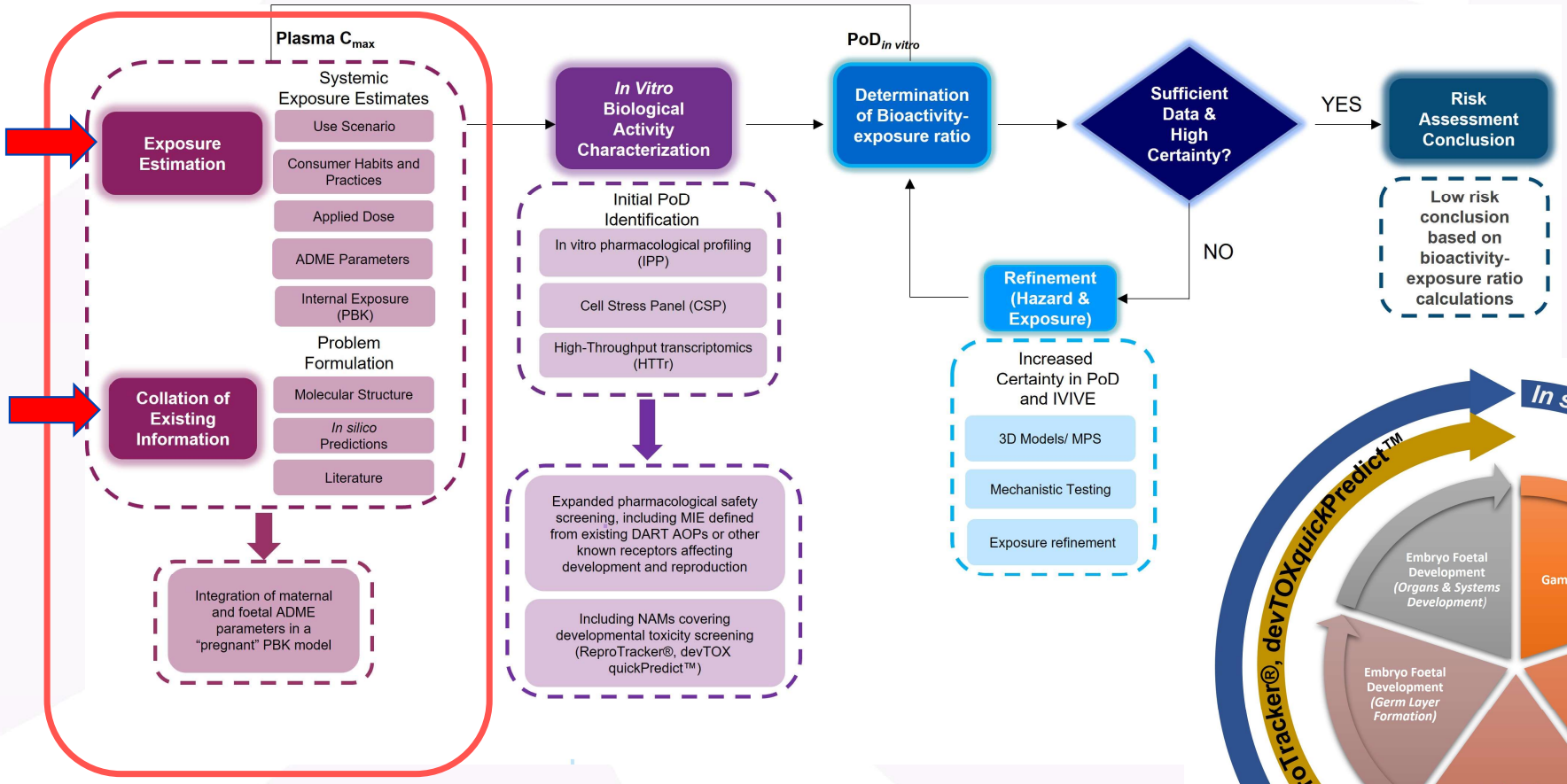
Unilever's NGRA Framework for DART

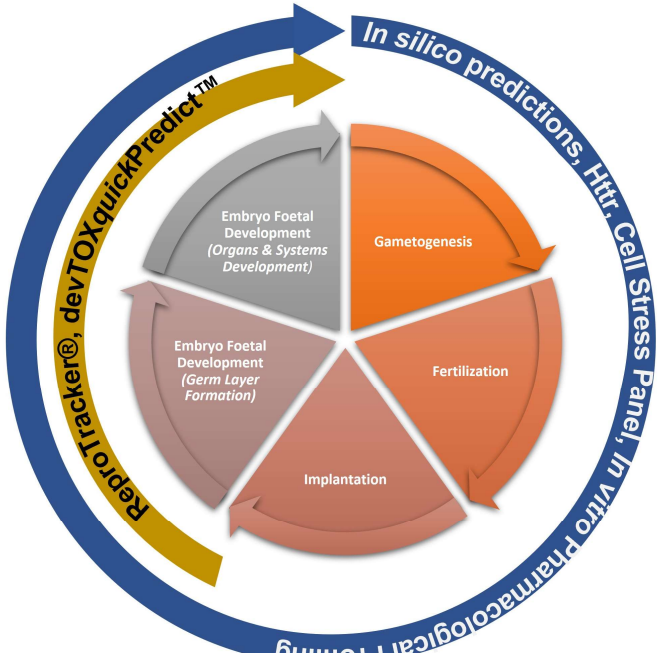
Unilever's NGRA Framework for DART – tiered approach





Unilever's NGRA Framework for DART – tiered approach





Systemic exposure estimates - PBK modelling

PBK modelling Framework

Level 0:

Characterise exposure scenario (who, where, how often, and how much)

Product & chemical information

Level 1:

Predictions from in silico only

parameterisation & sensitivity

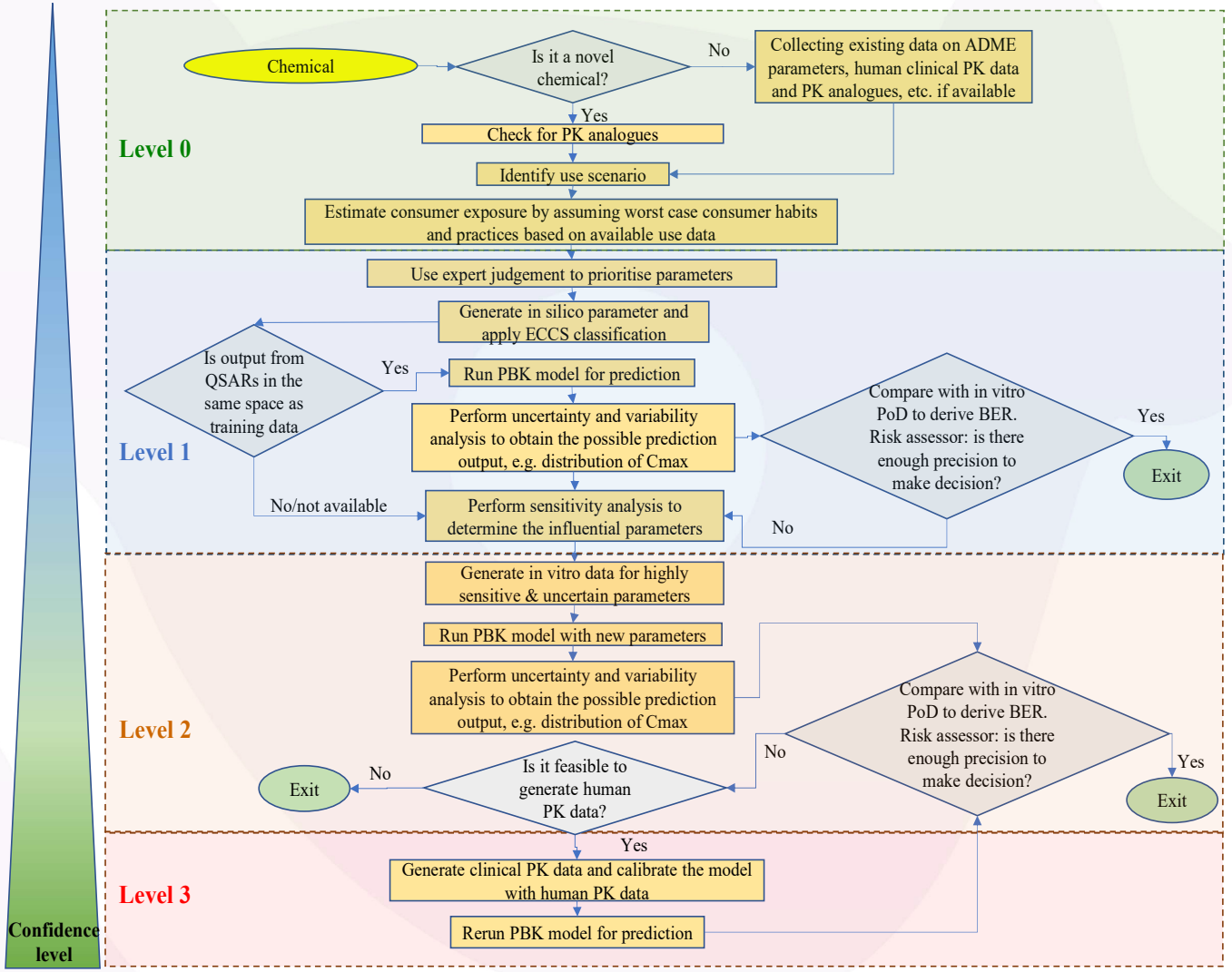
Level 2:

PBK modelling based on in vitro parameterisation

Level 3

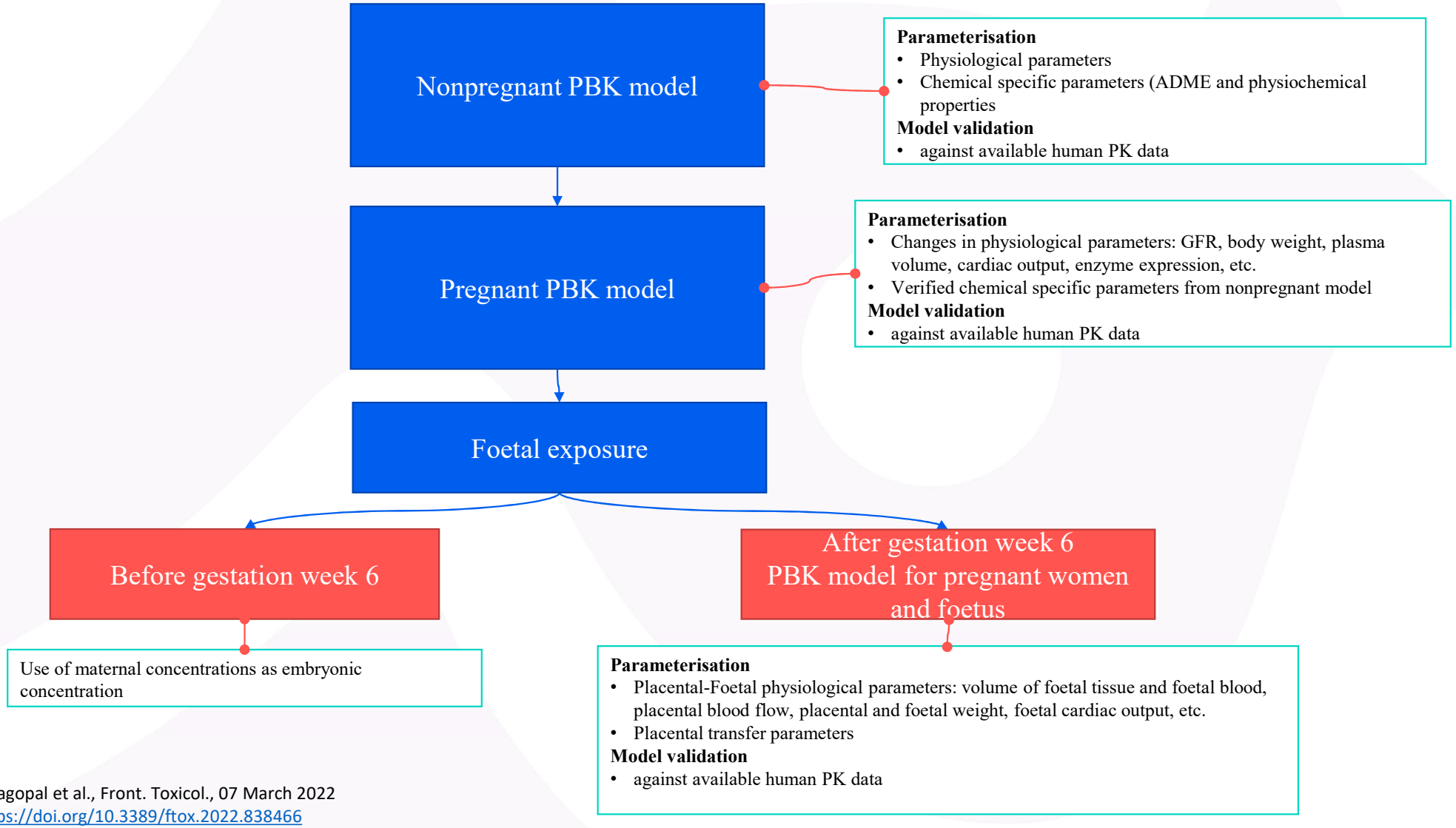
Generating human PK data for validation or/and calibration

- The progression between levels is closely related to the risk assessment process
- Use tools that are as complex as necessary to make the decision
- Move to more complex tools if more data is needed



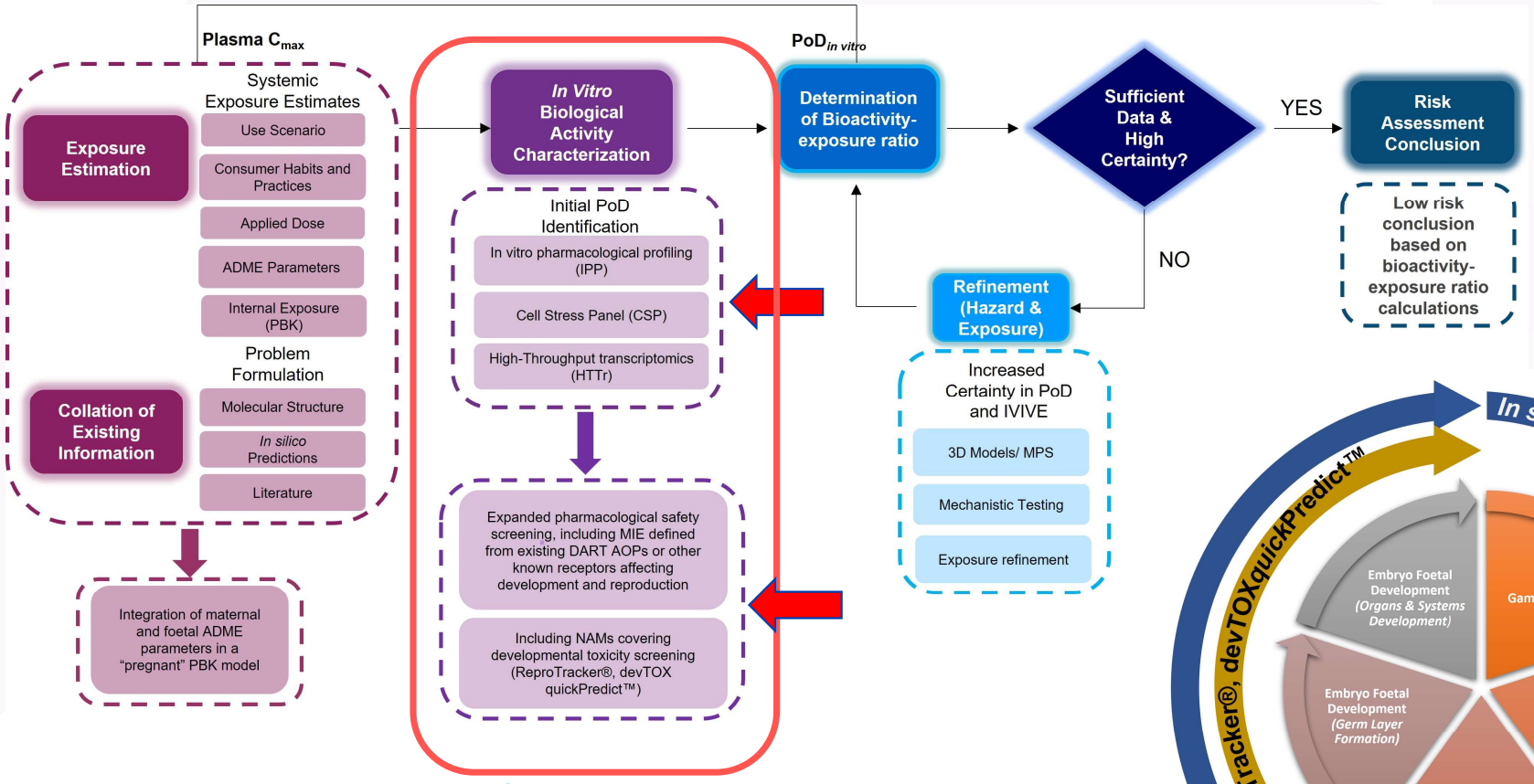
Li, Hequn et al. "PBK modelling of topical application and characterisation of the uncertainty of Cmax estimate: A case study approach" Toxicology and Applied Pharmacology, vol. 442, 2022, p. 11599.

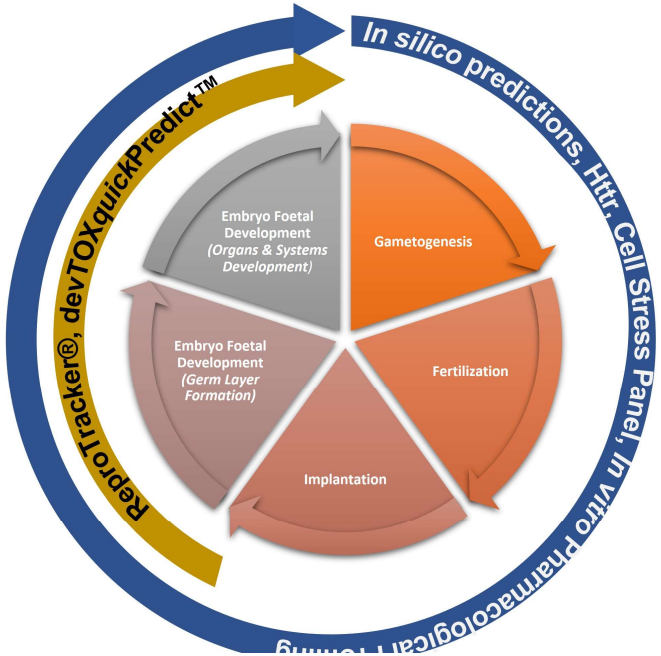
Systemic exposure estimates– pregnant PBK modelling



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

Unilever's NGRA Framework for DART – tiered approach

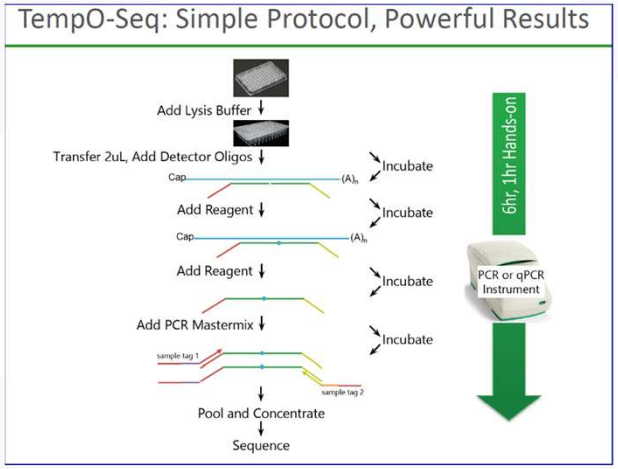


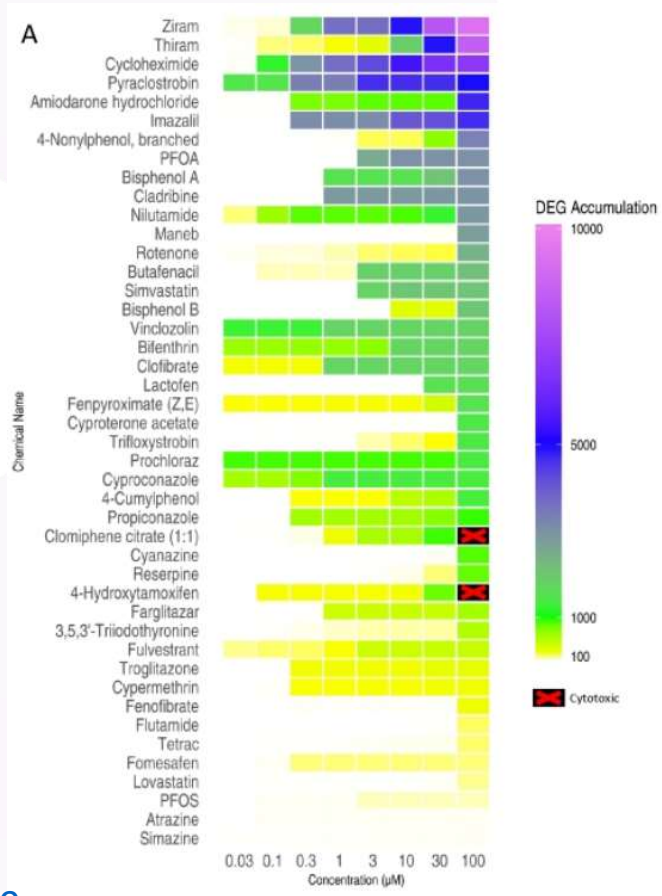


In vitro biological activity characterisation
-High throughput transcriptomics

Cells treated for 24h with 7 concentrations of each chemical to generate dose-response data (5 biological replicates). Three cell lines chosen to cover a range of biological diversity:

- **MCF-7** – human breast adenocarcinoma cell line
- **HepG2** – human liver carcinoma
- **HepaRG** – terminally differentiated hepatic cells that retain many characteristics of primary human hepatocytes

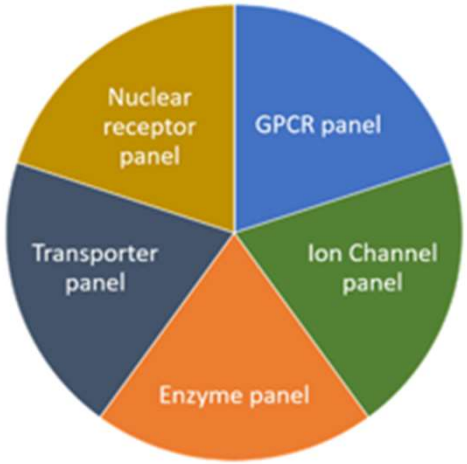




Toxicol Sci (2021) 181(1):68-89

In vitro biological activity characterisation
-*in vitro* pharmacological profiling

- The IPP panel contains **63 targets** with known **safety liabilities** that were tested in binding, enzymatic, coactivator recruitment and luciferase assays.
- 44 of the targets have been associated with ***in vivo* adverse drug reactions** (Bowes *et al.*, 2012) and a further 19 targets implicated in **developmental and reproductive toxicity** were added to the panel based on a literature search.



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PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

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

Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek, Arun Sridhar, Gareth Waldron and Steven Whitebread

Abstract | *In vitro* pharmacological profiling is increasingly being used earlier in the drug discovery process to identify undesirable off-target activity profiles that could hinder or halt the development of candidate drugs or even lead to market withdrawal if discovered after a drug is approved. Here, for the first time, the rationale, strategies and methodologies for *in vitro* pharmacological profiling at four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) are presented and illustrated with examples of their impact on the drug discovery process. We hope that this will enable other companies and academic institutions to benefit from this knowledge and consider joining us in our collaborative knowledge sharing.

safety testing of drug candidates and are designed to prevent serious ADRs from occurring in clinical studies.

The only *in vitro* pharmacology assay that is absolutely required by regulatory authorities is one that measures the effects of new chemical entities on the ionic current of native (I_{Kr}) or heterologously expressed human voltage-gated potassium channel subfamily H member 2 (KCNH2; also known as hERG)[3]. The mechanism by which blockade of hERG can elicit potentially fatal cardiac arrhythmias (torsades de pointes) following a prolongation of the QT interval is well characterized[3,4], and the seriousness of this ADR is one reason why this assay is a mandatory regulatory requirement. Receptor binding studies are also recommended as the first-tier approach for the assessment of the dependence potential of novel chemical entities[5].

However, current regulatory guidance does not describe which targets should constitute an *in vitro* pharmacological profiling panel and does not indicate the stage of the discovery process at which *in vitro* pharmacological profiling should occur.

Targets (gene)	Hit rate*		Main organ class or system	Effects	
	Binding	Functional or enzymatic		Agonism or activation	Antagonism or inhibition
G protein-coupled receptors					
Adenosine receptor A_{2A} (*ADORA2A*)	High	Low (agonist)	CVS, CNS	Coronary vasodilation; ↓ in BP and reflex; ↑ in HR; ↓ in platelet aggregation and leukocyte activation; ↓ in locomotor activity; sleep induction	Potential for stimulation of platelet aggregation; ↑ in BP; nervousness (tremors, agitation); arousal; insomnia
α_{1A}-adrenergic receptor (*ADRA1A*)	High	Low (agonist); high (antagonist)	CVS, GI, CNS	Smooth muscle contraction; ↑ in BP; cardiac positive ionotropy; potential for arrhythmia; mydriasis; ↓ in insulin release	↓ in smooth muscle tone; orthostatic hypotension and ↑ in HR; dizziness; impact on various aspects of sexual function
α_{2A}-adrenergic receptor (*ADRA2A*)	High	Low (agonist); medium (antagonist)	CVS, CNS	↓ in noradrenaline release and sympathetic neurotransmission; ↓ in BP; ↓ in HR; mydriasis; sedation	↑ in GI motility; ↑ in insulin secretion
β_1-adrenergic receptor (*ADRB1*)	Medium	NA	CVS, GI	↑ in HR; ↑ in cardiac contractility; electrolyte disturbances; ↑ in renin release; relaxation of colon and oesophagus; lipolysis	↓ in BP; ↓ in HR; ↓ in CO
β_2-adrenergic receptor (*ADRB2*)‡	High	Medium (agonist); medium (antagonist)	Pulmonary, CVS	↑ in HR; bronchodilation; peripheral vasodilation and skeletal muscle tremor; ↑ in glycogenolysis and glucagon release	↓ in BP

Bowes J, *et al.*, 2012 Reducing safety-related drug attrition: the use of in vitro pharmacological profiling. Nat Rev Drug Discov. 11(12):909-22.

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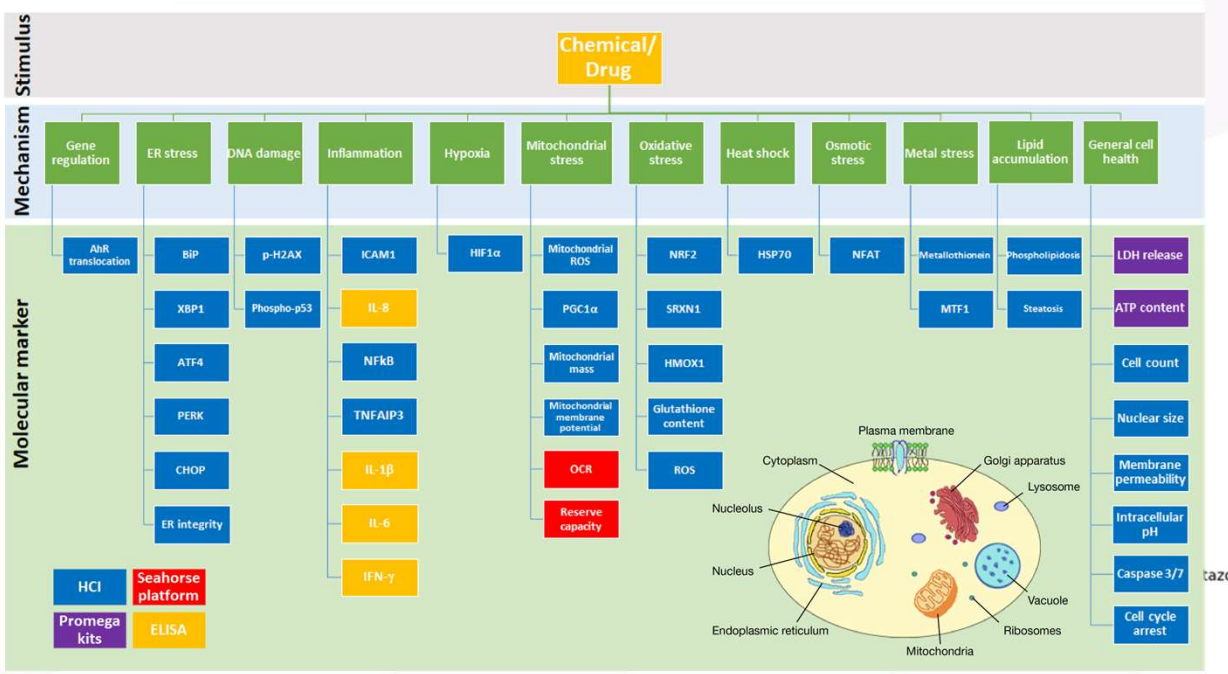
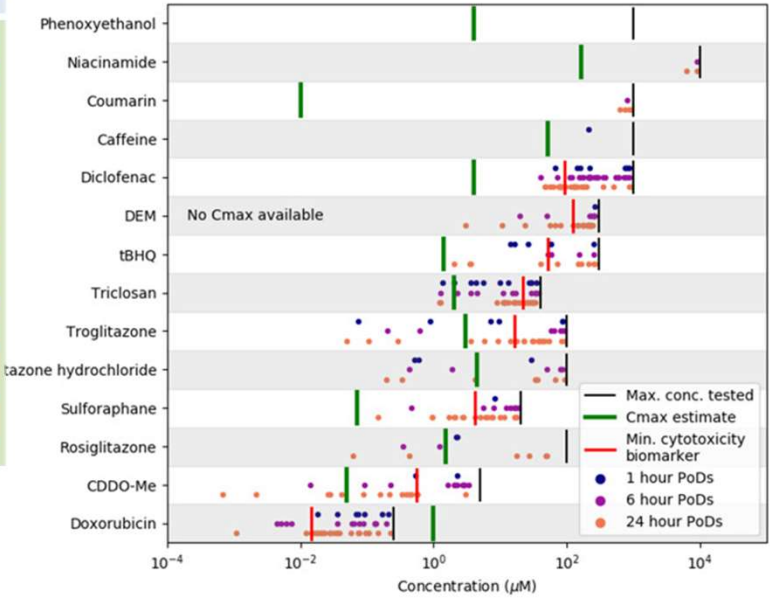
In vitro biological activity characterisation
-Cell stress panel



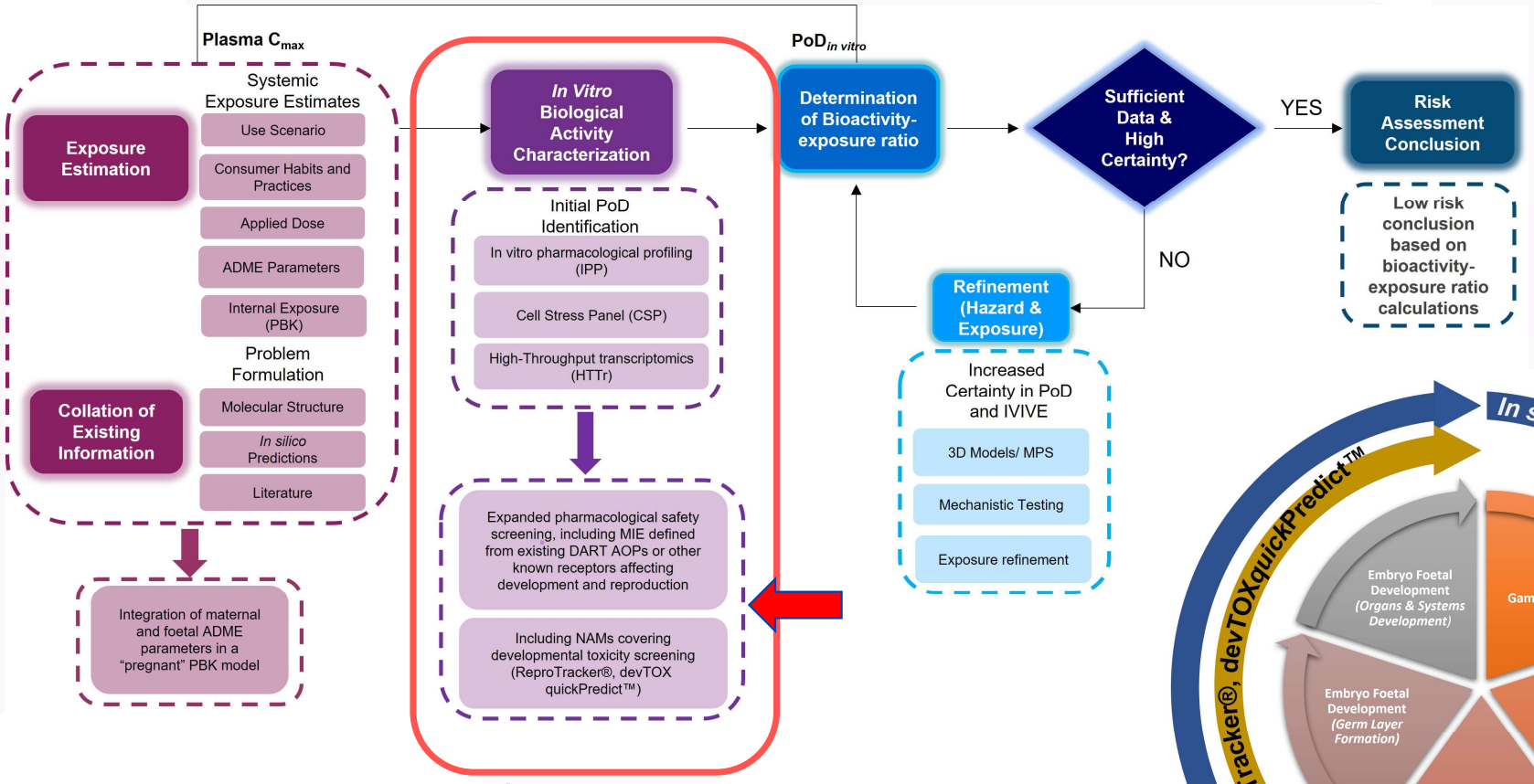
[Cell Stress Panel Assay (cyprotex.com)]

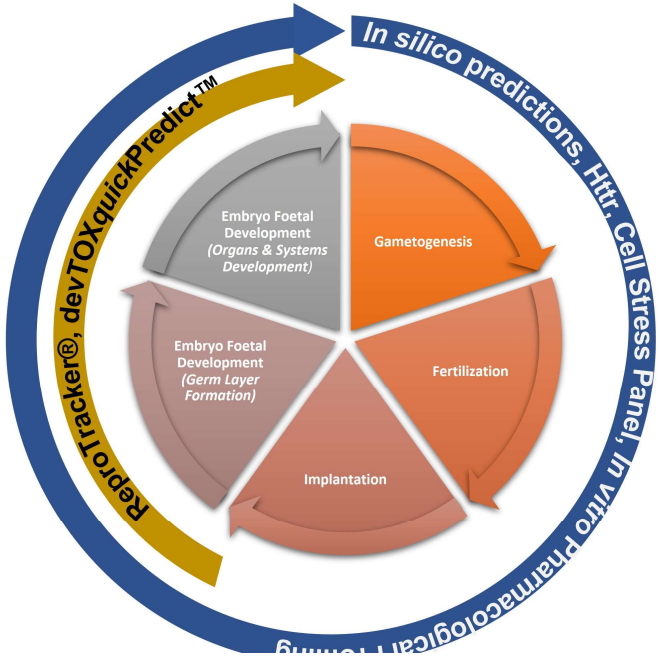
- 36 biomarkers, 3 cell lines (HepG2, HepaRG, MCF7), 3 timepoints, 8 concentrations



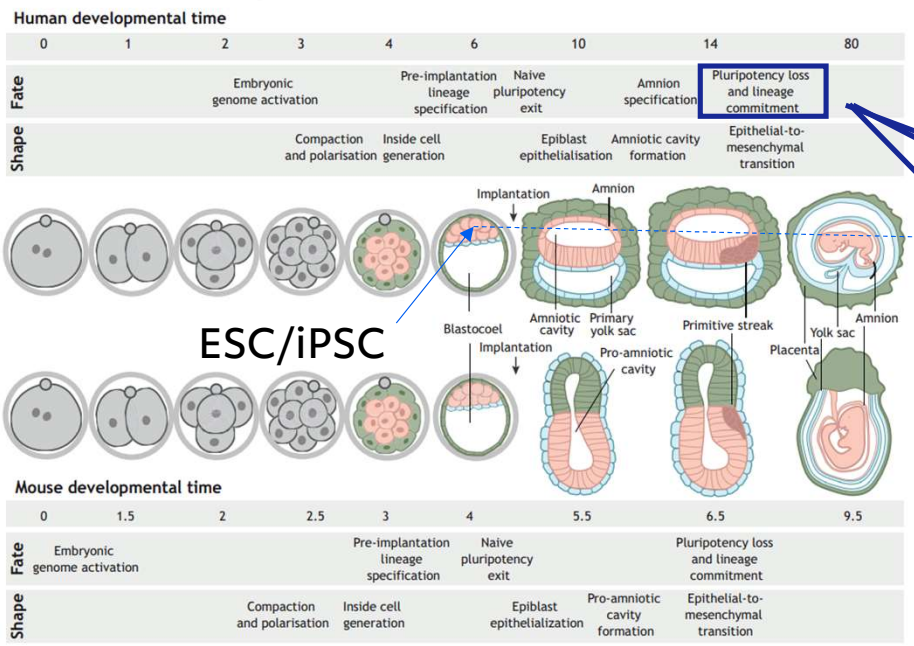
Toxicol Sci (2020) 176, 11-33

Unilever's NGRA Framework for DART – tiered approach



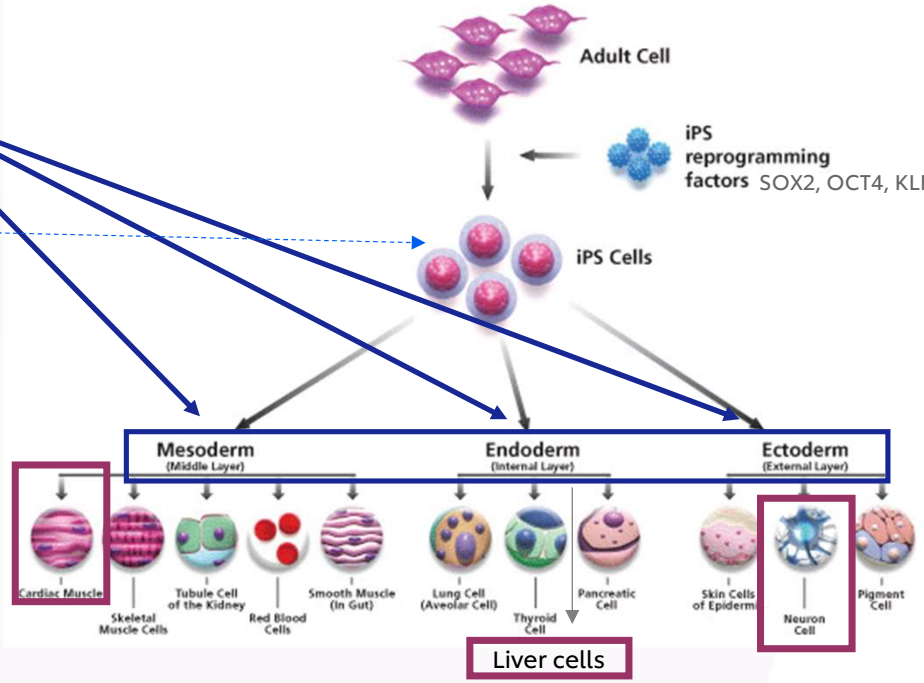


Induced pluripotent stem cells (iPSCs) to detect developmental toxicity



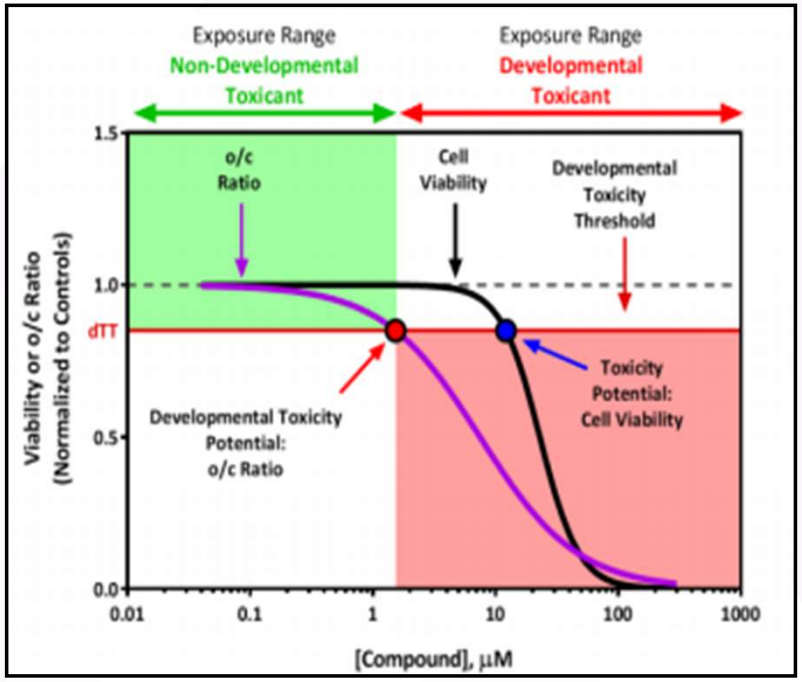
modified from Shahbazi, (2020) Development Jul 17;147(14):dev190629

Induced Pluripotent Stem Cell Differentiation Protocols | Sigma-Aldrich



- iPSCs can be used as a surrogate for embryonic stem cells
- Assays have been developed to either use iPSCs directly (devToxquickPredict™ platform; Stemina) or the differentiation into heart, liver and neuronal cells (ReproTracker®; Toxys) as NAMs for developmental toxicity

In vitro biological activity characterisation – devTOX quickPredict™



Published in final edited form as:
Toxicol Sci. 2020 April 01; 174(2): 189–209. doi:10.1093/toxsci/kfaa014.

Profiling the ToxCast Library With a Pluripotent Human (H9) Stem Cell Line-Based Biomarker Assay for Developmental Toxicity

Todd J. Zurlinden[*], Katerine S. Saili[*], Nathaniel Rush[*], Parth Kothiya[*], Richard S. Judson[*], Keith A. Houck[*], E. Sidney Hunter[†], Nancy C. Baker[‡], Jessica A. Palmer[§], Russell S. Thomas[*], Thomas B. Knudsen[*,1]

[*]National Center for Computational Toxicology (NCCT)

[†]National Health and Environmental Effects Research Laboratory (NHEERL), Office of Research and Development (ORD), U.S. Environmental Protection Agency (USEPA), Research Triangle Park, North Carolina 27711

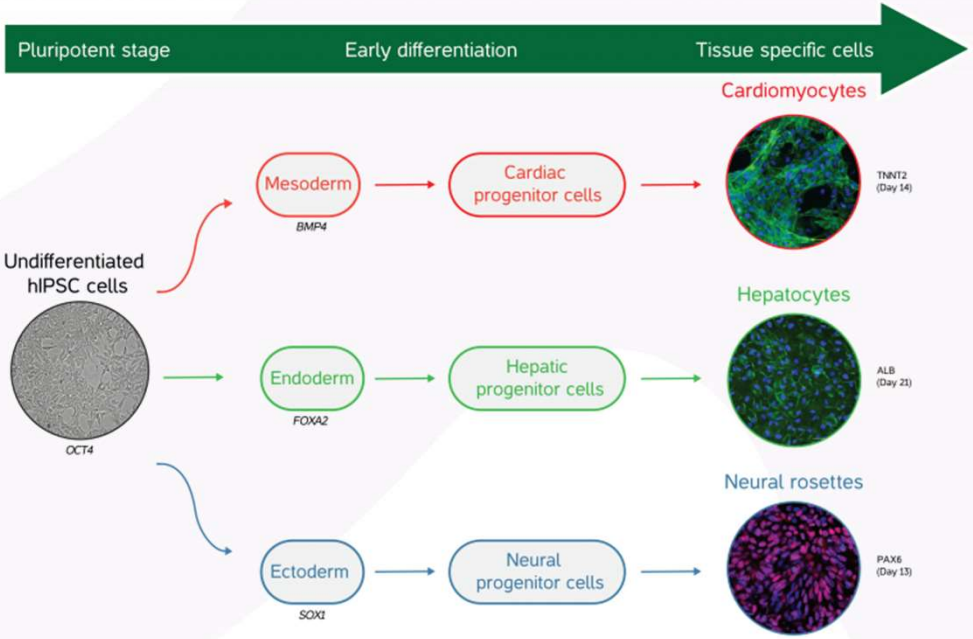
[‡]Leidos, Research Triangle Park, North Carolina 27711

[§]Stemina Biomarker Discovery, Inc, Madison, Wisconsin 53719

- **1065 chemicals tested, 19% showed a positive biomarker response**
- **biomarker performance in general reached accuracies of 79% to 82% with excellent to outstanding specificity (> 84%) but modest sensitivity (< 67%) when compared with in vivo animal models of human prenatal developmental toxicity**

summarised from Zurlinden et al., (2020) Toxicol Sci. Apr 1;174(2):189-209

In vitro biological activity characterisation – ReproTracker® assay



Model systems	Model accuracy (%)	References
ReproTracker	85%	A. Jamalpoor et al., submitted., 2021
Mouse EST	78%	A. Seiler et al., 2011
Whole Embryo Culture	68%	K. Augustine-Rauch et al., 2010
Micromass	70%	I. Wilk–Zasadna et al., 2009

Received: 18 November 2021 | Revised: 18 February 2022 | Accepted: 23 February 2022
DOI: 10.1002/bdr2.2001

Birth Defects Research | Society for Birth Defects Research & Prevention | WILEY

RESEARCH ARTICLE

A novel human stem cell-based biomarker assay for in vitro assessment of developmental toxicity

Amer Jamalpoor | **Sabine Hartvelt** | **Myrto Dimopoulou** | **Tom Zwetsloot** | **Inger Brandsma** | **Peter I. Racz** | **Torben Osterlund** | **Giel Hendriks**

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Funding information
EIT Health, Grant/Award Number: HS-2016-BENE-03; Netherlands Enterprise Agency

Abstract

Background: Testing for developmental toxicity according to the current regulatory guidelines requires large numbers of animals, making these tests very resource intensive, time-consuming, and ethically debatable. Over the past decades, several alternative in vitro assays have been developed, but these often suffered from low predictability and the inability to provide a mechanistic understanding of developmental toxicity.

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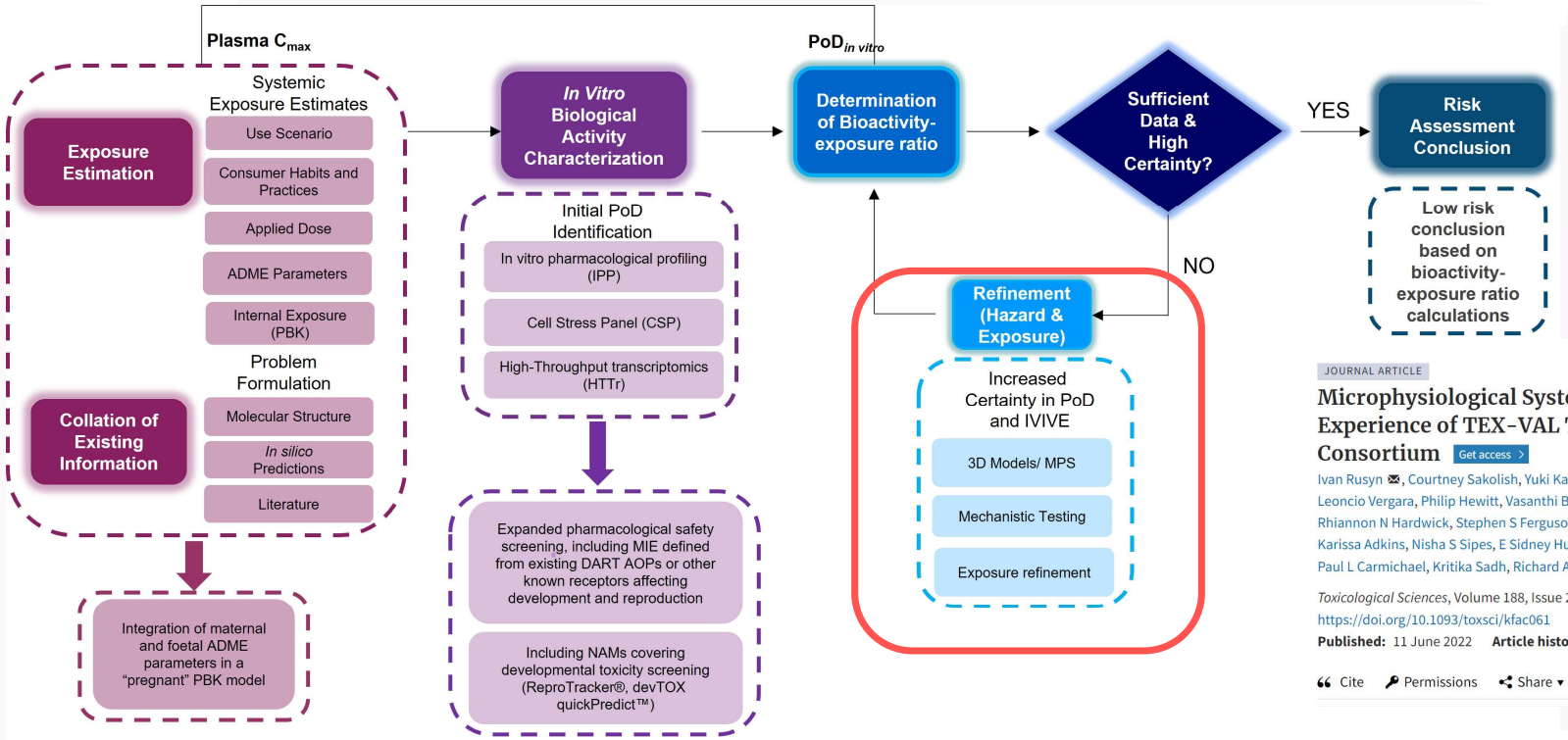
Refinement of Biological Activity and Exposure



- **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

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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

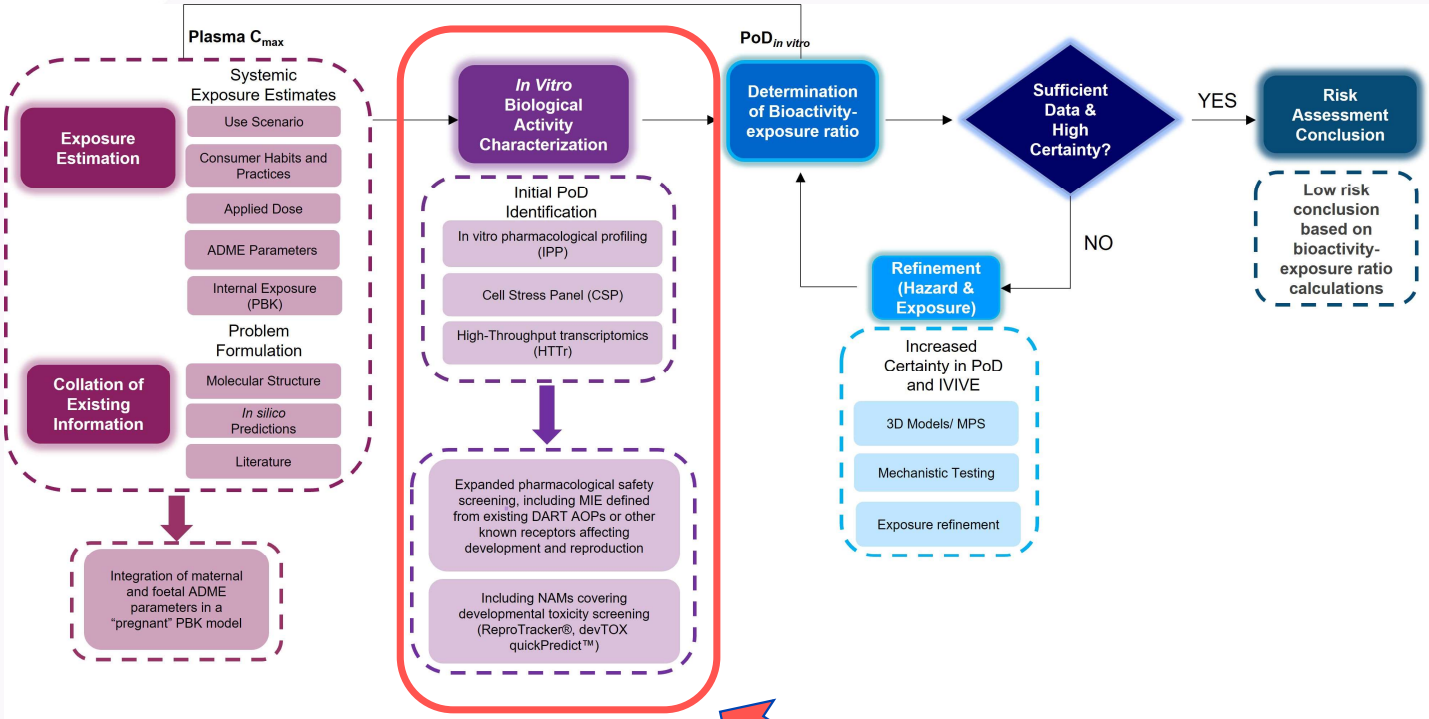
Scientific and Technical Challenges associated with NGRA

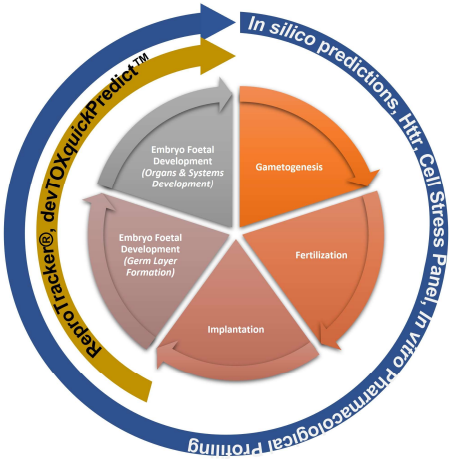
- Metabolic capacity of the framework (cell models, MPS, alginate technology, etc.)
- Short duration exposures and extrapolation to chronic effects
- Complex data interpretation and uncertainty analysis
- Spatio-temporal complexity of developmental and reproductive processes
- Coverage of important cellular and intercellular processes
- Chemical domain of applicability / case studies – need for a flexible and fit for purpose validation
- etc.

Unilever

Biological coverage of the NGRA Framework for DART

What is the biological coverage of the NGRA DART Framework?

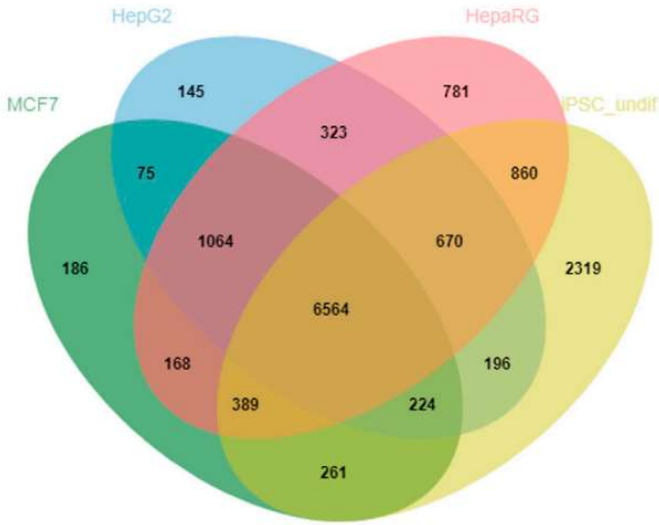


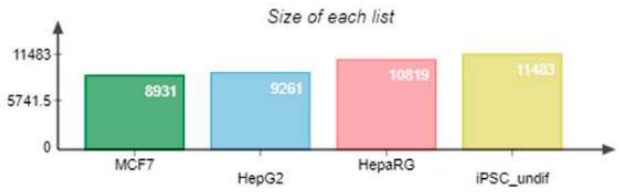


Does the Bioactivity Characterisation cover for important cellular and intercellular processes?

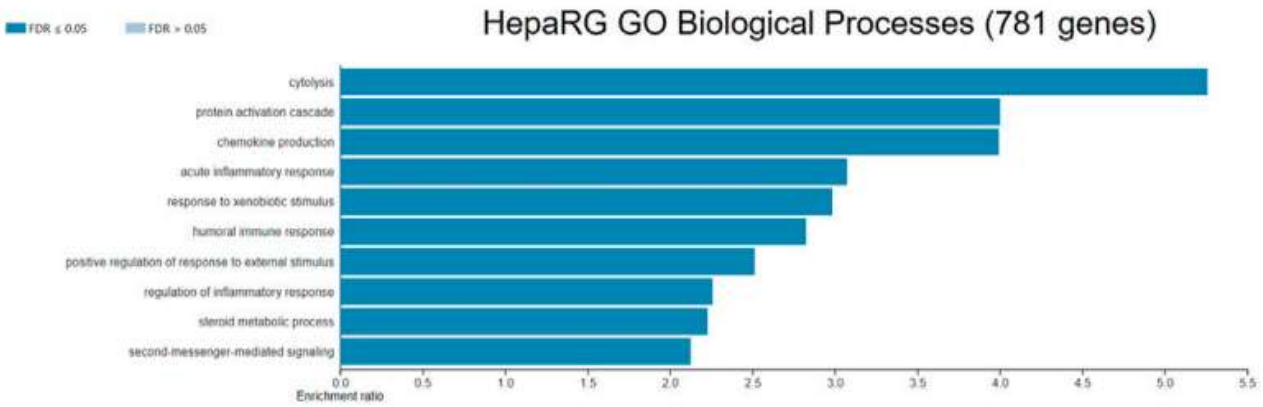
Baseline expression of the cell lines within the NGRA DART

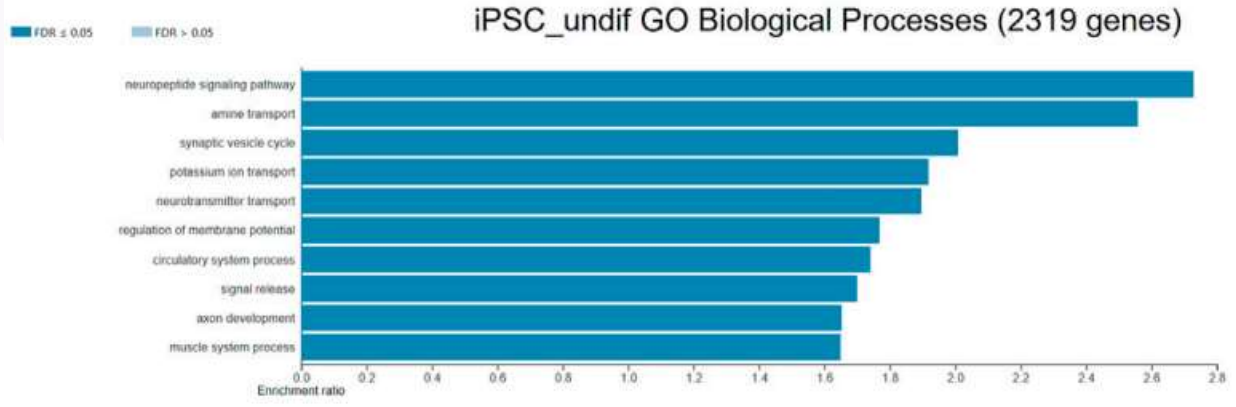
HepG2, MCF-7, HepaRG- Systemic Toolbox
hiPSCs- ReproTracker®, devTOXquickPredict™





14,225 genes in total





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

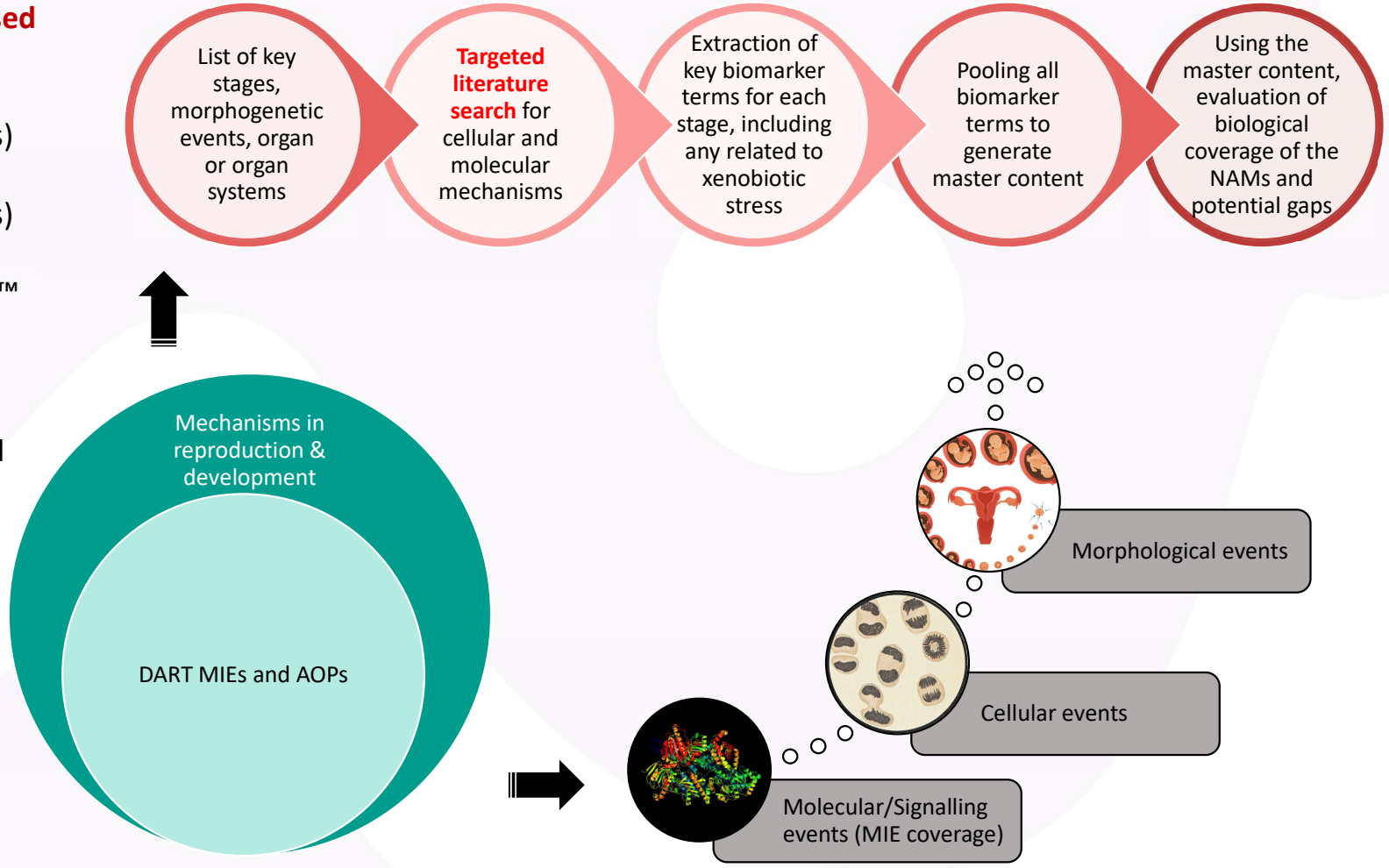
Key Biomarkers for DART - Systematic literature search

Chemicals & assays based approach

- ReproTect (10 chemicals, 14 assays)
- ChemScreen (12 chemicals, 31 assays)
- ReproTracker®
- devTOXquickPredict™
- ToxCast

AOPs based approach

- Eleven DART-related Adverse Outcome Pathways (AOPs) published in 2015
- Over 90 AOPs in AOPWiki related to DART
- Network AOPs



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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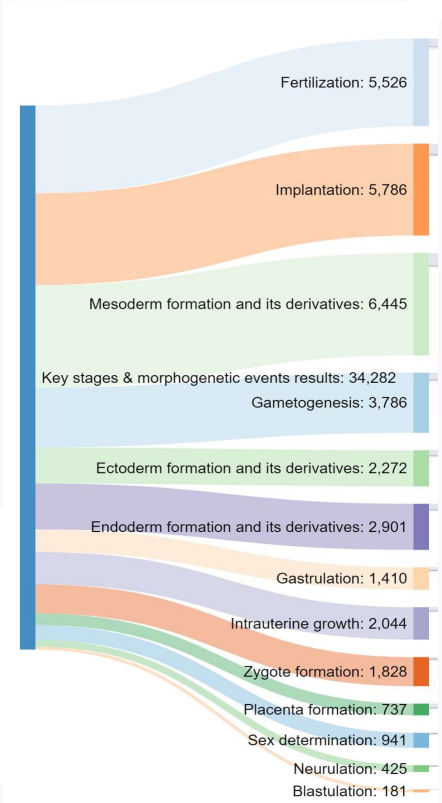
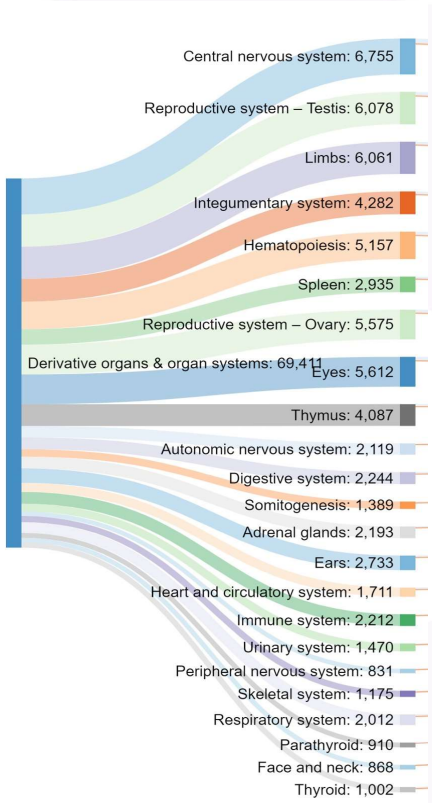
Overview of Literature Search and Extraction of Key Markers Information

Literature search
MeSH Ontology
37 million Articles

Validation and quality check of results; finalising the articles





34,308 articles on key stages and morphogenetic events

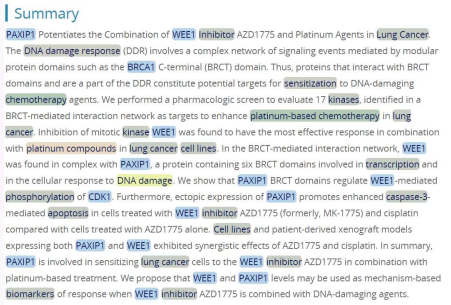
69,299 articles on organs and organ systems development

103,607 total articles

Pooling extractions, Thresholding of hit counts

Semantic enrichment using HGNC, miRNA and biological processes ontologies

Abstracts extracted and collated


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.

Overview of Literature Search and Extraction of Key Markers Information

Download Abstracts of Selected articles

Extract Genes

Vocabulary based on Hugo Gene Nomenclature Committee standard list of genes

Extract Cellular & Molecular Mechanisms

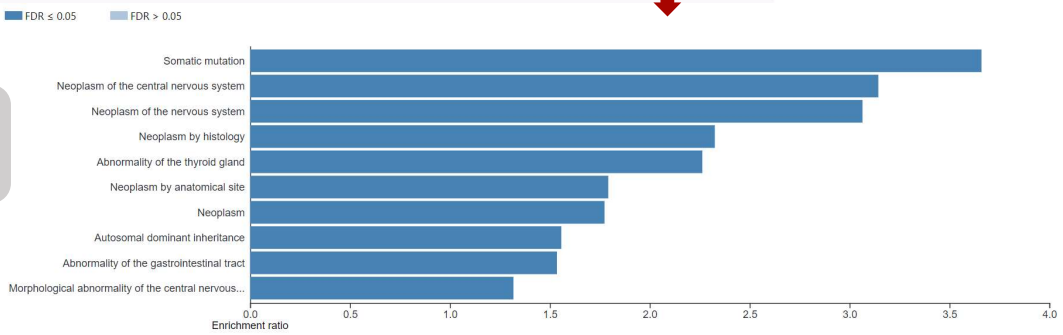
Extract miRNA

A	B	C	D	E	F	G	H	I
PMID	Pub Yr	Title	Authors	Journal		Issue	Pages	
34332630	2021	Brain organoid: a 3D technology for investigating cellu	Agboola OS, Hu X, Shan Z, Wu Y,	Stem cell research & therapy		1	430	
34325647	2021	Activation of microglial GLP-1R in the trigeminal nucle	Jing F, Zou Q, Wang Y, Cai Z, Tang	The journal of headache and pa	10.1	1	86	
34303983	2021	Exposure to cadmium induces neuroinflammation an	Huang Y, Dai Y, Li M, Guo L, Cao	The Science of the total environ	10.101		149043	
34253646	2021	Preclinical Evaluation of the Effects of Trazpiroben (TA	Whiting RL, Choppin A, Luehr G,	The Journal of pharmacology ar	10.1124/			
34249938	2021	The Altered Anatomical Distribution of ACE2 in the Br	Cui H, Su S, Cao Y, Ma C, Qiu W	Frontiers in cell and developme	10.3389/fi		684874	
34162530	2021	Regenerative medicine for neurological diseases-will r	Burns TC, Quinones-Hinojosa A	BMJ (Clinical research ed.)	10.1136/b		955	
34130715	2021	Programmed suppression of oxidative phosphorylatio	Chang RC, Thomas KN, Mehta N	Epigenetics & chromatin	10.1186/s	1	27	
34071978	2021	Brain-Derived Neurotrophic Factor Signaling in the Pa	Numakawa T, Odaka H	International journal of molecul	10.3390/ij	22		
34054129	2021	Biallelic and monoallelic variants in PLXNA1 are implic	Dworschak GC, Punetha J, Kalan	Genetics in medicine : official jo	10.1038/s41436-021-01126			
34040637	2021	Gene Environment Interactions in the Etiology of Neu	Finnell RH, Caiaffa CD, Kim SE, Li	Frontiers in genetics	10.3389/fg	12		
34019717	2021	Neurotrophic factor levels in the serum and cerebrosp	Wang S, Zou F, Wu S, Wu Y, Yue Y	Microbiology and immunology	10.1111/1348-0421.12918			
33946667	2021	Therapeutic Effects of hiPSC-Derived Glial and Neuror	Salikhova D, Bukharova T, Cherki	International journal of molecul	10.3390/ij	22	9	
33917816	2021	Potential Roles of the WNT Signaling Pathway in Amyc	Jiang X, Guan Y, Zhao Z, Meng F,	Cells	10.3390/c	10	4	
33804711	2021	Investigating Primary Cilia during Peripheral Nervous	Yusifov E, Dumoulin A, Stoecklii E	International journal of molecul	10.3390/ij	22	6	
33803024	2021	Microglia Development and Maturation and its Implic	Wurm J, Konttinen H, Andressen	International journal of molecul	10.3390/ij	22	6	
33801158	2021	Involvement of Bcl-xL in Neuronal Function and Devel	Bas J, Nguyen T, Gillet G	International journal of molecul	10.3390/ij	22	6	
33766226	2021	[Insulin-like growth factor 1 (IGF-1) promotes phagocy	Zhai L, Chen X, Lu S, Yang D, Li W	Xi bao yu fen zi mian yi xue za zl		37	3	199-204
33765252	2021	Neurospheres: a potential in vitro model for the study	da Silva Siqueira L, Majolo F, da	Molecular biology reports	10.1007/s	48	4	3649-3663
33734615	2021	Brain imaging features of children with Hoyeraal-Hreid	Zhang MJ, Cao YX, Wu HY, Li HH	Brain and behavior	10.1002/b	11	5	e02079
33727946	2021	The Neuroprotective Effect of Byu d Mar 25 in LPS-ind	Liu L, Zhang Y, Tang L, Zhong H,	Evidence-based complementary	10.1155/2	2021		8879014
33722788	2021	Berberine-loaded M2 macrophage-derived exosomes	Gao ZS, Zhang CJ, Xia N, Tian H,	Acta biomaterialia	10.1016/j	126		211-223
33705794	2021	Adult astrocytes from reptiles are resistant to proinfla	Du N, Li H, Sun C, He B, Yang T, S	The Journal of biological chemis	10.1016/j	296		100527
33679718	2020	Different Functions of Recombinantly Expressed Dom	Bijelić D, Adžić M, Perić M, Jakov	Frontiers in immunology	10.3389/fi	11		624612
33677027	2021	Early life stress exposure worsens adult remote micro	Catale C, Bisicchia E, Carola V, Vi	Brain, behavior, and immunity	10.1016/j	94		89-103
33670341	2021	Linear Skin Defects with Multiple Congenital Anomalii	Indrieri A, Franco B	Genes	10.3390/g	12	2	

CNS - 6757 Abstracts

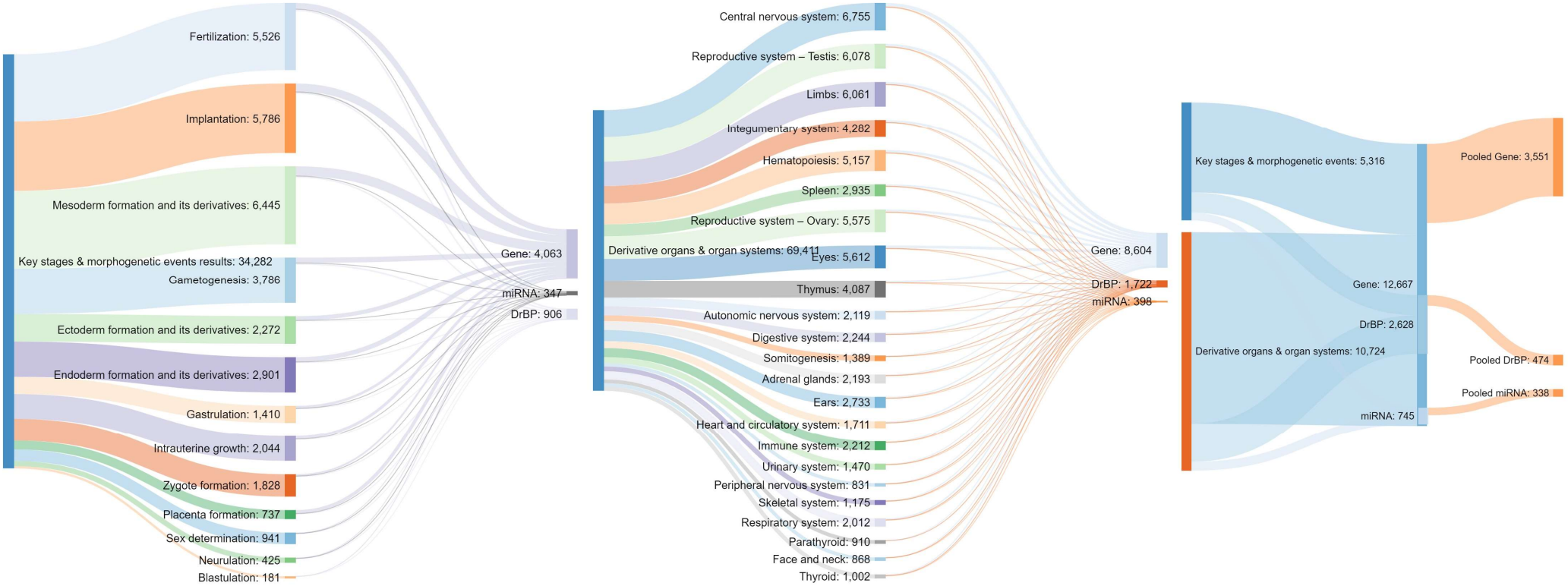
	A	B	C
1	Gene symbol	Name	HitCount
2	GFAP	glial fibrillary acidic protein	554
3	SHH	sonic hedgehog	505
4	WNT1	Wnt family member 1	441
5	BDNF	brain derived neurotrophic factor	379
6	AQP1	aquaporin 1 (Colton blood group)	360
7	NES	nestin	346
8	FGF2	fibroblast growth factor 2	345
9	IGF1	insulin like growth factor 1	341
10	GNRH1	gonadotropin releasing hormone 1	334
11	TH	tyrosine hydroxylase	329
12	NGF	nerve growth factor	327
13	CSPG4	chondroitin sulfate proteoglycan 4	295
14	MBP	myelin basic protein	294
15	PAX6	paired box 6	288
16	TGFB1	transforming growth factor beta 1	267
17	EGF	epidermal growth factor	232
18	TP53	tumor protein p53	193
19	INS	insulin	189
20	INSR	insulin receptor	186
21	NCAM1	neural cell adhesion molecule 1	182
22	TNF	tumor necrosis factor	180
23	CDKN2A	cyclin dependent kinase inhibitor 2A	176
24	APP	amyloid beta precursor protein	173
25	TNC	tenascin C	166
26	OLIG2	oligodendrocyte transcription factor 2	163
27	SOX2	SRY-box 2	163
28	CTNNB1	catenin beta 1	158
29	IL6	interleukin 6	154
30	RTN4	reticulon 4	142
31	VIP	vasoactive intestinal peptide	142

902 genes



Human Phenotype Ontology

Pooled List of DARS biomarkers



Rajagopal et al., Front. Toxicol., 2022

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

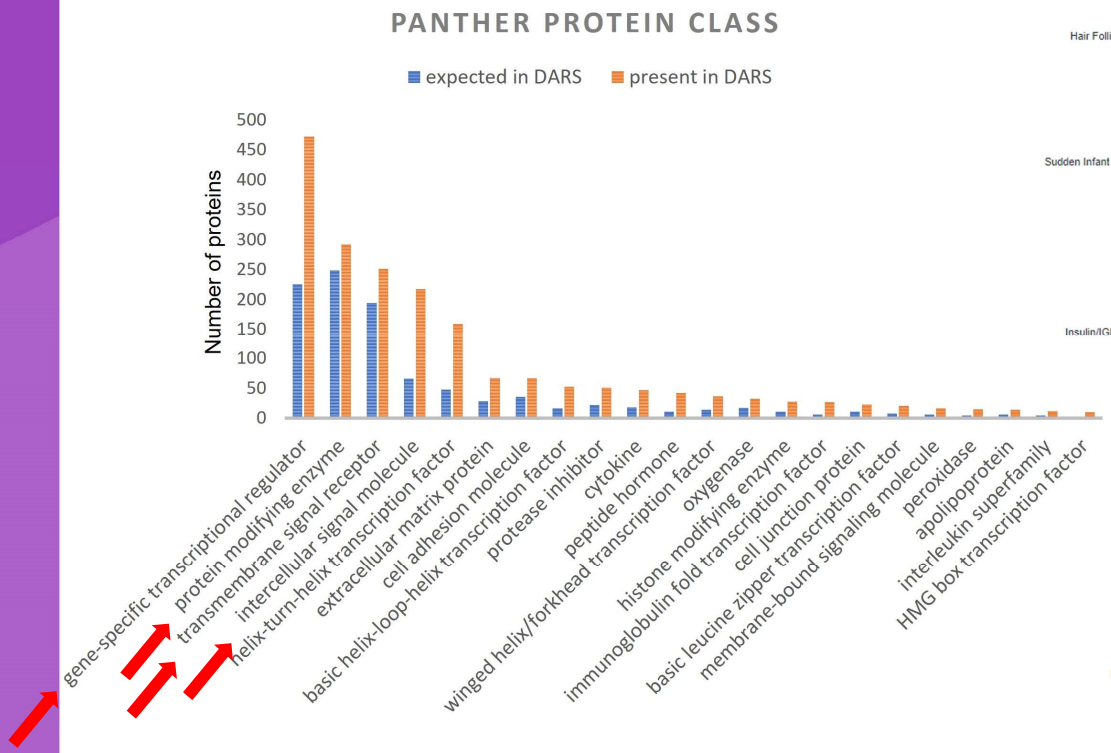
338 DARS miRNA

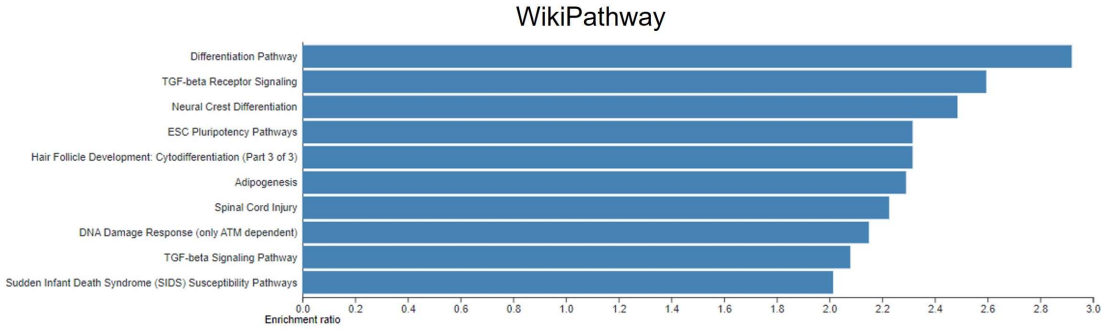
	A	B
1	HitID	HitCount
2	LET7	155
3	MIR-21	127
4	MIR-145	85
5	MIR-125B	73
6	MIR-17	73
7	MIR-17-92	65
8	MIR-1	64
9	MIR-302	62
10	MIR-124	56
11	MIR-29B	55
12	MIR-34C	52
13	MIR-34A	51
14	MIR-130B	51
15	MIR-375	49
16	MIR-200C	46
17	MIR-24	45
18	MIR-29A	44
19	MIR-429	41
20	MIR-223	41

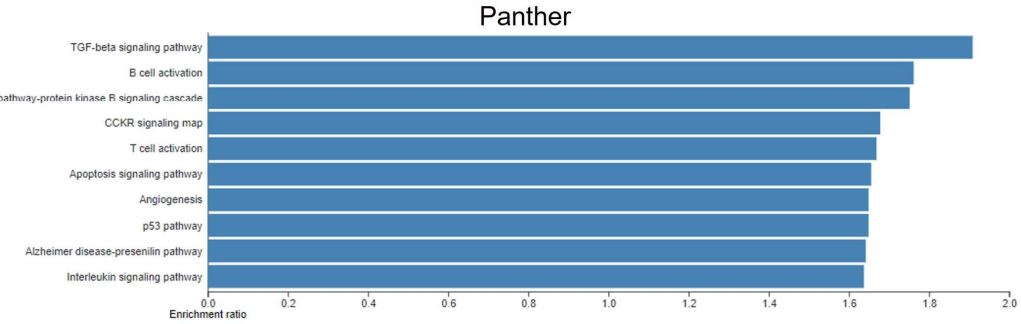
Unilever

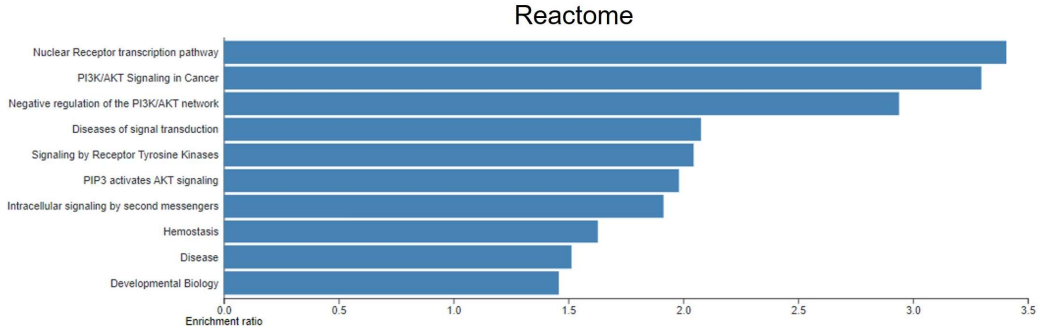
Rajagopal et al., Front. Toxicol., 2022

Protein classes and signalling pathways over-represented in DARS biomarkers







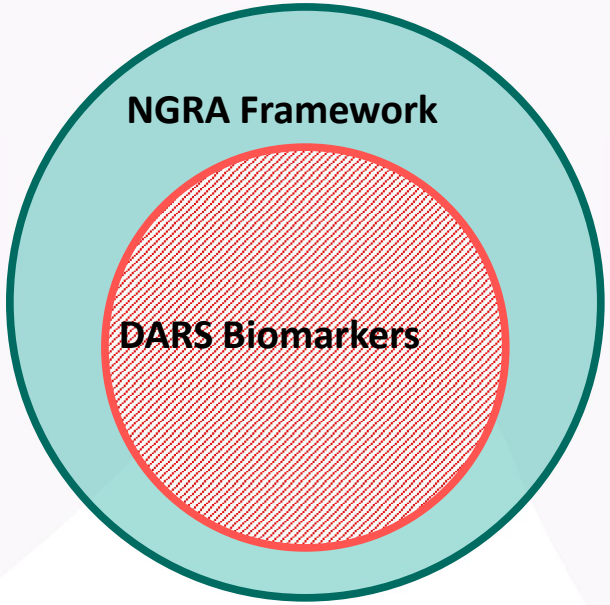


DARS BP: Signalling, cell cycle, cell death, DNA methylation, epithelial to mesenchymal transition, phosphorylation, cell differentiation, cell development, oocyte maturation and neurogenesis

DARS miRNA: LET-7, MIR-21 and MIR-145

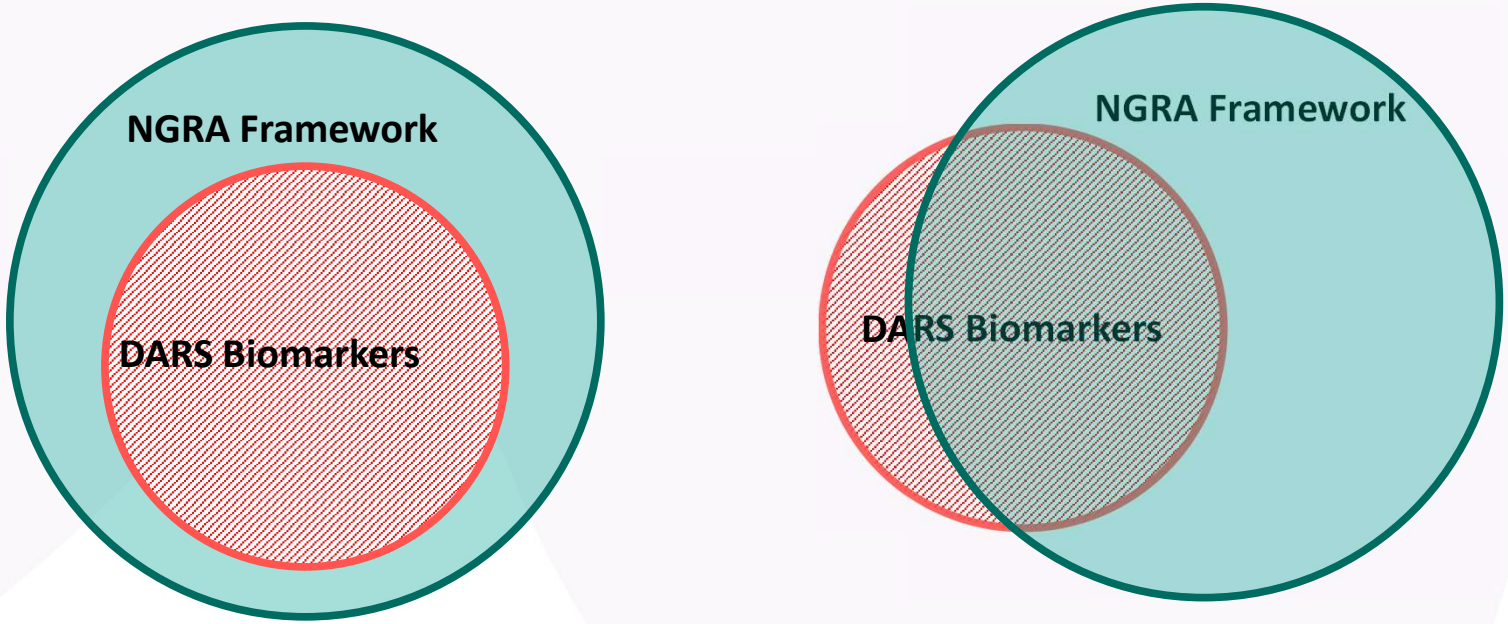
Rajagopal et al., Front. Toxicol., 2022

Biological coverage of the DARS biomarkers by the DART NGRA



Expectation

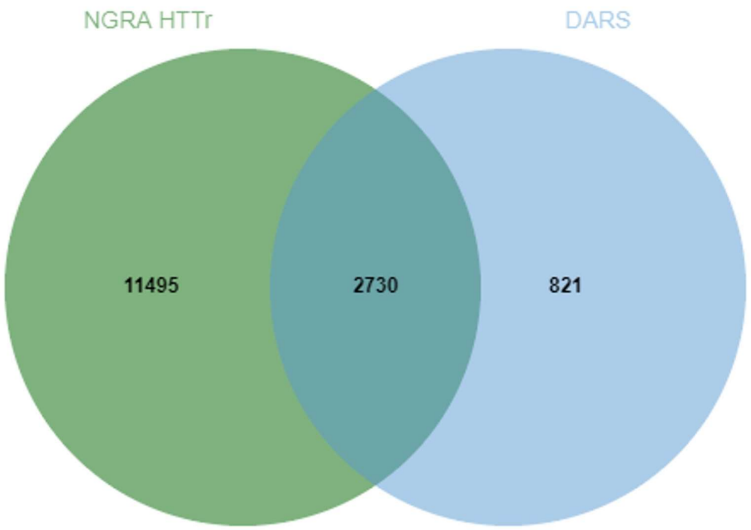
Biological coverage of the DARS biomarkers by the DART NGRA

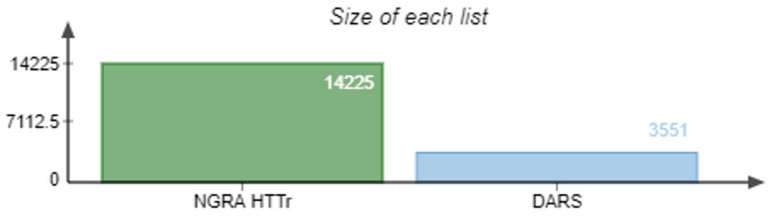


Expectation *versus* Reality

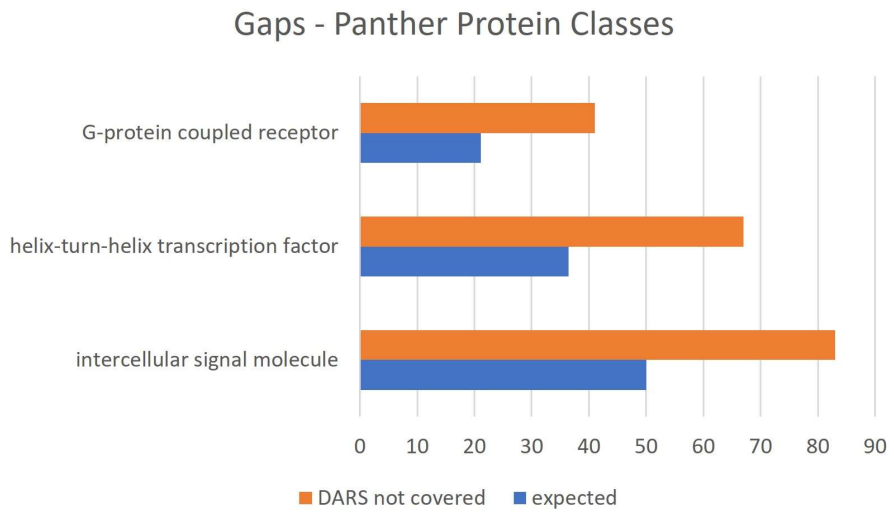
Biological coverage of the DARS biomarkers by the DART NGRA

Coverage





Gaps



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

Rajagopal et al., Front. Toxicol., 2022

Biological coverage of the DARS biomarkers by the DART NGRA

Coverage

General cellular & functional processes- cell survival, cytotoxicity

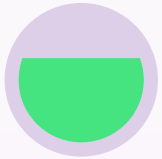
Receptor or enzyme activity- IPP covers about 13%

Signalling pathways- DARS genes

Specific differentiation- ReproTracker®

Specific cellular processes- devTOXQuickPredict™

Cellular stress- Cell stress panel assays

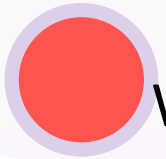


Gaps

Specific cellular processes- E.g. cytokine secretion or myelination or androgen biosynthesis

Specific functional processes- E.g. sperm motility or axon guidance or lymphocyte migration

Receptor or enzyme activity- E.g. receptor tyrosine kinases or receptor serine/threonine kinases or GPCRs



Weight of evidence

Integrating data from different NAMs

MIE -> KEs -> Adverse effects E.g. ADORA 2A binding, inhibition of PI3Kinase-AKT signalling, T cell development

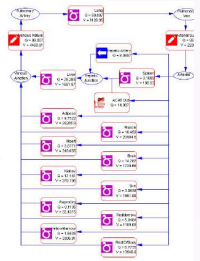
Rajagopal et al., Front. Toxicol., 2022

Case studies - flexible and fit for purpose validation of

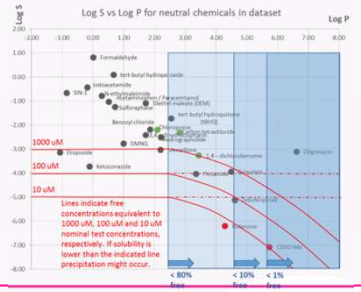
NGRA DART

DART NGRA Framework evaluation – decision making

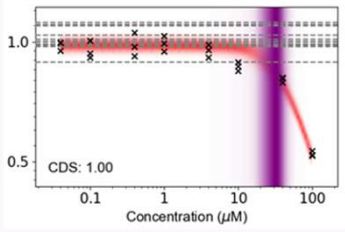
PBK models



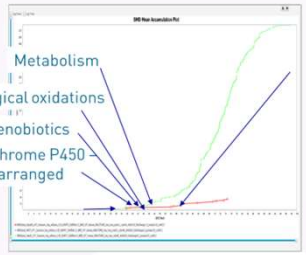
Free concentration



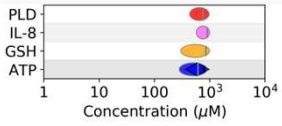
Conc. Resp. models



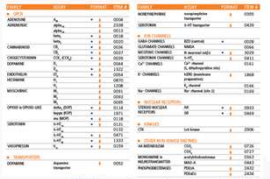
HTTr

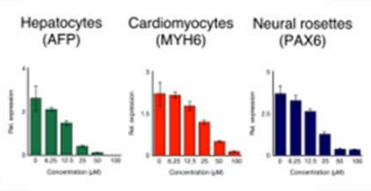


CSP

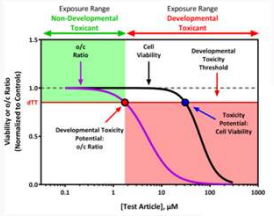


IPP+



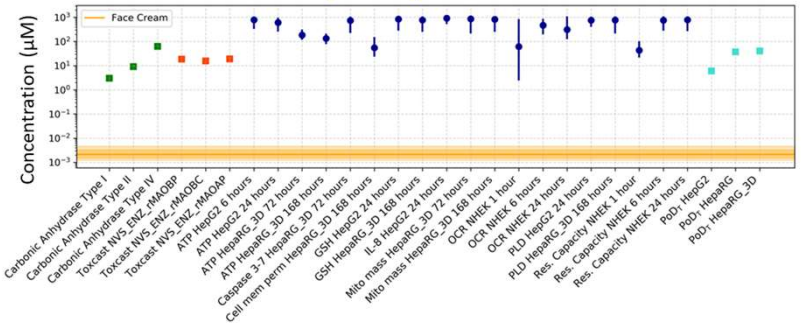


ReproTracker



devTox quickPredict™

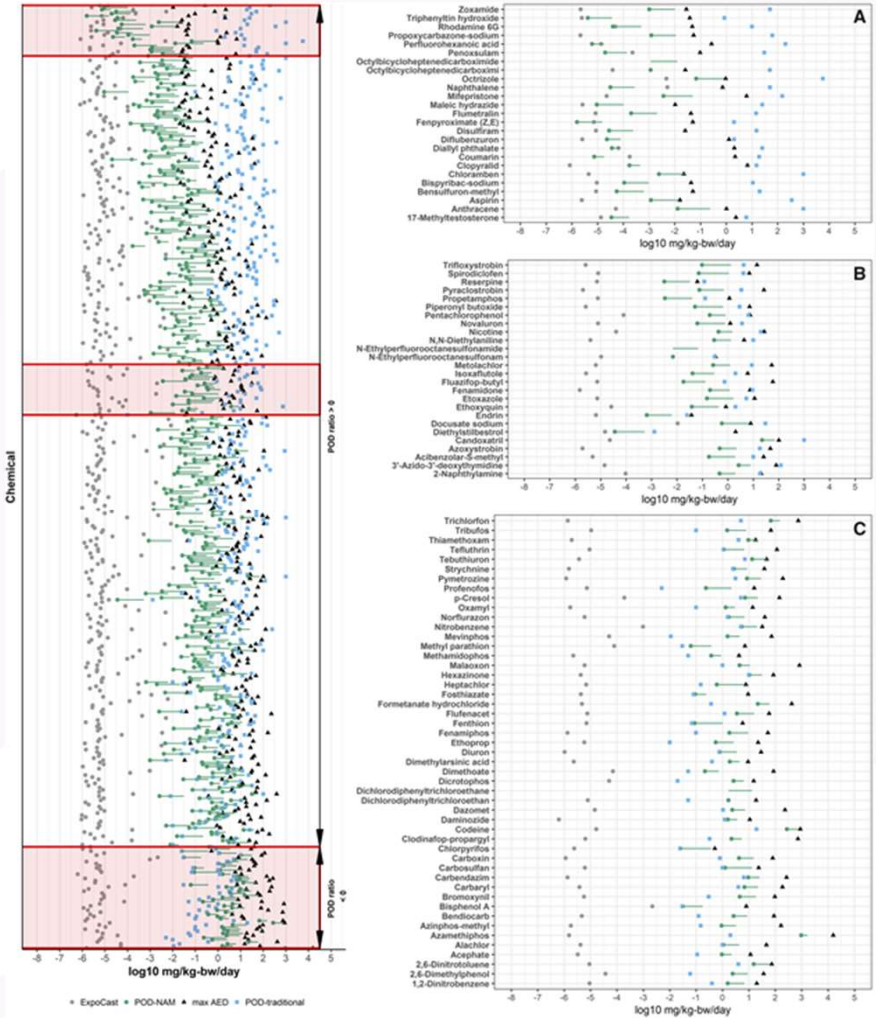
Bioactivity-Exposure Ratio



Inform safety decision

HTTr: High-throughput transcriptomics **CSP:** Cell Stress Panel **IPP:** In vitro pharmacological profiling

How PODs from NAMs compare to PODs coming from animal studies
-including chronic, developmental/reproductive studies



448 chemicals

APCRA
ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

"The primary conclusion of our work is that for 89% of the chemicals in this case study, the HTS approach to derivation of a $POD_{NAM,\ 95}$ for screening and prioritization purposes produced a value less than or equal to the $POD_{traditional}$ from *in vivo* toxicology studies."

Toxicol Sci, Volume 173, Issue 1, January 2020, Pages 202–225, https://doi.org/10.1093/toxsci/kfz201

Unilever

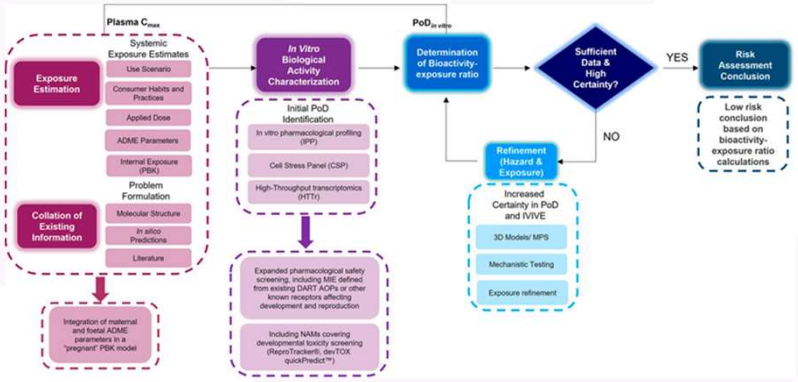
How PODs from NAMs compare to PODs coming from animal studies

"The purpose of this SciAD is to demonstrate that PODBioactivity can provide a lower bound estimate for in vivo based effect levels derived from oral repeat-dose, developmental, and reproductive studies considered under the Chemicals Management Plan (CMP). The PODBioactivity was lower than the lowest PODTraditional cited in the risk assessment for 43 of the 46 of the chemicals examined. These findings are consistent with other published case studies using similar methodology. This was done to demonstrate confidence in using in vitro bioactivity as a surrogate lower bound estimate of in vivo adverse effect levels." **From Health Canada**

Unilever

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

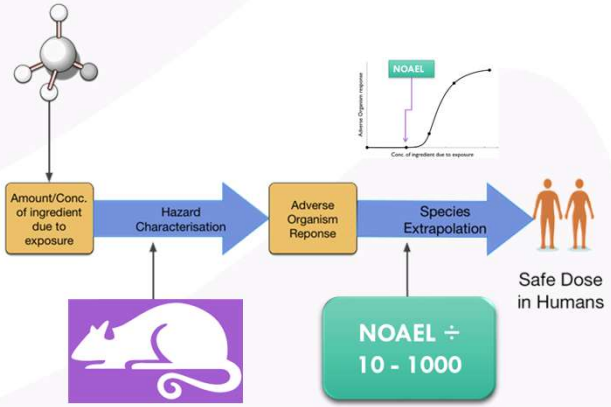
- Identification and filling of existing gaps (PBPK modelling e.g placenta transfer measurements, DNT, DIT, endocrine disruptors, multigenerational effects, studying epigenetics in germline development, advanced cell models for refinement)
- CLP/GHS hazard classification
- Use for regulatory purposes (REACH submission)

Unilever

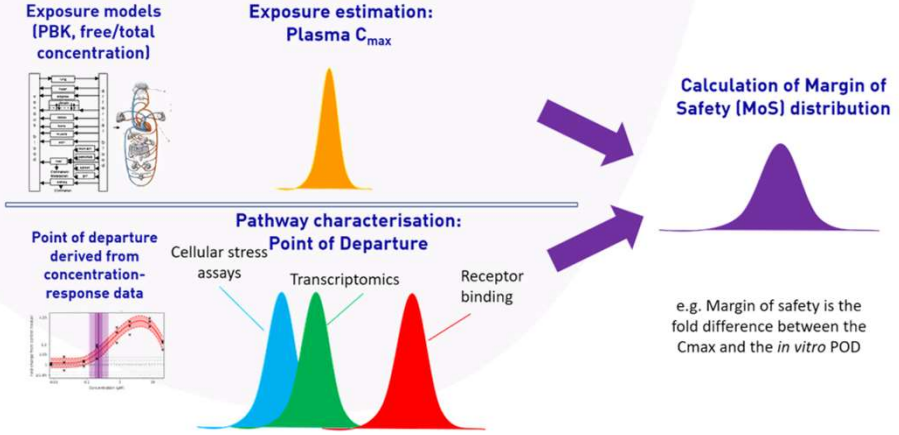
Conclusions – Regulatory challenge: Meeting requirements of REACH without compromising our stance on non-animal testing

- A paradigm shift is underway as use of non-animal safety science increases & safety assessment frameworks evolve to embed NAMs & NGRA
- Translation of NGRA concepts into chemical regulatory frameworks, strategic plans & guidance is moving forward steadily but needs to accelerate
- Plans to address current data gaps in REACH dossiers using non-animal approaches based on the use of NAMs and NGRA

'Traditional' Risk Assessment

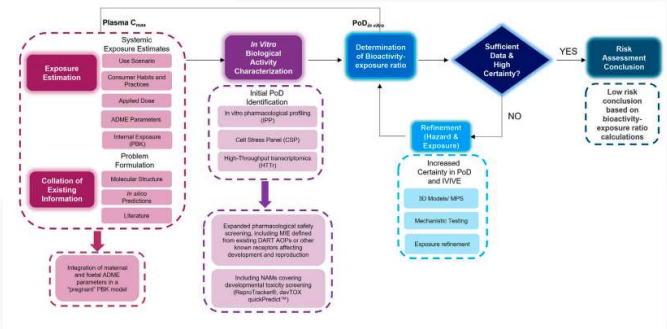


'Next Generation' Risk Assessment



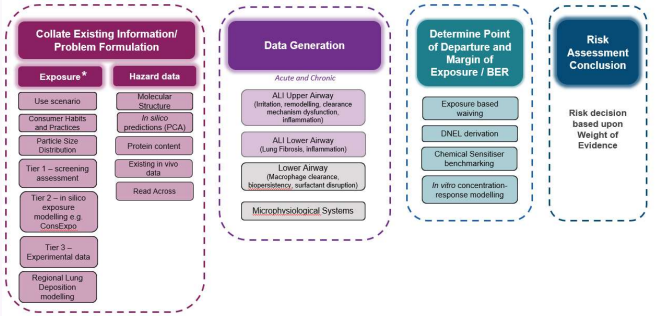
Unilever NGRA frameworks for Consumer Safety decisions

Developmental & Reproductive



Rajagopal et al (2022) *Frontiers in Toxicology*, doi: 10.3389/ftox.2022.838466

Inhalation



Ongoing Evaluations

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EPA and Unilever Announce Major Research Collaboration to Advance Non-animal Approaches for Chemical Risk Assessment

August 19, 2021

Contact Information
EPA Press Office (press@epa.gov)

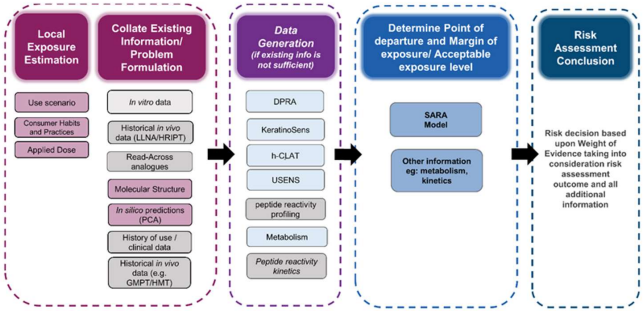
WASHINGTON – Today, the U.S. Environmental Protection Agency (EPA) and Unilever announced a collaborative agreement to explore better ways to assess chemical risks associated with consumer products. This agreement builds on prior cooperation between EPA and Unilever regarding New Approach Methods (NAMs), which are a promising alternative to conventional toxicity testing that are intended to reduce reliance on the use of animals.

EPA and Unilever have been jointly evaluating and using NAMs since 2015. This collaboration is helping EPA implement its New Approach Methods Work Plan and is the foundation for new efforts to demonstrate that these novel approaches can help decision makers better protect consumers, workers and the environment.

"EPA is a pioneer in developing and applying NAMs to identify and quantify risks to human health, while reducing the use of animals in chemical toxicity testing," said **H. Christopher Frey, Deputy Assistant Administrator for Science Policy in EPA's Office of Research and Development**. "We are excited to continue the collaboration with Unilever, which enhances the robustness of our mutual research to demonstrate the use of NAMs."

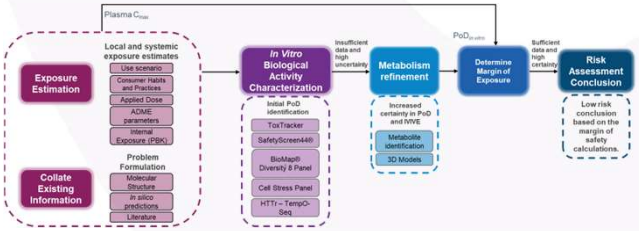
The new collaborative effort aims to establish a framework for the Next Generation of Risk Assessments based on NAMs. Such assessments are intended to quantify health risks to humans with sufficient scientific rigor to replace conventional animal-based methods and to support EPA's mission to protect human health and the environment.

Skin Sensitisation



Reynolds et al (2021) *Reg Tox Pharmacol*, **127**, 105075

Systemic



Baltazar et al (2020) *Toxicol Sci*, 176, 236-252

National Toxicology Program
U.S. Department of Health and Human Services

NICEATM News - 2021 Issue 25: May 27

In this Newsletter:

NICEATM to Collaborate with Unilever on Development of Predictive Model for Skin Sensitization

NICEATM to Collaborate with Unilever on Development of Predictive Model for Skin Sensitization

NICEATM has entered into an agreement with consumer products company Unilever to collaboratively test and further develop their Skin Allergy Risk Assessment (SARA) predictive model. SARA is a computational model that uses a variety of input data to estimate a probability that a chemical will cause an allergic skin reaction in humans. NICEATM will test the SARA model using a variety of chemical data sets, including chemicals of interest to U.S. and international regulatory agencies. NICEATM and Unilever will also work together to expand the SARA model to include data generated by NICEATM. The intent is to make the SARA model openly available for public use along with other NICEATM predictive models. Availability of the SARA model will help further reduce animal use for the endpoint of skin sensitization, and will improve upon existing efforts by providing points of departure for quantitative human risk assessment.

Information about other NICEATM projects to evaluate alternatives to animal use for skin sensitization is available at https://ntp.niehs.nih.gov/go/ACDtest.

Reference: Reynolds et al. Probabilistic prediction of human skin sensitizer potency for use in next generation risk assessment. Comput Toxiol 9:36-49. https://doi.org/10.1016/j.comtox.2018.10.004

Unilever

SEAC SAFETY & ENVIRONMENTAL ASSURANCE CENTRE
Scientific Excellence And Collaboration

OECD

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