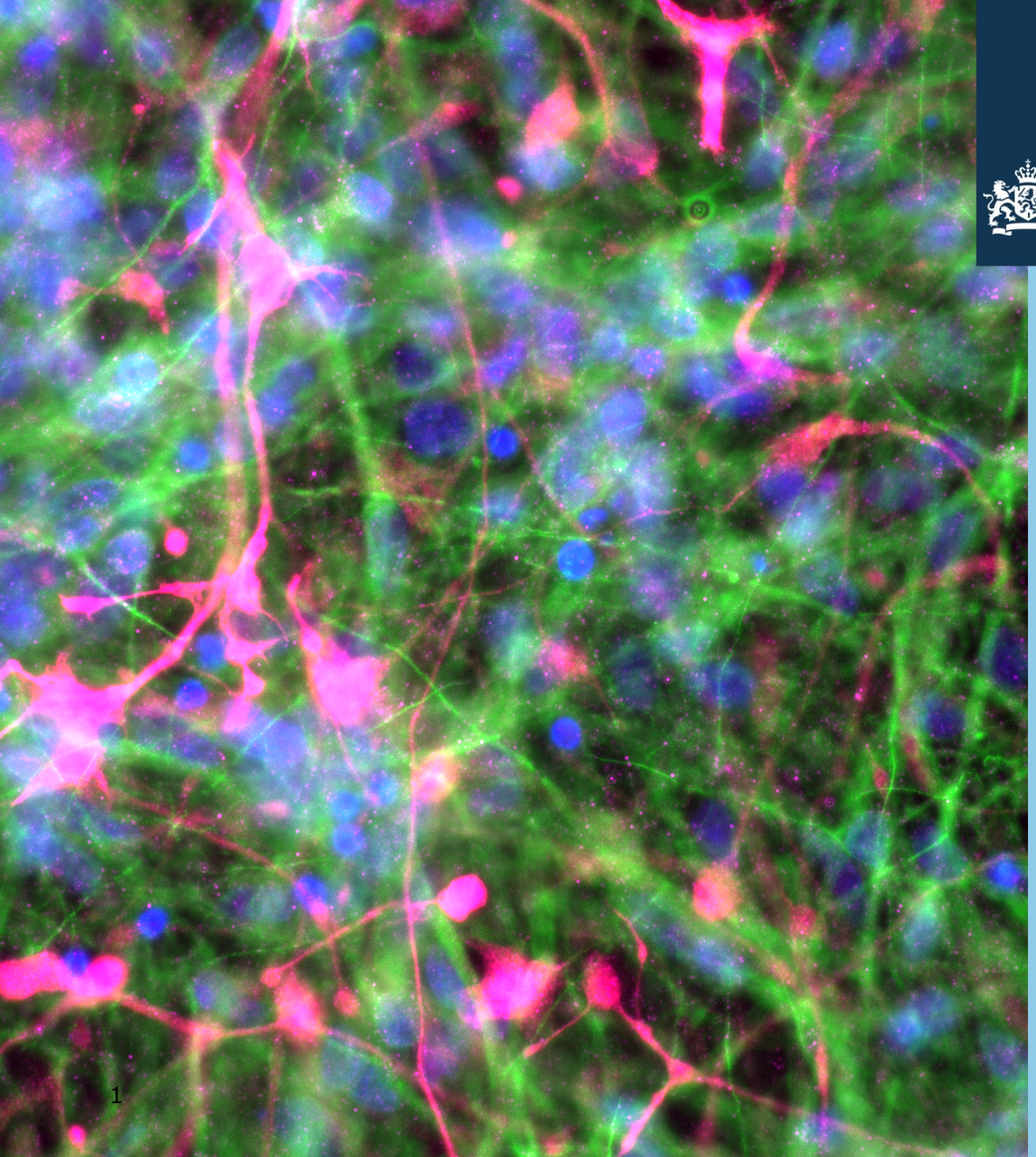


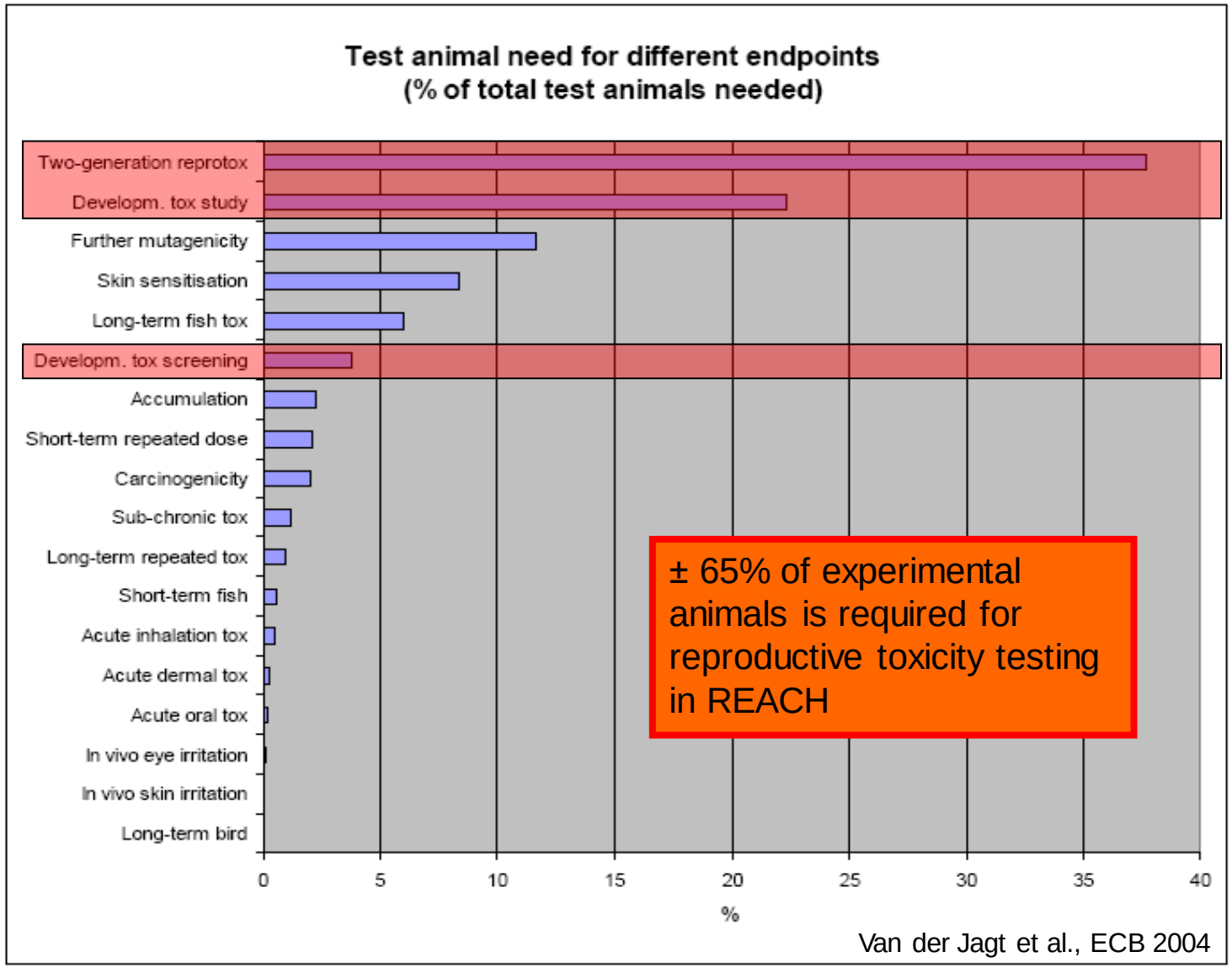
National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Reproductive Toxicology and the road to Animal-free Human Hazard and Risk Assessment

Aldert H. Piersma

Center for Health Protection
RIVM Bilthoven-NL

ECHA NAM Workshop, Helsinki, 31 May 2023



Van der Jagt et al., ECB 2004

~~OECD416~~ → OECD443
OECD414

OECD421

Current issues with reproductive toxicity testing

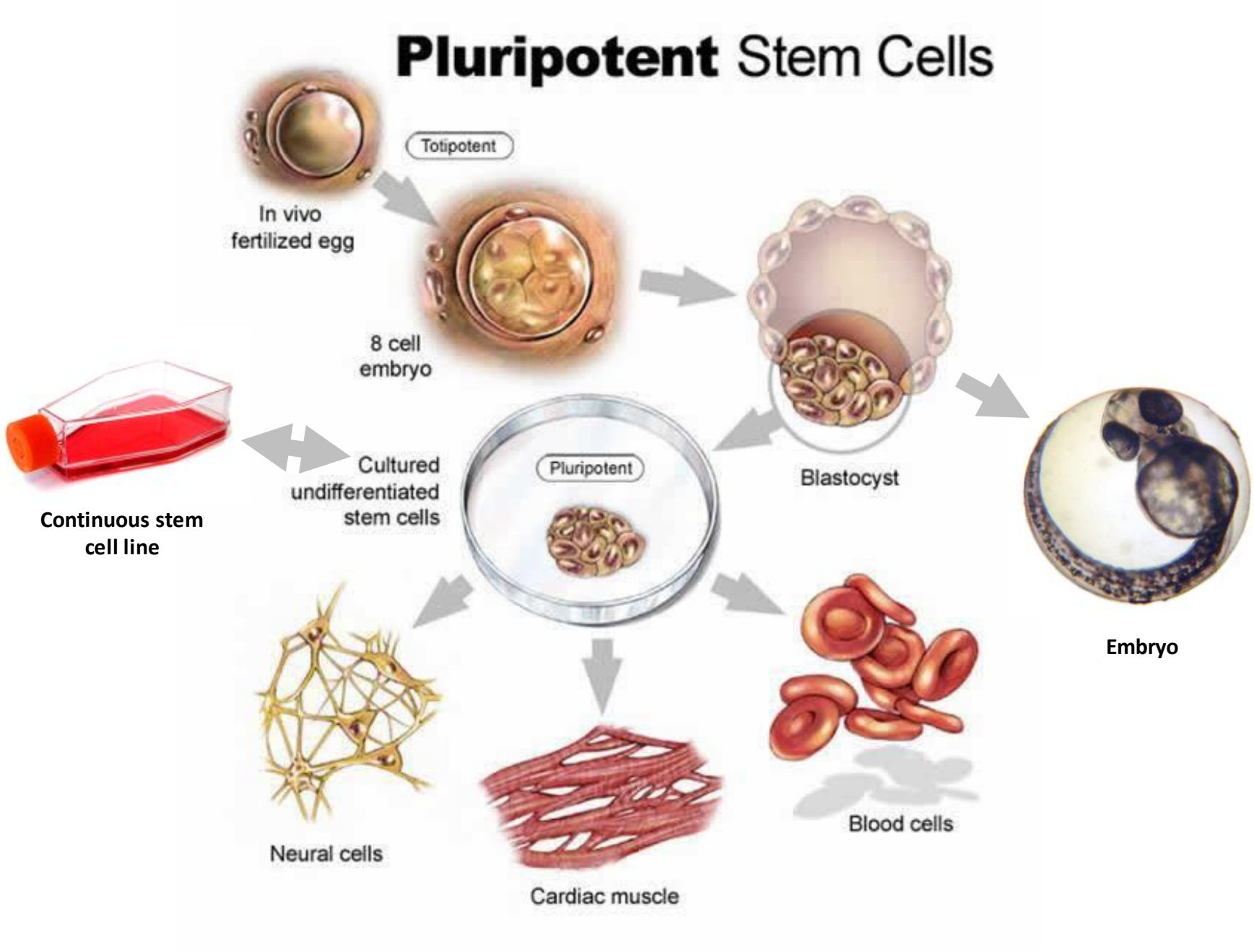
- The triggers for generating a second-generation offspring in OECD443
- The requirement for an OECD443 after observing thyroid changes in other studies
- The need to study endocrine disruption after observing liver enzyme induction effects
- The requirement for extreme high dosing for less potent compounds
- The need to accept extreme general toxicity in parental animals in order to find a LOAEL for fertility or developmental effects

Underlying issues:

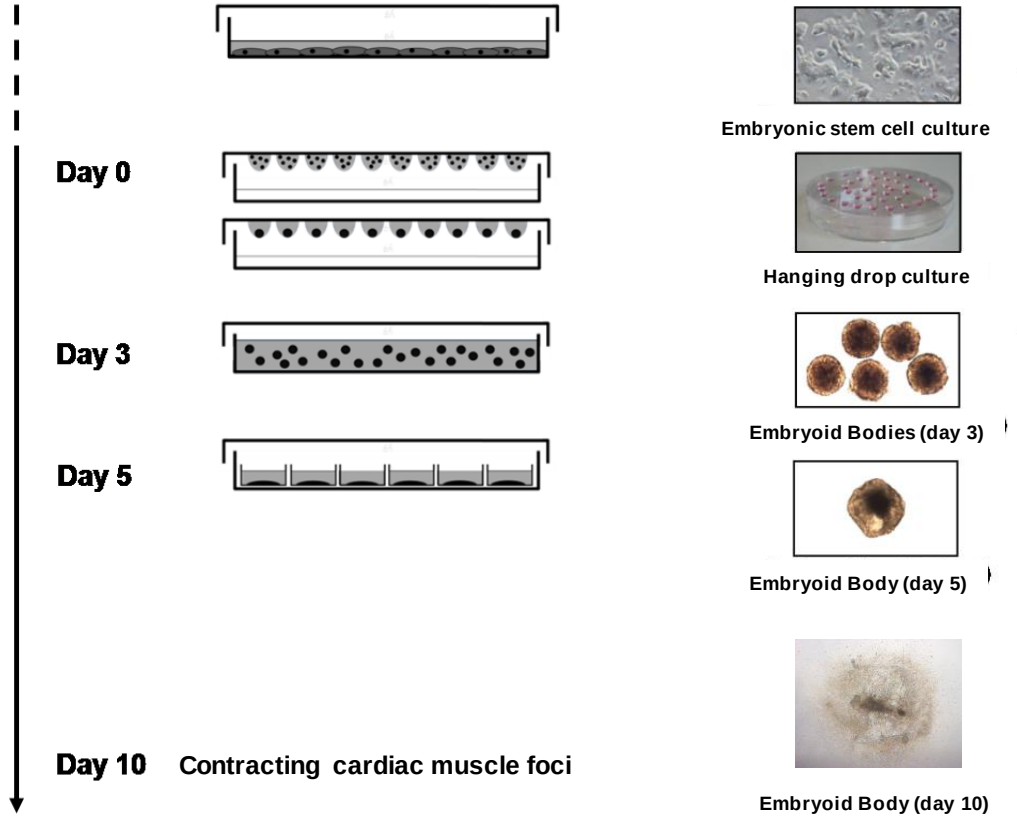
- Executing risk management based on hazard identification only, without consideration of realistic exposure scenarios and compound potency
- The tendency to consider changes in physiological parameters as an indication of adversity
- Distinguishing adaptive homeostatic changes from adverse health effects
- The ethical limits of animal welfare and suffering

Classic List of Alternatives in Developmental Toxicology

type of test	test	end points
continuous cell lines	HEPM	proliferation
	MOT	adhesion
	V79	metabolic cooperation
	N115	differentiation
	EC/EST	differentiation
primary cell cultures	brain	differentiation
	limb bud	differentiation
organ cultures	limb bud	development
embryo cultures	Hydra	regeneration
	frog	development
	rodent	development


Pluripotent Stem Cells
Totipotent
In vivo fertilized egg
8 cell embryo
Blastocyst
Embryo
Pluripotent
Cultured undifferentiated stem cells
Continuous stem cell line
Neural cells
Cardiac muscle
Blood cells

Embryonic stem cell test (cardiac)



Van Dartel et al., 2009

ATLA 30, 151–176, 2002 151

The ECVAM International Validation Study on *In Vitro*

EST ~80% predictivity

ATLA 37, 313–328, 2009 313

A Review of the Implementation of the Embryonic Stem Cell Test (EST)

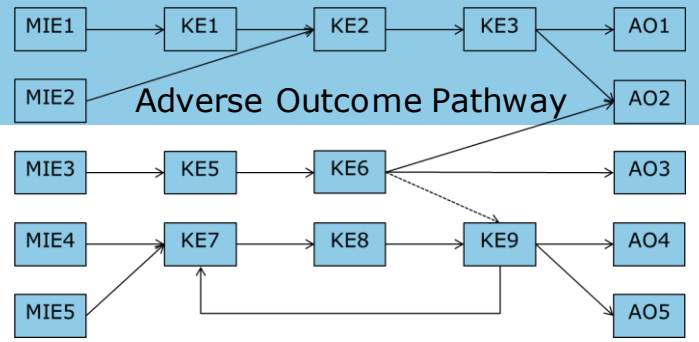
The report and recommendations of an ECVAM/ReProTect Workshop[a]

Philip Marx-Stoelting,[1b] Els Adriaens,[2] Hans-Jürgen Ahr,[3] Susanne Bremer,[4] Bernward Garthoff,[5] Heinz-Peter Gelbke,[6] Aldert Piersma,[7] Cristian Pellizzer,[4] Ulrike Reuter,[8] Vera Rogiers,[9] Barbara Schenk,[1] Silke Schwengberg,[10] Andrea Seiler,[11] Horst Spielmann,[11] Margino Steemans,[12] Donald B. Stedman,[13] Philippe Vanparys,[14] Joan Albert Vericat,[15] Miriam Verwei,[16] Femke van de Water,[17] Marc Weimer[18] and Michael Schwarz[1]

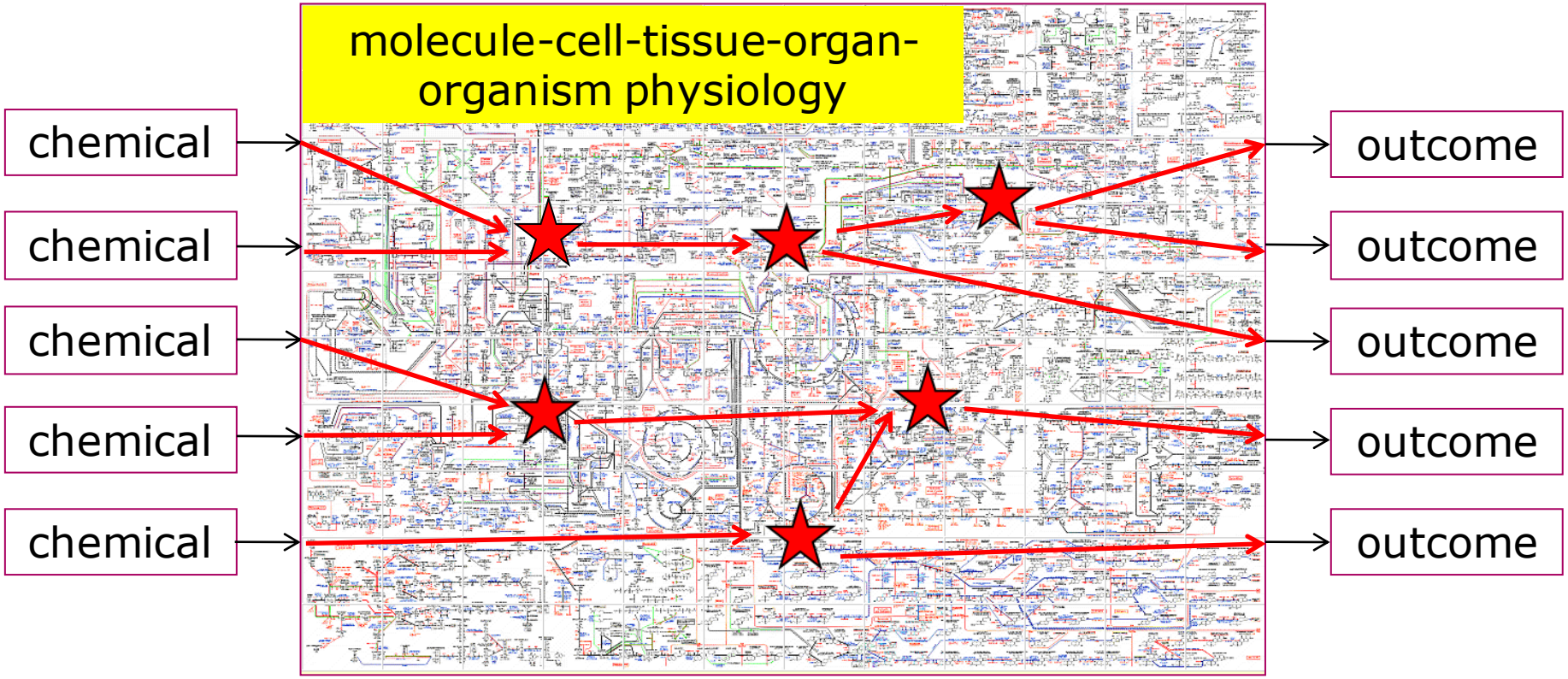
~40% false positives

Apr;83(2):104-11. doi: 10.1002/bdrb.20148.

Limited biological domain of any in vitro test precludes that it will correctly 'predict' all toxicants.

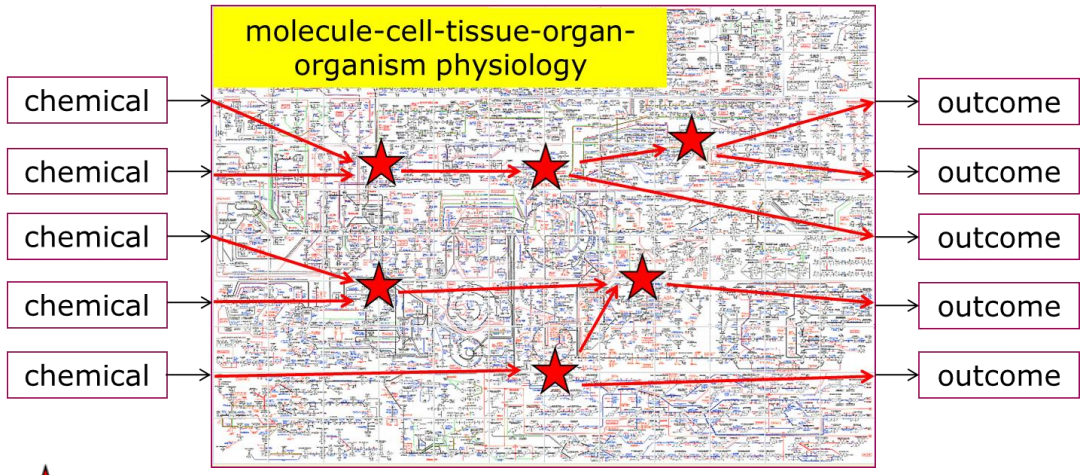


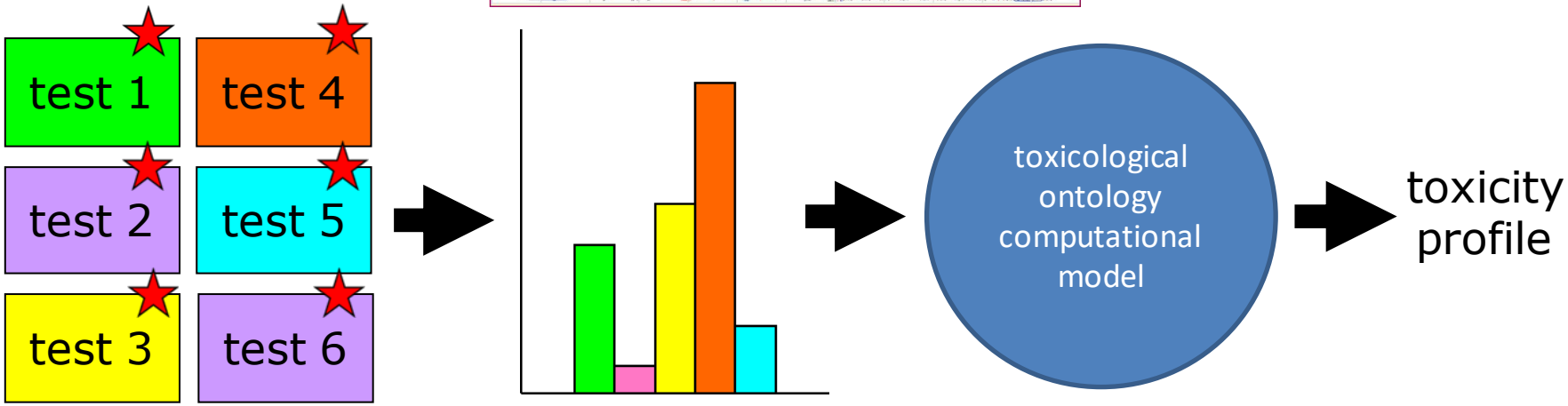
Toxicological Ontology



Cf. The Virtual Physiological Human: vph-institute.org

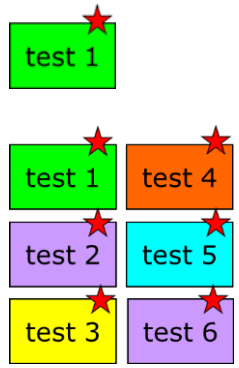
Animal-free hazard assessment



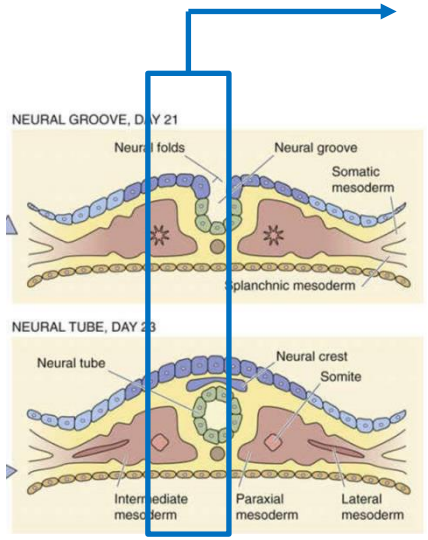


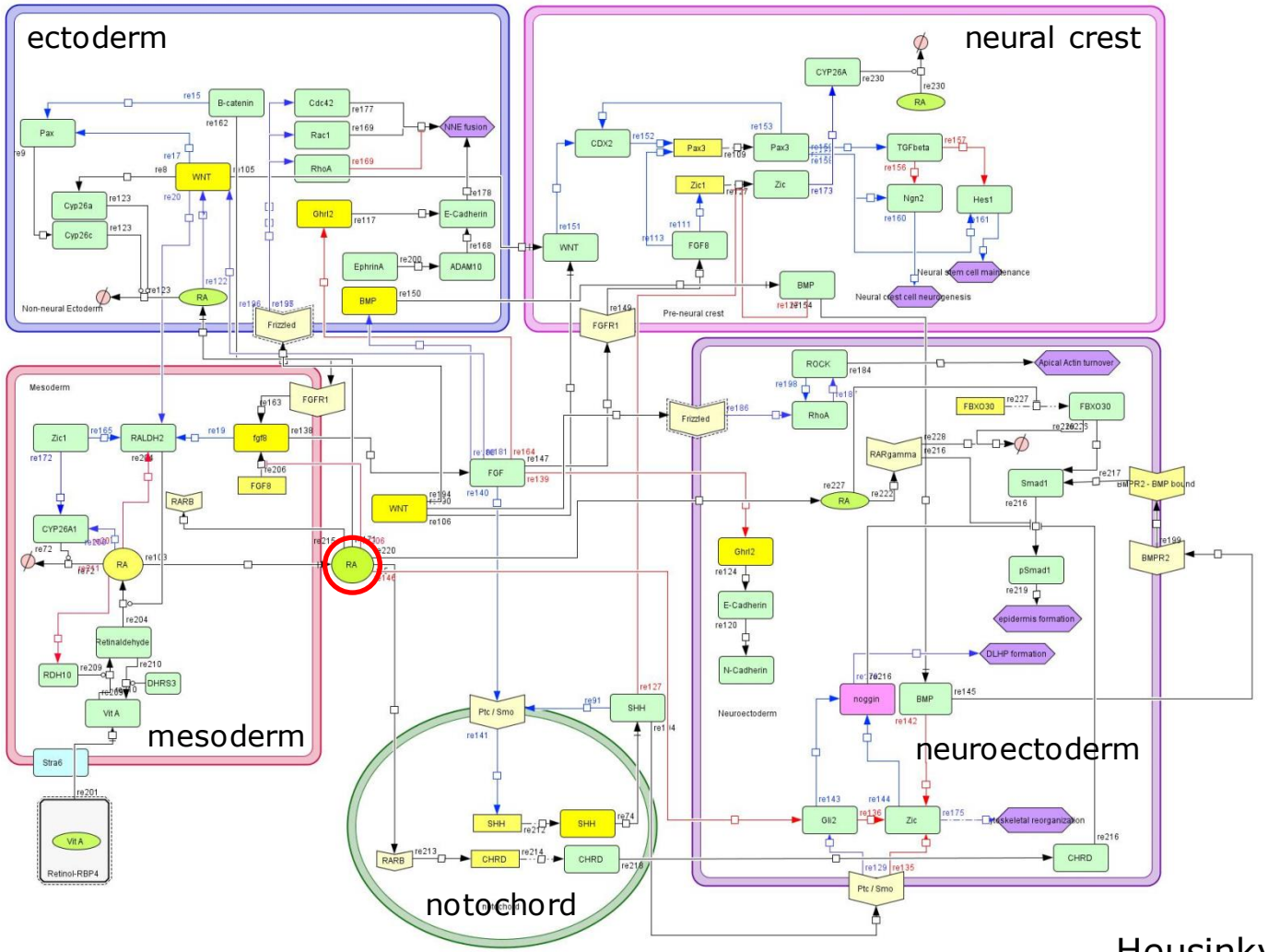
Test system requirements

- Biological domain
  - Describes the biology of the system in terms of MoA, AOP, key event(s) covered, and end point measured
- Technical performance
  - Standardization, variability, transferability
- Chemical domain
  - Solubility, volatility, …
- Sensitivity / specificity
  - Validate each individual test with known positives and known negatives against its biological domain only, "mechanistic validation" ★
  - Validate test battery as a whole against in vivo toxicity, based on sufficient mechanistic coverage of biology/toxicology



CellDesigner map for neural tube closure



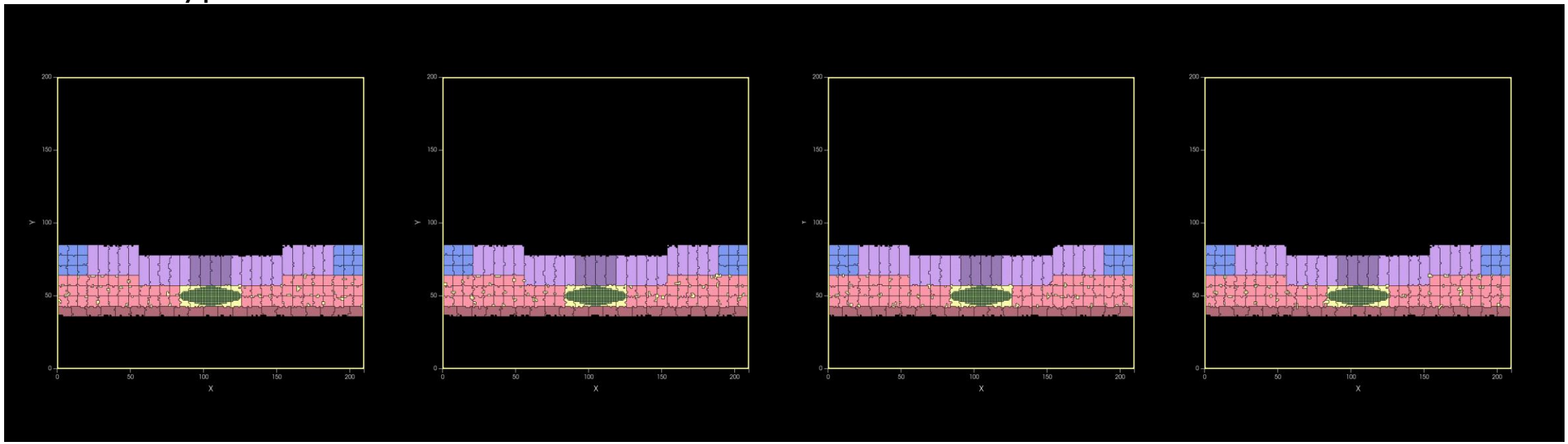


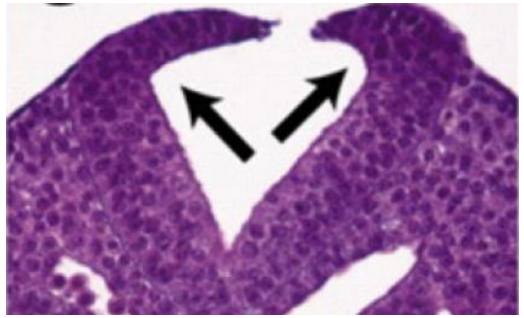
Heusinkveld, et al., 2020

Cf. US EPA Virtual Embryo Project

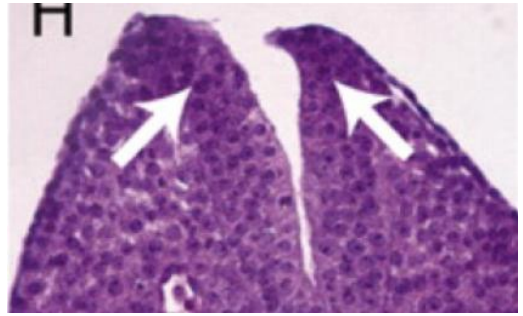
Synthetic dose-response BMP inhibition/activation

BMP Hypoactivation | Normal | BMP upregulation | BMP Hyperactivation

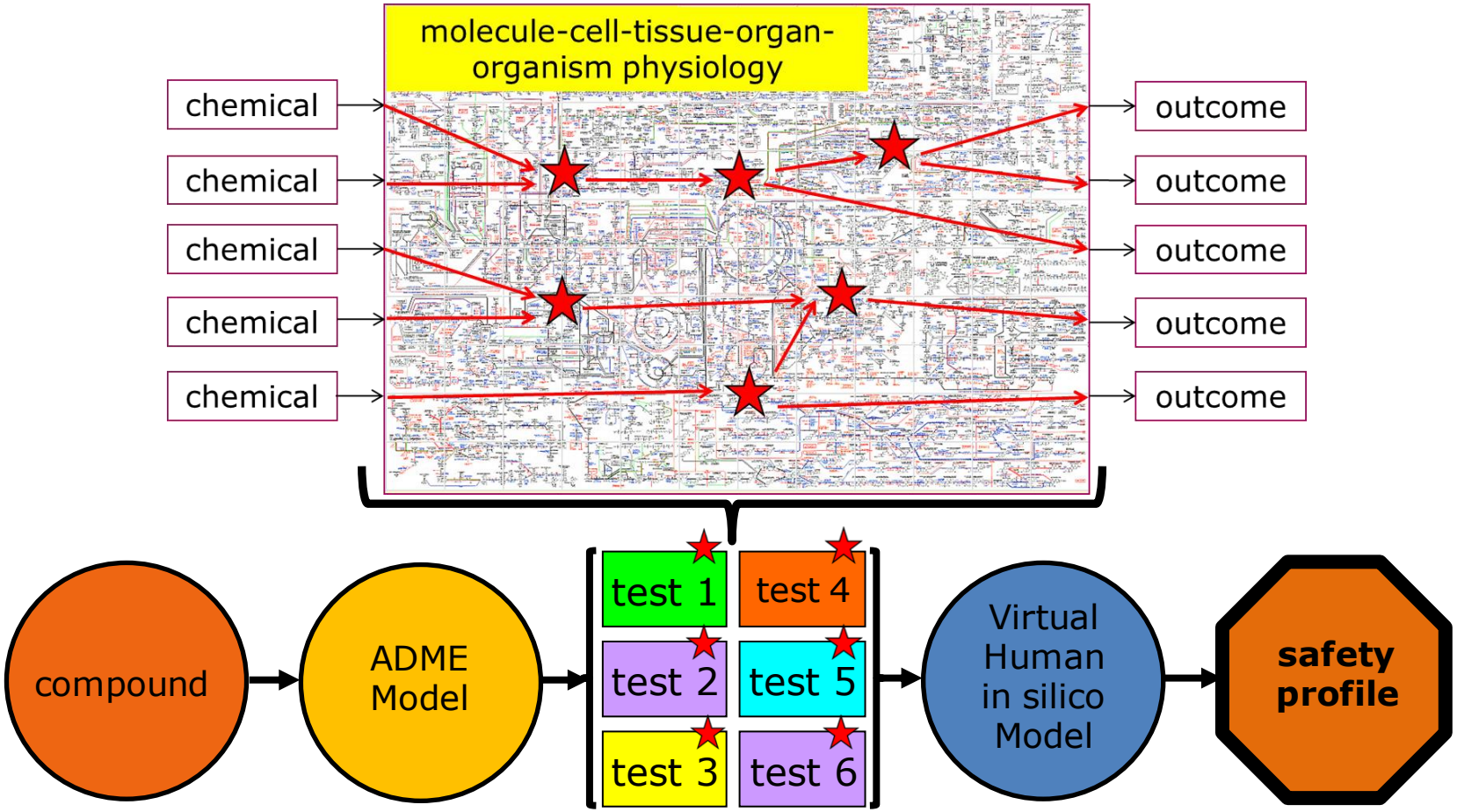




CompuCell3D animation
Berkhout et al., in progress



Animal-free human hazard assessment



Take home messages

- Current regulatory hazard-based risk management based on animal study protocols meets with serious issues regarding interpretation and animal welfare.

- Computational approaches based on the toxicological ontology offer innovative opportunities.
- Clinical diagnostics and treatment now successfully uses computational models using personalized patient parameter input.
- The US EPA Virtual Embryo project shows proofs of principle of computational prediction of dose-related compound-induced adverse health effects.
- The Virtual Physiological Human project provides the physiological basis for computational modelling.
- Test batteries comprehensively covering the AOP-network provide data input for computational models.
- Current international projects (ONTOX, VHP4Safety) exploit and build on this principle.

Selected references

- The Virtual Physiological Human: https://www.vph-institute.org/
- Computational diagnostics: Van Schalkwijk et al., 2010 Briefings in Bioinform. 11(4):403-416.
- US EPA Virtual Embryo: https://www.epa.gov/ , search term 'virtual embryo'
- ONTOX: https://ontox-project.eu/
- VHP4safety: https://www.sciencrew.com/c/6586?title=VHP4Safety
- Piersma et al., Toxicol In Vitro. 2018 Feb;46:163-165.
- Piersma et al., Current Opinion in Toxicology, https://doi.org/10.1016/j.cotox.2019.03.009.
- Heusinkveld et al., Reprod Toxicol. 2021 Jan;99:160-167.
- Piersma et al., Curr Res Toxicol. 2022 May 13;3:100074.
- Piersma et al., Current Opinion in Toxicology 2023, 33:100386.
- Bridges et al., Regulatory Toxicology and Pharmacology 139 (2023) 105356.

VPH Institute
Building the Virtual Physiological Human

v-Embryo

ONTOX

VHP4SAFETY
virtual human platform for safety assessment