AN IN VITRO BATTERY ADDRESSING DEVELOPMENTAL NEUROTOXICITY

Ellen Fritsche

20 years BfR

Berlin, November 4th 2022

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LEIBNIZ-INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG

Mitglied der Leibniz-Gemeinschaft

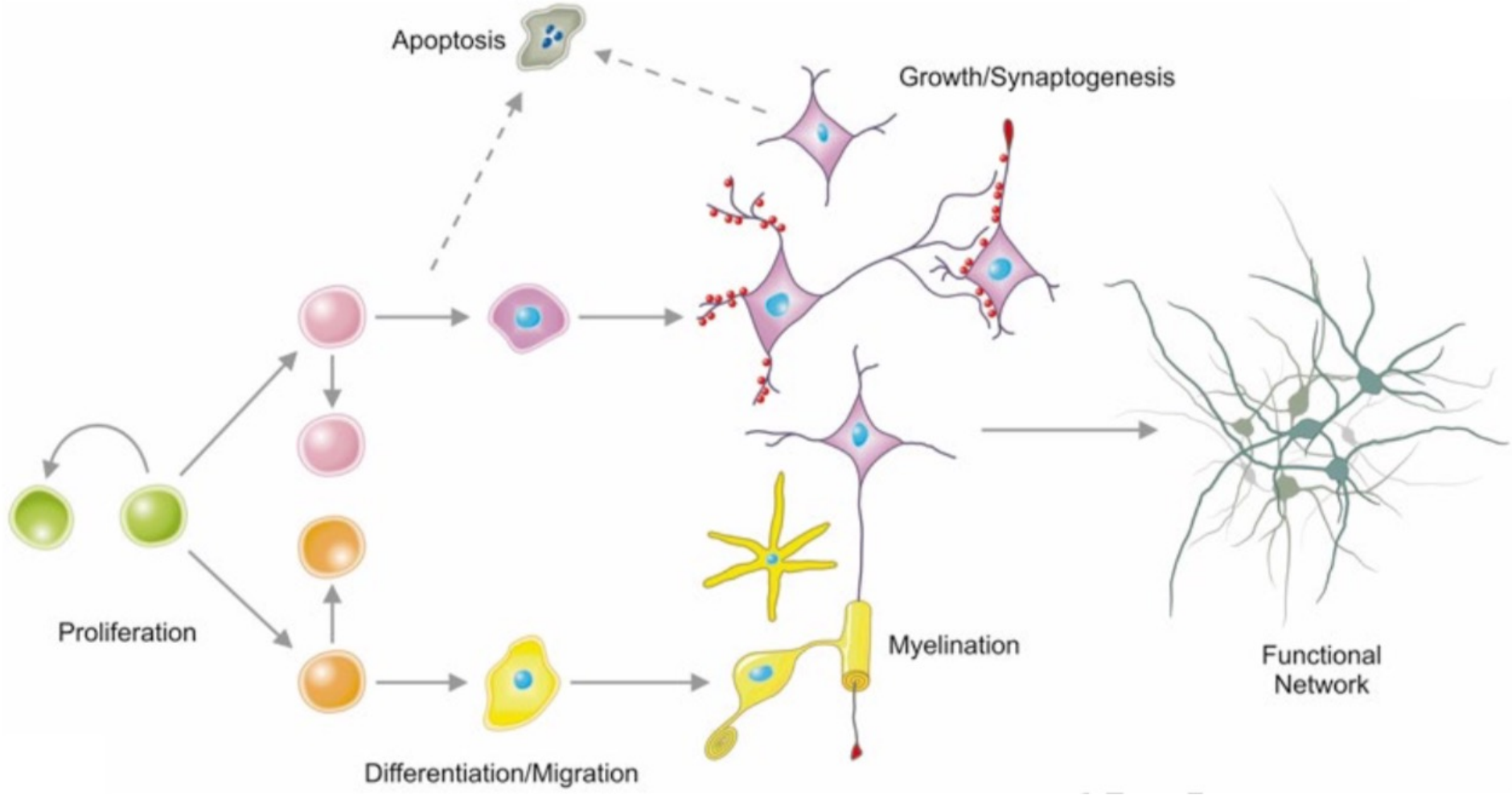
DNTOX

International DNT Activities

Fritsche OECD 2016

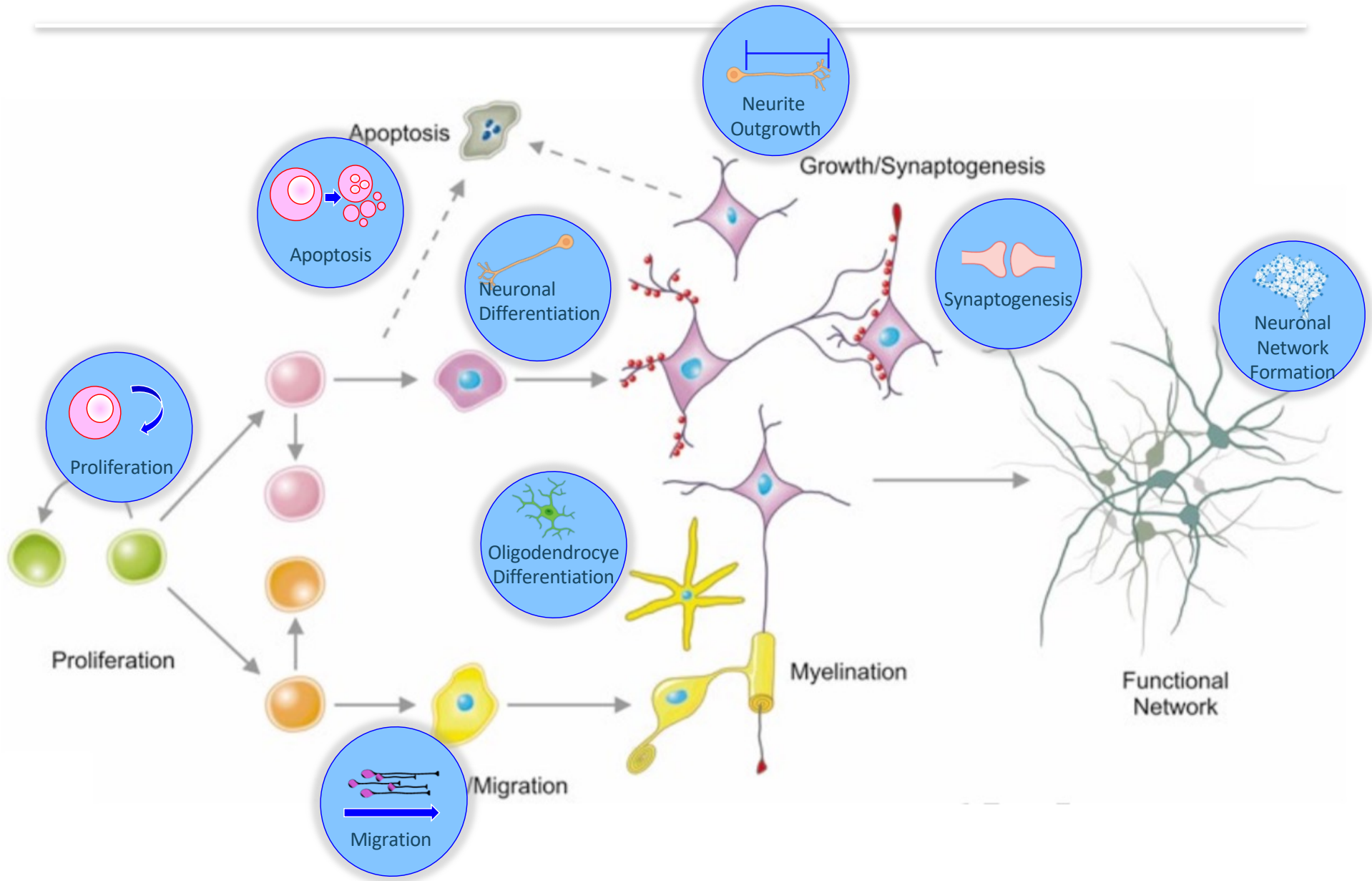
Fritsche et al. ALTEX 2017

Key Neurodevelopmental Processes



Kindly provided by **William Mundy, U.S. Environmental Protection Agency** and **John Havel, SRA International, Inc.**

Key Neurodevelopmental Processes



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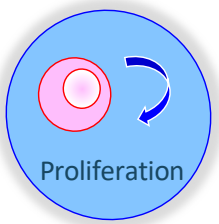
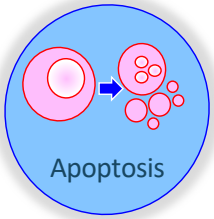
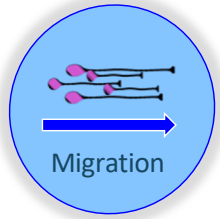


NPC1 - hNPC

hNP1 - hNPC



hNP1 - hNPC

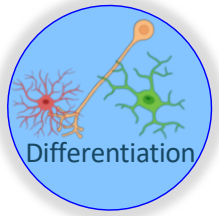


UKN2 - hNCC

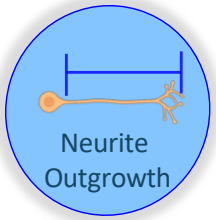
NPC2a - hRG

NPC2b - hNeu

NPC2c - hOligo



NPC3 - hNeu



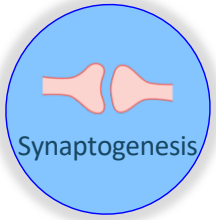
NPC4 - hNeu

UKN4 - hNeu

UKN5- hNeu

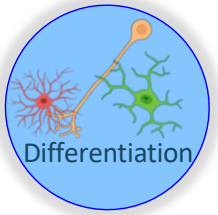
hN ini - hNeu

Cortical ini - rNeu

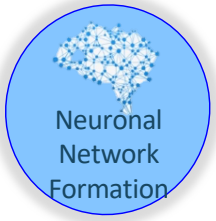


rCortical matur

rSynaptogenesis



NPC5 - hOligo

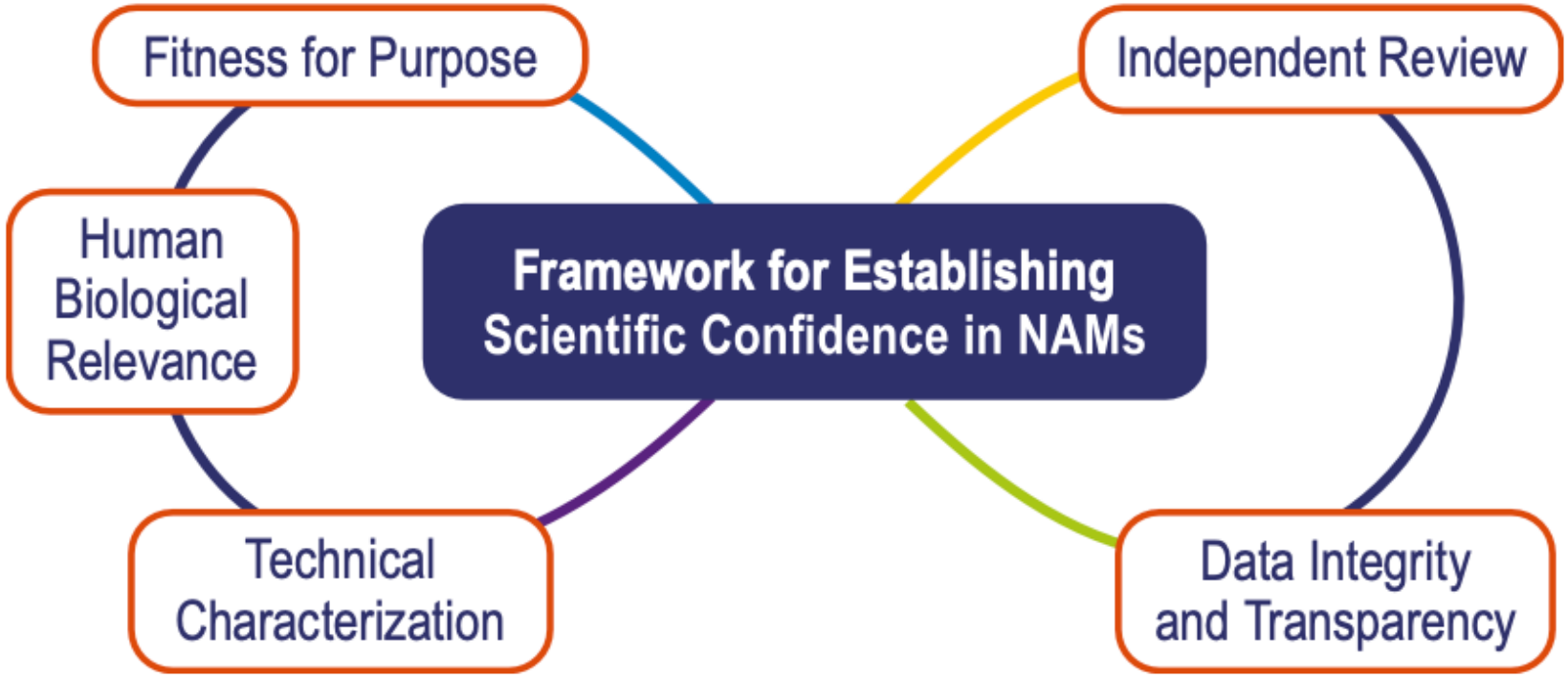


rCortical MEA

h-human; r-rat; NPC-neural progenitor cell; NCC-neural crest cell; RG-radial glia; Neu-neuron; Oligo-oligodendrocyte; ini-initiation; matur-maturation; MEA-microelectrode array

Crofton & Mundy 2021, Table 2.3

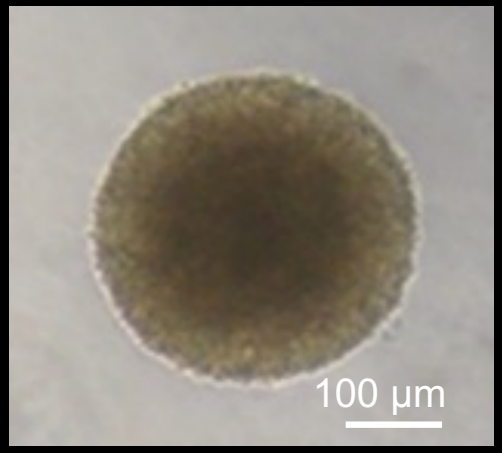
Establishment of Scientific Confidence in NAMs*

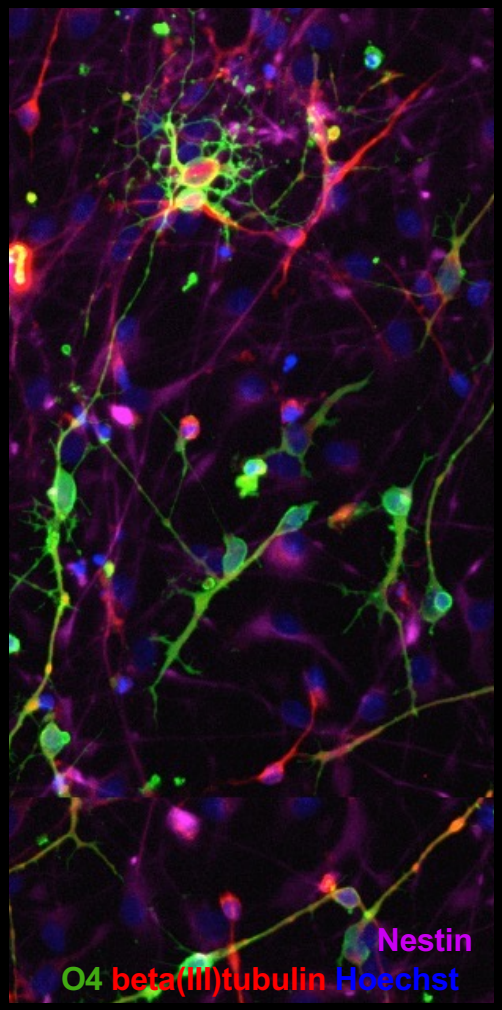


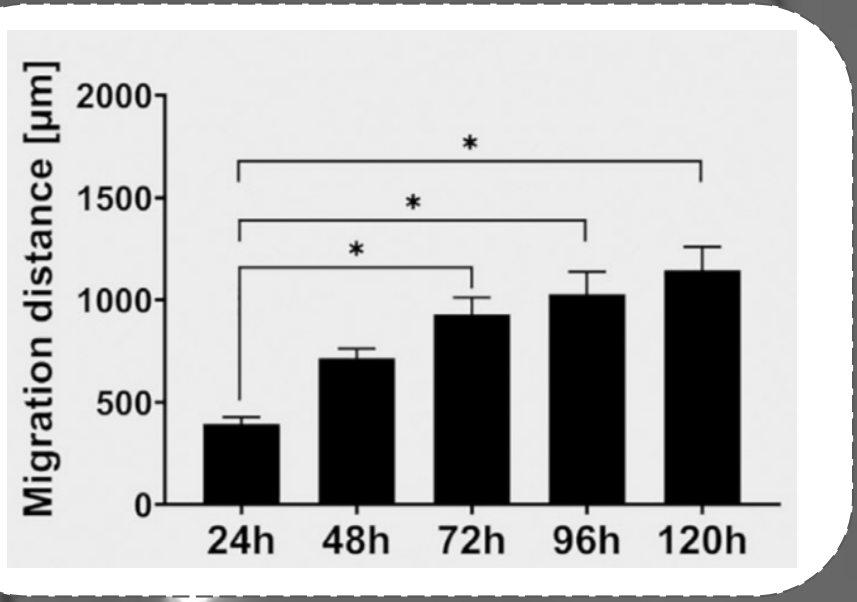
Van der Zalm et al. 2022

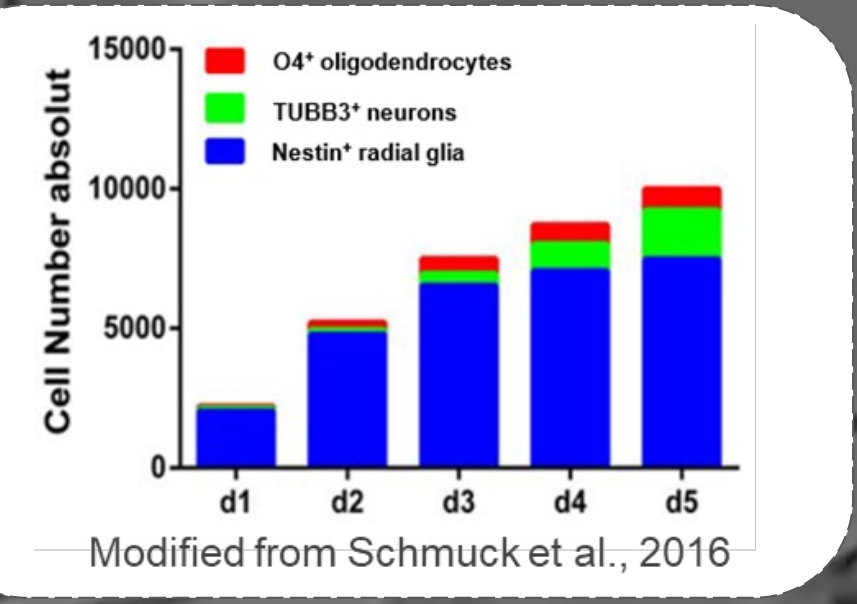
*NAMs - New Approach Methods

Human Neurospheres





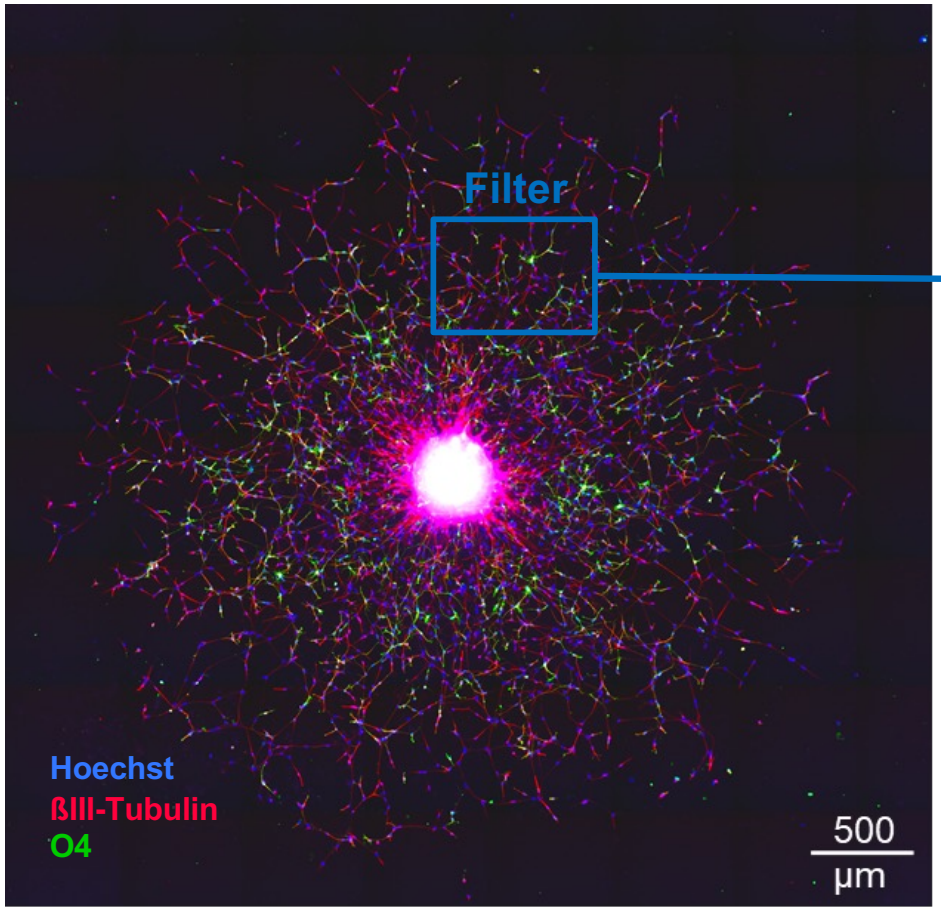




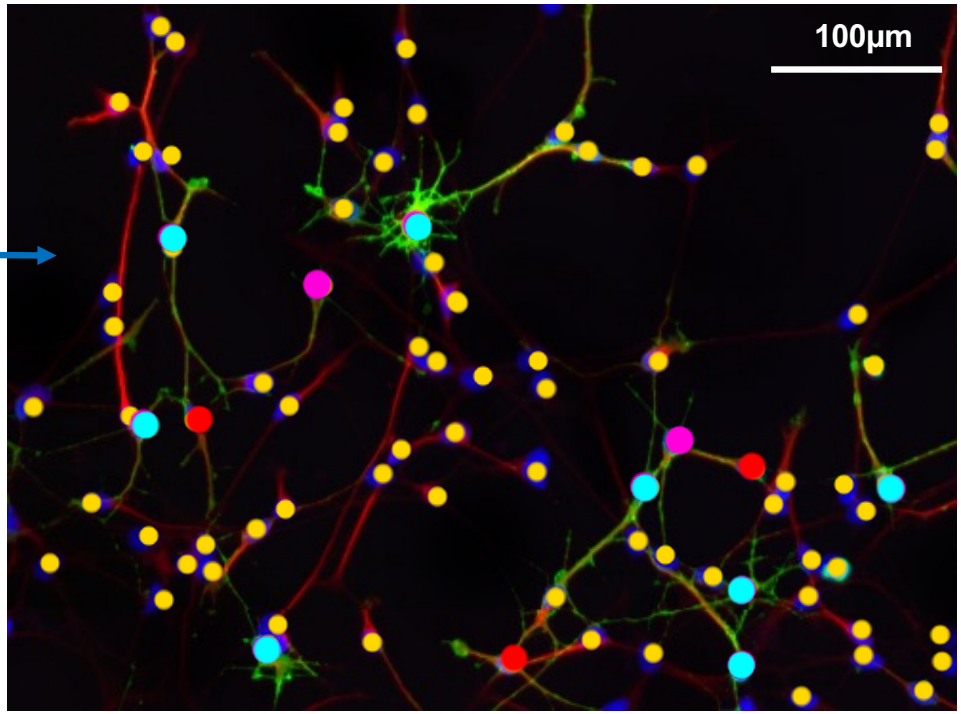
Modified from Schmuck et al., 2016

AI*-based Cell Identification

Multichannel Neurosphere Image



AI learns from human ground truth



- Cellomics Nuclei
- Human annotations = Ground Truth

New run with different parameters

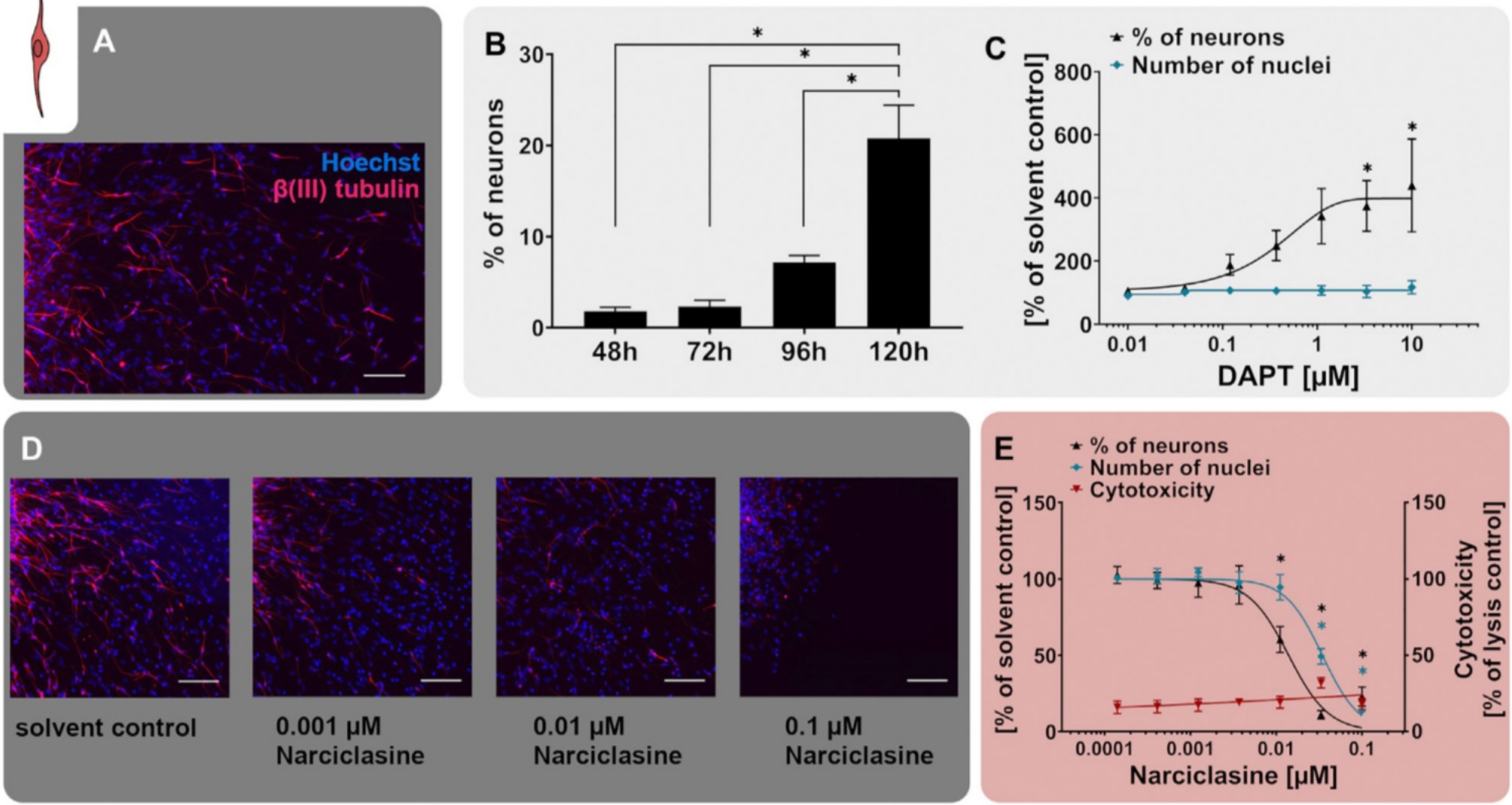
Comparing Results

- True Positives (noted by human + AI)
- False Positives (only noted by AI)
- False Negatives (only by human)
- True Negatives (not noted by either)

*AI – Artificial Intelligence

Förster et al. 2022

Differentiating NPC – NPC3 Assay



Technical Characterization of DNT Test Methods

Bench Marks!

Template for the Description of Cell-Based Toxicological Test Methods to Allow Evaluation and Regulatory Use of the Data

Alice Krebs[1,2], Tanja Waldmann[1], Martin F. Wilks[3], Barbara M. A. van Vugt-Lussenburg[4], Bart van der Burg[4], Andrea Terron[5], Thomas Steger-Hartmann[6], Joelle Ruegg[7], Costanza Rovida[8], Emma Pedersen[9], Giorgia Pallocca[1,8], Mirjam Luijten[10], Sofia B. Leite[11], Stefan Kustermann[12], Hennicke Kamp[14], Julia Hoeng[14], Philip Hewitt[15], Matthias Herzler[16], Jan G. Hengstler[17], Tuula Heinonen[18], Thomas Hartung[8,19], Barry Hardy[20], Florian Gantner[21], Ellen Fritsche[22], Kristina Fant[9], Janine Ezendam[10], Thomas Exner[20], Torsten Dunkern[23], Daniel R. Dietrich[24], Sandra Coecke[11], Francois Busquet[8,25], Albert Braeuning[26], Olesja Bondarenko[27], Susanne H. Bennekou[28], Mario Beilmann[29] and Marcel Leist[1,2,8]

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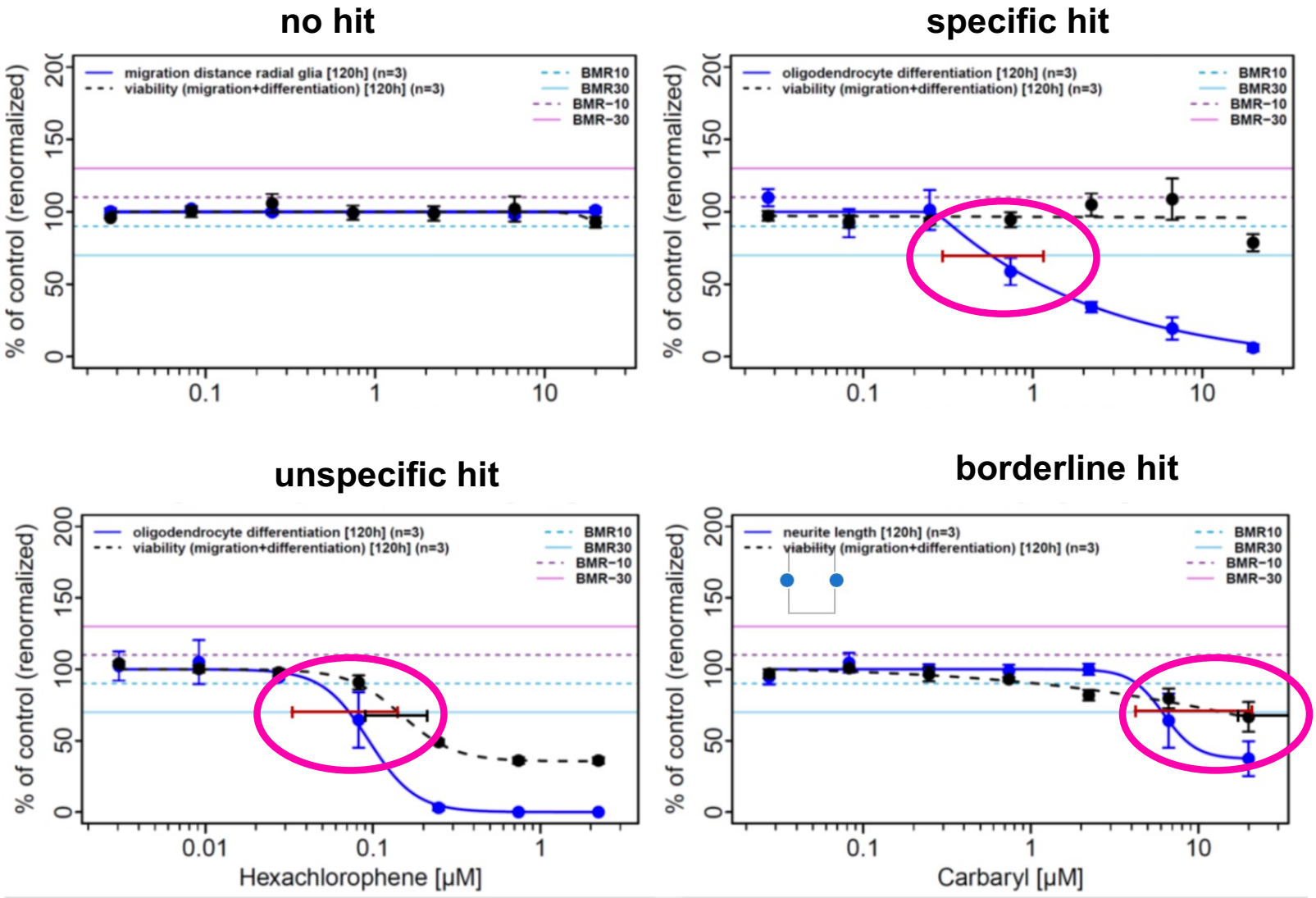
Classification Models



Blum et al. Chemosphere accepted Masjosthusmann et al. EFSA supp publ 2020

Keßel et al. submitted to ALTEX; Bioarchiveshttps://doi.org/10.1101/2022.10.18.512648

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Predictive Capacity – DNT EU-IVB

Positive controls	specific + brdl.	specific
Cadmium chloride	TP	TP
Chlorpyrifos	TP	FN
Dexamethasone	TP	TP
Hexachlorophene	TP	TP
Lead (II) acetate trihydrate	TP	TP
Manganese (II) chloride	TP	TP
Methylmercury chloride	TP	TP
PBDE 47	TP	TP
PBDE 99	TP	FN
(±) Ketamine hydrochloride	FN	FN
5,5-Diphenylhydantoin	FN	FN
Acrylamide	TP	TP
all-trans-Retinoic acid	TP	TP
Chlorpromazine hydrochloride	TP	TP
Deltamethrin	TP	TP
Domoic acid	FN	FN
Haloperidol	TP	TP
Maneb	TP	FN
Methylazoxymethanol acetate	TP	TP
Nicotine	FN	FN
Paraquat dichloride hydrate	TP	TP
PFOA	FN	FN
PFOSK	TP	TP
Sodium valproate	TP	TP
Tebuconazole	TP	TP
Tributyltin chloride	TP	TP
Trichlorfon	TP	TP
Triethyl-tin bromide	TP	FN

Negative controls	Acetaminophen	TN	TN
	Amoxicillin	TN	TN
	Aspirin	TN	TN
	Buspirone	TN	TN
	Chlorpheniramine maleate	TN	TN
	D-Glucitol	TN	TN
	Diethylene glycol	TN	TN
	D-Mannitol	TN	TN
	Doxylamine succinate	TN	TN
	Famotidine	TN	TN
	Ibuprofen	TN	TN
	Metformin	TN	TN
	Metoprolol	TN	TN
	Penicillin	TN	TN
	Saccharin	TN	TN
	Sodium benzoate	TN	TN
	Warfarin	TN	TN

Performance [%]	Sensitivity	82	68
	Specificity	100	100
	Accuracy	89	80
	Balanced accuracy	91	84
	PPV	100	100
	F1 score	91	84
	MCC	80	67

Blum et al. accepted

First Draft OECD IVB Guidance Document

EXTERNAL SCIENTIFIC REPORT

APPROVED: 18 October 2021

doi:10.2903/sp.efsa.2021.EN-6924

External Scientific Report on the Interpretation of Data from the Developmental Neurotoxicity In Vitro Testing Assays for Use in Integrated Approaches for Testing and Assessment

Kevin M Crofton[1] and William R. Mundy[2],

EFSA supporting publication 2021:EN-6924

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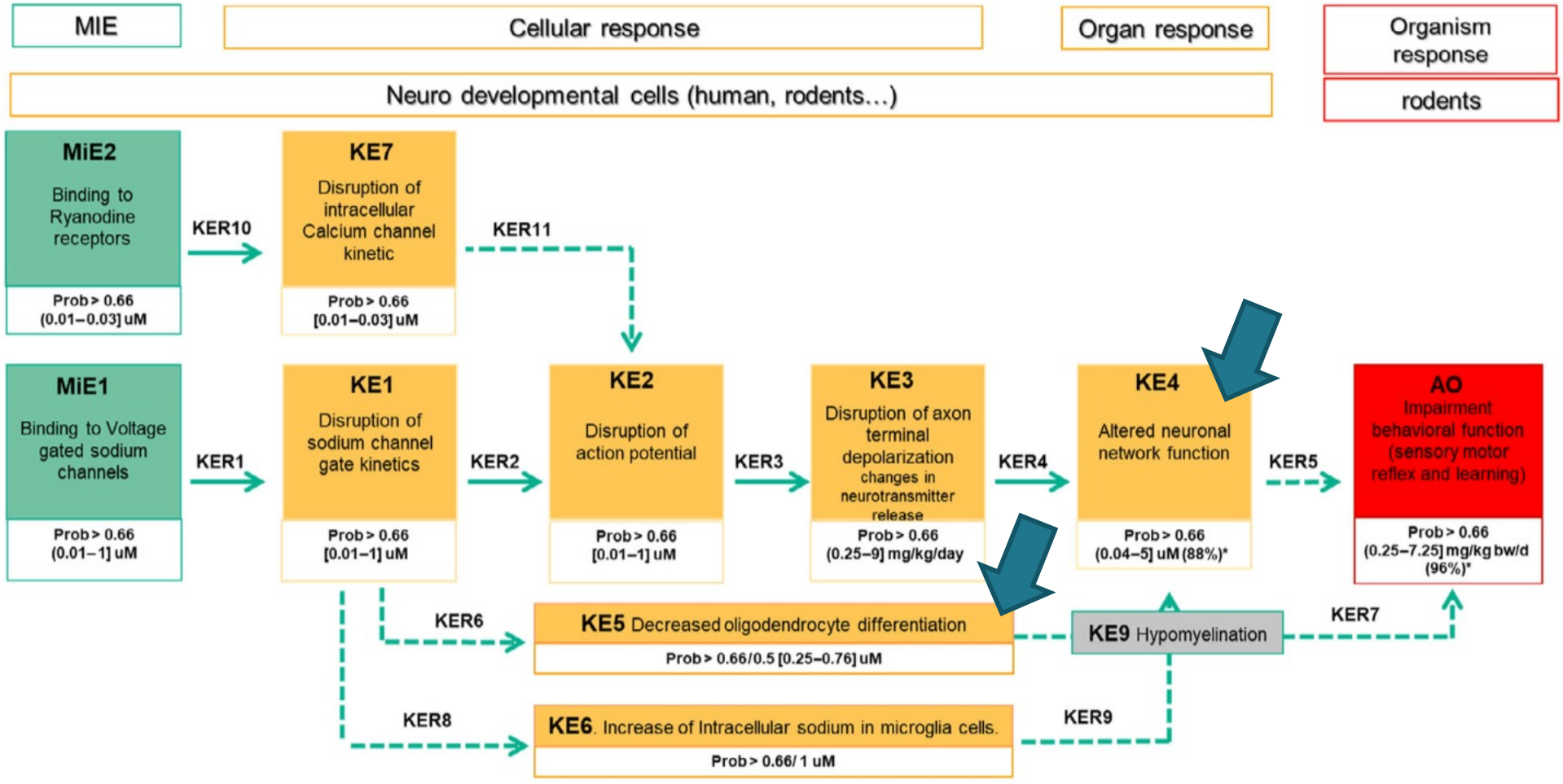
Case study I – Hazard assessment

Endpoint (Test method; BMR)	Deltamethrin (µM)	Flufenacet (µM)
NCC migration (UKN2; BMC25)	18.4[s]	>100
Radial glia migration (NPC2a, 120h; BMC10)	16.3[us]	>20
Neurite length (NPC4; BMC30)	14.9[us]	>20
Neurite area (NPC4; BMC30)	15.9[us]	>20
Oligodendrocyte differentiation (NPC5; BMC30)	0.6[s]	17.8[us]
Neurite are (UKN5; BMC25)	112.8[us]	>100
Rat neuronal network formation (rNNF; BMC50)	0.5[s]	>20
Human neuronal network formation (hNNF; BMC50)	4.1[s]	>20

s = specific hit; us = unspecific hit

Masjosthusmann et al. EFSA supp publ 2020

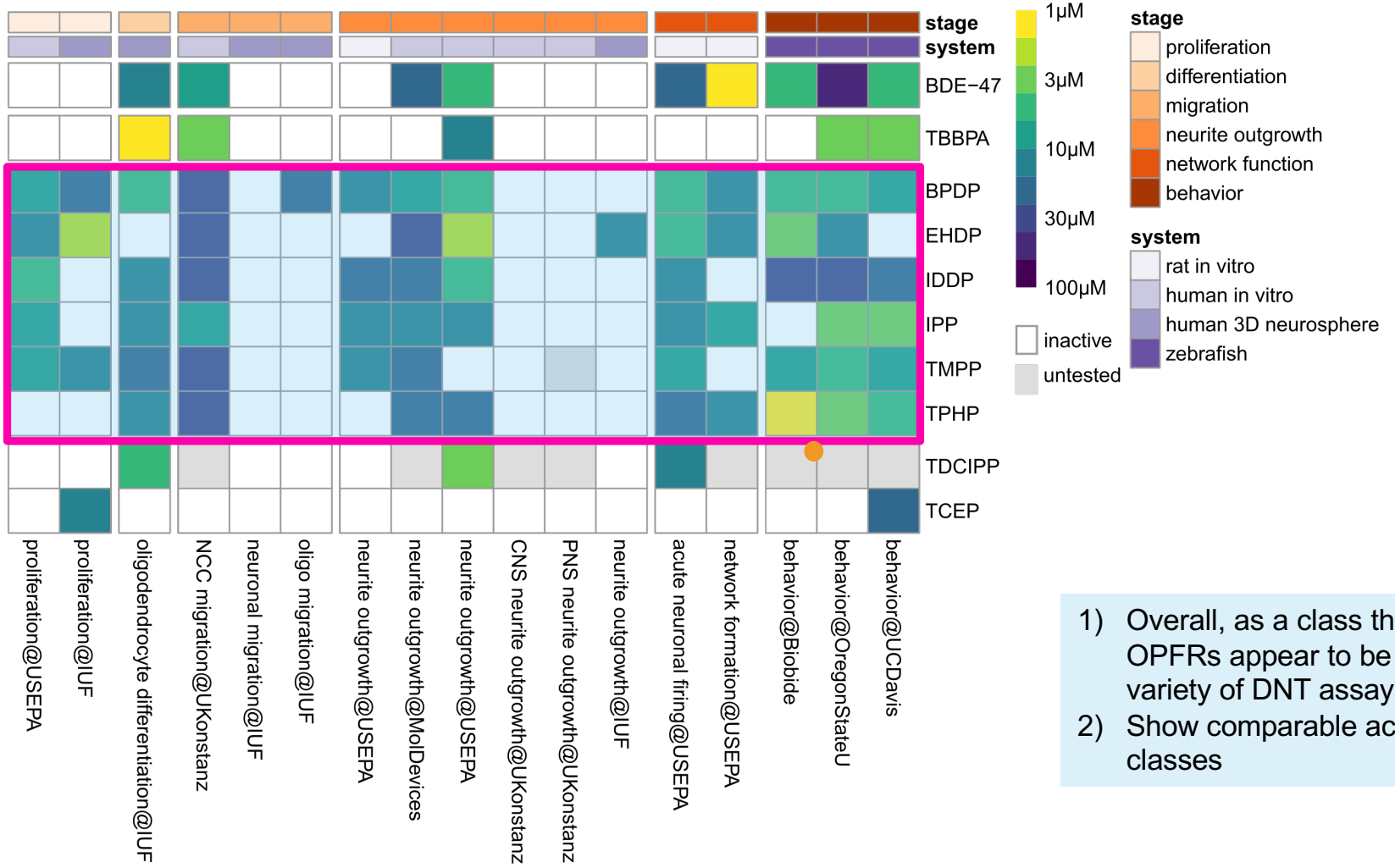
Case study I – Hazard assessment



AOP-informed IATA

Hernandez et al. EFSA J 2021

Case study II – Screening and Prioritization



US-NTP

1) Overall, as a class the aromatic OPFRs appear to be active in a variety of DNT assays
2) Show comparable activity to other classes

kindly provided by Helena Hogberg, NIEHS

Updated Blum et al., 2019, Environ. Sci. Technol.
Klose et al., 2021, Cell. Biol. Toxicol.

Crofton & Mundy 2021, Appendix

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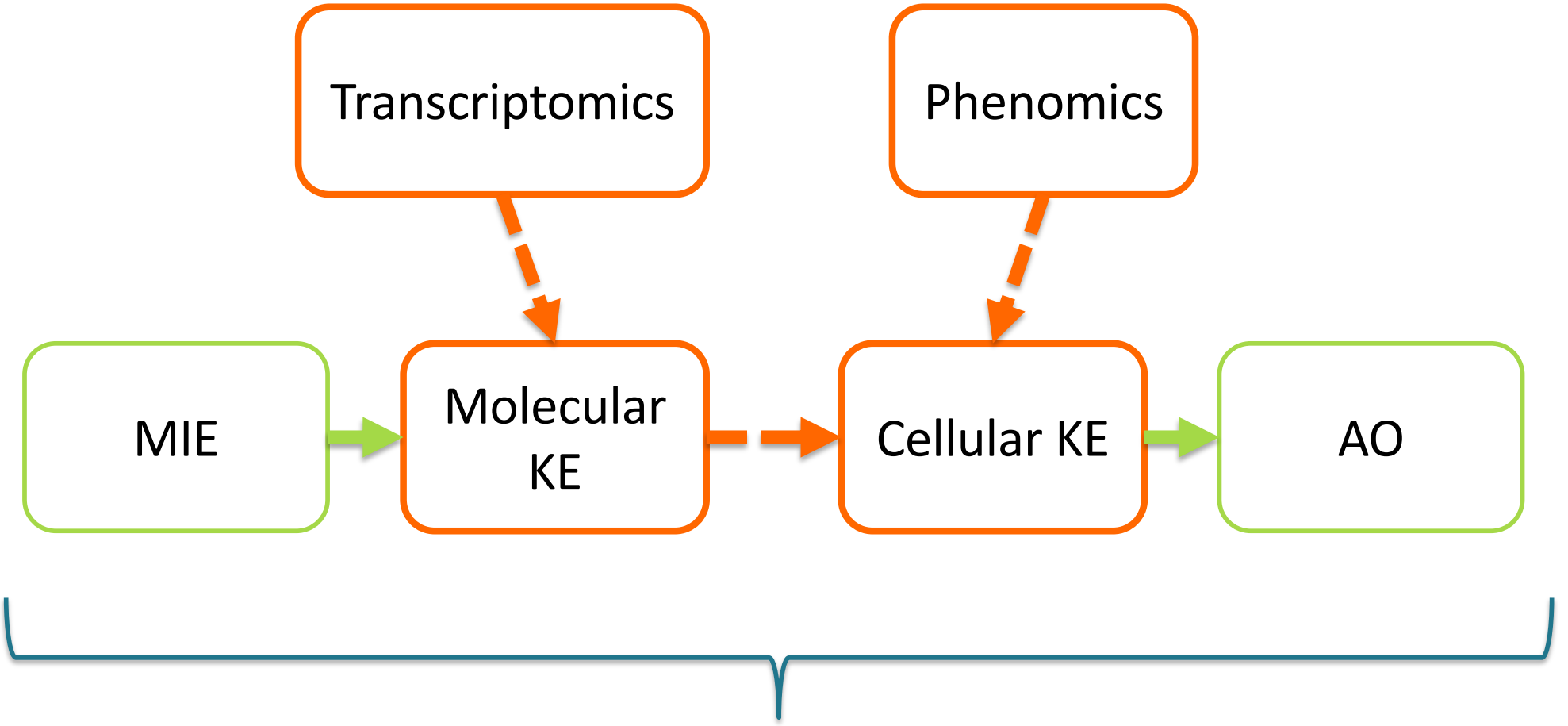
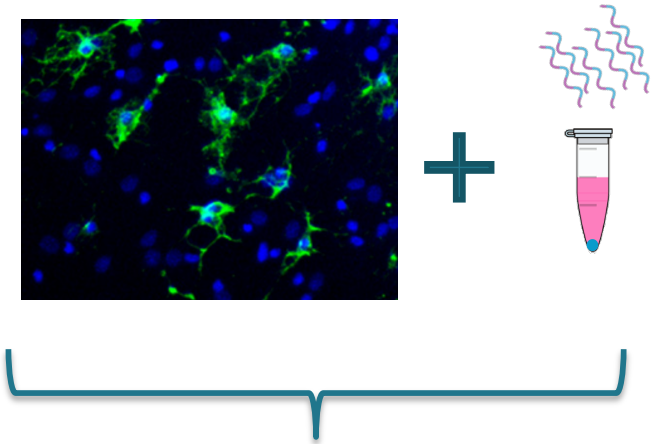
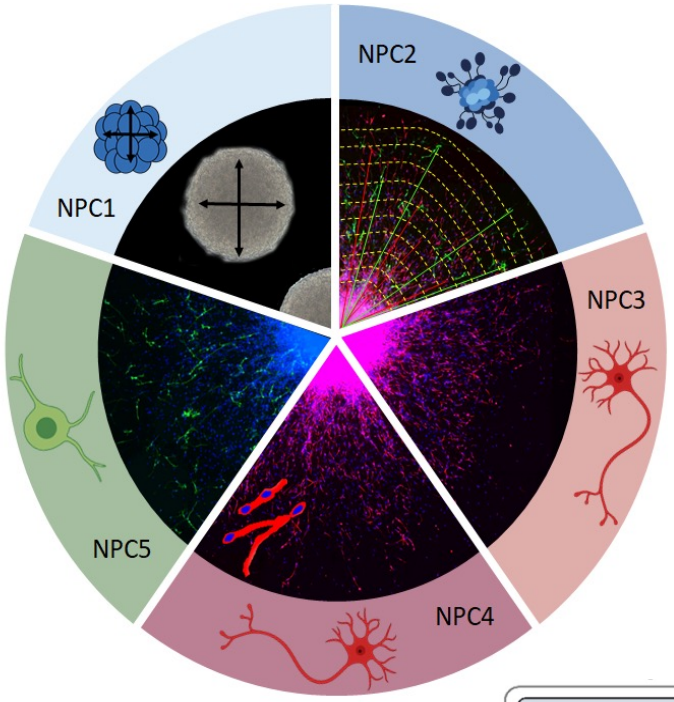
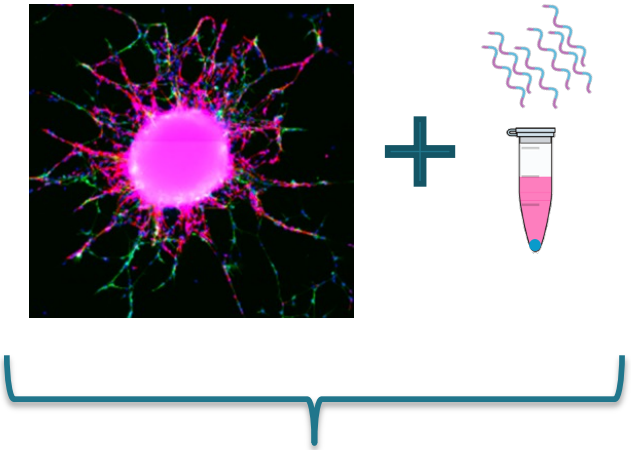
Phenomics and Transcriptomics for AOP development

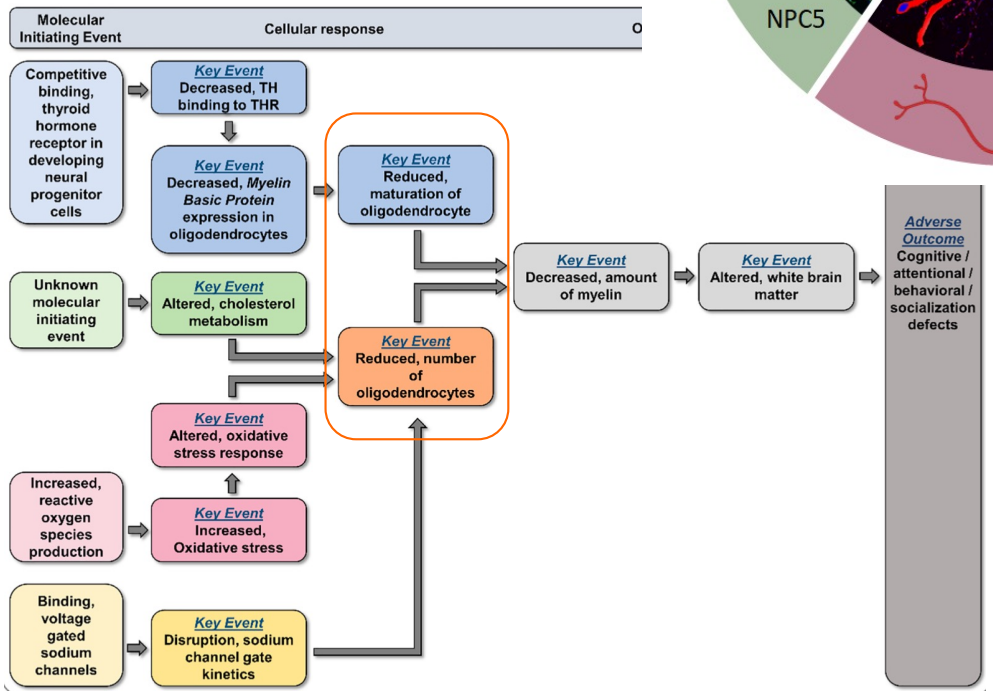


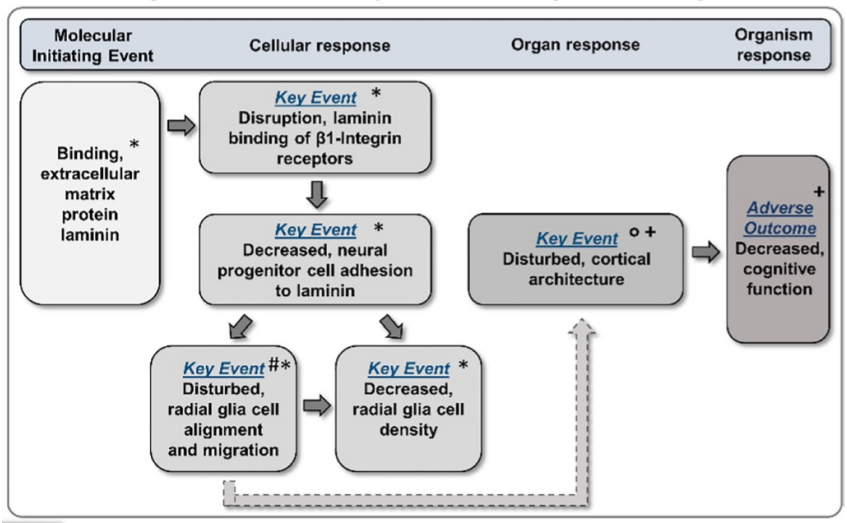
Phenomics and Transcriptomics for AOP development











Klose et al., 2021, Cell. Biol. Toxicol.

Klose et al., 2021, ALTEX

Klose et al., 2022, Cell. Biol. Toxicol.

Barenys et al., 2016, Arch. Toxicol.

DNT IVB in current International Projects

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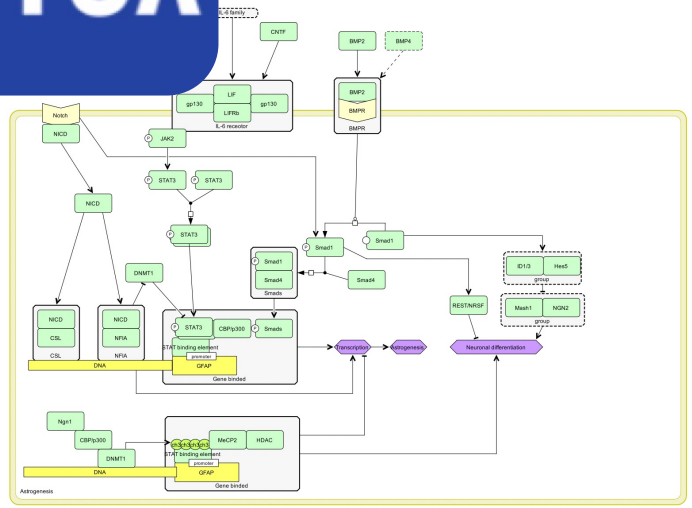
ONTOX

RISK HUNT3R

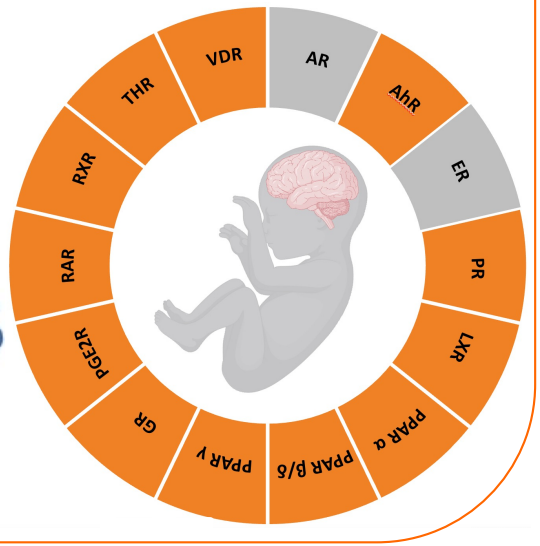


PARC

US-NTP

HORIZON EUROPE

ENDpoiNTs



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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DNT-IVB

Marcel Leist

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Universität

Guidance Document on DNT *in vitro* Testing

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Thank you for your attention!

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