

DEDICATION TO THE USE OF IN VITRO ALTERNATIVE TECHNIQUES TO STUDY TOXICOLOGICAL MECHANISMS: CASE STUDY OF DEVELOPMENTAL NEUROTOXICITY

Ellen Fritsche

2023 SOT In Vitro Lecture and Luncheon
March 20th 2023

IUF

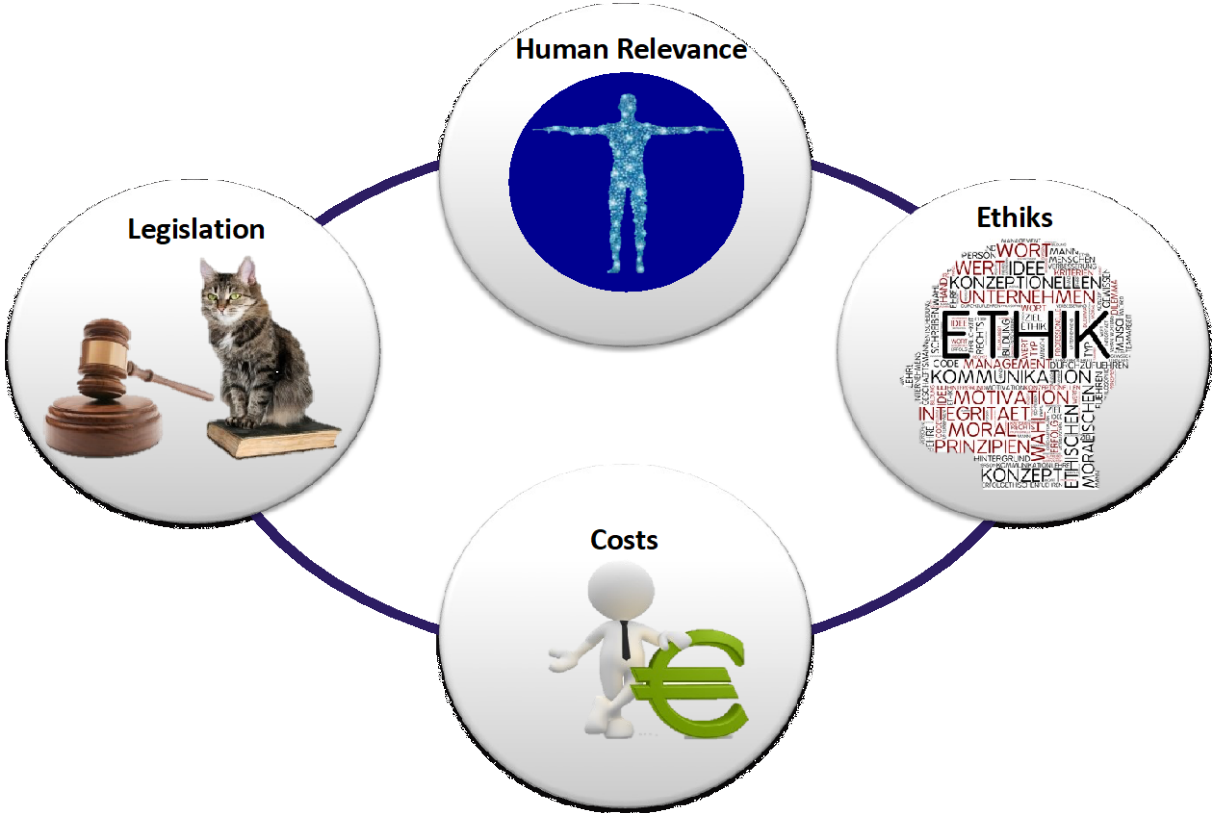
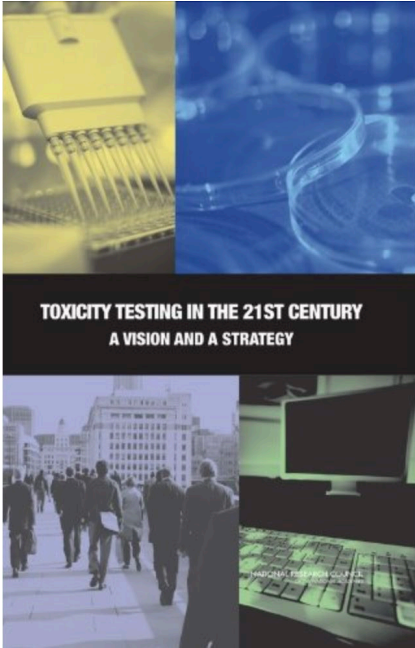
LEIBNIZ-INSTITUT
FÜR UMWELT-
MEDIZINISCHE
FORSCHUNG

Mitglied der

Leibniz
Leibniz-Gemeinschaft

Motivations for Developing Alternative Methods

Tox21 – a multi-agency approach to the use of new approach methodologies



William Russel und
Rex Burch



The '3R' Concept Replace, Reduce, Refine

The principles of the **3R** in animal research



Research with animals

Replace

Achieve a research objective by avoiding or replacing the use of animals.

Reduce

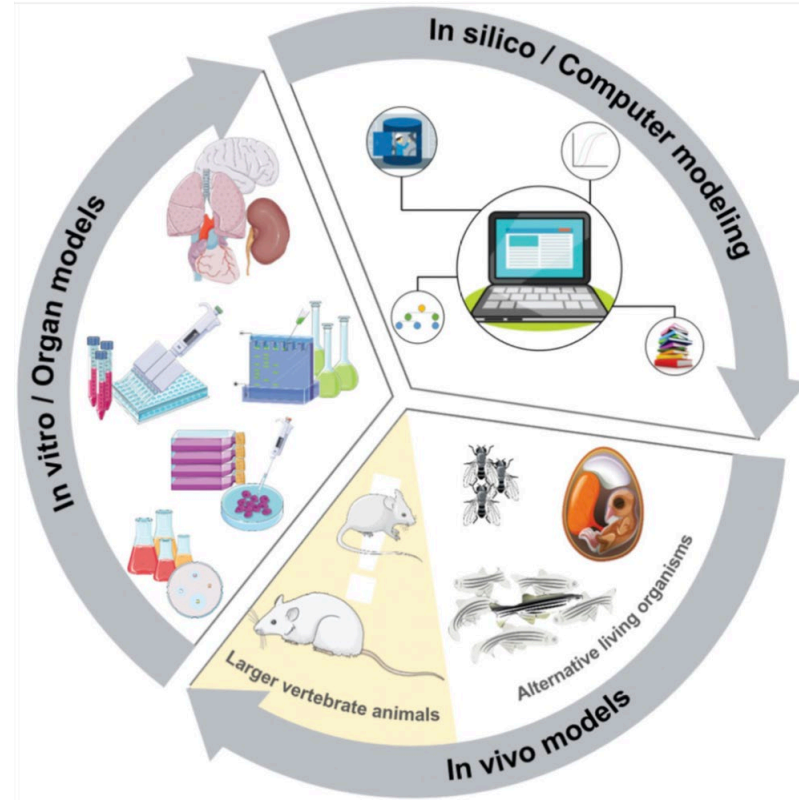
As many experiments as necessary, but as few laboratory animals as possible.

Refine

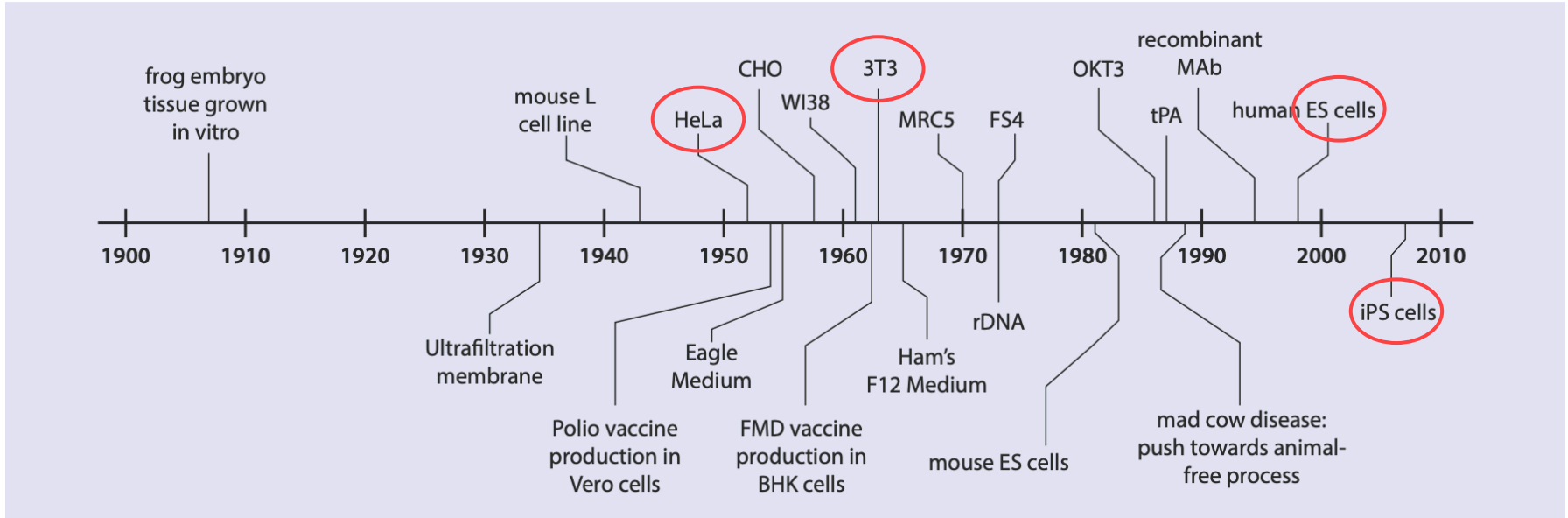
Minimize the potential suffering and stress of laboratory animals and enhance their wellbeing.

The '3R' Concept Replace, Reduce, Refine

- in silico
- in chemico
- in vitro
- in vivo



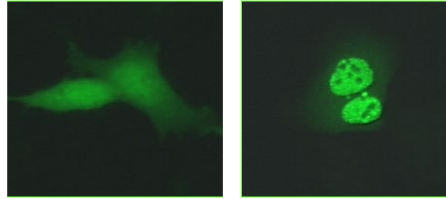
In vitro is not in vitro.....



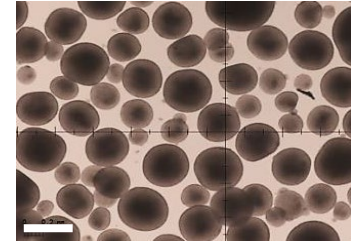
- 2D monolayer
- 3D spheres
- 3D organoids
- 3D bioengineered tissues

- Tumor cell lines
- Primary cells
- Stem cells

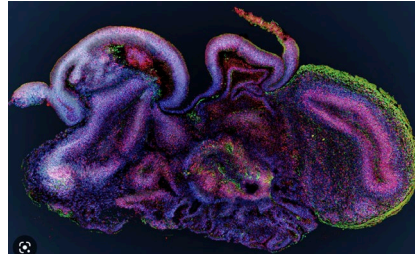
2D monolayer, GFP tagged line



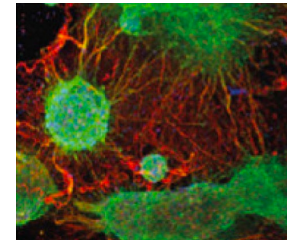
3D spheres



3D organoids

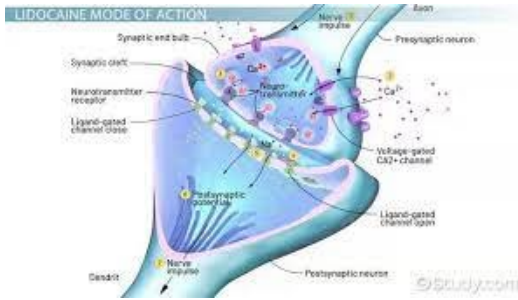


3D engineered tissues

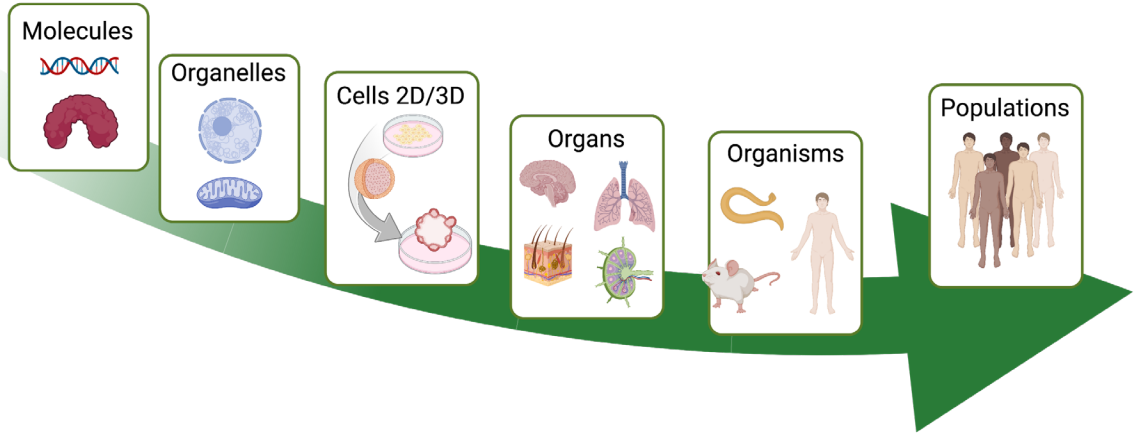


The choice of model solely depends on the research question!

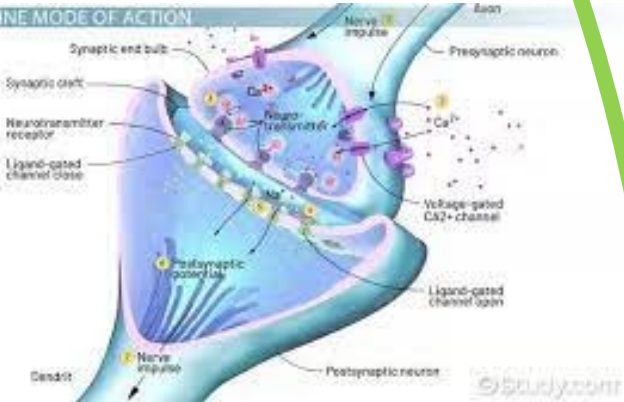
Considerations for planning an experiment using NAMs



- What is the underlying research question?
- Which model is suitable for answering my question?



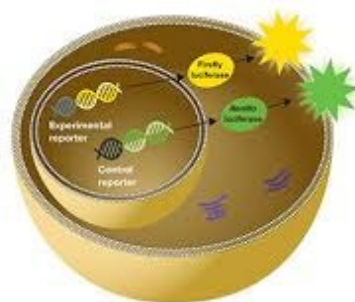
Mode-of-action Analyses



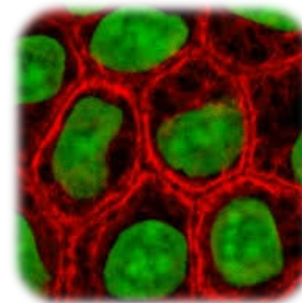
E.g. ToxCast Assays for MIE identification



MIE – Molecular Initiating Event



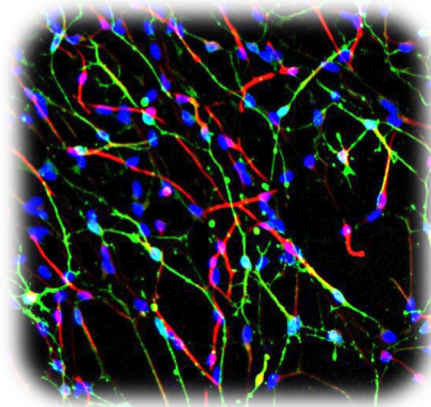
Reporter Gene Assays



Mono Cell Cultures



easy to study/evaluate



Mixed Cell Cultures

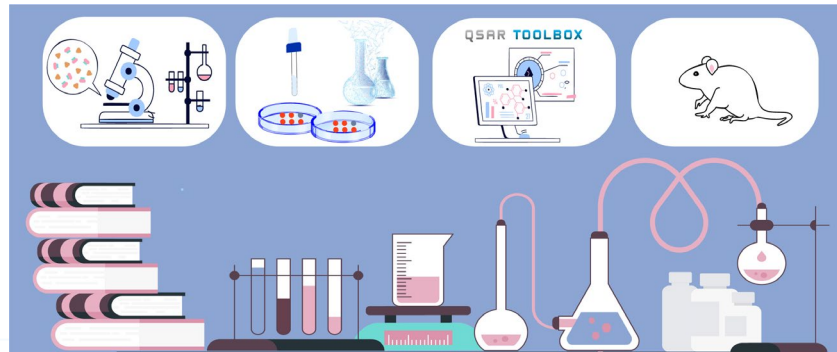


more difficult to study/evaluate



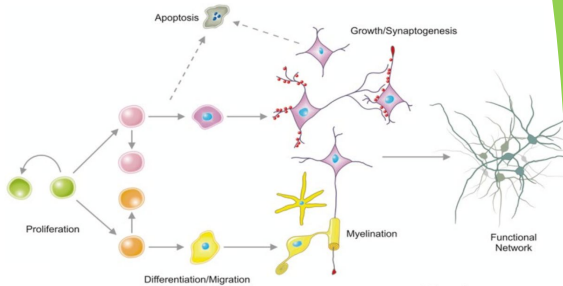
Guideline Study


- From apical endpoints to mode-of-action-based analyses
 - More complex questions in the whole organism in vivo context
 - A battery of in vitro/in silico tests is needed
 - Quantitative understanding is necessary (QIVIVE)
 - The IATA context is desired





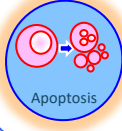
IATA – Integrated Approaches to Testing and Assessment



The current OECD/EFSA/EPA **DNT IVB** based on key neurodevelopmental processes

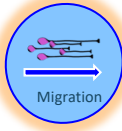



 NPC1 - hNPC
hNP1 - hNPC



LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG



 hNP1 - hNPC



LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG



 UKN2 - hNCC




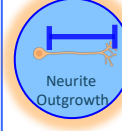

LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG



LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG



LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG


 NPC3 - hNeu



LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG


 NPC4 - hNeu

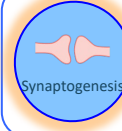

LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG



UNIVERSITÄT KONSTANZ



UNIVERSITÄT KONSTANZ

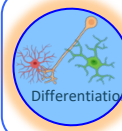

LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG



LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG

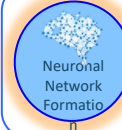
 rCortical matur
rSynaptogenesis



LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG


LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG

 NPC5 - hOligo

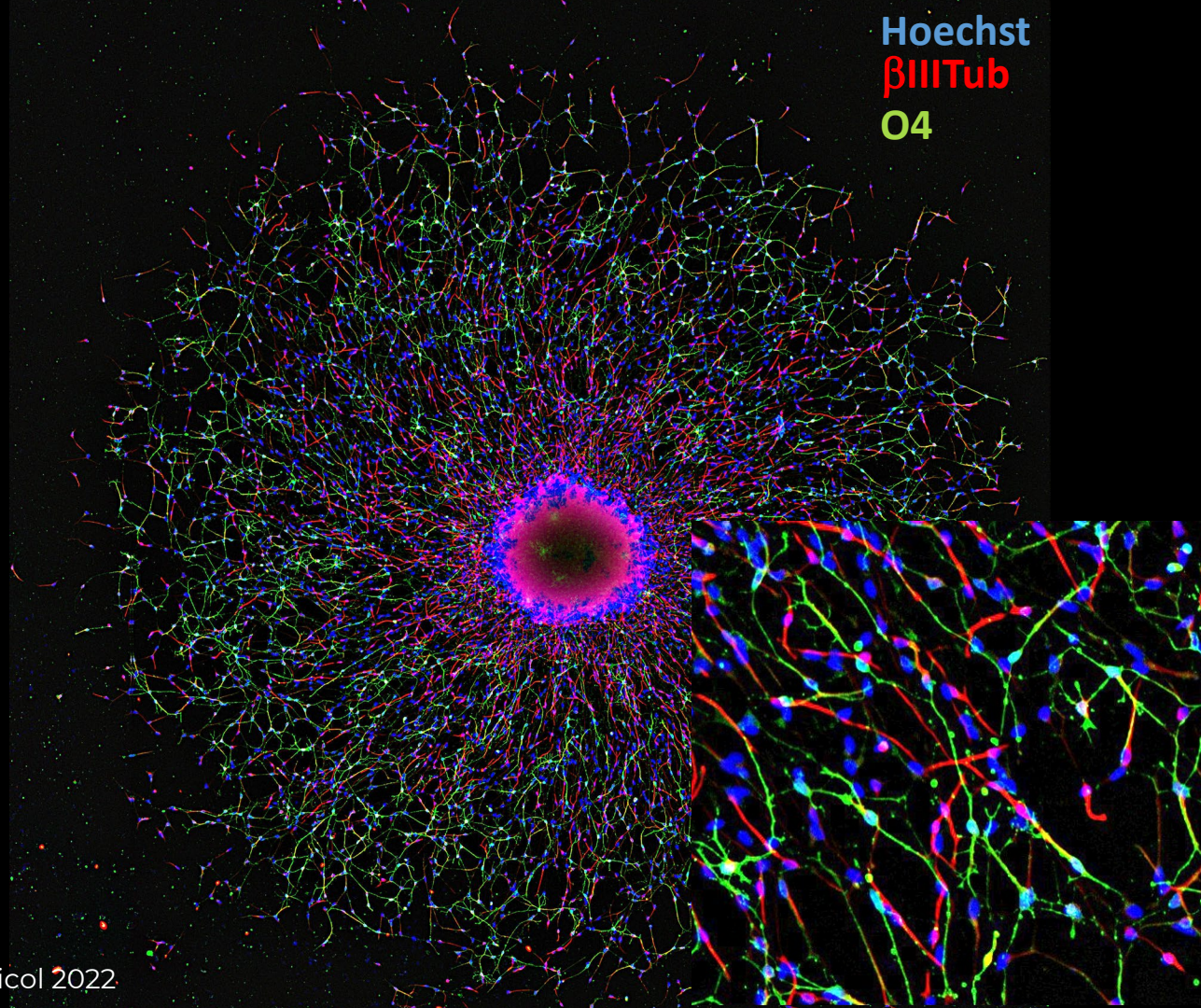
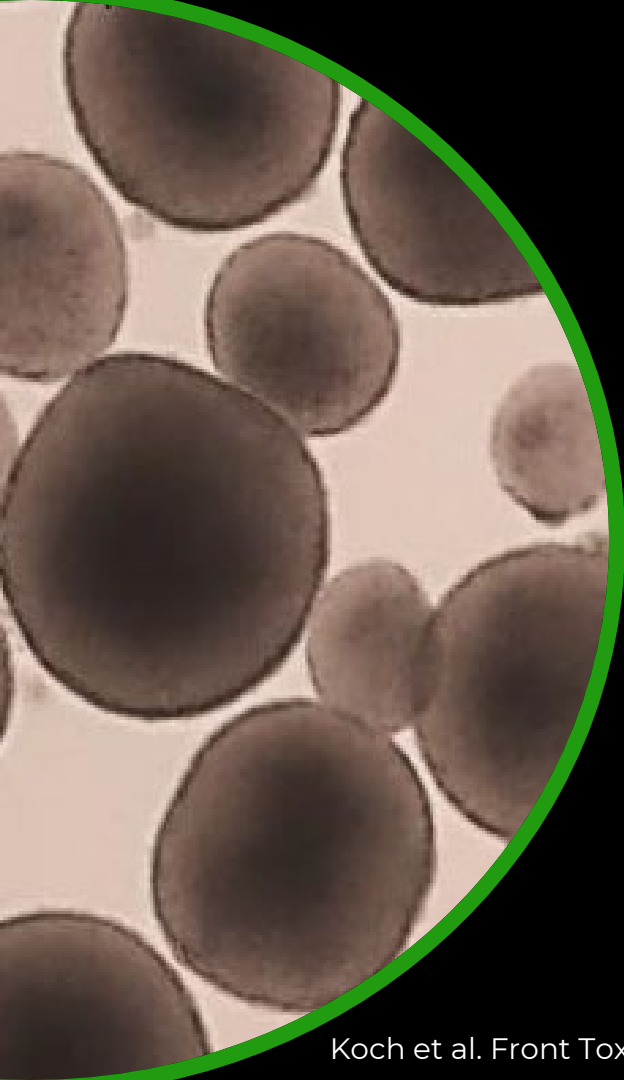

LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG

 rCortical MEA


LEIBNIZ INSTITUT FÜR UMWELT-MEDIZINISCHE FORSCHUNG

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



Hoechst
 β III Tub
O4

Data Evaluation Workflow DNT EU-IVB & Classification Models

Experimental background

Pre-processing

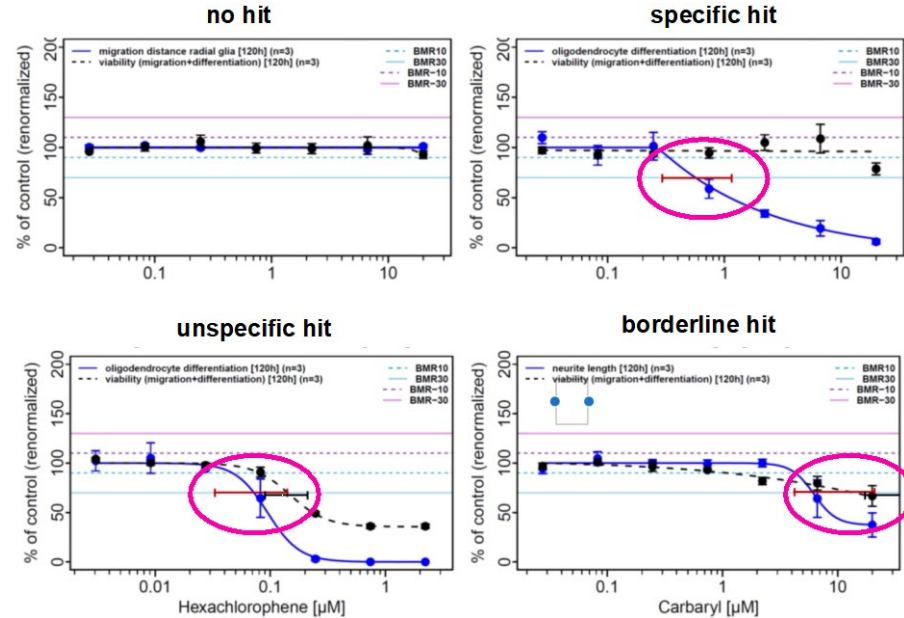
Re-normalization

Best Fit curve fitting

Data summary by median

BMC and CI estimation – confidence bands

Classification



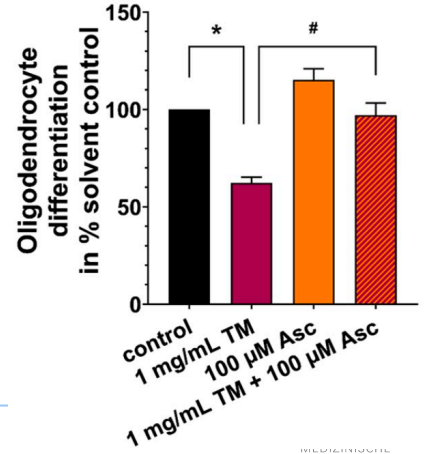
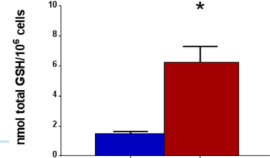
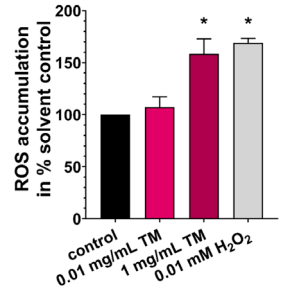
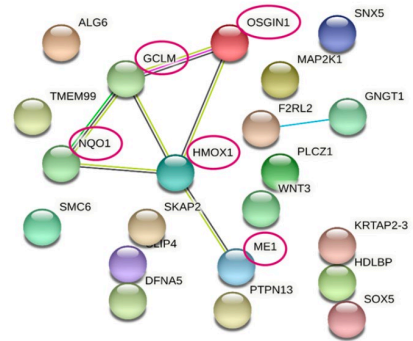
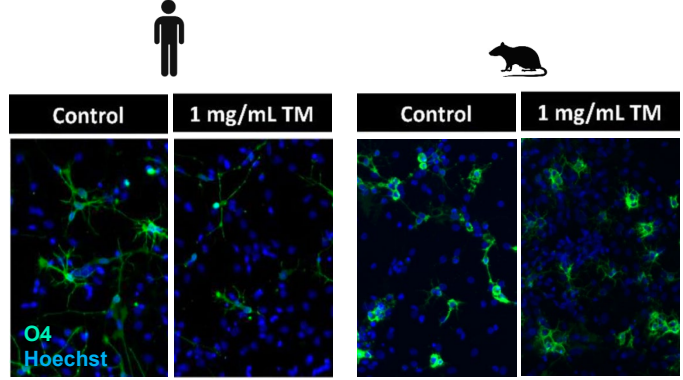
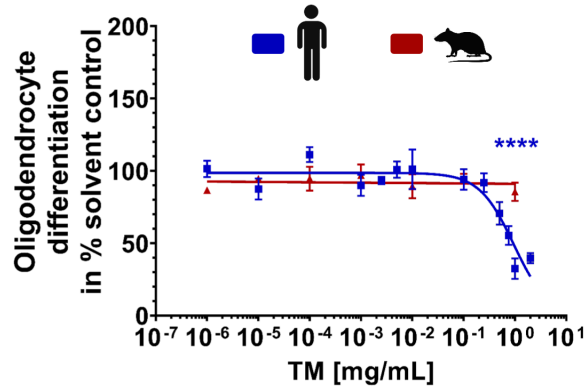
<https://github.com/iuf-duesseldorf/fritsche-lab-CRStats>

Libriz

The **species-**
overarching 3D
multicellular,
organotypic
neurosphere method
 is set up for screening
 and hazard
 characterization



Libriz



Klose et al. Cell Biol Toxicol 2022

What are benefits and limitations of New Approach Methodologies (NAMs)?



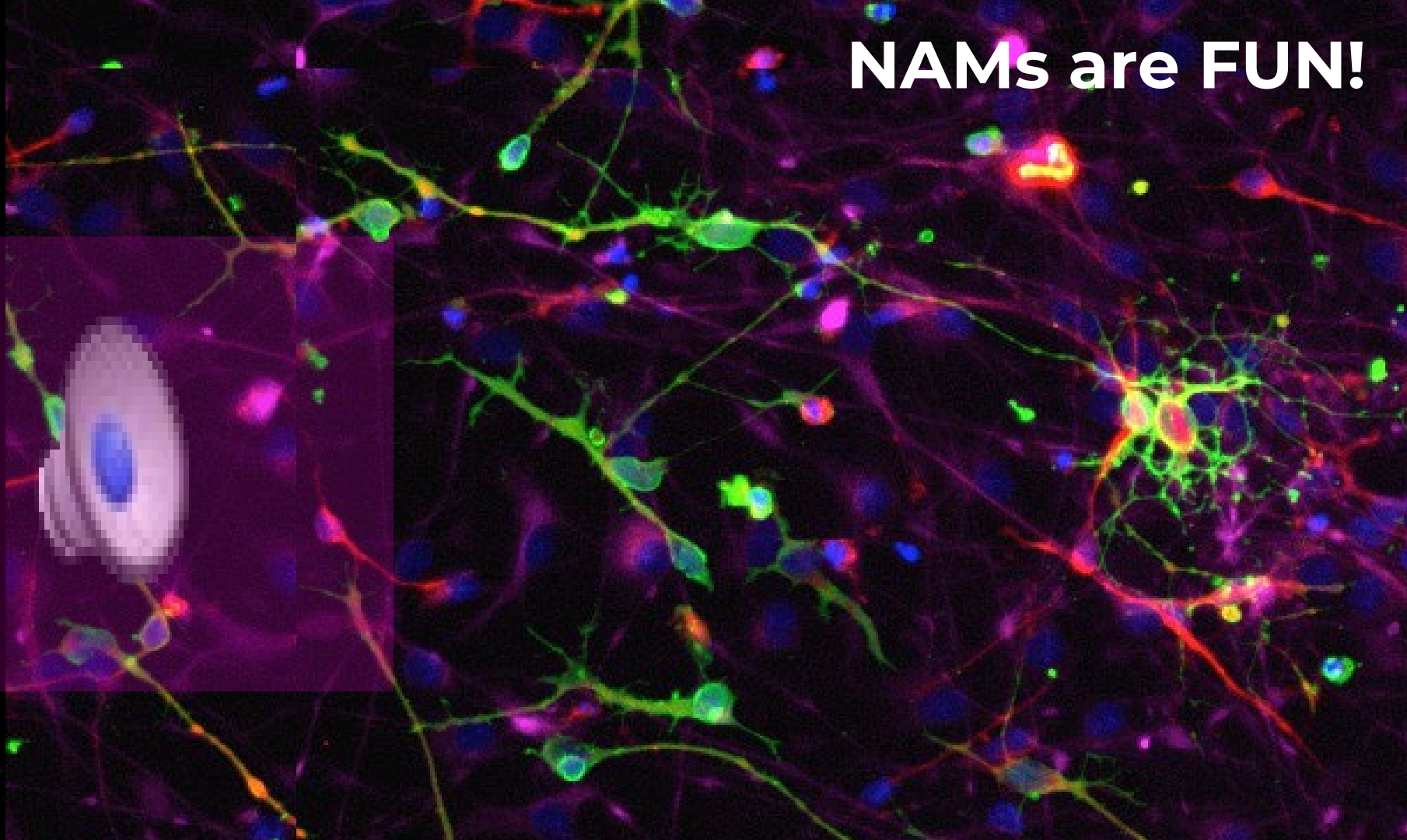
Benefits:

- correct species
- species comparison possible
- physiologically relevant – especially with organotypic NAMs
- faster
- higher throughput
- more cost-effective

Limitations:

- not the whole organism
- organ crosstalks are not necessarily assessed
- usually lack of immune system
- usually lack of vasculature

NAMs are FUN!



Lab