IS THE CURRENT PARADIGM SUITABLE FOR TESTING THE OVERALL CHEMICAL UNIVERSE?*

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*An Analysis of the Limitations and Uncertainties of In Vivo Developmental Neurotoxicity Testing and Assessment to Identify the Potential for Alterative Approaches, **Martin Paparella, Susanne Hougaard Bennekou, Anna Bal-Price 2020**; https://doi.org/10.1016/j.reprotox.2020.08.002

The scientific views presented here are those of the presenter alone and do not necessarily reflect official views of the Medical University of Innsbruck or the European Food Safety Agency

current paradigm

OECD TG 426
OECD TG 443 with DNT cohort
OPPTS 870.6300
NAFTA Guidance
https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/developmental-neurotoxicity-study-guidance

dose-response & NOAEL/LOAEL or BMD

- with P and F1 generation of rats
- clinical signs related to neurotoxicity
- behavioral ontogeny
- motor activity
- motor and sensory function
- learning and memory (not in TG 443)
- brain weight; brain morphometrics, neuropathology of CNS and PNS

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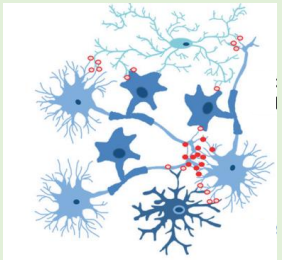
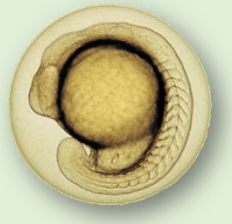
NAM Approaches



human induced pluripotent stem cells
primary human neurprogenitor cells
rat cortical cultures, see e.g. EFSA PPR Panel et al. 2021, https://doi.org/10.2903/j.efsa.2021.6599

- exposure up to ca. 1 month
- dose-response & BMDs for
  - proliferation
  - migration
  - differentiation
  - neurite outgrowth
  - synaptogenesis
  - network formation and function

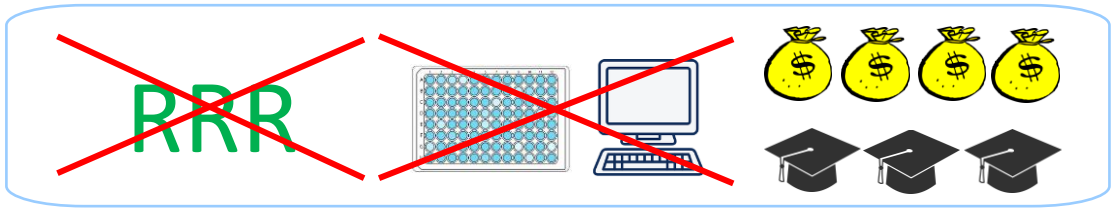


- zebrafish-embryos < 5 dpf
  - behavior
- …

Images for rat from pixabay.com; for neuronal network from Bal-Price et al. 2018, https://doi.org/10.14573/altex.1712081; for fish-embryo from Thomas Braunbeck

rodent based DNT testing and assessment

practicality



> 800 animals/ test (>160 just DNT cohort in TG 443)

> 1 year (40 days for testing)

> € 1.000.000 / test

>100.000 chemicals on market

~160 rodent DNT tests/30 years

gain/maintain historical negative and positive control database

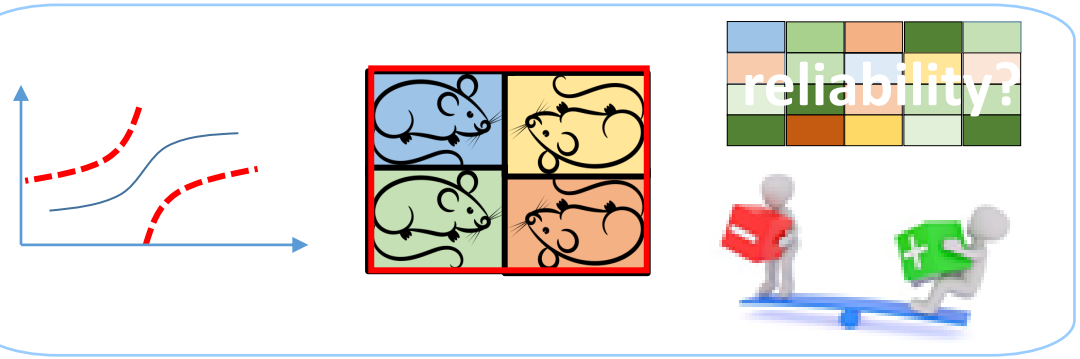
gain/maintain expertise for testing and assessment

huge effort to manually assess study reports

https://doi.org/10.1016/j.reprotox.2020.08.002

rodent based DNT testing and assessment

uncertainty of variability



- FOB: 50-100% concordance, similarity of slope 0.3-0.94
- ASR, MA, L&M?
- CVs 20% to >140%
- statistical significance vs. biological relevance?
- variable test designs -> variable dynamic range -> critical effect size cannot be generalized
- positive controls & historical negative controls needed (but often not available) to demonstrate lab´s proficiency & dynamic range / critical effect size for their test design
- positive controls usually for acute exposure – can dev. tox. reduce dynamic range? Δ controls/exposure -> Δ range?
- different test selection -> different results?
- high complexity in testing & no standard assessment “pipeline”
- in my practice often incomplete method & data description, inadequate conduct, inadequate statistics

- e.g. for non-DNT animal tests:

  variance in systemic LOAELs ~0.5 log10-mg/kg/bw (Pham et al. 2020)

  >20% qualitative discordance between replicate tests

  GHS category reliability 60-80%

  40% discordant ADI derivations by different expert groups

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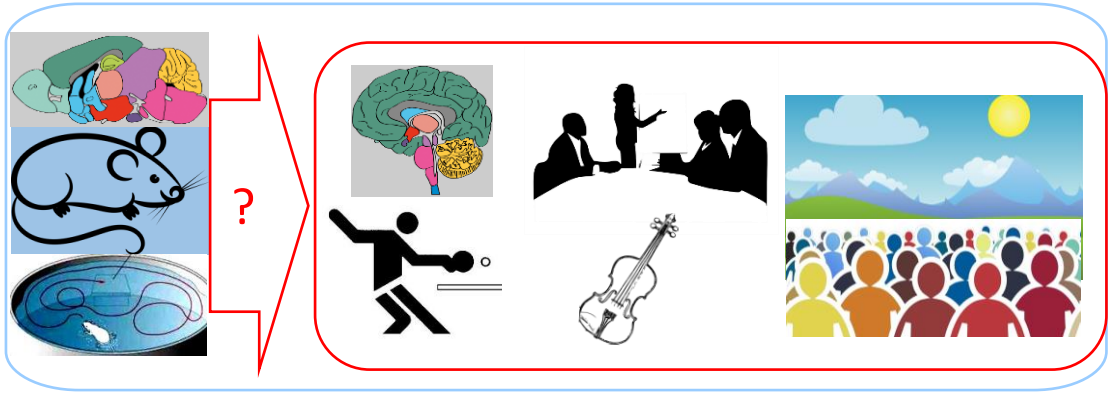
rodent based DNT testing and assessment

uncertainty of relevance



- ~20 chemicals DNT positive in human and rodents
- no quantification for human sensitivity/specificity
- extrapolation uncertainties: functional, anatomical, metabolism & kinetics, cellular

- e.g. for non-DNT related animal tests:
  - > 20% qualitative discordance between species
  - unknown confidence interval for human limit value aiming for high protection levels, e.g. 1:10,000 or 1: 1 million

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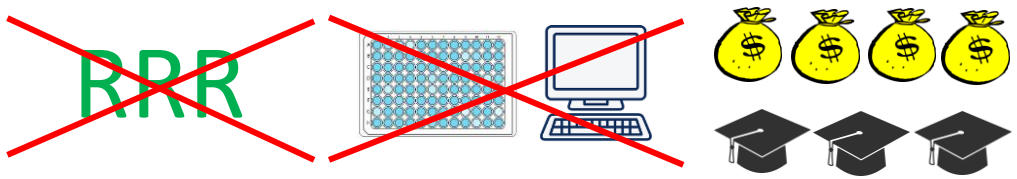
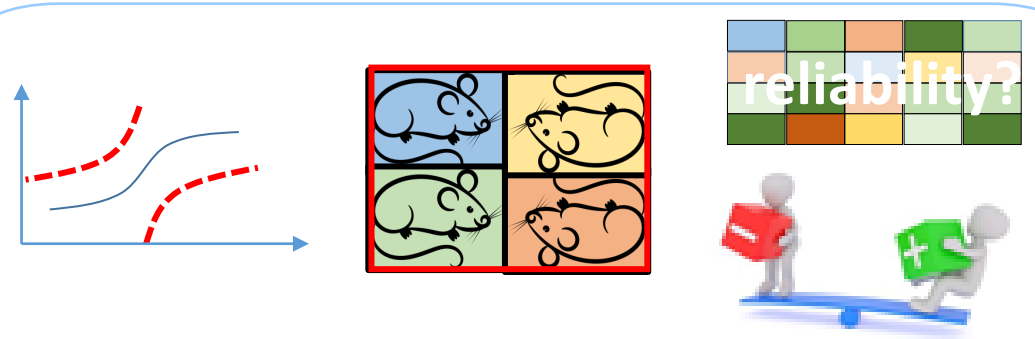
rodent based DNT testing and assessment

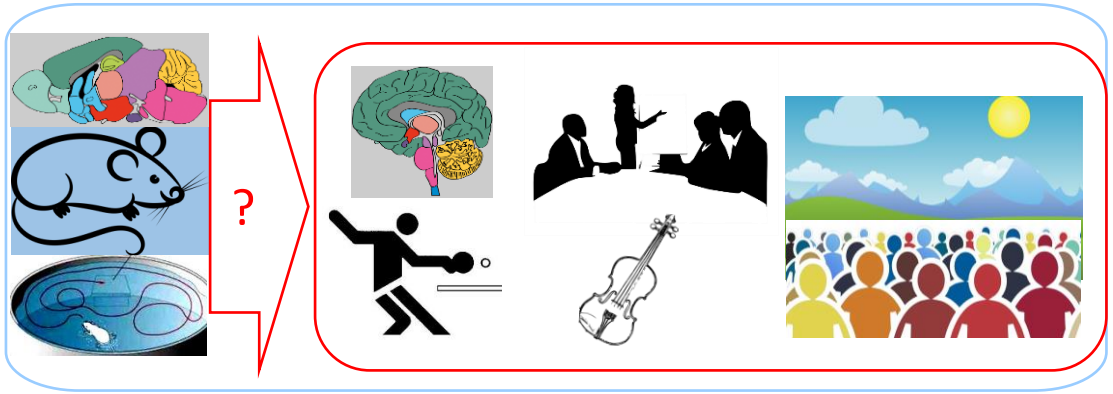
practicality



uncertainty of variability



uncertainty of relevance



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NAM based DNT testing and assessment

↑ 3Rs, ↑ throughput, ↓ costs, to assess more chemicals & new green chemistry, & maintain training for ↑overall human safety

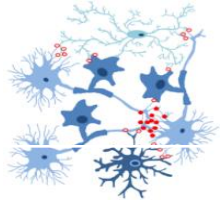
↑replicates, study internal positive control, ↑ concentration range w/o 3R conflict for ↑ reliability

↑ standardization of testing and assessment & ↑ validation w/o 3R conflict for ↑ global comparability of results

early, sensitive, mechanistic, human relevant, complementary indicators of toxicity for ↑ human safety and ↑ mixture extrapolation

QIVIVE, using points of departure from NAMs, within an IATA may provide, eventually context dependent, at least the same level of protection

uncertainties of NAMs perceived as “new” are conceptually similar for in vivo approaches



definitive vs. indicative adversity	• MIEs, KEs may be compensated or not at organism level • human world modifiers (diet, life-style ect.) may compensate or increase deficits	• also transient increase or decrease and change in variability are considered • human world modifiers (diet, life-style ect.) may compensate or increase deficits
different testing & assessment designs	• methods details affect BMD/BMR • standard data assessment pipeline in progress	• methods details affect BMD/BMR • no standard data assessment pipeline
mechanistic validity	• KEs & KERs to AOs are incomplete • AOPs are work in progress	• rodent MA, ASR, L&M endpoints are not aligned with functionally similar neurological effects/tests in humans • reading, planning, organizing, advanced sportive, artistic activity – not covered
biotransformation	• limited to isolated test system • may be dissimilar to human in vivo	• species variability • may be dissimilar to human
“positive” more reliable than “negative”	see above	• see above
need for modelling	In vitro kinetics and QIVIVE	• kinetic species extrapolation particularly uncertain for repro-studies • limit value derivation is uncertain

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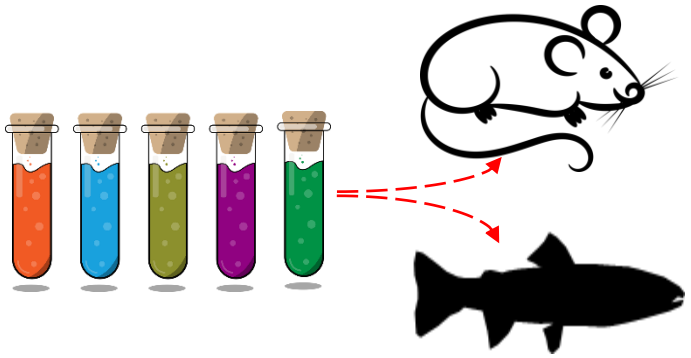
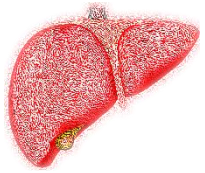
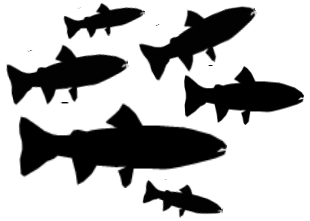
YESTERDAY







mortality
reproduction
motor & sensory
tissue damage behavior
learning & memory
malformation

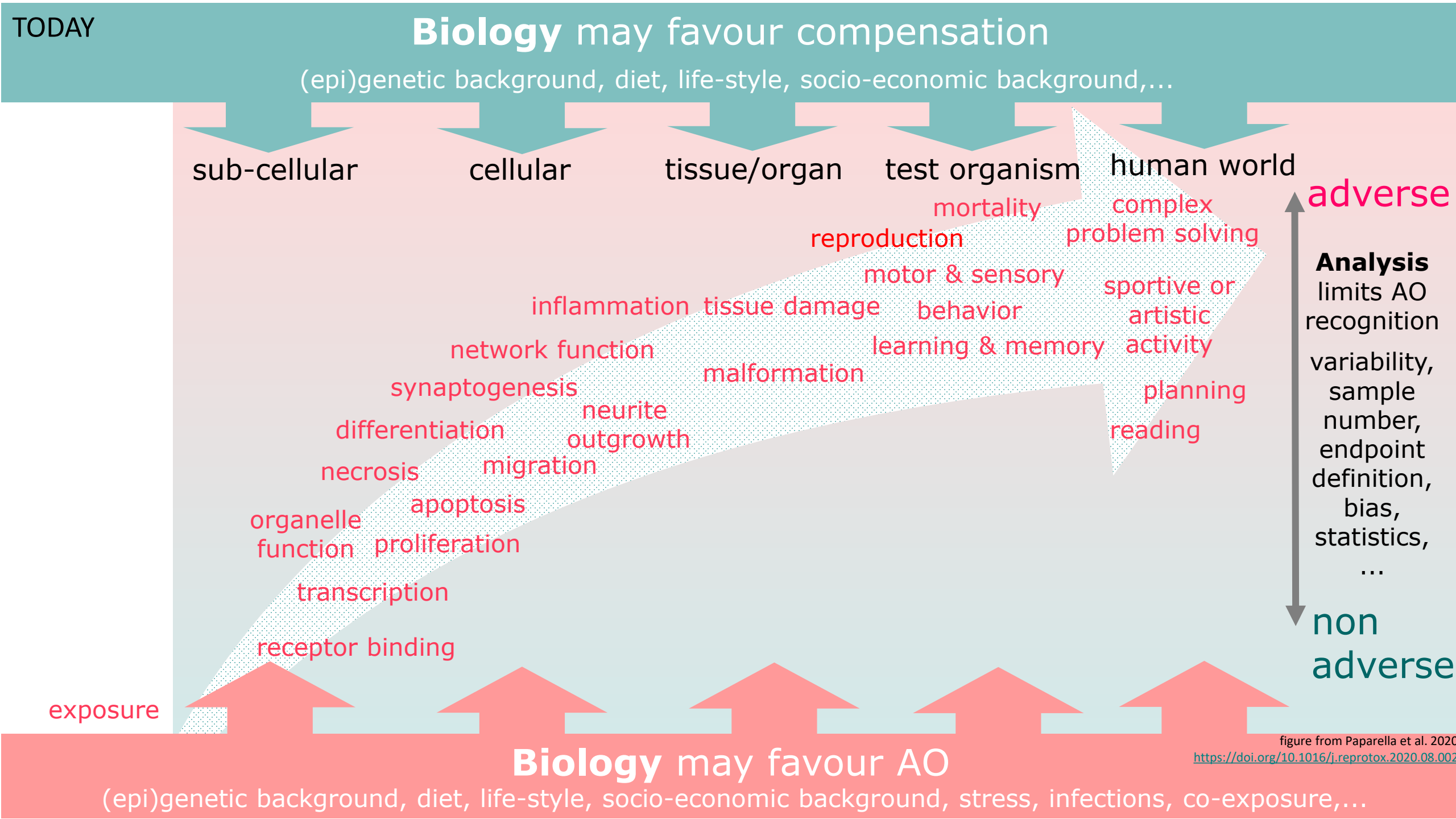


figure from Paparella et al. 2020
https://doi.org/10.1016/j.reprotox.2020.08.002

TOMORROW

Biology may favour compensation

(epi)genetic background, diet, life-style, socio-economic background,...

sub-cellular | cellular | tissue/organ | test organism | human world

adverse

in vitro MoA hazard class?

inflammation
network function
synaptogenesis
differentiation
neurite outgrowth
necrosis
migration
apoptosis
organelle function
proliferation
transcription
receptor binding
QIVIVE

tissue damage
malformation

mortality
reproduction
motor & sensory behavior
learning & memory

complex problem solving
sportive or artistic activity
planning
reading

Analysis limits AO recognition

variability, sample number, endpoint definition, bias, statistics, ...

non adverse

exposure

Biology may favour AO

(epi)genetic background, diet, life-style, socio-economic background, stress, infections, co-exposure,...

Paparella et al. 2020
https://doi.org/10.1016/j.reprotox.2020.08.002

summary

- ✓ The current estimation of developmental neurotoxicity based on in vivo test guidelines bears various critical practical regulatory limitations and scientific uncertainties and for achieving the final goal of protection of men and environment from hazardous chemicals
- ✓ NAMs may relieve practical limitations, uncertainties for data variability and may provide – within IATAs- at least similar human relevance
- ✓ To fully exploit the potential of NAMs, the focus of regulatory toxicology needs to shift from individual WoE based substance assessment towards development and harmonization of IATAs. These should be built on highly standardized NAMs supported by computational approaches.

- ✓ All this is conceptually similar for other in vivo TGs and the potential for NAMs
- ✓ Acceptability of risk & uncertainty requires socio-ecologic-economic justification

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