SACATM 2024

INTERNATIONAL EFFORTS IMPLEMENTING NAMS FOR ASSESSING DEVELOPMENTAL NEUROTOXICITY IN CHEMICAL RISK ASSESSMENT

Iris Mangas,

Regulatory Toxicologist

Pesticides Peer Review Unit

EUROPEAN FOOD SAFETY AUTHORITY

DEVELOPMENTAL NEUROTOXICITY (DNT)

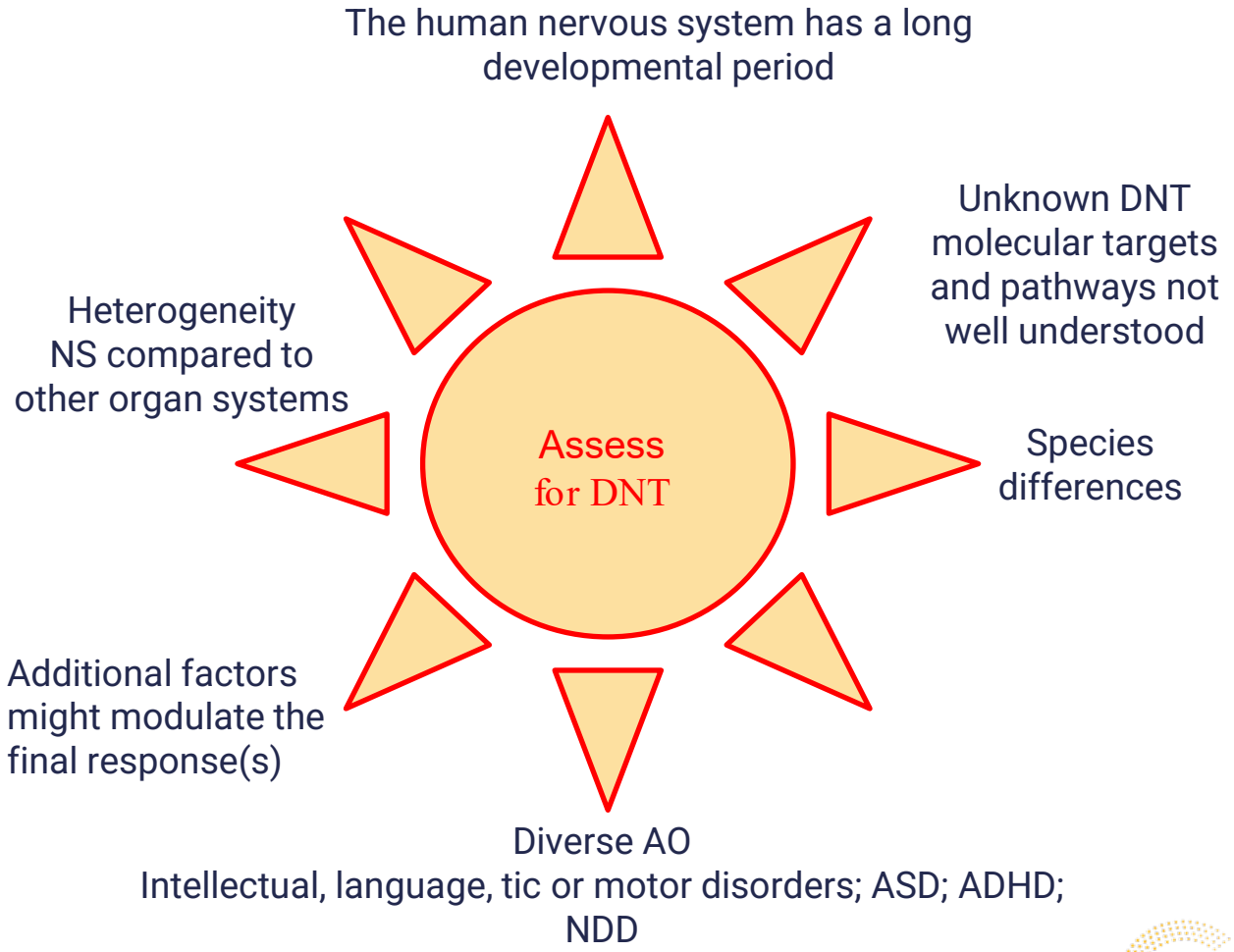
DNT chemicals: diverse set of substances that have the potential to interfere **with the normal development of the nervous system**, which, if perturbed **without compensation**, may lead to adverse effects on nervous system **structures and/or functions.**

Evaluating DNT as one of the endpoints to assess chemical safety is embedded in various regulations and several TGs are available for DNT in vivo screening.

However, use have been limited, potentially due to their methodological and interpretation complexity, ethical concerns and high costs.

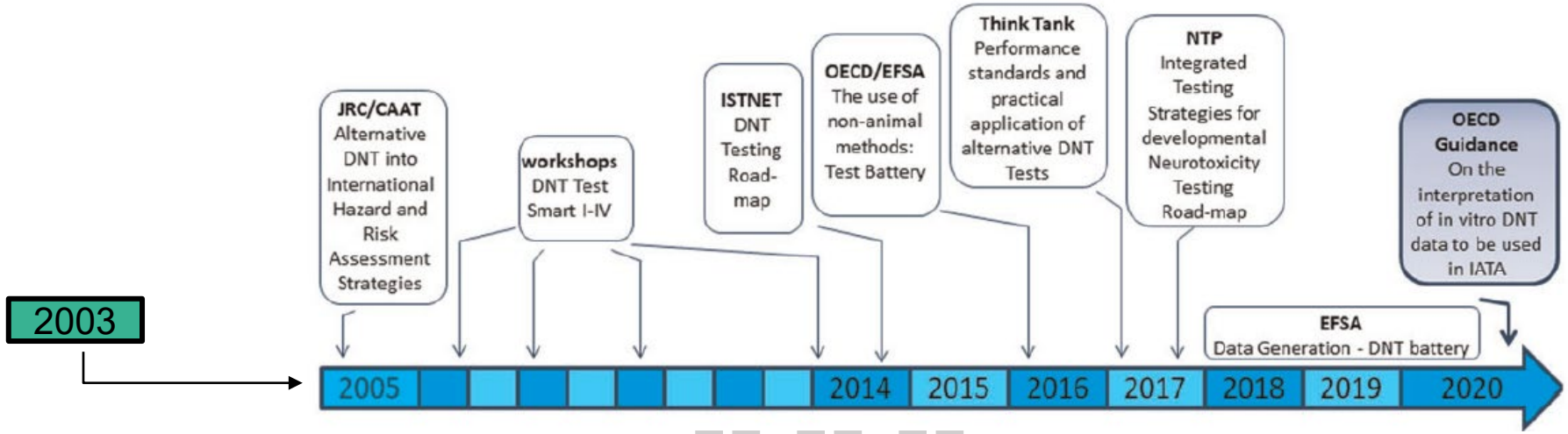
OECD N377., Key success factors:

- Collaborative effort
- Use the data in real case studies

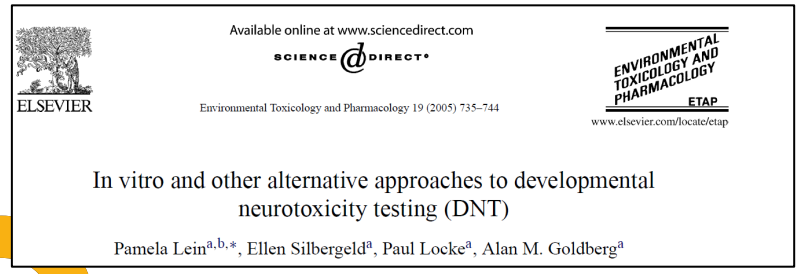


Developing and identifying an appropriate test system for DNT testing is challenging.

BUILDING THE DNT IVB

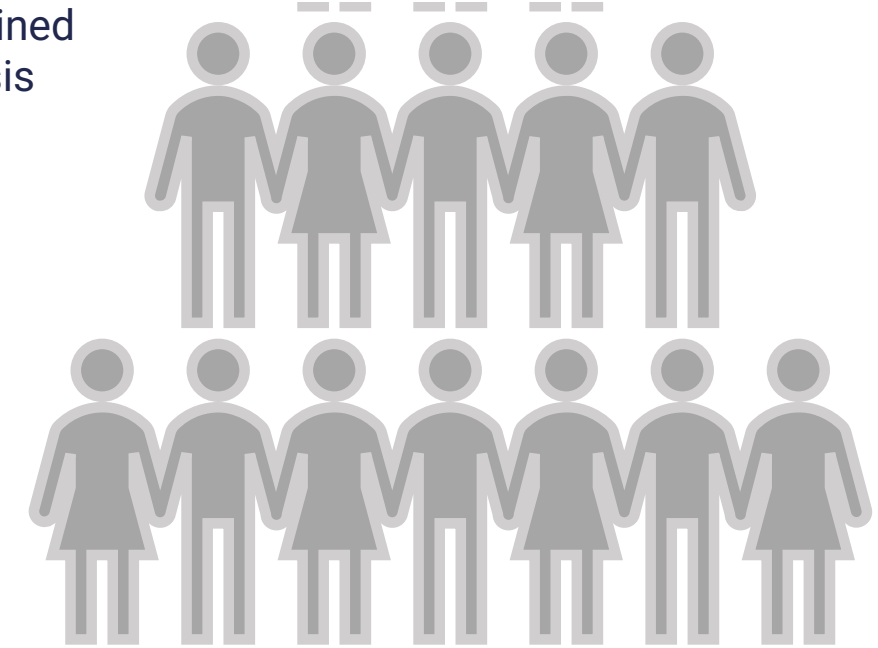


Advances using in vitro neural models combined with technology for higher throughput analysis has fostered the development of assays.


Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Environmental Toxicology and Pharmacology 19 (2005) 735–744
ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY
ETAP
www.elsevier.com/locate/etap

In vitro and other alternative approaches to developmental neurotoxicity testing (DNT)

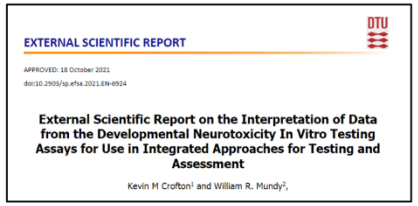
Pamela Lein[a,b,*], Ellen Silbergeld[a], Paul Locke[a], Alan M. Goldberg[a]

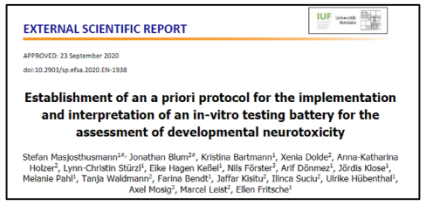


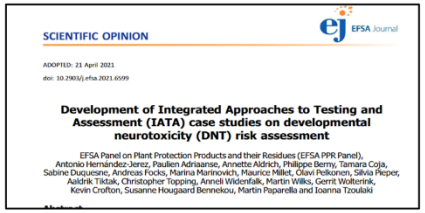
*Bringing together **basic researchers** in the fields of developmental neurobiology and mechanistic toxicology with policy analysts and **decision makers***

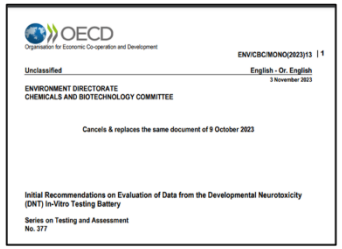
Adapted from Tim Shafer slide

EFSA SUPPORT TO THE INTERNATIONAL EFFORT









OECD DNT IVB guidance
OECD TGs

Regulatory NEED 2013

2015 ● Systematic literature review of available methods

Readiness criteria

2016 ● SPSF OECD EG Inclusion WNT plan

Better understating

2020 ● Experimental Work

2021 ● IATA case studies

2022 ● European Stakeholders Workshop

2023 ● OECD WNT approval

2024 ● Launching DNT IVB EFSA pesticides implementation projects

2027 ●

Regulatory Action

Can NAMs completely ~~replace~~ a DNT paradigm ?

Can NAMs be useful in refining and reducing animal testing and in producing a more informative risk assessment?

Transferability to GLP/ reproducibility

Produce high quality data

Use the data: AOP IATA

Interpretative guidance PoD: QiVIVE

COMMON NEEDS: THE OECD NO. 377

OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2023)13 | 1

Unclassified — English - Or. English

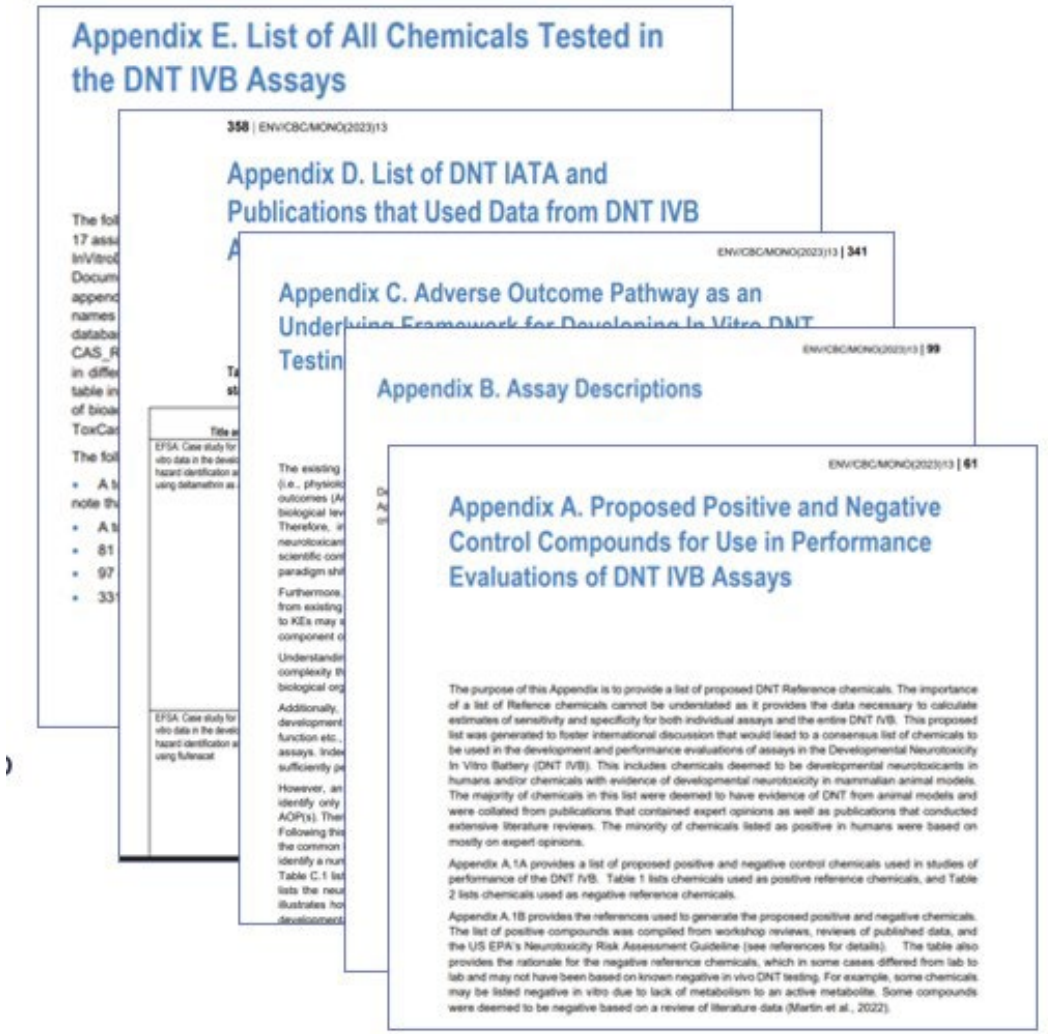
3 November 2023

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Cancels & replaces the same document of 9 October 2023

Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery

Series on Testing and Assessment
No. 377

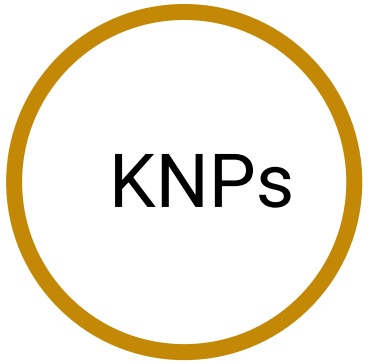


- 1. Review on the science on DNT IVB and provide GD on how to evaluate the DNT IVB and its relevance for assessing DNT
- 2. Context (AOP informed IATA) and description DNT IVB. + Appendix B
- 3. WoE considerations
- 4. Integration of evidence. Standardize use.

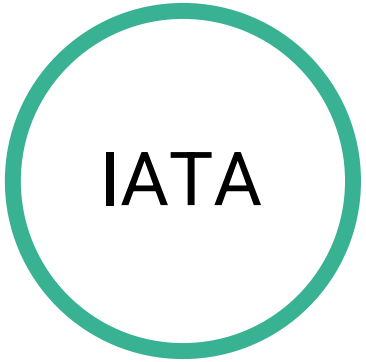
THE OECD DNT IVB INITIAL RECOMMENDATIONS DOCUMENT NO.377

- The purpose is to provide guidance on **how to evaluate *in vitro* data** from the assays comprising the DNT IVB (e.g., hit vs non-hit, biological coverage). Understand the uncertainties in the assays and the data outputs.
- It is not intended to provide interpretative guidance on the use of results in human risk assessments. Specific criteria for such use will likely be developed by regulatory agencies who will determine acceptability based on their needs.

1. A battery of assays judged ready for use by international working groups
2. Tested a common set of chemicals 81
3. Assay descriptions expanded version of OECD GD211



1. Assays are designed to recapitulate basic processes of neurodevelopment
2. AOPs as a Framework for In Vitro DNT. Review of existing DNT AOPs



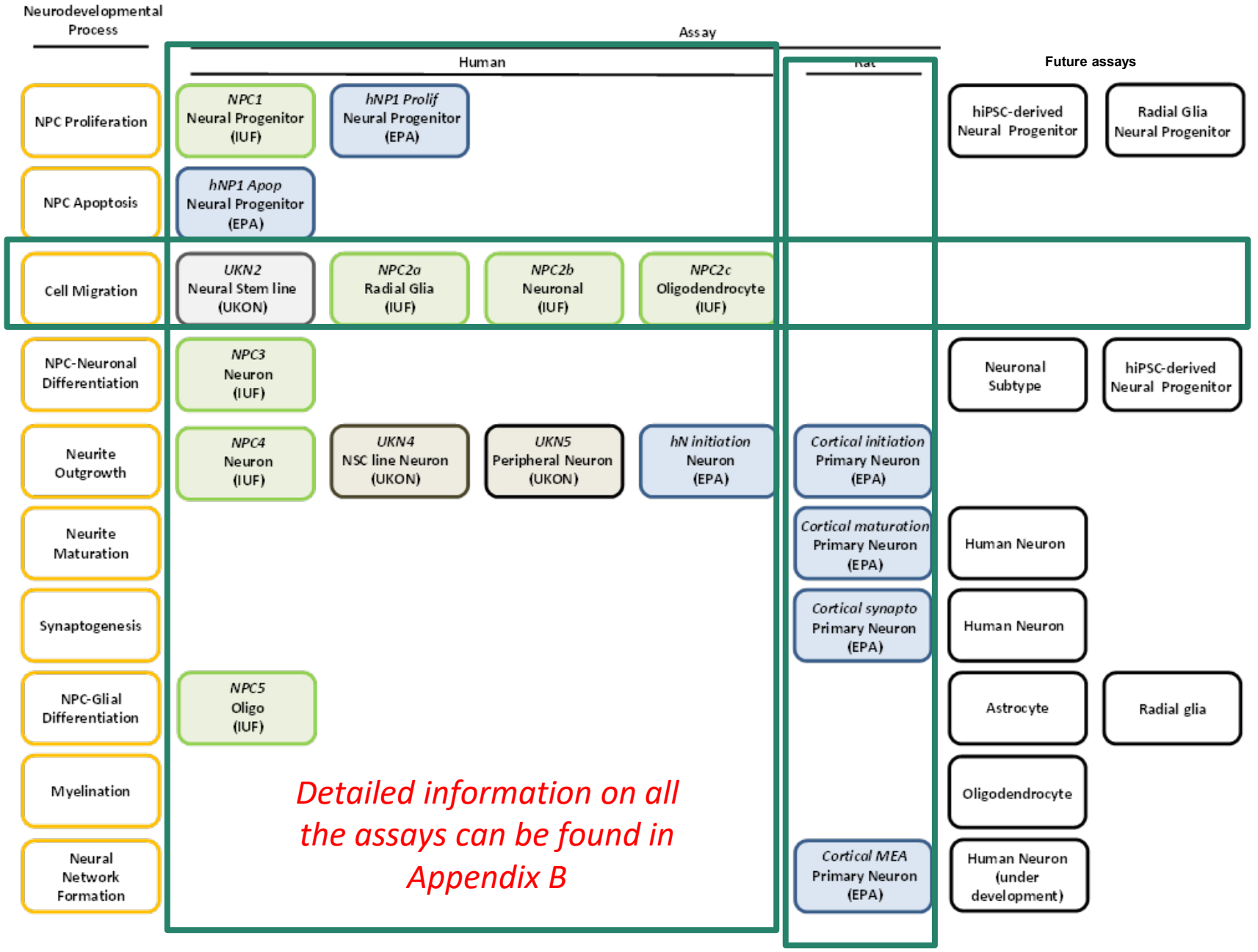
1. As a tool to integrate it in an WoE with all other available data.
2. Balancing the uncertainty in the data, with the uncertainty acceptable for the regulatory decision.
3. Series of proof of concept case studies **different CoU**

Figure 2.3. Assays in the DNT IVB

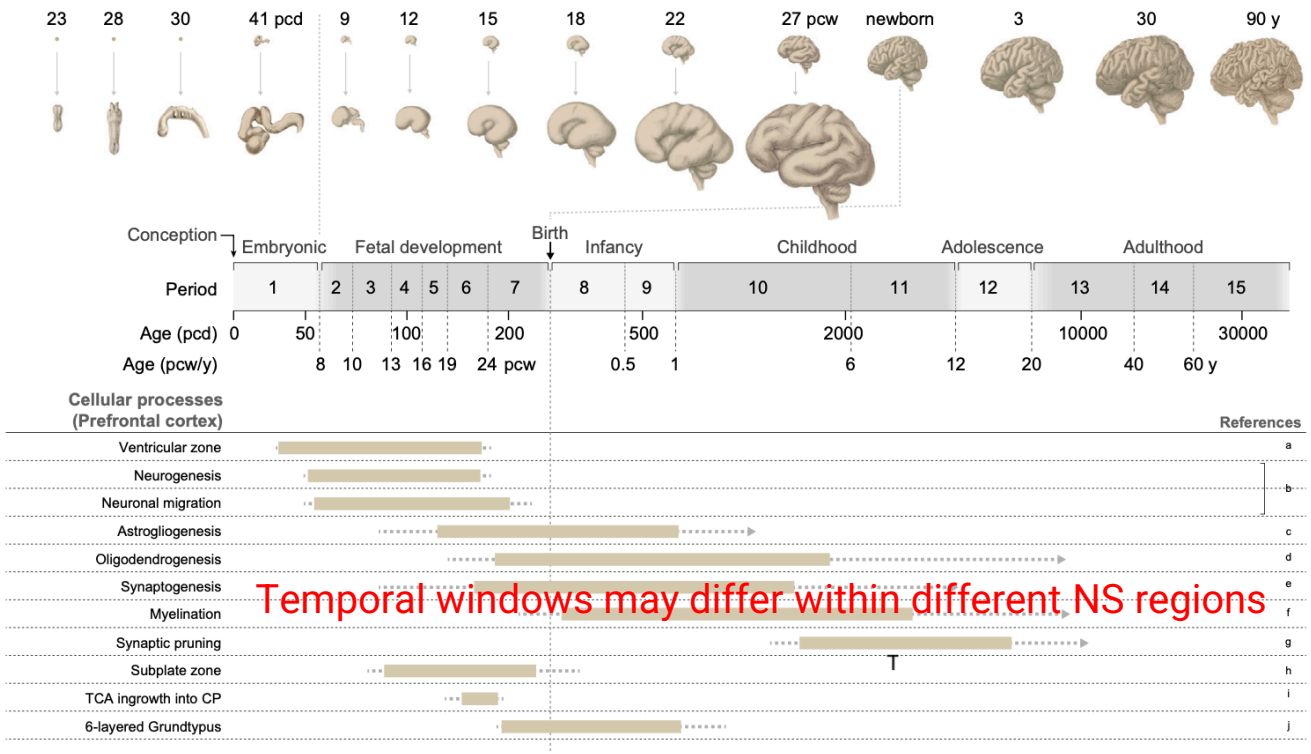
- Assays are not based on MiE (e.g., receptors).

- The assays in the DNT IVB instead measure neurodevelopmental **processes that are critical for normal development of the nervous system** and based on the following assumptions:

1. Changes in these cellular processes will reflect the integration of chemical disruptions in multiple up-stream molecular events.
2. Chemicals affecting one or more of these neurodevelopmental processes in vitro have the potential to do so in vivo if exposure.



WHAT DO THEY MEASURE? KNPS



Brain development is a highly complex procedure that covers time and a delicate balance of a large variety of key neurodevelopmental processes

Silbereis et al. 2016

Biological processes at cellular level necessary for NS development. Multiple cellular pathways.

DNT IVB KNDP	DEVELOPMENTAL TIMING test system	
	Embryonic	Fetal
NPC proliferation		X
NPC apoptosis		X
NPC migration		x
Radial glia migration		X
Neuronal migration		X
Oligodendrocyte migration		X
Neuronal differentiation	X	X
Neurite outgrowth	X	X
Neuronal maturation	X	X
Synaptogenesis	X	X
Oligodendrocyte differentiation		X
Neuronal network formation	X	X

Overview of neurodevelopmental timing of the current DNT IVB assays

ELEMENTS FOR WOE IATA

1. Generic issues for all in vitro assays.
2. Issues specific for DNT IVB assays (unknown metabolic competence, applicability domain, nominal concentrations,
3. Transparency in data analyses
4. Individual assay interpretation
5. Evaluation of data from the entire battery of assays

- Treat the data from the DNT IVB like all other data used in a WoE in hazard assessments

*This is the longest section in the GD

ACCEPTABILITY EFSA DNT IATA CASE STUDY PROJECT
EU PESTICIDES REGULATORY CONTEXT OF USE AS AN EXAMPLE

May 2019 — Establishment of EFSA PPR Panel IATA WG

6 WG experts + hearings

Observers: EC JRC, ECHA, MS, industry, NGO

June 2021 — April 2021 Submission to OECD IATA case study program

EFSA PPP Panel endorsed DLM and Flufenacet IATAs

PPR Panel agreed with the approach for DNT assessment of pesticides

Sep 2022 — WPHA endorsement and publication flufenacet and deltamethrin IATA case studies

Reviewed by Canada, Germany, Italy, Australia, Netherlands, OECD, BIAC.

Jan 2023 — EC mandate Acetamiprid : Test an existing IATA*

June 2023 — MS experts endorsed the IATA conclusions during the Peer Review Meeting for deltamethrin RMS Austria

EFSA IATA on acetamiprid published in EFSA Journal and accepted by EC

April 2024 — EFSA PPP Panel endorsed IATA on acetamiprid

MS experts endorsed the IATA conclusions for acetamiprid

Launching outsourcing projects

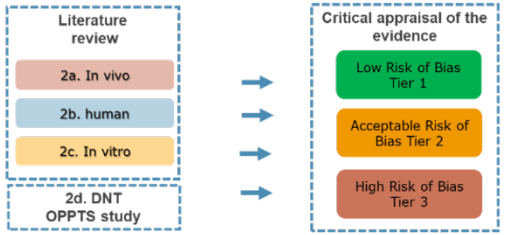
EFSA acetamiprid IATA WG

EFSAAOP INFORMED IATA CASE STUDIES WOE WORKFLOW: EVIDENCE BASED

Uncertainty Analysis

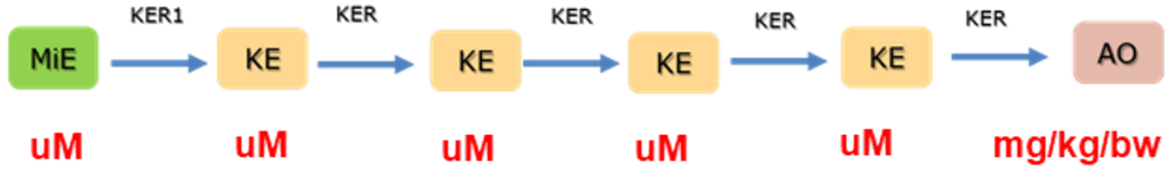
DNT HAZARD IDENTIFICATION PROBLEM FORMULATION

Step 1. Systematic literature review and critical appraisal to assess data quality (reliability and reporting)



In the context of the European pesticides Regulation (EU) 283/2013 and 1107/2009

Step 2. Data integration: AOP framework



- Dose and temporal concordance considerations

Step 3. Data gap and uncertainty analysis

Step 4. AOP informed IATA conclusion and Regulatory conclusion

OECD IATA CS 362 and Cs 363.
https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/cbc/mono(2022)24&doclanguage=en .

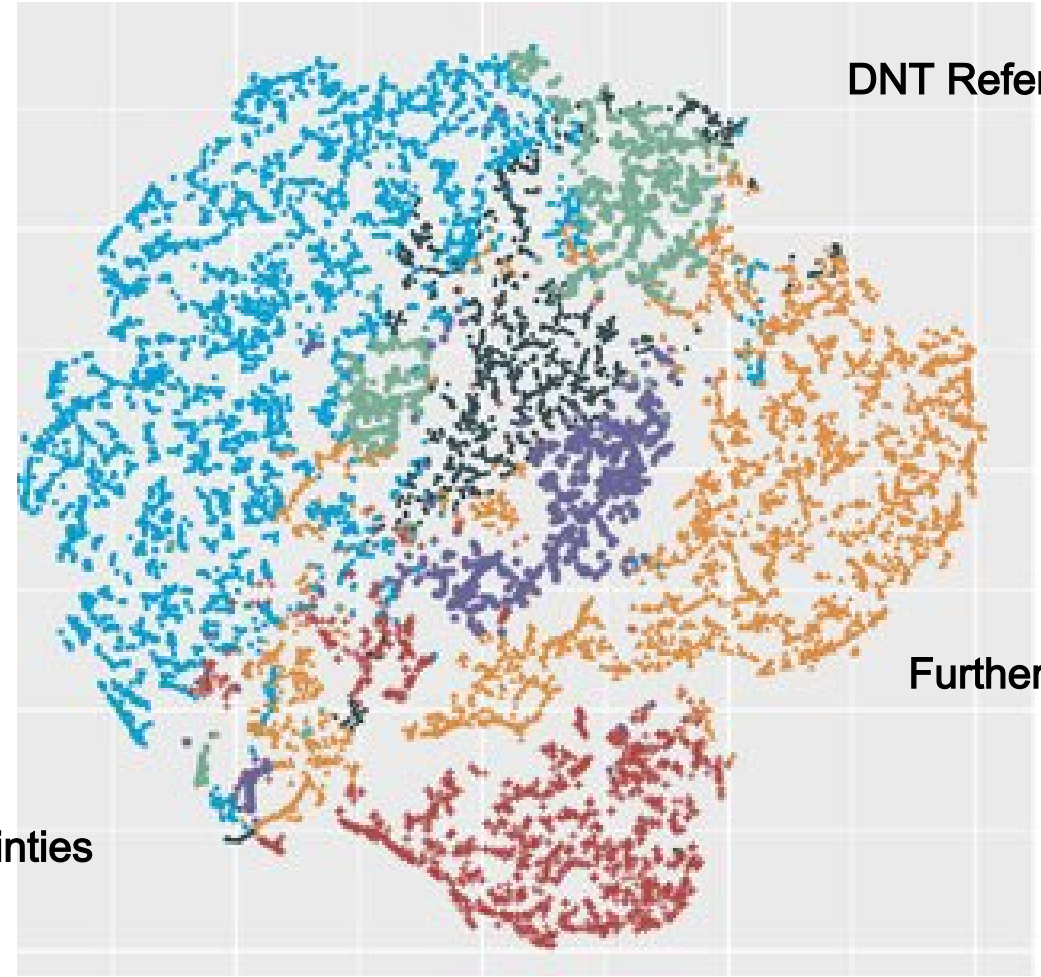
MOVING FORWARD: REGULATORY IMPLEMENTATION

Validation

Optimization SOPs
and
Lab to lab transfer

Tiered Approach/es

Biological uncertainties



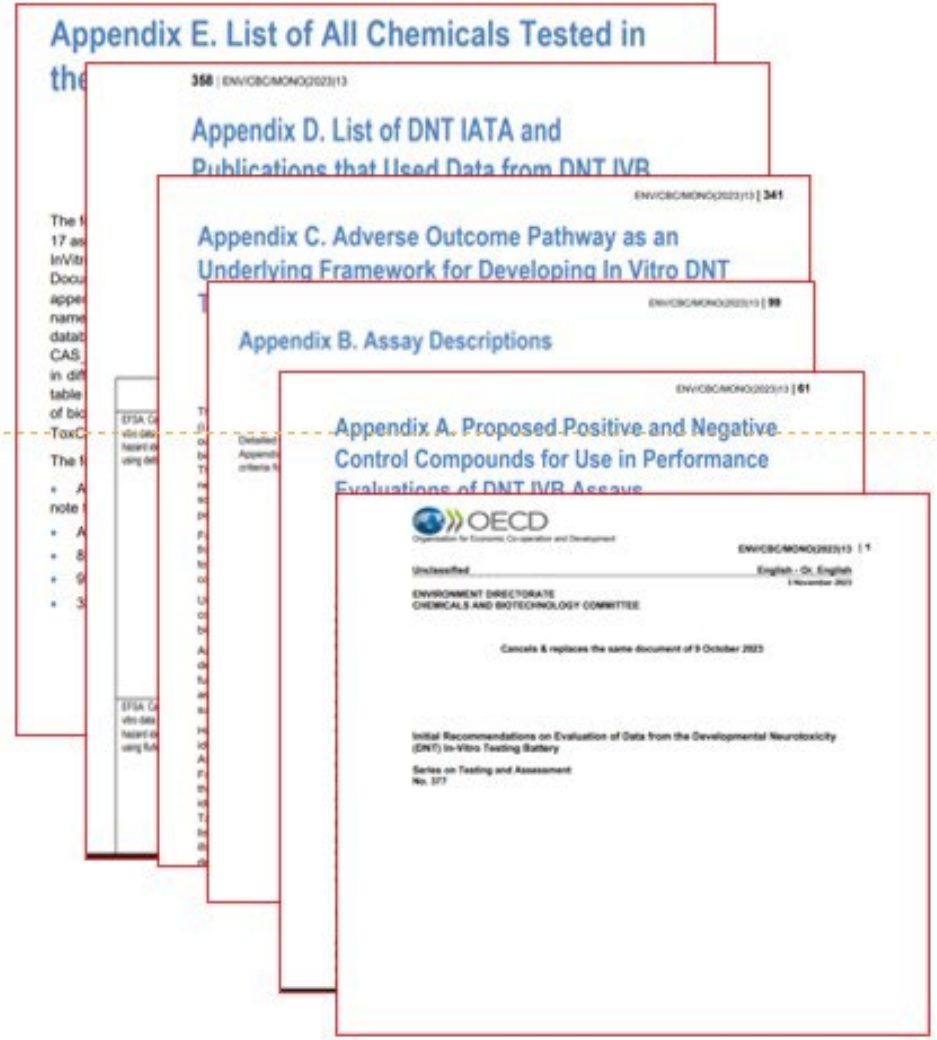
DNT Reference Chemical list

QIVIVE with PBK for DNT

Further interpretative guidance

From EFSA Stakeholders Workshop 2022 and OECD WNT commenting phase 2023

TOWARDS AN OECD GUIDANCE: ONGOING WORK REFERENCE CHEMICAL LIST



Current OECD DNT IVB EG efforts

Appendix A: OECD Guidance document provided a draft list.
Ongoing update: developing an OECD consensus list

A soon to be released EFSA report

"Recommended DNT Reference Chemical Test Set For In Vitro Assay Development"

Mundy and Crofton., 2024

Updates and prioritizes a list of 164 chemicals

TOWARDS AN OECD GUIDANCE: ONGOING WORK COMMON AGREED ANALYTICAL PIPELINE

There are a number of on -going efforts to begin analyses of all the data in the DNT IVB that will foster understanding of the worth of the assays and the battery.

Research Article

The Impact of Biostatistics on Hazard Characterization Using *In Vitro* Developmental Neurotoxicity Assays

Hagen Eike Keßel[1], Stefan Masjosthusmann[1], Kristina Bartmann[1], Jonathan Blum[2], Arif Dönmez[1], Nils Förster[4], Jördis Klose[1], Axel Mosig[4], Melanie Pahl[1], Marcel Leist[2], Martin Scholze[#,5] and Ellen Fritsche[#,1,3]

[1]IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany; [2]In vitro Toxicology and Biomedicine, Dept inaugurated by the Doerenkamp-Zbinden Foundation, University of Konstanz, Konstanz, Germany; [3]Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany; [4]Bioinformatics Group, Ruhr University Bochum, Bochum, Germany; [5]Brunell University, London, UK

Abstract

In chemical safety assessment, benchmark concentrations (BMC) and their associated uncertainty are needed for the toxicological evaluation of *in vitro* data sets. A BMC estimation is derived from concentration-response modelling and results from various statistical decisions, which depend on factors such as experimental design and assay endpoint features. In current data practice, the experimenter is often responsible for the data analysis and therefore relies on statistical software, often without being aware of the software default settings and how they can impact the outputs of data analysis. To provide more insight into how statistical decision-making can influence the outcomes of data analysis and interpretation, we have developed an automated platform that includes statistical methods for BMC estimation, a novel endpoint-specific hazard classification system, and routines that flag data sets that are outside the applicability domain for an automatic data evaluation. We used case studies on a large dataset produced by a developmental neurotoxicity (DNT) *in vitro* battery (DNT IVB). Here we focused on the BMC and its confidence interval (CI) estimation as well as on final hazard classification. We identified five crucial statistical decisions the experimenter must make during data analysis: choice of replicate averaging, response data normalization, regression modelling, BMC and CI estimation, and choice of benchmark response levels. The insights gained are intended to raise more awareness among experimenters on the importance of statistical decisions and methods but also to demonstrate how important fit-for-purpose, internationally harmonized and accepted data evaluation and analysis procedures are for objective hazard classification.

JOURNAL ARTICLE

Integrating Data From *In Vitro* New Approach Methodologies for Developmental Neurotoxicity FREE

Kelly E Carstens, Amy F Carpenter, Melissa M Martin, Joshua A Harrill, Timothy J Shafer, Katie Paul Friedman ✉

Toxicological Sciences, Volume 187, Issue 1, May 2022, Pages 62–79,
https://doi.org/10.1093/toxsci/kfac018
Published: 16 February 2022

PDF Split View Cite Permissions Share

Abstract

In vivo developmental neurotoxicity (DNT) testing is resource intensive and lacks information on cellular processes affected by chemicals. To address this, DNT new approach methodologies (NAMs) are being evaluated, including: the microelectrode array neuronal network formation assay; and high-content imaging to evaluate proliferation, apoptosis, neurite outgrowth, and synaptogenesis. This work addresses 3 hypotheses: (1) a broad screening battery provides a sensitive marker of DNT bioactivity; (2) selective bioactivity

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

Concentration Response BMC by Lab Integrative Analyses Resources

Developmental NeuroToxicity Data Integration and Visualization Enabling Resource (DNT-DIVER)

Research shows that a child's developing nervous system is far more vulnerable to chemical exposures than an adult nervous system. Recent increases in the rise of neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD), dyslexia, and autism spectrum disorder have prompted scientific interest in the potential contribution of environment toxicants to these disorders.

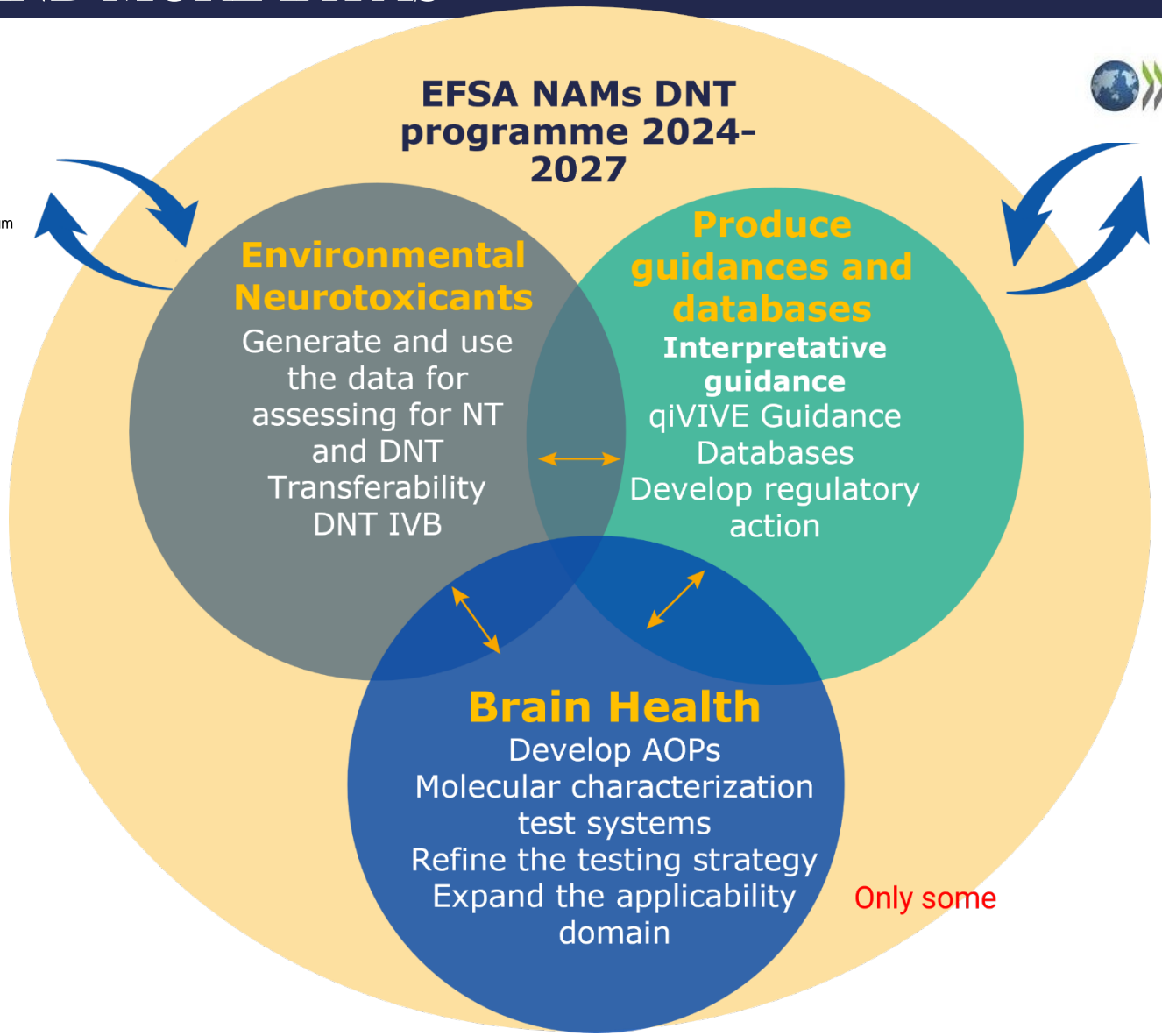
Traditional animal, or *in vivo*, studies provide important information about developmental neurotoxicity (DNT) but they are time and resource intensive. NTP has also developed more rapid screening tools that use human cell-based, or *in vitro*, assays, as well as alternate animal models such as zebrafish and planaria to identify toxicants with potential for DNT. Multiple tests, or assays, are often required to represent the complexity of the developing nervous system, but that can make it challenging to compare and summarize results.

NTP designed the Developmental NeuroToxicity Data Integration and Visualization Enabling Resource (DNT-DIVER) to analyze, compare, and visualize multiple DNT assays in an interactive web-application.

Standardize and simplify analysis of data

Current OECD DNT IVB EG efforts

TOWARDS AN OECD GUIDANCE: ONGOING EFFORTS TRANSFERABILITY, TESTING AND MORE IATAS



Using only pesticides approved in EU

Test system accesibility standarization

Only some

Implementation/standardization of biokinetic data in the DNT IVB and PBK models is needed

There are a number of on-going efforts to transfer the DNT IVB or some of the assays to “naïve” laboratories and to develop new assays. Here EFSA plan to contribute to this international and collaborative efforts is summarized

LESSONS AND LEARNINGS

- The DNT IVB successfully used, accepted and trusted by the stakeholders. The DNT IVB demonstrated to provide relevant, high-quality data on information regarding DNT potential of chemicals that are perturbing early cellular processes that are difficult to measure in vivo.
- The DNT-IVB data is publicly available and should be integrated in the regulatory pesticides risk assessment in EU. The available data for approved pesticides in EU is limited. To make a further regulatory action (change in data requirements) the **transferability to naïve laboratories able to conduct the assays under GLP and make them commercially available is needed.**
- AOP informed IATA is the EFSA current recommended framework to integrate the DNT IVB mechanistic data in pesticides RA since allows identification and quantification of uncertainties and transparent report.
- The final aim is to have an internationally agreed **interpretative guidance** and an **agreed standard workflow** facilitating the mutual use in the regulatory frameworks for quantitative risk assessment while retaining necessary flexibility depending on the context of use and advancement of science.
- This future work will continue to imply international and collaborative effort paving the way also for a broader use of NAMs.

ACKNOWLEDGMENTS

OECD DNT IVB EG

Member States Competent Authorities Pesticides peer-review experts

EFSA Plant Protection Products Panel

EFSA IATA DNT WG experts

- **Kevin Crofton (former US EPA)**
- Antonio Hernández-Jerez (University of Granada)
- Anna Price (JRC)
- Martin Paparella (University Innsbruck)
- Tamara Coja (AGES)
- Jerome Jenri (ANSES)

EFSA Methodological Support Unit

- Laura Martino
- Irene Munoz Guajardo

Pesticides Peer Review Unit Mammalian Toxicology Team

- **Andrea Terron**
- Iris Mangas
- Martina Panzarea
- Jochem Louisse

EFSA WG hearing experts:

- **Magdalini Sachanna (OECD)**
- Tim Shafer (US EPA)
- Mary Gilbert (US EPA)
- Kelly Carsten (US EPA)
- Marcel Leist (U Konstanz)
- Ellen Fritsche (SCAHT)
- Katharina Koch (IUF)

EFSA contractors DNT:

Universität Konstanz

IUF LEIBNIZ RESEARCH INSTITUTE FOR ENVIRONMENTAL MEDICINE

NIPH

DNTOX

MEDIZINISCHE UNIVERSITÄT INNSBRUCK

THANK YOU VERY MUCH
STAY CONNECTED

Contact me at:
Iris.MANGAS@efsa.europa.eu

LISTEN TO OUR PODCAST
Science on the Menu –Spotify, Apple Podcast and YouTube

FOLLOW US ON TWITTER
@efsa_eu @methods_efsa
@plants_efsa @animals_efsa

FOLLOW US ON LINKEDIN
Linkedin.com/company/efsa

FOLLOW US ON INSTAGRAM
@one_healthenv_eu

CONTACT US
efsa.europe.eu/en/contact/askefsa

efsa
EUROPEAN FOOD SAFETY AUTHORITY