AOP INFORMED IATA CASE STUDIES FOR DNT RISK ASSESSMENT

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BRIEF BACKGROUND

- **A challenging target:**
  - The goal is to assess any pesticide (regulated chemical) for DNT using an integrated approach (IATA) and minimize the request of DNT in vivo guideline studies.
- **A challenging scenario:**
  - Limited in vivo DNT testing, high costs, interpretative uncertainties and the need to reduce animal use, has fostered calls for the development of NAMs focused on screening chemicals for developmental neurotoxicity
  - Recent advances using in vitro neural models combined with technology for higher throughput analysis has fostered the development of assays to rapidly screen chemicals for impacts on critical neurodevelopmental processes
- **An effective science-policy interface is necessary**.
  - This is a common NAM issue to define a common goal and level of acceptance of the uncertainties
- **A road map is needed to bridge** the different scientific methods, approaches and forms of data and evidence to regulatory processes/legislations.

THE PATHWAY: TO AN INTEGRATED DNT TESTING STRATEGY

Scientific Opinion on the **developmental neurotoxicity potential of acetamiprid and imidacloprid**


SCIENTIFIC OPINION

Scientific Opinion on the developmental neurotoxicity potential of acetamiprid and imidacloprid[1]

EFSA Panel on Plant Protection Products and their Residues (PPR)[2,3]
European Food Safety Authority (EFSA), Parma, Italy

This Scientific Opinion, published on 21 February 2014, replaces the earlier version published on 17 December 2013[1].

ABSTRACT

The European Food Safety Authority asked the Panel on Plant Protection Products (PPR) and their residues to deliver a Scientific Opinion on the developmental neurotoxicity (DNT) potential of the neonicotinoid insecticides acetamiprid and imidacloprid. An *in vitro* study (Kimura-Kuroda et al., 2012) suggested that excitation and/or desensitisation of nicotinic acetylcholine receptors (nAChRs) by these compounds might affect developing mammalian nervous systems as occurs with nicotine. To evaluate the DNT potential of acetamiprid and imidacloprid, the PPR Panel scrutinised the open literature, the draft assessments reports and dossiers submitted for approval. The Panel concludes that both compounds may affect neuronal development and function, although several methodological limitations have been identified. Considering the available DNT studies for imidacloprid and acetamiprid, important uncertainties still remain and further *in vivo* studies following OECD test guideline (TG) 426 are required to robustly characterise a DNT potential and dose-response relationships, particularly for acetamiprid. The Panel considers that current ARfDs may not be protective enough for the possible DNT of acetamiprid and imidacloprid. [illegible] as regards the ADI for acetamiprid. More conservative [illegible] of the existing toxicological data. However, the [illegible] Kimura-Kuroda et al. (2012) prevent its current [illegible] of clear and consistent [illegible] Development [illegible]

PPR Panel Recommended the development of an integrated DNT testing strategy.

2013

efsa European Food Safety Authority

OECD

TER POLICIES FOR BETTER

Composed of **robust, reliable and validated in vitro** assays

2016

BETTER UNDERSTANDING


SCIENTIFIC REPORT

Establishment of an a priori protocol for the implementation and interpretation of an in-vitro testing battery for the assessment of developmental neurotoxicity

Stefan Masjosthusmann[1#], Jonathan Blum[2#], Kristina Bartmann[1], Xenia Dolde[2], Anna-Katharina Holzer[2], Lynn-Christin Stürzl[1], Eike Hagen Keßel[1], Nils Förster[2], Arif Dönmez[1], Jördis Klose[1], Melanie Pahl[1], Tanja Waldmann[2], Farina Bendt[1], Jaffar Kisitu[2], Ilinca Sucki[2], Ulrike Hübenthal[1], Axel Mosig[3], Marcel Leist[2], Ellen Fritsche[1]

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#authors contributed equally

Abstract

In this project we set up a human cell-based DNT *in vitro* testing strategy that is based on test methods with high readiness and data generated therefrom. The methods underwent a fit-for-purpose evaluation considered four key elements: 1. The test system, 2. the exposure scheme, 3. the assay and an endpoint(s) and 4. the classification model. This testing battery was challenged with 119 chemicals with toxicological information was available (for some of them also on their DNT hazard) performed in 5 test systems measuring 10 DNT-specific endpoints and additional derived parameters. For approximately half of the compounds, additional [illegible] tests was added by the US-EPA. This extended battery was [illegible] test methods of this current DNT *in vitro* battery [illegible] showed low redundancy. Battery performance [illegible]

2020

PPR Panel established the IATA DNT WG AIM to develop IATA case studies on DNT Risk Assessment

AIM to assess the applicability of the IVB in the pesticides RA in the context of the European pesticide Regulation (EU) 283/2013 and 1107/2009

COLLABORATIVE CASE STUDIES

2020

DATA GENERATION


OPINION

Development of Integrated Approaches to Testing and Assessment (IATA) case studies on developmental neurotoxicity (DNT) risk assessment

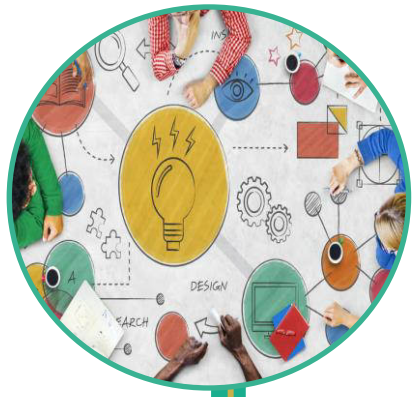
EFSA Panel on Plant Protection Products and their Residues (EFSA PPR Panel), Antonio Hernandez-Jerez, Paulien Adriaanse, Annette Aldrich, Philippe Berny, Tamara Coja, Sabine Duquesne, Andreas Focks, Marina Marinovich, Maurice Millet, Olavi Pelkonen, Silvia Pieper, Aaldrik Tiktak, Christopher Topping, Anneli Widenfalk, Martin Wilks, Gerrit Wolterink, Kevin Crofton, Susanne Hougaard Bennekou, Martin Paparella and Joanna Tzoulaki

Abstract

The EFSA Panel on Plant Protection Products and their Residues (PPR) has developed, as a self-task mandate (EFSA-Q-2019-00100), two adverse outcome pathway (AOP)-informed integrated approach to testing and assessment (IATA) case studies to answer a developmental neurotoxicity (DNT) hazard identification and characterisation problem formulation that could support the regulatory decisions for the pesticide active substances deltamethrin and flufenacet. The IATA were developed to assess the applicability of the DNT *in vitro* testing battery (IVB), designed to explore fundamental neurodevelopmental processes, in the regulatory risk assessment of pesticides. For this purpose, evidence-based-approach methodology was applied: 1) systematic literature review and critical appraisal of the evidence i.e. human observational studies, *in vivo* data from rodent models and new approaches (NAMs, i.e. *in vitro* studies including high-throughput testing from IVB and literature) for both case studies; 2) a quantitative uncertainty analysis of the expert knowledge elicitation (EKE) and a probabilistic approach; 3) integration of the evidence in a formal framework. This stepwise approach resulted in the [illegible] of the case studies. A probabilistic quantification [illegible] allowed the assessment

2021

Stakeholders Workshop



DNT IVB OECD GUIDANCE 2023

2022

PURPOSE OF THE OECD GUIDANCE DOCUMENT

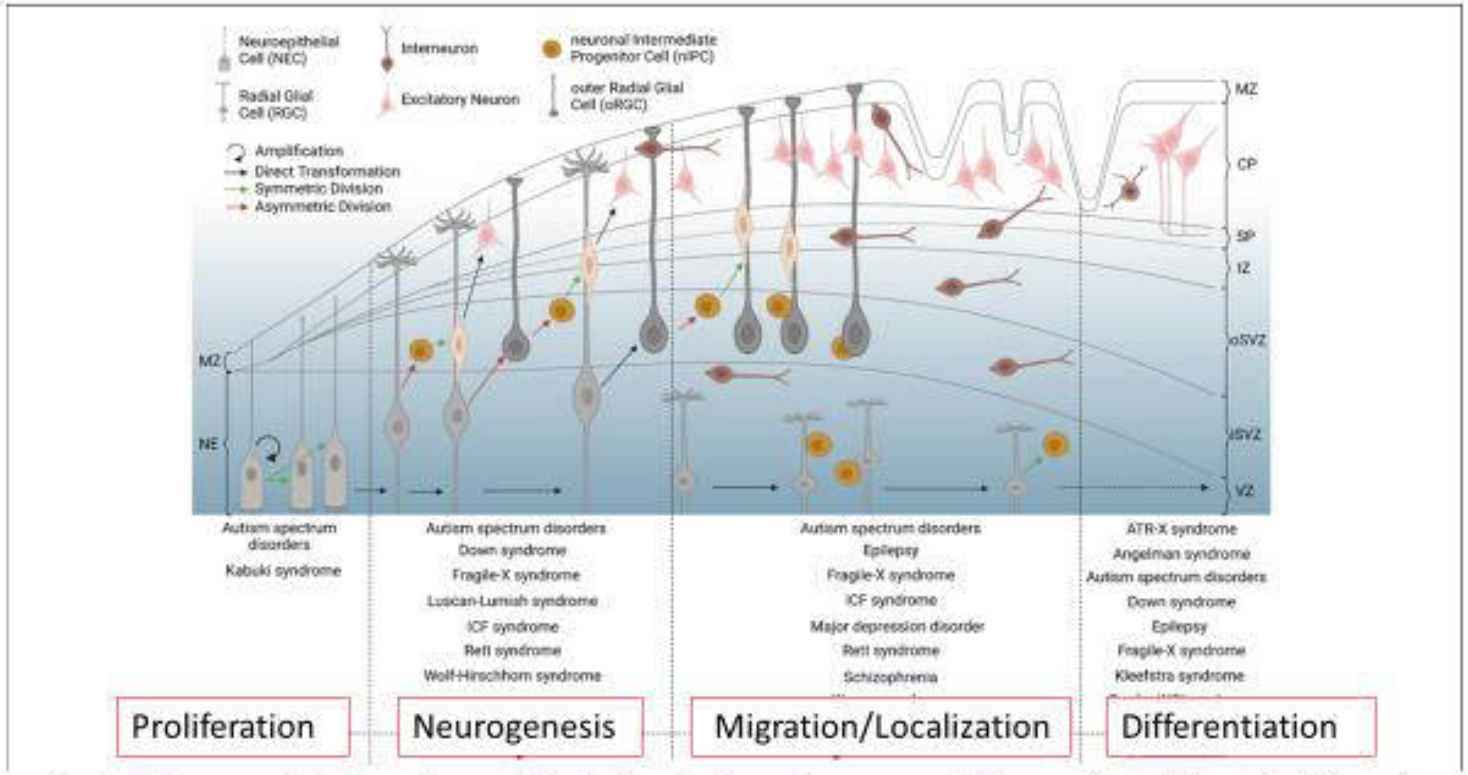
- "The purpose is to provide guidance on **how to evaluate *in vitro* data** from the assays comprising the battery (e.g., hit vs non-hit, uncertainties, biological coverage). It is not intended to guide the use of results in human risk assessments. Specific criteria for such use will likely be developed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

1. Judged ready for use in screening and prioritization by international working groups
2. Tested a common set of chemicals
3. Assay descriptions expanded version of OECD GD211

- AOPs as a Framework for In Vitro DNT.
- Review of existing DNT AOPs

- Integrate it with all other available data
- Balancing the uncertainty in the data, with the uncertainty acceptable for the regulatory decision
- Series of proof of concept case studies.

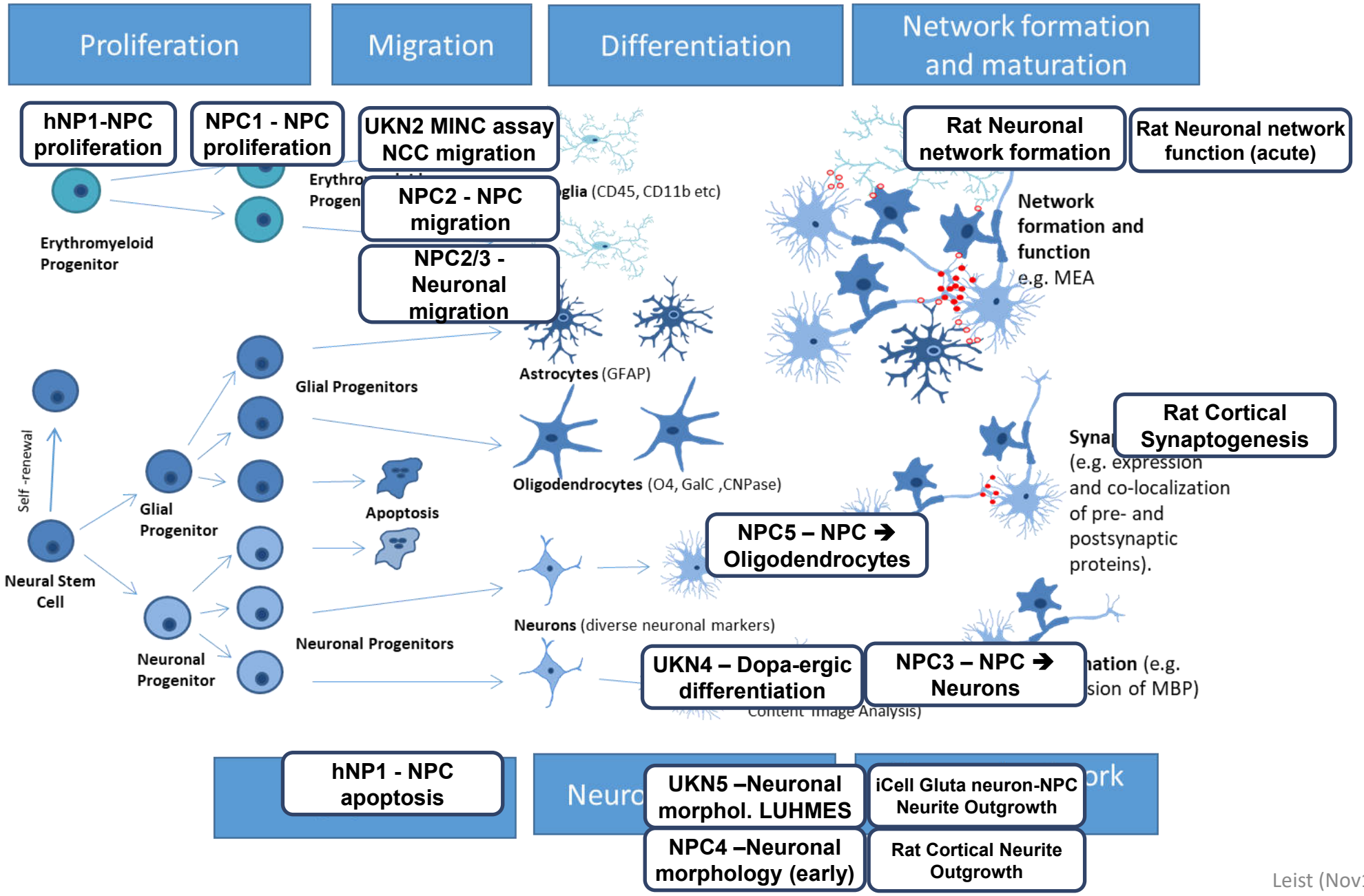
Neurodevelopment: Complex Temporal and Spatial Orchestration of Critical Milestone Events



Reichard and Zimmer-Bensch, Frontiers NS, 2021

- Different developmental disorders with distinct phenotypes result from disruption of different milestones
- Thyroid and sex hormones modulate these events
- This is only cortical development!

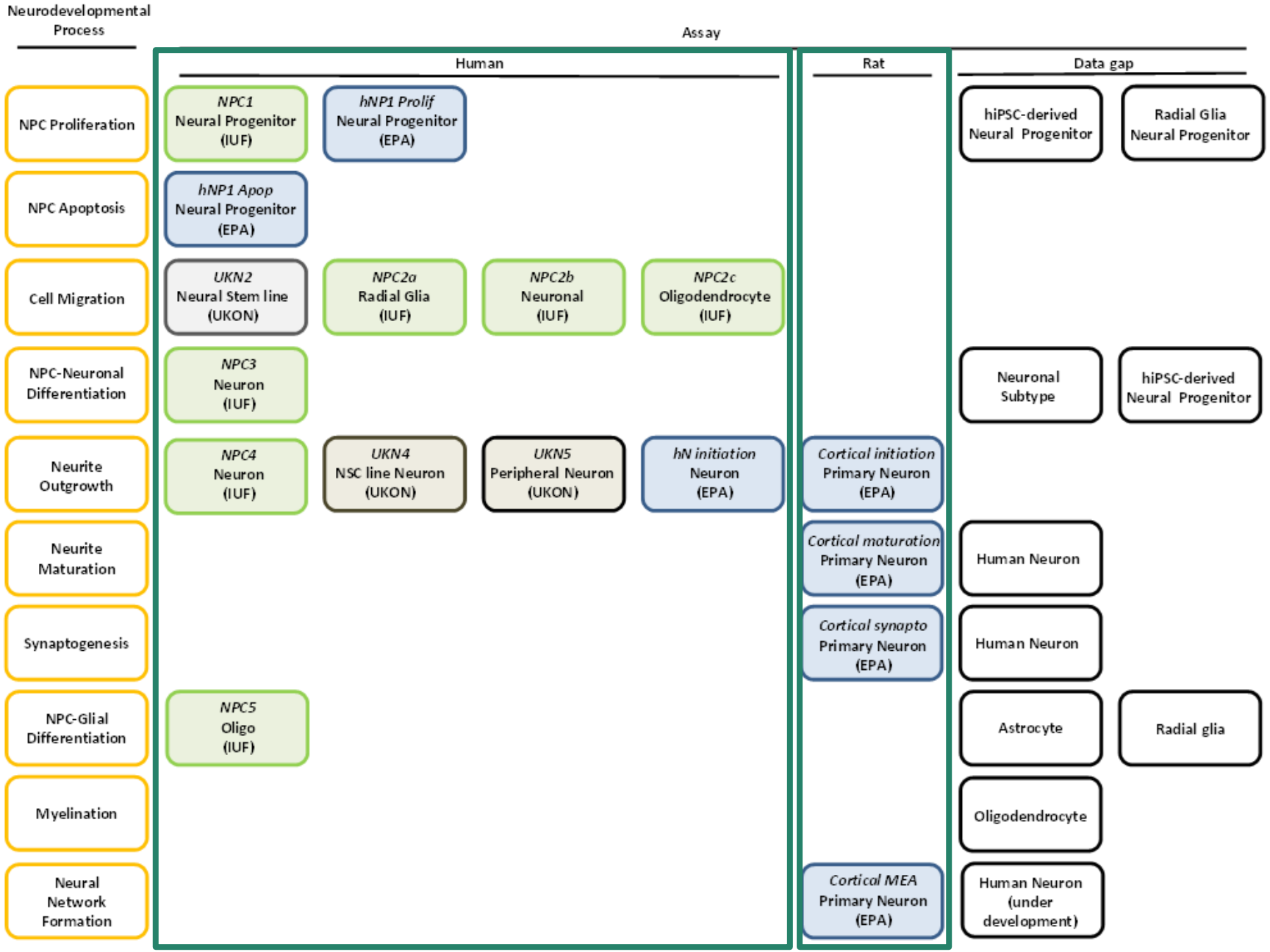
Key neurodevelopmental processes: mapping of *in-vitro* assays



Leist (Nov18)

Assays in the current DNT IVB

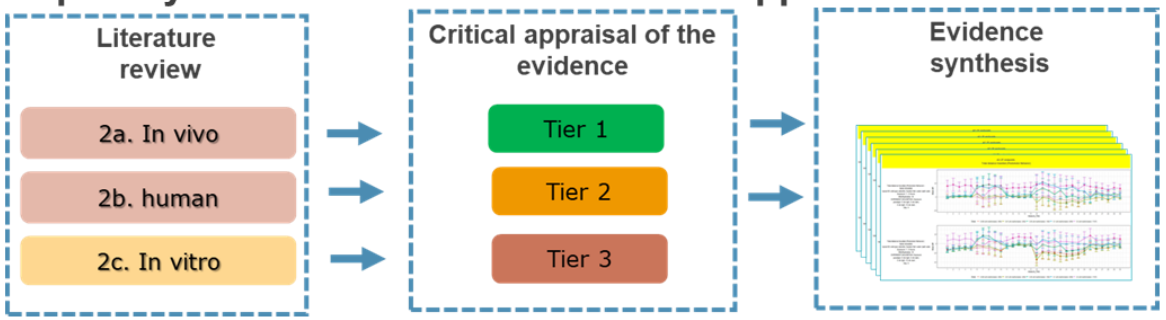
- **Some are human and some are rodent cell based**



EFSA IATA CASE STUDIES: PROOF OF CONCEPT

Step 1. Problem formulation: Single Chemical DNT hazard assessment.

Step 2. Systematic data collection and appraisal



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

Step 3. Data integration. AOP framework

Step 4. Filling the data gap. IVB

Step 5. Data analysis and interpretation in the light of the uncertainty analysis

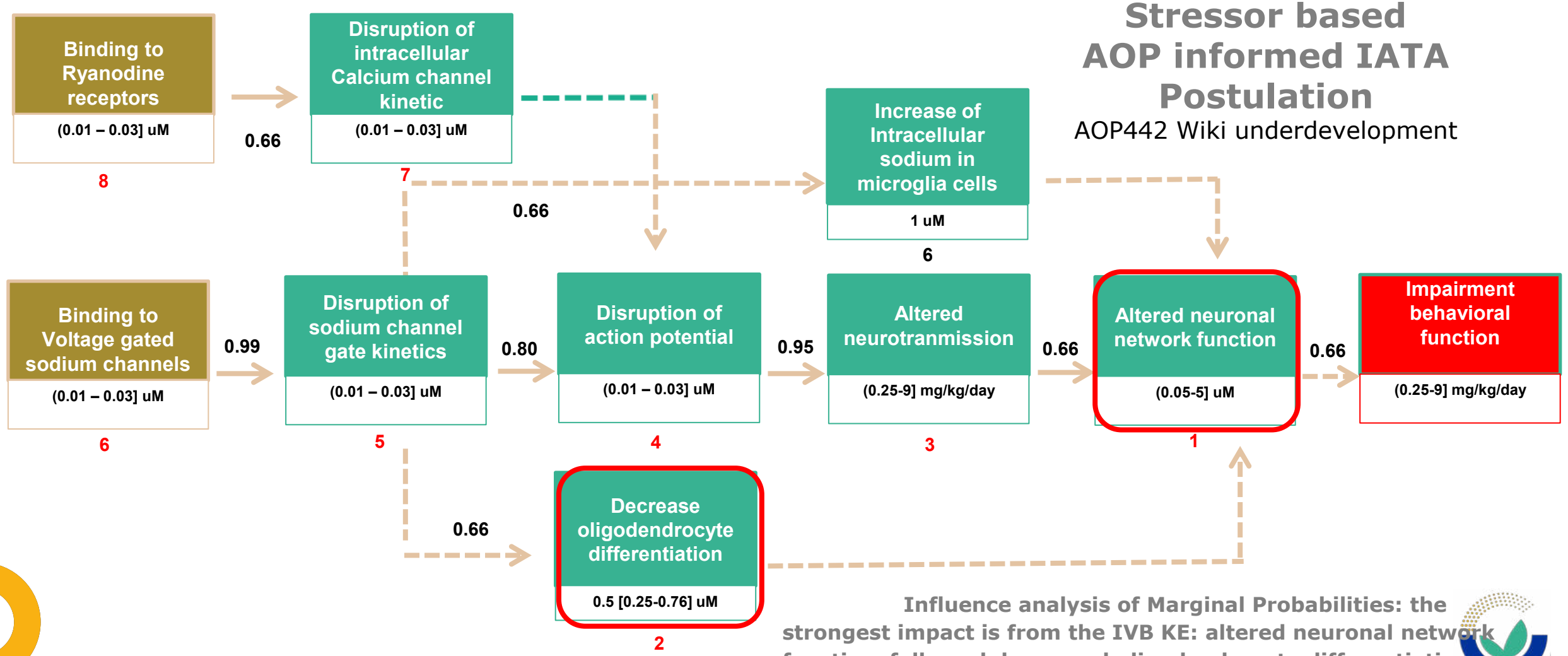
Step 6. Probabilistic assessment of the IATA-AOP Network.

UNCERTAINTY ANALYSIS

Hazard identification using a probabilistic approach.
Hazard characterization by Expert Knowledge elicitation

To quantify the impact of the DNT-IVB on the mechanistic understanding to conclude on the IATA case study.

THE RESULTS: DELTAMETHRIN CASE STUDY



Influence analysis of Marginal Probabilities: the strongest impact is from the IVB KE: altered neuronal network function followed decreased oligodendrocyte differentiation.

EFSA journal 2021;19(5):6599, 67 pp.; OECD CS 262.

AOP TIME AND DOSE CONCORDANCE ANALYSIS

	MIE	KE1	KE2	KE3	KE4	AO
	Binding to VGSC	Disruption of sodium channel gate kinetics	Disruption of action potential	Disruption of axon terminal depolarisation; changes in neurotransmitter release	Altered neuronal network function	Impairment behavioural function (sensory motor reflex and learning)
Concentration/dose	[0.01–1] µM [1]	[0.01–1] µM [1]	[0.01–1] µM [1]	*In vitro* [0.01–1] µM [2] *In vivo* [0.25–9] mg/kg [3]	0.04–5 µM (0.04 µM, corresponds to 19.3 ng of deltamethrin per gram of brain, 5 µM corresponds to 2,4 µg of deltamethrin per gram of brain) [4]	0.25–7.25 mg/kg bw/day oral gavage doses of 0.25–1 mg/kg/day in pups at PND 15 by gavage (single dose) [5] correspond to a brain concentration of 10.7 to 42.8 ng/g of brain assuming linearity.

THE RESULTS: FLUFENACET CASE STUDY

MiE

KE

KE

KE

AO

In vitro data from the systematic literature review

In vivo rodents /HOS

?

IVB integration. Overall this IATA supports the **conclusion, derived from in vitro, that there is no evidence that flufenacet is a direct developmental neurotoxicant.**

Changes Quantitative morphometrics evaluation

Caudate putamen diagonal and caudate putamen transverse
PND72 females

Flufenacet Evidence; hazard was identified from the systematic review and OECD 426 study in the light of uncertainties (Prob > 66%). 137 references were screened. For in vitro evidence 3 papers selected, for HOS 0 publications selected; for in vivo 0 publications selected.
OECD CS 263.

LESSONS LEARNT

- The case studies are the first European experience integrating the DNT-IVB for regulatory purposes and **showed the relevance** of the DNT-IVB in an AOP informed IATA **for regulatory decision-making**.
- This collaborative effort, will build **confidence in the use of NAM** in regulatory decisions.
- The proposed DNT-IVB **scientific validation is appropriate** for the inclusion of NAMs in risk assessment using an AOP informed IATA approach.
- The AOP informed IATA approach is **fit for purpose for hazard identification and characterization in the RA process** e.g. equivocal in-vivo studies, additional information on MIEs or different test systems.
- Lack of existing AOPs is a limitation and postulating and developing AOPs is resource demanding.
- Implementation of PBK models is a necessary step for using DNT NAM as PoD

THE WAY FORWARD: EFSA DNT PROGRAMME

- The **2 EFSA new projects (2023-2027)** are intended to foster the experience matured over the last years.

PROJECT : **Advancing Environmental Neuroscience**

Budget 3.6 M €

Objectives:

1. Test pesticides in the DNT-IVB.
2. Produce transferability/reproducibility data for the DNT-IVB.
3. EFSA in vivo DNT Database (DNT IVB-in vivo concordance analysis).
4. Collaborative IATA CASE STUDIES RISK ASSESSMENT.
5. Enlarge to the development of NAMs for Parkinsonian syndromes AO (NT).
6. 4 Calls will be launched in spring 2023

PROJECT : **Development of AOPs and NAMs that address data gaps**

Budget up to 5 M €

Objectives:

1. Development of AOPs/AOP networks (AOPs) and NAMs (including Risk Assessment case studies) that address toxicity to glial cells in the context of developmental sensory or motor DNT.
2. Outline proposals are being evaluated; successful Outline Proposals will be invited to submit a full proposal in Step 2 (1st Quarter 2023).

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