08 March 2022

EFSA perspective: IATA case studies on developmental neurotoxicity risk assessment

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Trusted science for safe food

European Food Safety Authority

Outline

- Background
- Problem formulation and workflow
- Deltamethrin IATA
- Flufenacet IATA
- Conclusions and PPR Panel recommendations

Moving forward to the implementation of DNT testing for regulatory purposes

European Food Safety Authority

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

ABSTRACT

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

2013

Workshop in DNT non animal test methods for regulatory purposes

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

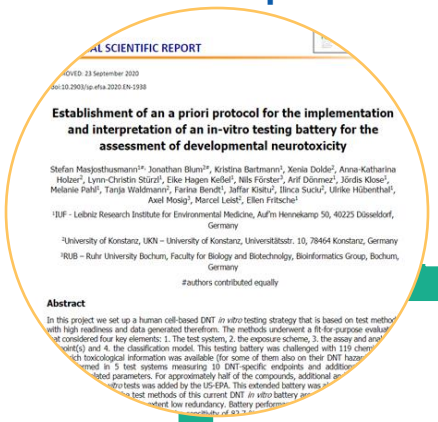
2016

Pesticide Unit submitted to OECD SPSF

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

2019

IVB DNT Scientific report


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

Abstract

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

2020

Development of IATA case studies on DNT Risk Assessment

2 Case studies Approved in the 7th CSP IATA OECD., Nov 2022


SCIENTIFIC OPINION

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

Abstract

2021

Stakeholders Workshop

DNT IVB OECD GUIDANCE

2022

Terms of Reference

Problem formulation: DNT hazard identification

1. *How certain are we that the pesticide active substance* ***deltamethrin OR flufenacet is a developmental neurotoxicant*** *in humans based on the data collected, appraised, synthesised and integrated using an operational protocol in line with the IATA and adverse outcome pathway (AOP) framework?* **in the context of the European pesticide Regulation (EU) 283/2013 and 1107/2009.**

2. *To what extent do the results of thein vitro testing battery (Masjosthusmann et al., 2020) on substance deltamethrin OR flufenacet influence the level of uncertainty as assessed in point 1?*

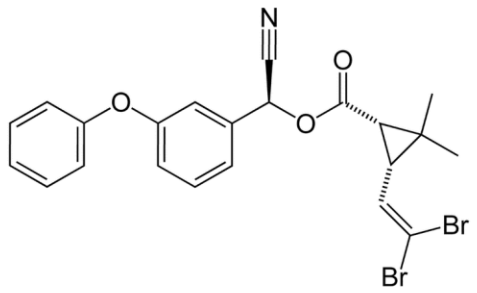
Target chemicals:

DNT IV-B data available →

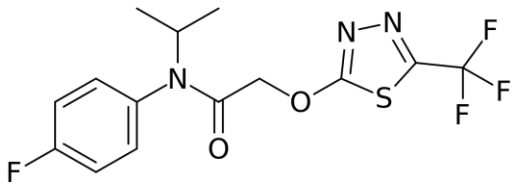
DNT TRIGGERED OECD 426 STUDY AVAILABLE →

Approved pesticides active substances →

DELTAMETHRIN



FLUFENACET



Neurotoxic pesticide MoA: bind to and disrupt voltage-gated sodium channels (VGSC) of insect nerves
Concern from public literature for DNT

Non neurotoxic pesticide MoA: inhibition of the biosynthesis of very long chain fatty acids resulting in inhibition of cell division and cell growth
No concern from public literature

EFSA journal 2021;19(5):6599, 67 pp. doi:10.2903/j.efsa.2021.6599

Evidence based AOP-informed IATA workflow

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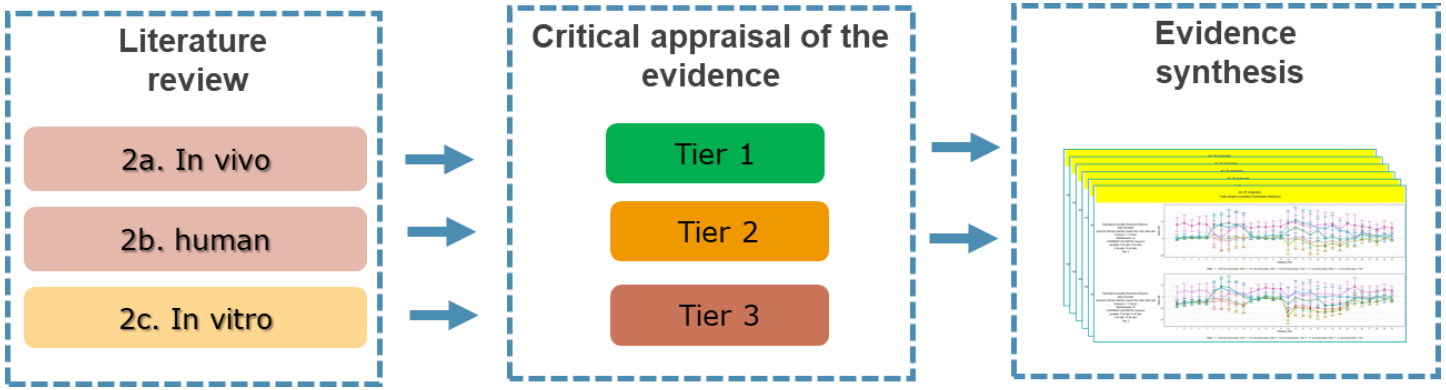
Flufenacet

Step 1. Problem formulation. Establishment of a Protocol.

Step 2. Systematic data collection and appraisal



Step 3. Data integration. AOP framework

Step 4. Filling the data gap. IVB

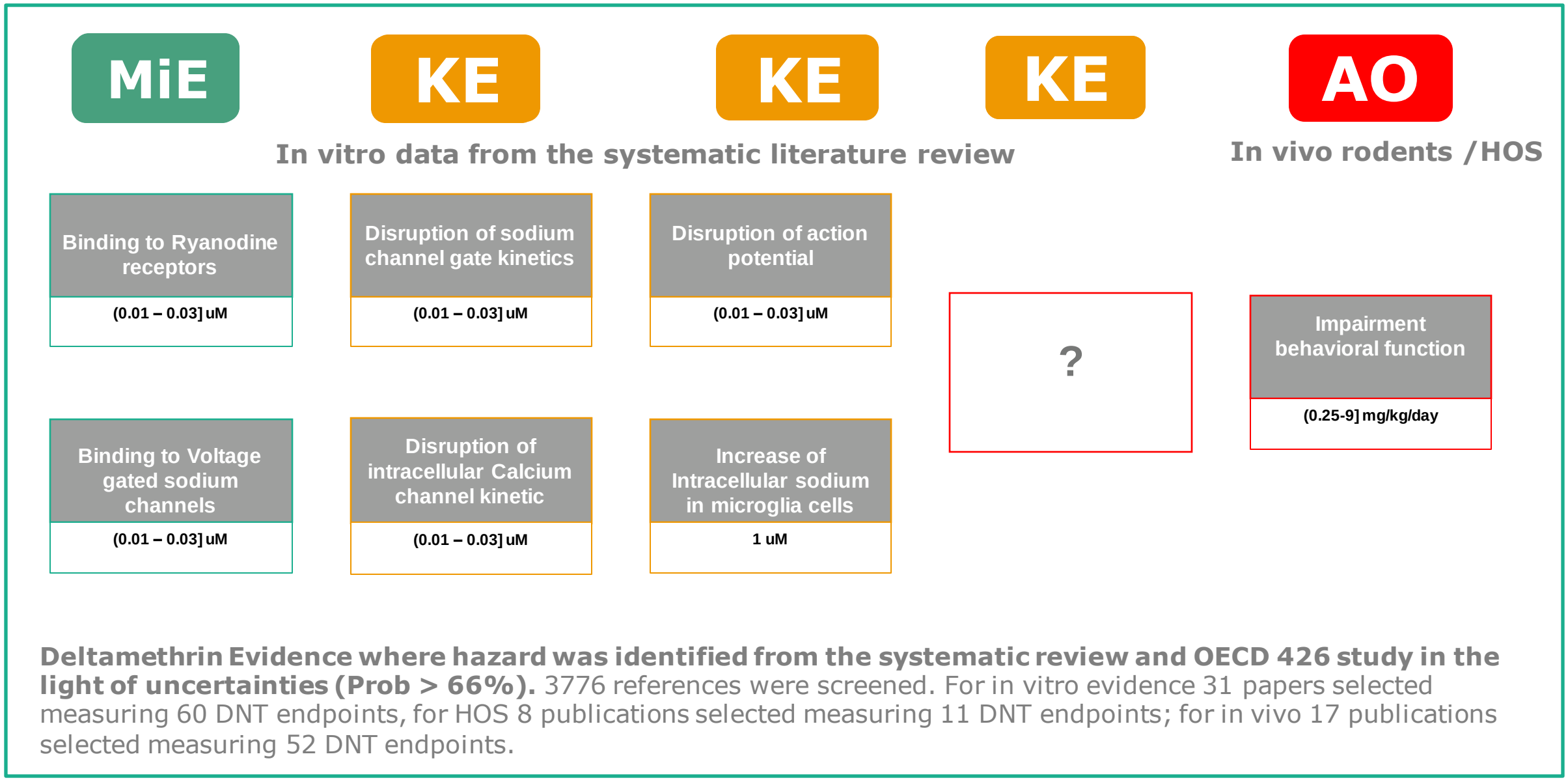
Step 5. Data analysis and interpretation in the light of the uncertainty analysis

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.

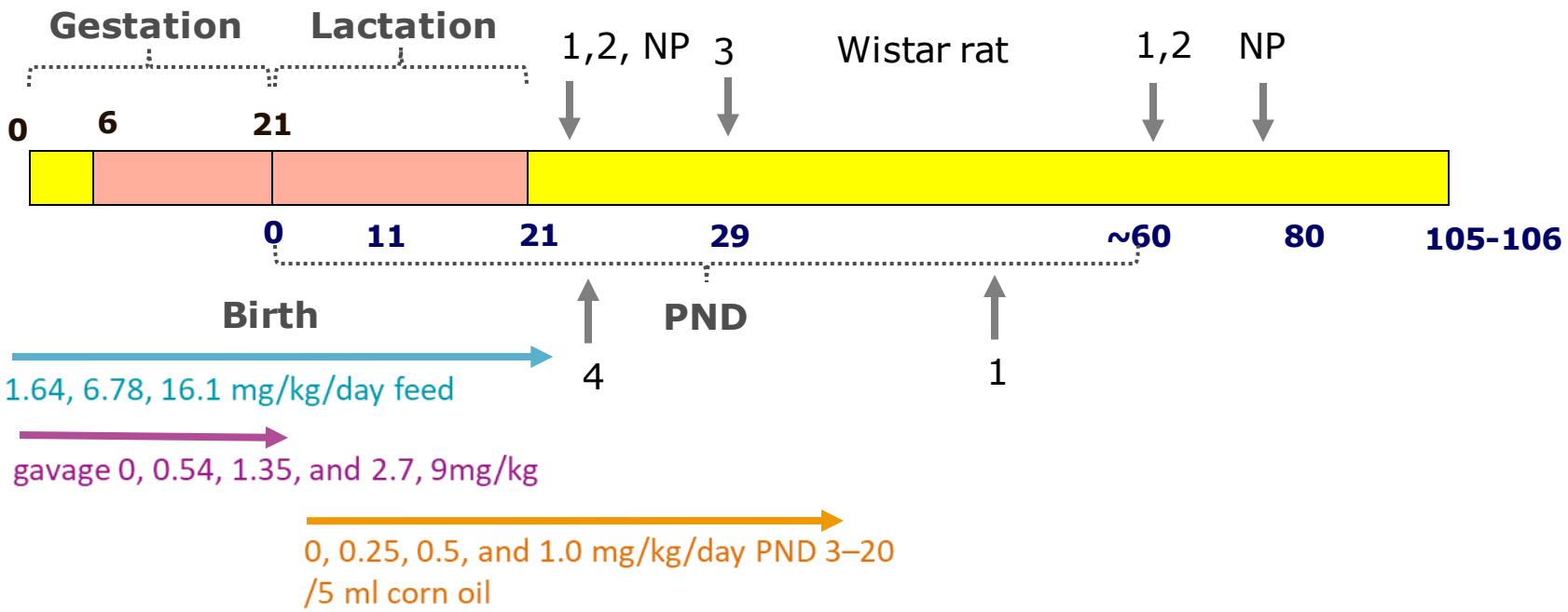
Step 3. Data integration. AOP framework



Deltamethrin Evidence where hazard was identified from the systematic review and OECD 426 study in the light of uncertainties (Prob > 66%). 3776 references were screened. For in vitro evidence 31 papers selected measuring 60 DNT endpoints, for HOS 8 publications selected measuring 11 DNT endpoints; for in vivo 17 publications selected measuring 52 DNT endpoints.

Deltamethrin, the DNT in vivo data

1. OECD 426: [1]Startle acoustic/[2]M-WM/[3]passive avoidance/[4]motor activity
2. Zhang et al., 2018,[4] Water Morris Maze
3. Pitzer 2019,[1], Acoustic and tactile startle response, Cincinnati Water Maze, Water Morris Maze, Conditioned Freezing Test



1. *No statistically significant and biologically relevant effect.*
2. *↑ escape latency and swimming distance to find the hidden platform (effect in acquisition phase) NOAEL/LOAEL= 2.37 /1.35 mg/kg bw d.*
3. *Acoustic and tactile startle response, ↑ Peak amplitude. NOAEL/LOAEL = 0.25/0.5 mg/kg bw d Cincinnati Water Maze Test. ↑ acquisition latency and related increase in number of errors to find the exit in a labyrinth in males pups gavage NOAEL/LOAEL= 0.5 / 1 mg/kg bw d.*

*In vivo experimental studies from literature yielded **apparent equivocal results** compared with the OECD TG 426 study. Experimental differences, reviewed in detail , suggests that the lack of positive outcomes in the OECD study likely resulted from both kinetic and dynamic differences between it and the two other studies.*

Deltamethrin, the human data relevance and limitations

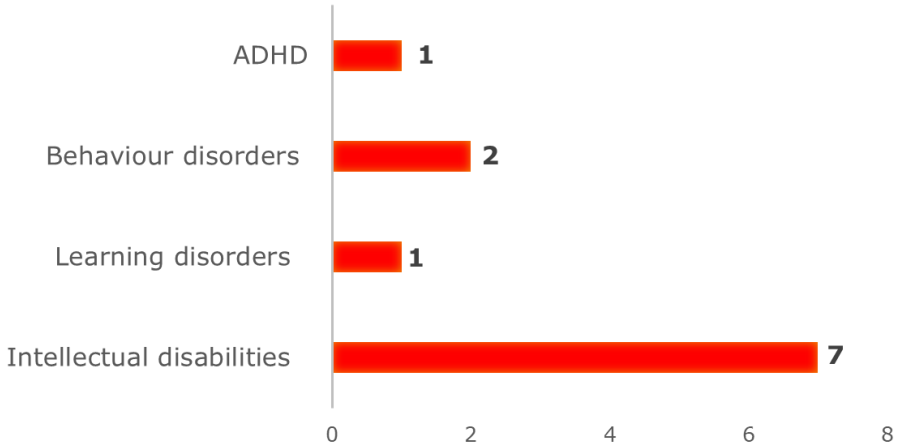
For pesticides, Regulation (EC) 1107/2009 ***limits the use of human data to observational.***

- They are concerned with an AO of interest in a population of interest.
- They are a reflection of 'real life' (e.g heterogeneous populations; relevant routes of exposure; cumulative exposure including relevant co-exposures)

Limitations and challenges

The level of probability for a causal association of deltamethrin exposure with all DNT outcomes was lower than 66%, principally due to 'probably' or 'definitively high' RoB for exposure measurements in the studies.
Difficulties to contextualize this in the RA.

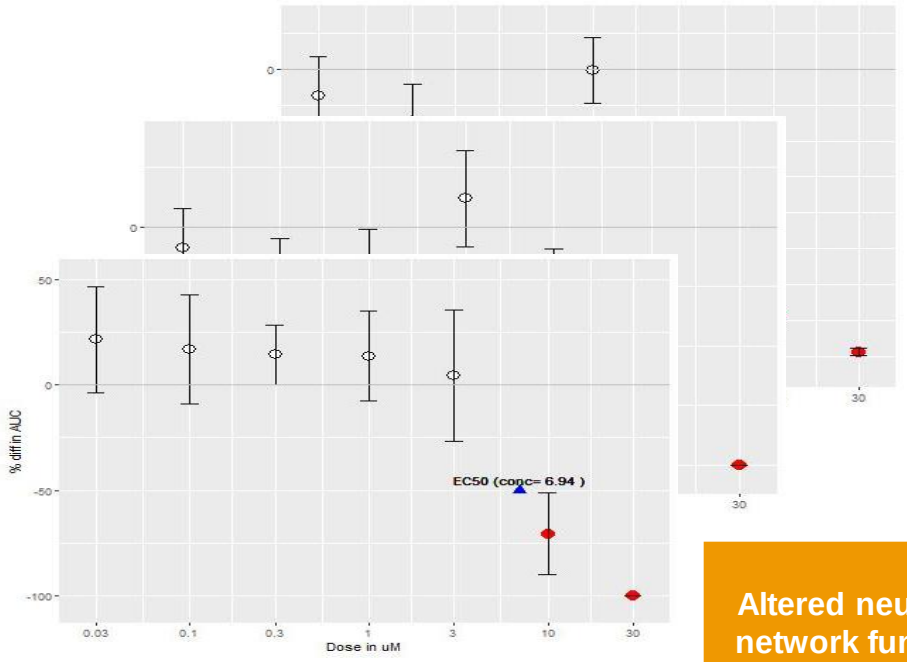
Human observational studies: 8 publications (cohorts), 11 endpoints



HOS data showed a potential DNT concern for the pyrethroid chemical class (using maternal urinary levels of 3-PBA and DCCA as exposure measurement)

Step 4. Filling the data gap. In vitro testing battery integration

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



DNT IVB integration

Altered neuronal network function

(0.05-5] uM

Decrease oligodendrocyte diferentiation

0.6 uM

Prob > 0.66

From Masjosthusmann et al.EFSA Supp Publ 2020

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

AOP informed IATA postulation

Binding to Ryanodine receptors
(0.01 – 0.03] uM

Disruption of intracellular Calcium channel kinetic
(0.01 – 0.03] uM

Binding to Voltage gated sodium channels
(0.01 – 0.03] uM

Disruption of sodium channel gate kinetics
(0.01 – 0.03] uM

Disruption of action potential
(0.01 – 0.03] uM

Altered neurotranmission
(0.25-9] mg/kg/day

Altered neuronal network function
(0.05-5] uM

Impairment behavioral function
(0.25-9] mg/kg/day

Increase of Intracellular sodium in microglia cells
1 uM

Decrease oligodendrocyte diferentiation
0.5 [0.25-0.76] uM

Prob > 0.66

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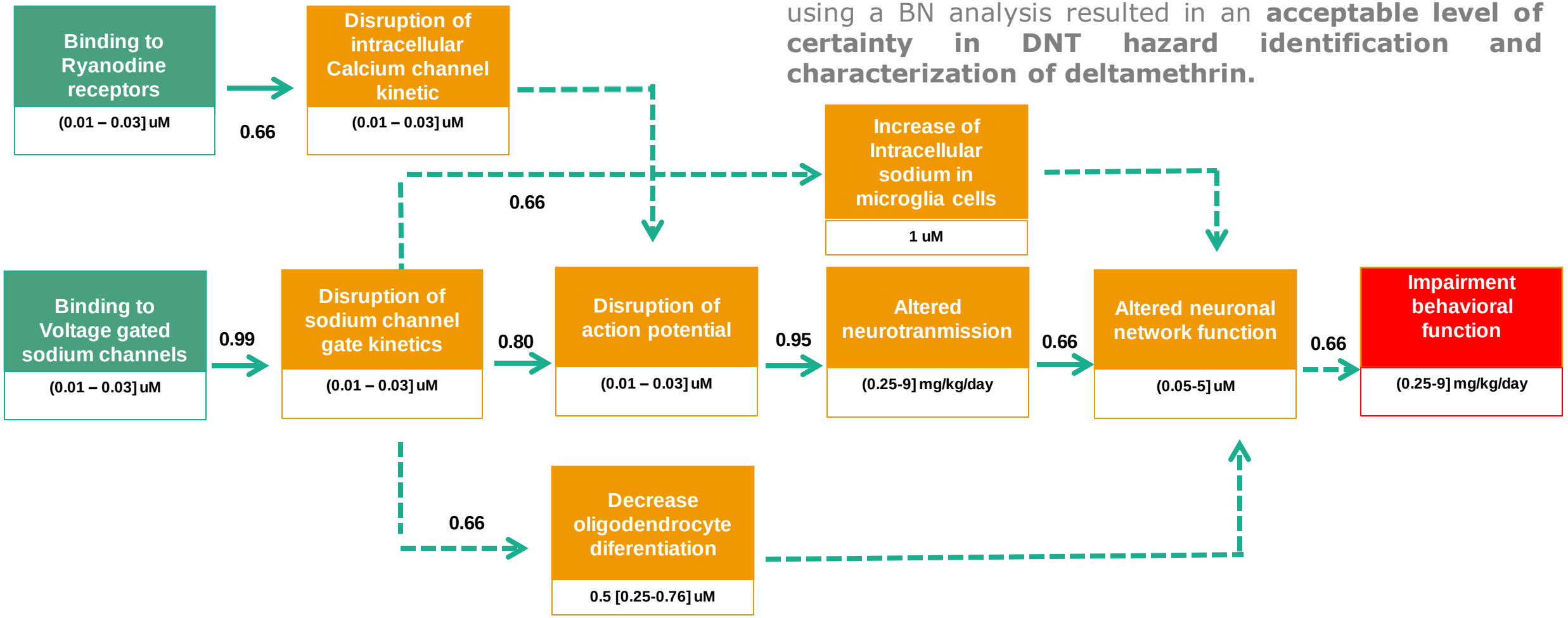
Step 5. Data analysis and interpretation in the light uncertainty analysis.

The quantitative WoE assessment of the AOP network using a BN analysis resulted in an **acceptable level of certainty in DNT hazard identification and characterization of deltamethrin.**



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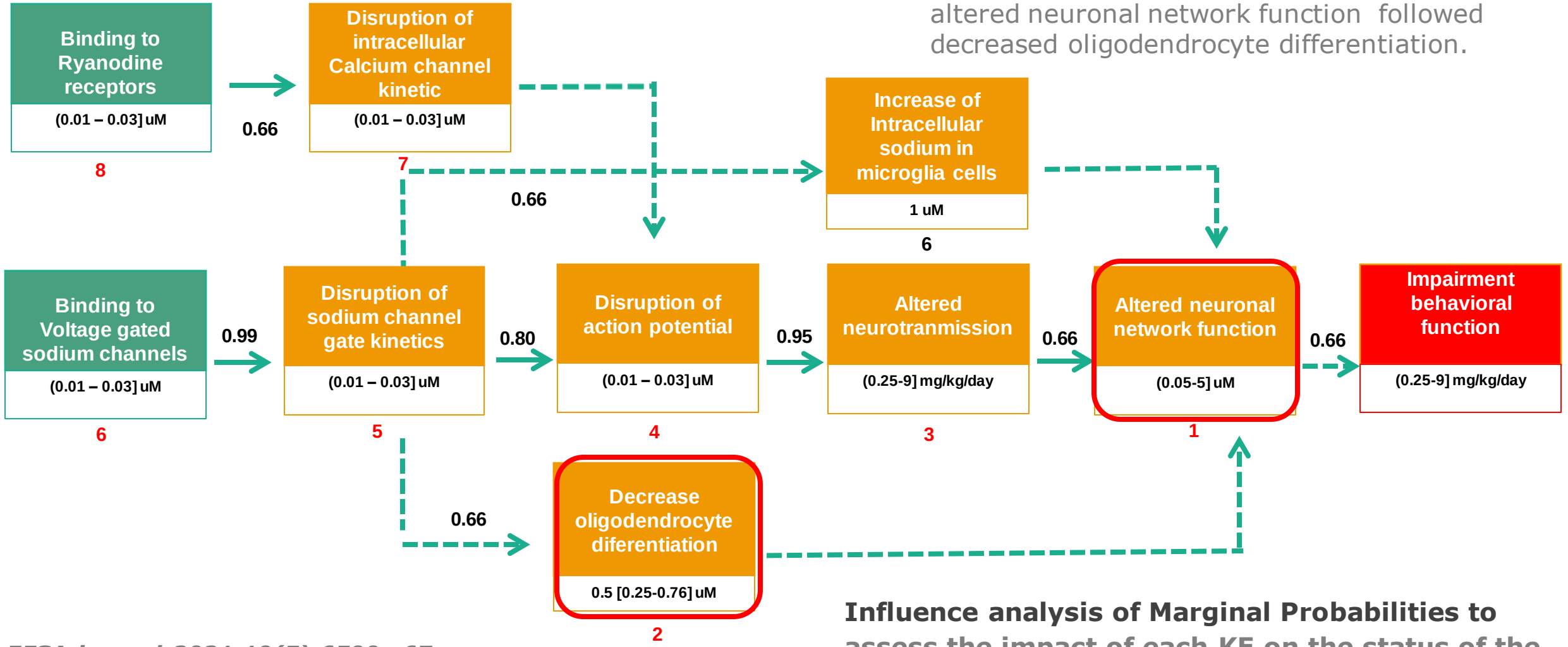
Quantitative WoE AOP Bayesian Analysis

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

The strongest impact is from the IVB KE: altered neuronal network function followed decreased oligodendrocyte differentiation.



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Influence analysis of Marginal Probabilities to assess the impact of each KE on the status of the AO.

Flufenacet. Step 3. Data integration. AOP framework

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 were 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.

Conclusions

- The approach taken **allowed conclusions to be drawn with an acceptable level of certainty in DNT hazard identification and characterisation** of deltamethrin and that flufenacet is not a direct developmental neurotoxicant.
- The case studies developed **showed the applicability of the in vitro battery (IVB) for developmental neurotoxicity (DNT)** in the context of the European pesticide Regulation (EU) 283/2013 and 1107/2009 and illustrate the usefulness of an AOP-informed IATA for regulatory decision making.
- The integration of the in vitro data, including the DNT-IVB, exemplifies how data from the DNT IVB can be **incorporated as part of the WoE** within the AOP-informed IATA framework. **The integration of the DNT IVB in the IATAs was essential to conclude.** For deltamethrin was key to develop the AOP and thus critical to inform the probability assessment on the potential for deltamethrin to cause DNT. For flufenacet was key to conclude that that there is no evidence that flufenacet is a direct developmental neurotoxicant.
- For deltamethrin, the overall activity led to **improved interpretation of human data** by providing a plausible mechanistic link to adverse outcomes, which would support their contextualization in the risk assessment process.

PPR Panel recommendations for the implementation DNT-IVB in the Risk Assessment

1. Considering the strength of the mechanistic understanding in the IATA case studies, **the inclusion of a DNT-IVB is recommended for DNT hazard characterization of pesticide active substances**. This can also be triggered when concerns exist from in vivo experiments or HOS. Similarly, the outcome of the DNT-IVB should be considered as a trigger for additional DNT investigations. The PPR Panel also supports to develop a GD explaining the uses and interpretation of the DNT-IVB data for regulatory toxicology assessments in line with the OECD programme. For this purpose, available information on the limitations and uncertainties of animal tests assessing DNT should be considered to the same degree as for the IVB.

2. The PPR Panel considers that **the potential of NAMs should be fully exploited** to allow efficient regulation with a long-term sustainable socioeconomic return of investment in terms of environmental health protection. Therefore, NAMs should be used in next-generation risk assessment (OECD GD 275; OECD, 2017; Dent et al., 2018; Desprez et al., 2018; Smith et al. 2016; Hatherell et al., 2020).

3. **The use of corrected nominal concentrations in the in vitro testing should be considered for estimating the effective concentration to be used for exposure extrapolation.** The uncertainty analysis provided in this scientific opinion for the in vitro test indicated that several points should be considered. These include, but are not limited to, predicted or empirical data on partitioning of the chemical with plastic, lipid and protein, intracellular concentration and accumulation where the chemicals are added multiple times to the test system

4. Based on these principles, implementation of the DNT-IVB methods for DNT endpoints assessment as a potential data requirement for pesticide active substances, and possibly for other chemicals, should be discussed with risk managers and stakeholders in the appropriate context.

Thank you very much

- EFSA IATA DNT WG appointed by the PPR Panel.

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