Workshop

March 7th 2022

EFSA DNT in-vivo database: DNT In-vivo in-vitro preliminary concordance analysis

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Trusted science for safe food

European Food Safety Authority

Outline

- The DNT in vivo database
- In vivo in vitro preliminary concordance analysis
- Analysis by chemical class: pyrethroids, neonicotinoids
- Uncertainties and next steps

The EFSA DNT in vivo database scope

1. Develop a standardized nomenclature that could be used for all in vivo DNT guideline studies.
2. Develop a database structure consistent with ToxRefDB.
3. Populate the database with a limited number of DNT studies (currently n=59) to allow follow-up work comparing results from the DNT IVB data to in vivo DNT guideline studies.

Overview EFSA DNT Database Snapshot

59 in vivo DNT guideline studies (OPPTS 870.6300, OECD 426 conducted 1991-2005)

15 no DNT effect observed;
38 DNT endpoint observed; 6 unacceptable

DNT endpoints common nomenclature.

ToxRefDB data model

Data source:
US EPA DER
EFSA conclusion
-33 substances both sources -

41 from there tested also in the IVB outsourced by EFSA (27 also in MEA).
Preliminary correlative analysis

34 from there tested in MEA (hNNF or/and rNNF) 6 also US EPA (Harril et al., 2018)

Status at February 2022 EFSA aims to continue database development

Data sources

- Public available Data Evaluation Reports (EFSA RAR, US EPA DER).
- Preliminary assessment of 33 substances from both sources:
  - Different assessments were conducted by EFSA and US EPA for 8 substances of 33 (25%) with the same study (8 as negative by EFSA and 7 positive by US EPA and 1 unacceptable).
- EFSA DNT database: includes the assessment from all US EPA DERs that are publically available.
- EFSA actions included:
  - Collaborations with US EPA and ECHA
  - DNT training.

DNT database endpoints: standard nomenclature

FOB

Autonomic: body temperature; urination; other; lacrimation; defecation; pupil size pupil function; ptosis; salivation

Grip strength : forelimb force; hindlimb force

Behaviour: Abnormal; Clonic-tonic convulsions; Vocalization; Excitability; Arousal; Reactivity; Activity; Tremors; Analgesic reflex; Coordination; Ear flick; Righting reflex; Gait posture; Stereotypies repetitive;
Grooming; Muscle tone; Activity rears

Learning and memory

Water maze acquisition

Water maze retention

Water maze motor function

Dry maze acquisition

Dry maze retention

Passive avoidance acquisition

Passive avoidance retention

Active avoidance acquisition

Active avoidance retention

Morphometrics and pathology

Morphometrics: Cerebellum; Corpus Callosum; Caudate Putamen; Cerebrum; Hippocampus; Germinal layer; Pons; brain height; brain width; brain length; Thalamus; Commisure; Fornix; Midbrain; Straitum; Morphometrics Other; Brainstem; Hypothalamus; olfactory bulbs; Thickness cortex; Radial Thickness Cortex; Height dentate hilus; length ventrical limb dentate hilus
Pathology gross: brain weight absolute; brain weight relative; brain; brain width

Motor activity

Activity automyted: Distance; Time; Horizontal; Vertical; Ambulatory (Note: Locomotor Activity); Total;
Habituation Ontogeny (Note: develops in control animals between PND 17 and 21);
Habituation within Session (Note: at all ages); Rears; Swim speed

Auditory startle response

Acoustic startle automated:
amplitude; latency; habituation; Prepulse inhibition

Tactile startle automated:
amplitude; latency; habituation; Prepulse inhibition

Other endpoints in the DB

- General systemic
- Developmental
- Clinical signs
- Cholinesterase
- Sexual landmark
- Not in list

Endpoint type; endpoint target; Endpoint Category. ToxRefDB nomenclature updated by extracting terms used in 33 DNT guideline study reports from 11 different testing laboratories

Database structure



Species	Strain or in vitro model	Animals /sex/group	Sex (administration)	Purity (%)	Route of admin	Vehicle-Volum	Doses tested (GD/LD)	Lowest dose tested (GD/	Highest dose tested (GD/	Dose unit	Duration of exposure	Duration unit	Generation/Life stage	Sex (effect do	Lowest Effect do	ENDPOINT TYPE	ENDPOINT TARGET	ENDPOINT CATEGORY	EFFECT DESCRIPTION	Effect description free text	Effect determination (time point)	Effect direction	NOAEL/ NOEL of the st	LOAEL/L OEL of the st	Unit	Endpoint generation	
Rat	Hsd Brl Han:Wist	24	F	99.78	Feed		0, 0.8-0.9, 4.2-8-0.9/1.2-1.	-10.5/13.7-19	mg/kg bw/day	GD6-LD10	Days	Parental (P)	F	8-10.5/13	In_life_Observatio	morbidity_mortality	Systemic	description	1 dam and her litter were k	daily	Increase	5.6	13.7	mg/kg bw/da	Maternal	t
Rat	Hsd Brl Han:Wist	24	F	99.78	Feed		0, 0.8-0.9, 4.2-8-0.9/1.2-1.	-10.5/13.7-19	mg/kg bw/day	GD6-LD10	Days	Parental (P)	F	8-10.5/13	In_life_Observatio	behavior_reactivity	Neurological	reactivity_to_handlir	Increased reactivity to hai	daily	Increase	1.2	5.6	mg/kg bw/da	Offspring	t
Rat	Hsd Brl Han:Wist	24	F	99.78	Feed		0, 0.8-0.9, 4.2-8-0.9/1.2-1.	-10.5/13.7-19	mg/kg bw/day	GD6-LD10	Days	Parental (P)	F	4.2-4.6/5	In_life_Observatio	clinical_signs	Systemic	other	Smaller changes in reactiv	daily	Change					
Rat	Hsd Brl Han:Wist	24	F	99.78	Feed		0, 0.8-0.9, 4.2-8-0.9/1.2-1.	-10.5/13.7-19	mg/kg bw/day	GD6-LD10	Days	Parental (P)	F		In_life_Observatio	behavior_activity_rears	Neurological	rearing_activity	Arena observations, inclu	daily	No effect					
Rat	Hsd Brl Han:Wist	24	F	99.78	Feed		0, 0.8-0.9, 4.2-8-0.9/1.2-1.	-10.5/13.7-19	mg/kg bw/day	GD6-LD10	Days	Parental (P)	F	8-10.5/13	In_life_Observatio	body_weight	Systemic	body_weight	statistically significant de	GD20, LD1 and LD11	non statisti					
Rat	Hsd Brl Han:Wist	24	F	99.78	Feed		0, 0.8-0.9, 4.2-8-0.9/1.2-1.	-10.5/13.7-19	mg/kg bw/day	GD6-LD10	Days	Parental (P)	F	8-10.5/13	In_life_Observatio	body_weight	Systemic	body_weight_gain	statistically significant de	GD6-GD20	Decrease					
Rat	Hsd Brl Han:Wist	24	F	99.78	Feed		0, 0.8-0.9, 4.2-8-0.9/1.2-1.	-10.5/13.7-19	mg/kg bw/day	GD6-LD10	Days	Parental (P)	F	8-10.5/13	In_life_Observatio	body_weight	Systemic	body_weight_gain	While high dose dams wei	LD1-11, LD1-21	No effect					
Rat	Hsd Brl Han:Wist	24	F	99.78	Feed		0, 0.8-0.9, 4.2-8-0.9/1.2-1.	-10.5/13.7-19	mg/kg bw/day	GD6-LD10	Days	Parental (P)	F	0.8-0.9/1.	In_life_Observatio	food_consumption	Systemic	food_consumption		GD6-GD20 and LD1-11, LD1-2	No effect					
Rat	Hsd Brl Han:Wist	24	F	99.78	Feed		0, 0.8-0.9, 4.2-8-0.9/1.2-1.	-10.5/13.7-19	mg/kg bw/day	GD6-LD10	Days	Parental (P)	F	4.2-4.6/5	In_life_Observatio	food_consumption	Systemic	food_consumption		GD6-GD20 and LD1-11, LD1-2	No effect					
Rat	Hsd Brl Han:Wist	24	F	99.78	Feed		0, 0.8-0.9, 4.2-8-0.9/1.2-1.	-10.5/13.7-19	mg/kg bw/day	GD6-LD10	Days	Parental (P)	F	8-10.5/13	In_life_Observatio	food_consumption	Systemic	food_consumption	statistically significant de	GD 10-13, 14-17 and 18-19	Decrease					

- ToxRefDB structure was used.
- One Excel file to collect all the substances
- Data extracted by dose and by endpoints.
- Each row corresponds to an endpoint type

Three main sections

Preliminary Analysis of In vivo-in vitro concordance Data sources

efsa European Food Safety Authority

In vitro data

EFSA IVB (Masjosthusmann., 2020)

Danish EPA IVB hNNF

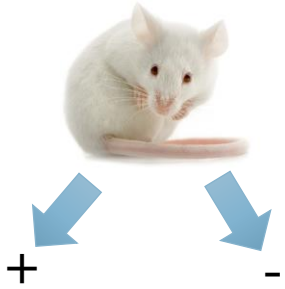
US EPA IVB (Frank.,2019; Shafer., 2019; Harril 2018)

all pos	Mas sma 2020	[24h]	UKN2 [24h]	[24h]	UKN4 [24h]	[24h]	UKN5 [24h]	BrdU [72h]	area [72h]	ation) [72h]	(prolifer ation)	distance radial	distance radial	distance neurons	distance oligode	differen tiation	[120h]	[120h]	e differen	[120h]	(migrati on)	(migrati on+diffe	on+diffe rentiatio	p	hNP1	hNP1	initiati on	initiati on	matur ation	(rat cortical	hNNF	2019_E C50	2018_EC 50	IN v pos
N	N	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	NT							not tested	not tested	not tested	
N	N	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	NT							no hit	not tested	no activit	
Y	Y	no hit	no hit	no hit	no hit	no hit	no hit	7.7	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	19	NT							no hit	not tested	not tested	
Y	N	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	N	inactive	inactive	inactive	inactive	inactive	inactive	no hit	not tested	0.66-9.88	
Y	Y	10.3225	16.2	no hit	8.7	39.1	22.2	no hit	no hit	no hit	no hit	15.9	15.4	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	NT							not tested	not tested	not tested	Una
Y	Y	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	6.4	no hit	no hit	no hit	no hit	NT							per networ	not tested	not tested	
Y	Y	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	1.1	no hit	no hit	no hit	no hit	NT							per networ	not tested	not tested	
Y	N	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	NT							not tested	4.25-15.57	not tested	
Y	Y	19	9.6	80	15	5	5	no hit	no hit	no hit	no hit	7.9	6.9	no hit	no hit	no hit	7.6	10	no hit	no hit	no hit	no hit	4.1	NT							Frequency	not tested	0.08-17.1	
Y	?	47.1	37	no hit	102.4	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	Y	64	inactive	inactive	inactive	inactive	inactive	no hit	not tested	1.4-22.12	Una
N	N	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	NT							no hit	not tested	not tested	
Y	Y	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	9.7	no hit	no hit	no hit	no hit	no hit	no hit	NT							no hit	not tested	not tested	
N	N	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	NT							not tested	no activit	not tested	
Y	Y	no hit	17.1	no hit	no hit	86.2	107.6	no hit	no hit	no hit	no hit	0.9	no hit	4	no hit	no hit	12	no hit	0.4	no hit	1.6	9.5	no hit	N	inactive	inactive	inactive	inactive	-	-	per Networ	not tested	0.5-23.5	
N	N	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	NT							no hit	not tested	not tested	
N	N	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	NT							no hit	not tested	not tested	
N	N	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	NT							no hit	not tested	not tested	
N	N	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	NT							no hit	no activity	not tested	
Y	Y	1.2	1.1	1.5	1.7	1.3	0.9	1	1.9	1.9	2.1	2.3	1.7	2.1	1.9	3.3	2.3	2.5	0.2	1.6	0.8	0.4	1.6	NT							not tested	not tested	0.01-0.32	
Y	Y	no hit	3.86	41.7	24	no hit	no hit	no hit	no hit	no hit	no hit	8.4	no hit	no hit	no hit	no hit	no hit	no hit	0.5	no hit	no hit	no hit	no hit	NT							not tested	4.335-19.46	not tested	
Y	N	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	NT							no hit	2.35-13.33	not tested	
N	N	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	no hit	NT							not tested	not tested	not tested	Una
Y	Y	18.9	15	no hit	10.6	no hit	no hit	no hit	no hit	no hit	no hit	13	10.3	no hit	no hit	12.9	11.5	14.1	3.5	17	no hit	no hit	14.7	NT							Frequency BMC-5	not tested	1.33-23.88	

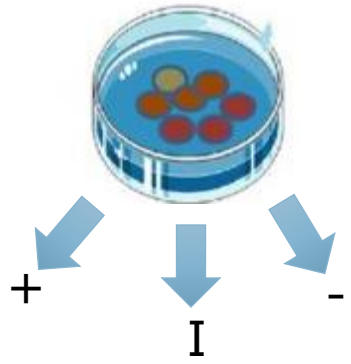
In vivo data EFSA DB

Preliminary Analysis
In vivo-in vitro concordance: criteria

In vivo data EFSA DB



In vitro data IVB



DNT +: A DNT endpoint has been assessed as being affected by the active substance, a LOEL has been set based on an acceptable DNT study. *

DNT -: In a guideline accepted study no DNT endpoint has been assessed as affected by the active substance.*

DNT inconclusive: Inconclusive/unacceptable studies not used for the comparison at this stage.

*As concluded by the by the regulatory authority.

DNT+: There is a hit (i.e., a hit that is DNT endpoint-specific as compared to cytotoxic) in at least one of the DNT assays tested (minimum Masjosthusmann., 2020).

DNT -: There is no hit in any of the DNT assays tested (minimum Masjosthusmann., 2020).

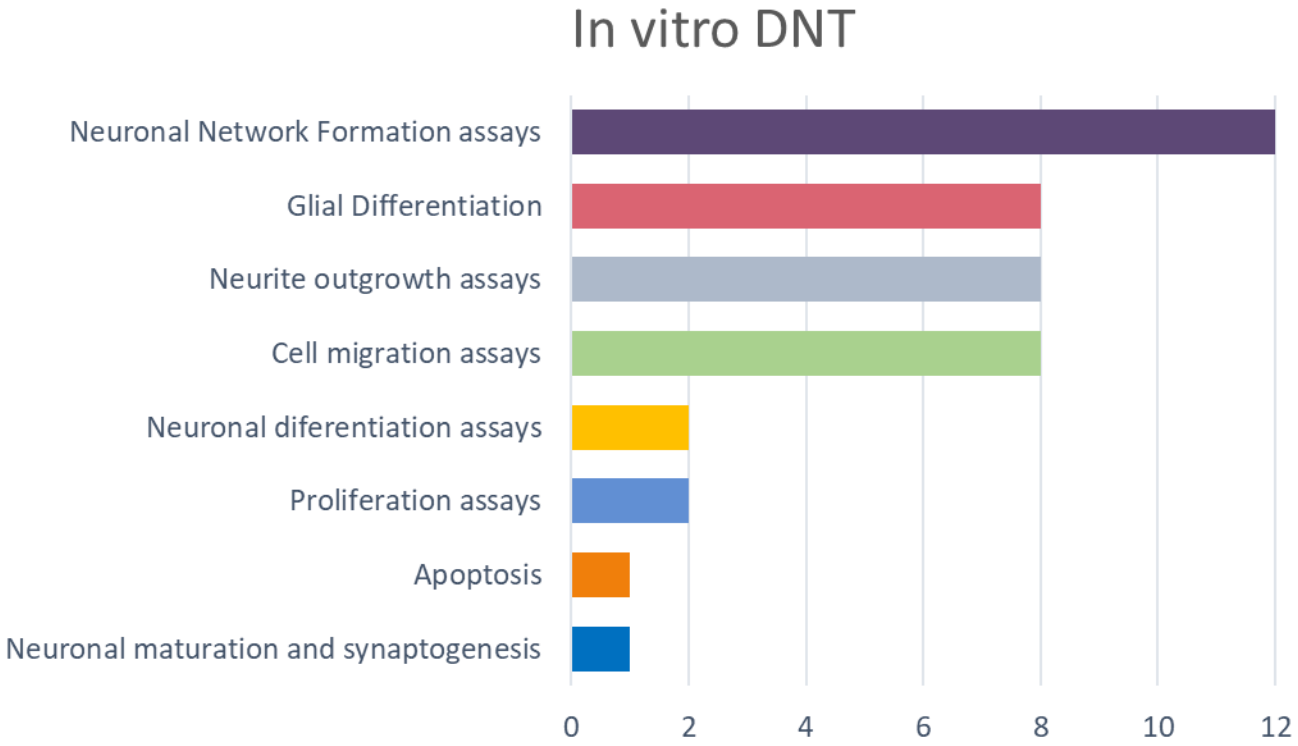
DNT inconclusive: there is at least one borderline hit, an unspecific hit or a non-selective hit observed h in any of the DNT assays tested and the rest of DNT assays are negative
As interpreted by the authors in Masjosthusmann., 2020; Shafer et al., 2019; Frank et al., 2018 and Harril., 2018.

Preliminary in vivo-in vitro concordance analysis

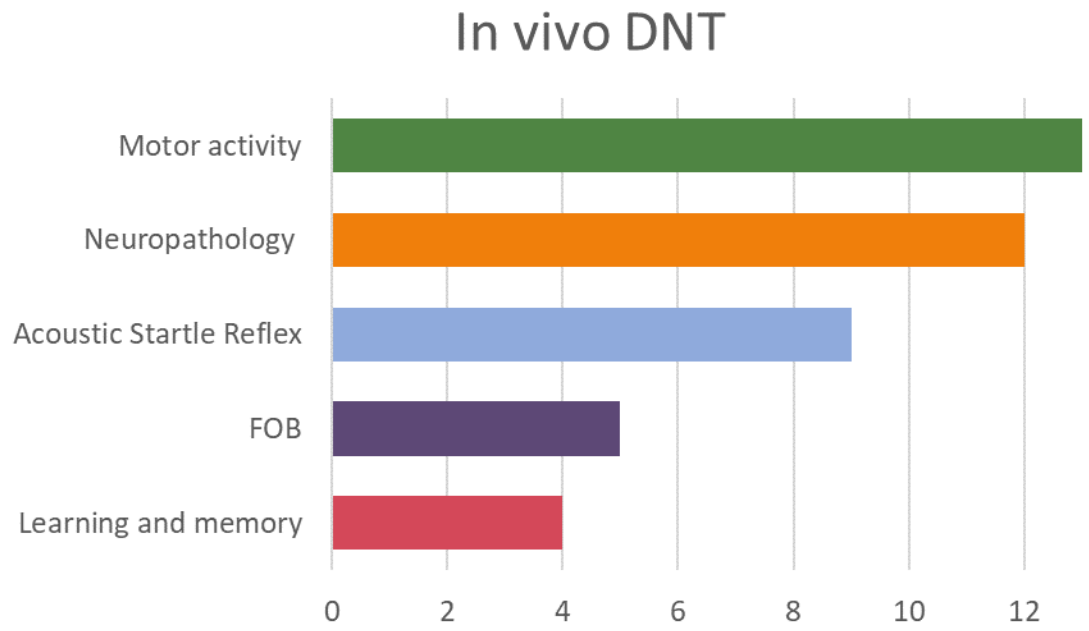
In Vivo GD	IVB (Masjosthusmann., 2020)				IVB (all data)			
	+	I	-	Total	+	I	-	Total
+	15	3	13	31	21	1	9	31
-	3	1	6	10	3	1	6	10
Total	18	4	19	41	24	2	15	41
Sensitivity	49 %				68 %			
Specificity	60 %				60 %			
Concordance	51 %				66 %			

- Two preliminary concordance analyses conducted with the available data for 41 pesticides active substances., February 2022.
- IVB all data: Masjosthusmann., 2020; Shafer et al., 2019; Frank et al., 2018 and Harril et al., 2018.
- Results in line with previous analysis sensitivity 61-87% (Draft OECD Guidance., 2021)

21 pesticides active substances in vivo +/ in vitro +



In vitro DNT neurodevelopmental processes with effects



In vivo DNT endpoint categories with effects

Can we link the in vivo findings with one or more of the disturbed in vitro neurodevelopmental processes?

Active substances In vivo - vitro + or inconclusive

- How to assess the non-concordant?

Active substance	Compound class	In vivo max dose tested (mg/kg bw per day)	In vivo Route of administration	In vivo LOAEL (mg/kg bw per day)	IVB positive	MIN conc affected (uM)	Comments From DNT Database
Endosulfan	Organochlorine	29.8	Diet	3.74	Oligodendrocyte differentiation	0.5	In vivo study does not satisfy the requirement from the guideline (OPPTS and OECD 426) due to the pending review of the positive control data.
Indoxacarb	Organochlorine	3	Gavage Polyethylene glycol (MW 400) - 2 mL/kg bw per day	3	Oligodendrocyte differentiation Migration Proliferation	0.5	
Mancozeb	Carbamate	30	Diet	30	Borderline Hit Neurite Area UKN5	37.4	
Flubendiamide	Organofluorine	979.6	Diet	99.5	Oligodendrocyte differentiation	3	

- Are these 4 substances false positive/borderline in vitro or false negative in vivo?
- Use may require an uncertainty analysis in an IATA.

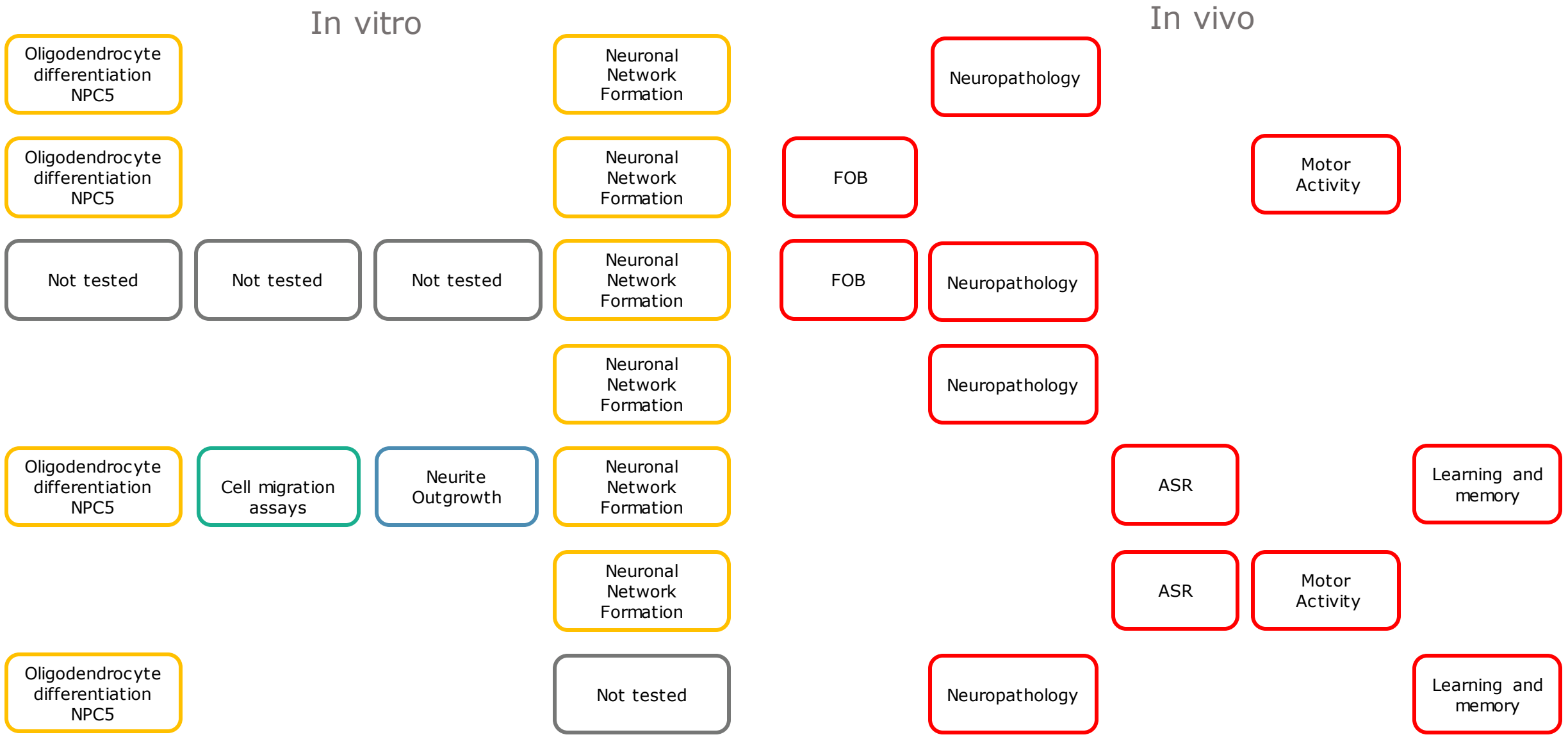
Active substances In vivo + vitro - or inconclusive

- How to assess the non concordant?

Active substance	Compound class	In vivo DNT LOEL (mg/kg bw per day)	In vivo Route of administration	Most sensitive Endpoint (mg/kg bw per day)	MAX concentration tested IVB (uM)	Borderline, unspecific hits	Data gaps current IVB
Acetamiprid	Neonicotinoid	10.0	Dams gavage	ASR	30	NO	1
Cymoxanil	Oxime ethers	5.0	Dams gavage	LM	20	NO	1, 3
Diazinon	OP	24.2	Diet	LM	20	NO	1, 2
Dimethoate	OP	0.5	PND 11-21 offspring gavage / water	MA	20	NO	1, 2
Methamidophos	OP				20	NO	1, 2
Tembotrione	Aromatic ketone	16.3	diet	NP	20	NO	1, 2, 3
Thiacloprid	Thiazolidine	25.6	diet	LM	20	NO	1
Thiamethoxam	Neonicotinoid		diet		20	NO	1
Topramezone	Other	8	diet	ASR, NP	8.56	NO	1,2,3,
Tri-allate	Tertiary amine	60	diet	MA, LM	20	YES (12.9 uM)	1,2

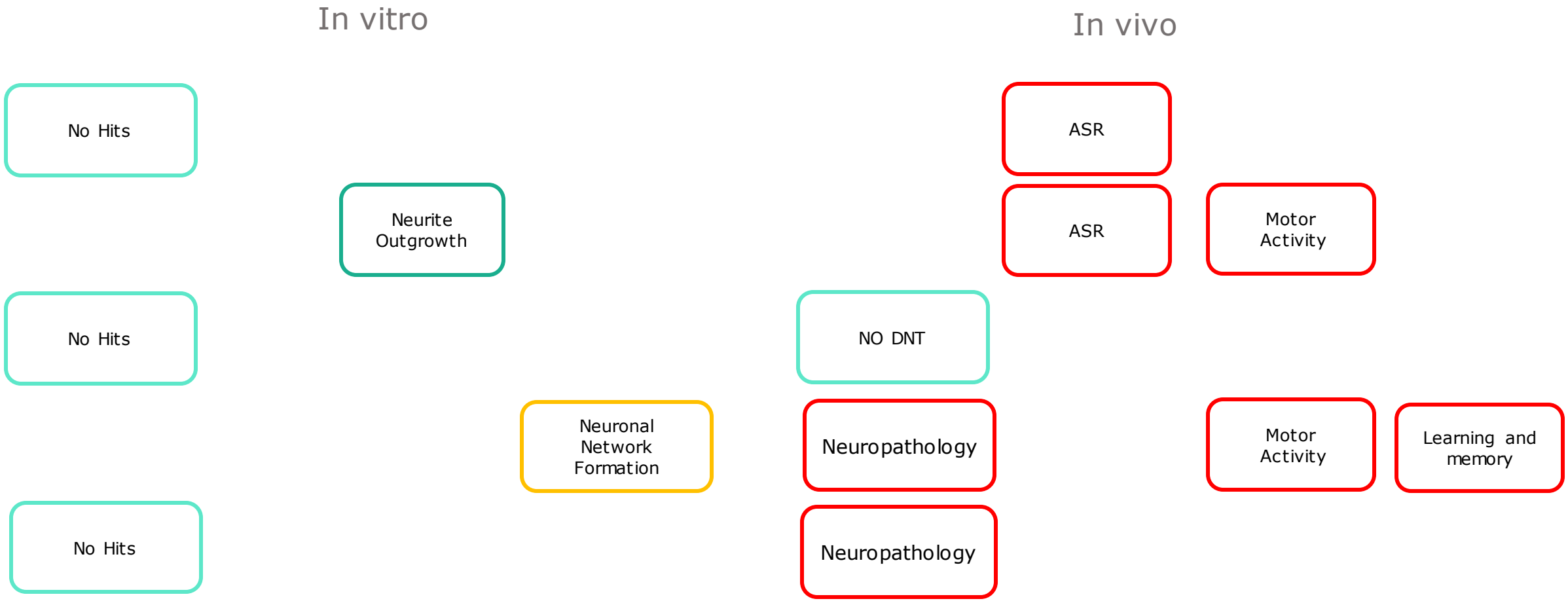
1. No data at the time of the analysis from US EPA IVB apoptosis, proliferation hNP1, neurite initiation hN2, Neurite maturation, synaptogenesis (Harril., 2018)
2. No data at the time of the time of the analysis from rat MEA (Frank., 2018; Shafer., 2019)
3. No data at the time of the time of the analysis from hNNF No data at the time of the time of the analysis from hNNF

Landscape by chemical class analysis: Pyrethroids



100% correlation + In vivo/+In vitro 7 substances

Landscape by chemical class analysis: Neonicotinoids



5 neonicotinoids

Uncertainties and limitations

- Number of substances is small, thus concordance analysis is only preliminary.
- IVB assays data are not available for most of the substances tested in vivo (only 28 substances in both IVB + Neurite Network function assays)
- Lack of systematic reviews: only one in vivo study included by active substance.
- A more comprehensive analysis is needed for both in vivo and in vitro data (e.g IATA, TK data to be included, mechanistic understanding).
- Species differences not considered in the correlation analysis (IVB mainly based in human-based in vito test systems)
- DNT endpoints results were compared in isolation. No other data has been considered in the analysis: TK data in vivo, physio-chemical properties of the substances, MoA or chemical class of the substances, other tox studies of the data package, thyroid disruption or other secondary DNT effect by metabolites (e.g. flufenacet case study).
- Intrinsic in vivo uncertainties: extrapolation to human, Tk issues for the negative.
- Cytotoxicity/viability interpretation in vitro.
- Different analysis algorithms by the three testing laboratories. Difficult to treat, pragmatic approach as in vitro inconclusive.
- Intrinsic in vitro uncertainties: gaps in the coverage of the DNT IVB for all critical neurodevelopmental processes, ADME processes not covered, and metabolites not included, secondary DNT not covered…

Take home message

- For the 41 substances for which an in vivo acceptable study and a conclusion from a regulatory authority is available, and DNT-IVB data is are available, a similar prediction outcome was evident for 27 substances (**66%)** (+ in vivo/+ in vitro or - in vivo/- in vitro).

- We do, however, understand that in vitro-in vivo correlative considerations or **performance analysis** for DNT remain complex and agreement on how to proceed with this and which **uncertainties** should be considered is still a **matter of discussion**.

- Therefore, at this time, it is in the opinion of EFSA that using this database for the interpretation of the performance of the DNT IVB should be done with **caution**.

Moving forward: next steps

- Incorporate the new IVB data from the ToxCast US EPA data (expected 3Q of 2022).
- Keep the dataset alive by including more in vivo DNT data.
- Test more substances in vitro that are present in the in vivo database.
- Continue collaboration EFSA with US EPA and US-NTP for DNT data collection, analysis and interpretation.
- More IATA case studies.
- Priority will be given for doing IATAs to the non-concordant substances (eg flufenacet assessed as no DNT by EFSA and after the IATA UA some DNT endpoints were considered as being affected).

The team

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