



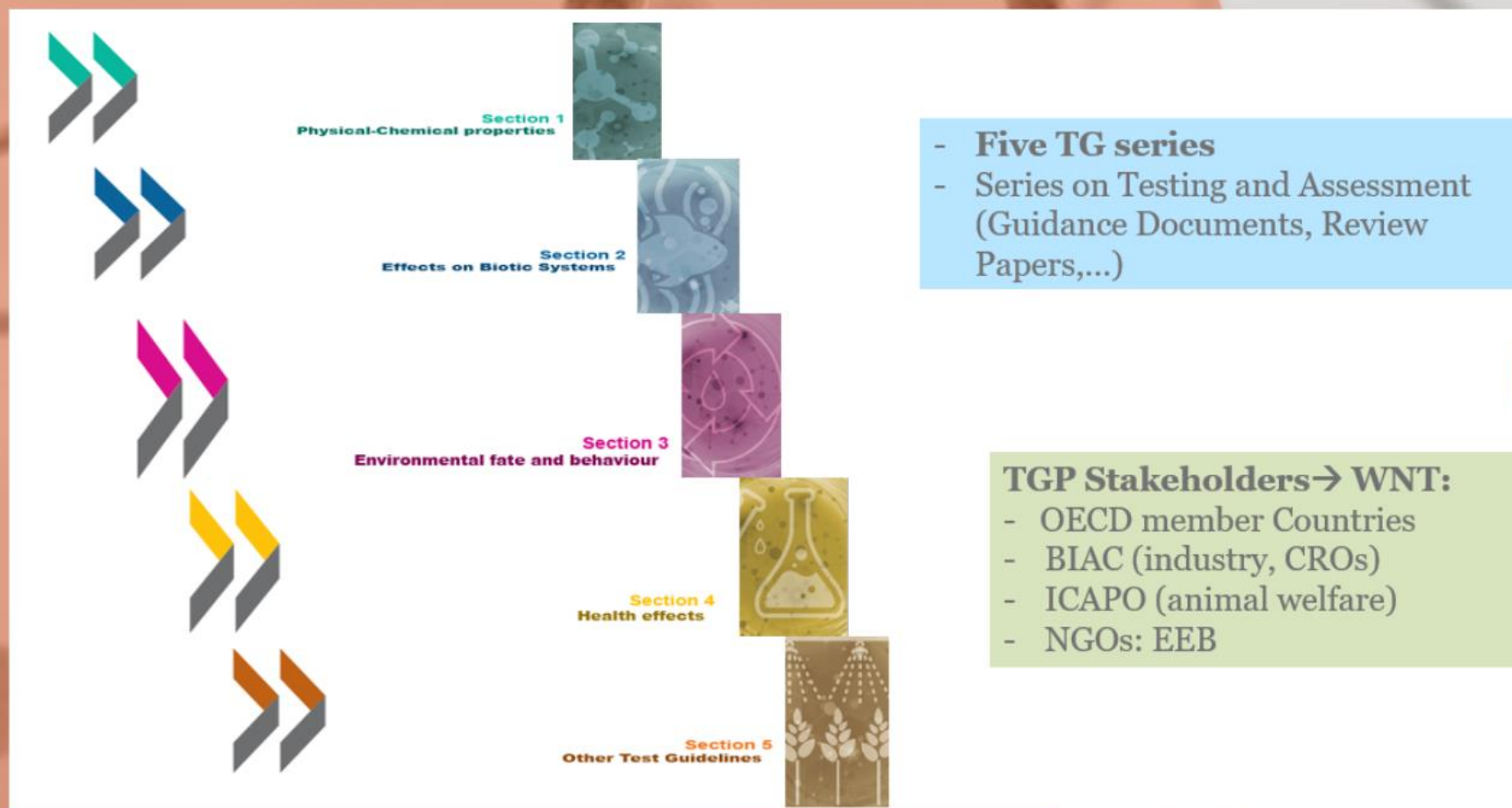
# OECD FRAMEWORK FOR A DNT TESTING BATTERY AND CASE STUDIES

Magda Sachana  
10<sup>th</sup> Berlin Workshop on Developmental Toxicology  
19-20 February 2020



# OECD TGs PROGRAMME

# OECD Test Guidelines Programme



*The OECD Mutual  
Acceptance of Data  
(MAD)*

## ***The OECD Mutual Acceptance of Data (MAD)***



### ***✓ Tested once –accepted for assessment everywhere***

- A safety test conducted in one country, in line with OECD standards, must be accepted by all OECD Members and 6 non-Member Adherents (Argentina, Brazil, India, Malaysia, Singapore and South Africa) for assessment purposes

- ✓ Avoids duplicative testing
- ✓ Avoids non-tariff barriers to trade
- ✓ Saves animal lives



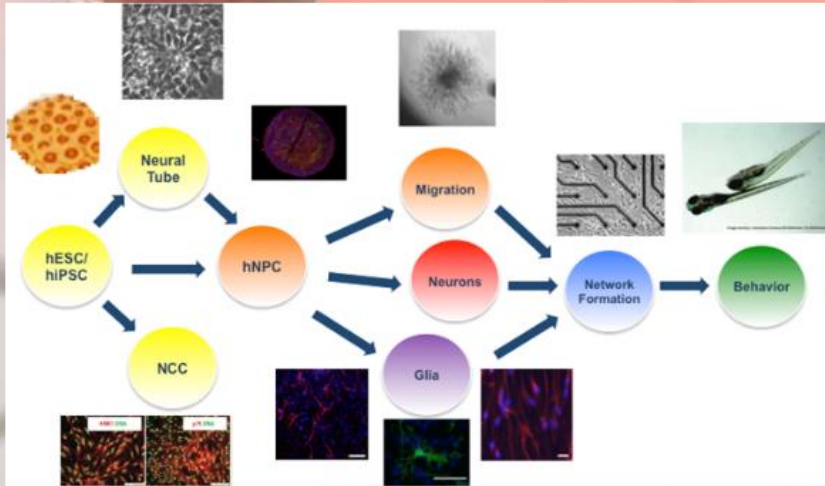
# OECD DNT PROJECT



# Background

Workshop	Year	Reference
<b>ECCVAM In Vitro Alternative Methods for DNT - Ispra</b>	2005	Coecke et al., 2007
<b>CAAT DNT TestSmart I - Reston</b>	2006	Lein et al., 2007
<b>CAAT DNT TestSmart II - Reston</b>	2008	Crofton et al., 2011
<b>DNT TestSmart III - Varese</b>	2011	Bal-Price et al., 2012
<b>DNT TestSmart IV - Philadelphia</b>	2014	None
<b>ISTNET DNT #1 - Zurich</b>	2014	Crofton et al., 2014 Bal-Price et al., 2015
<b>t4 DNT Reference Chemicals - Zurich</b>	2014	Aschner et al., 2017
<b>DENAMIC Workshop - Amsterdam</b>	2015	None
<b>OECD/EFSA Workshop - Brussels</b>	2016	OECD/EFSA, 2017; Fritsche et al., 2017
<b>ISNET DNT #2 - Konstanz</b>	2017	Bal-Price et al., 2018
<b>NTP Integrated DNT Testing Workshop - RTP</b>	2017	Behl et al., 2018

# Project Intro



**2014:** the EFSA PPR Panel recommended the development of a DNT in vitro testing battery to be used as a first tiered approach

**2015:** EFSA's external literature review on in vitro and alternative DNT testing methods

**2016:** OECD/EFSA workshop on DNT: the use of non-animal test methods for regulatory purposes

**2017:** the WNT accepted the inclusion of the DNT project proposal in its work plan

*Leading  
Team*

*Goal*

## *Leading Team*

**Andrea Terron**

**Pesticides Unit  
EFSA**



**Tim Shafer**

**US EPA**



**Anna Bal-Price**

**JRC**



**Susanne Hougaard  
Bennekou**

**Danish EPA**



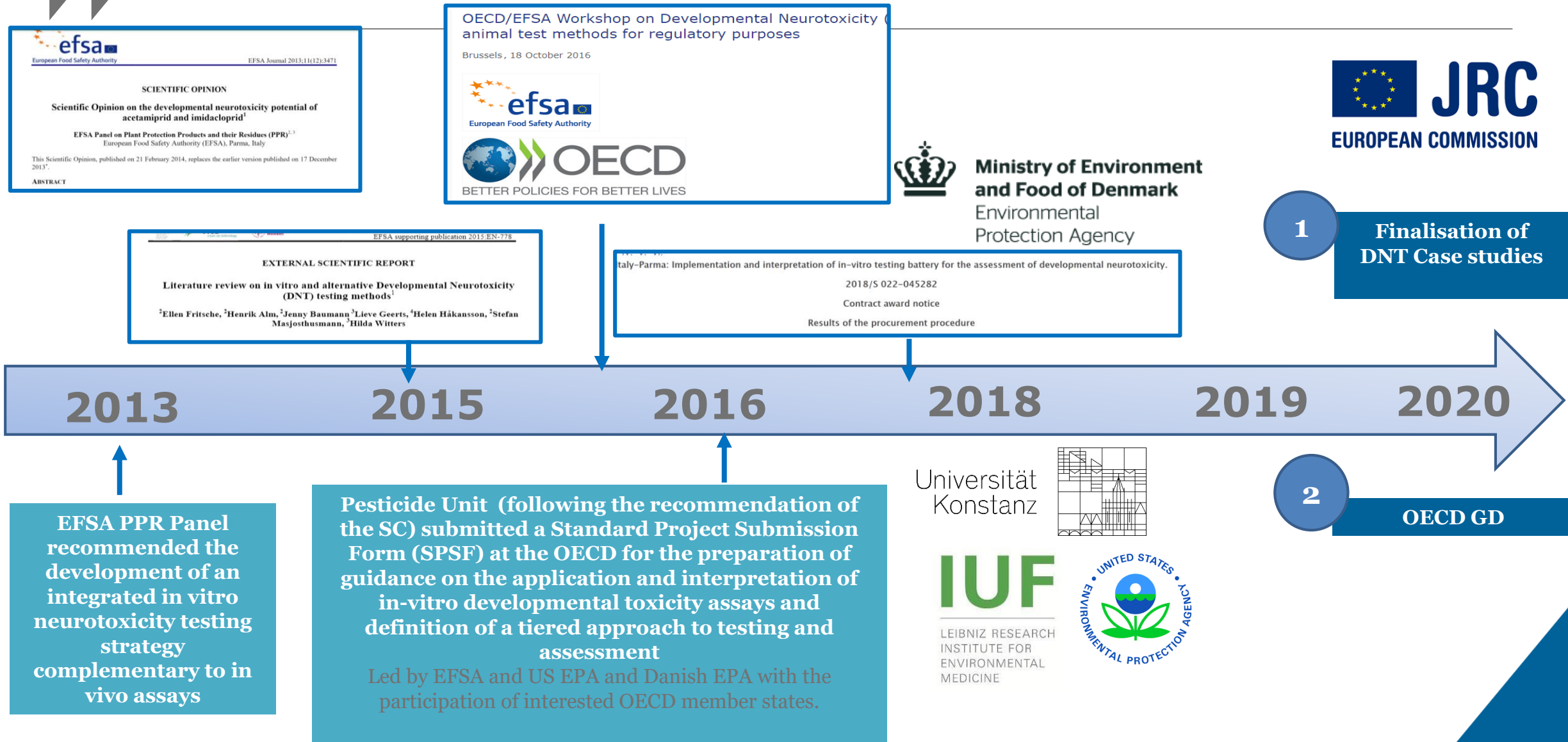


# Goal

- To develop a Guidance on application and interpretation of in-vitro DNT assays
- To provide a flexible and tailored DNT testing battery to address different regulatory needs, identify the current suitability of different assays, and provide elements for an integrated approach to testing and assessment (IATA) of DNT modalities not related to endocrine systems
- The guidance will be applicable to all chemicals



# Timelines – DNT project





# DNT IATA CASE STUDIES

# *DNT IATA case studies*

**1**

## **Application domain**

- screening
- hazard characterisation

**2**

## **Illustrate the practical use of the GD**

**3**

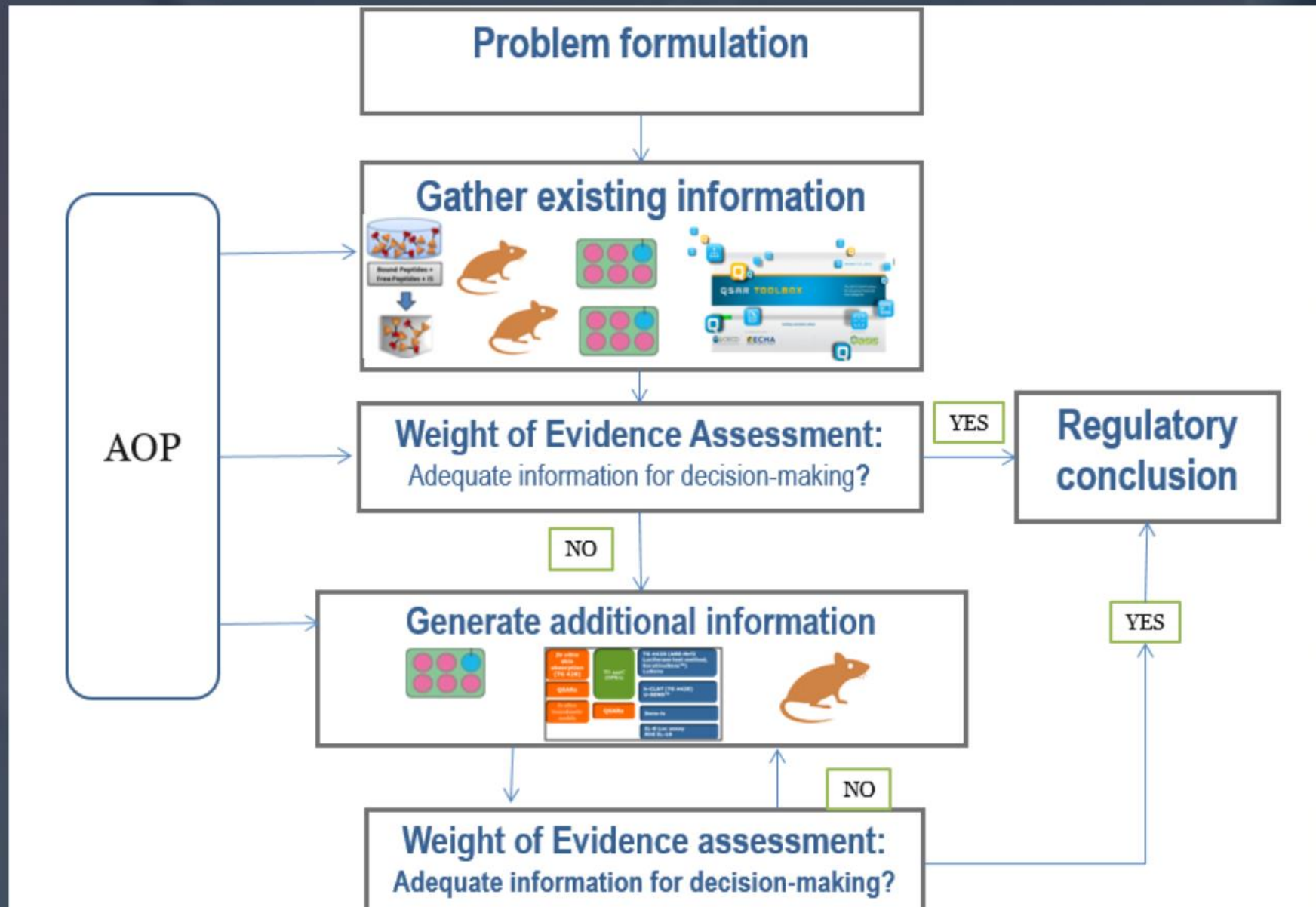
## **Facilitate data interpretation**

**4**

## **Harmonise reporting**



# Integrated Approaches to Testing and Assessment





# DNT CSs: Work in progress

- Interpretation of DNT *in-vitro* assays based on IATA case studies. E.g.
  - US DNT-NTP: DNT screening and prioritization of Organophosphorus flame retardants.
  - EU-ToxRisk: DNT hazard characterization of neonicotinoid pesticides based on NAMs.
  - BIAC: In vitro assays and in silico models to screen compounds for potential DNT activity.
  - EFSA: IATA development for DNT for hazard characterization of pesticides.
- Will use the outcome of the in-vitro testing battery and the outcome of additional work conducted in the **zebrafish** model.



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How might data from in vitro assays be used for DNT testing?

Scenario #1:

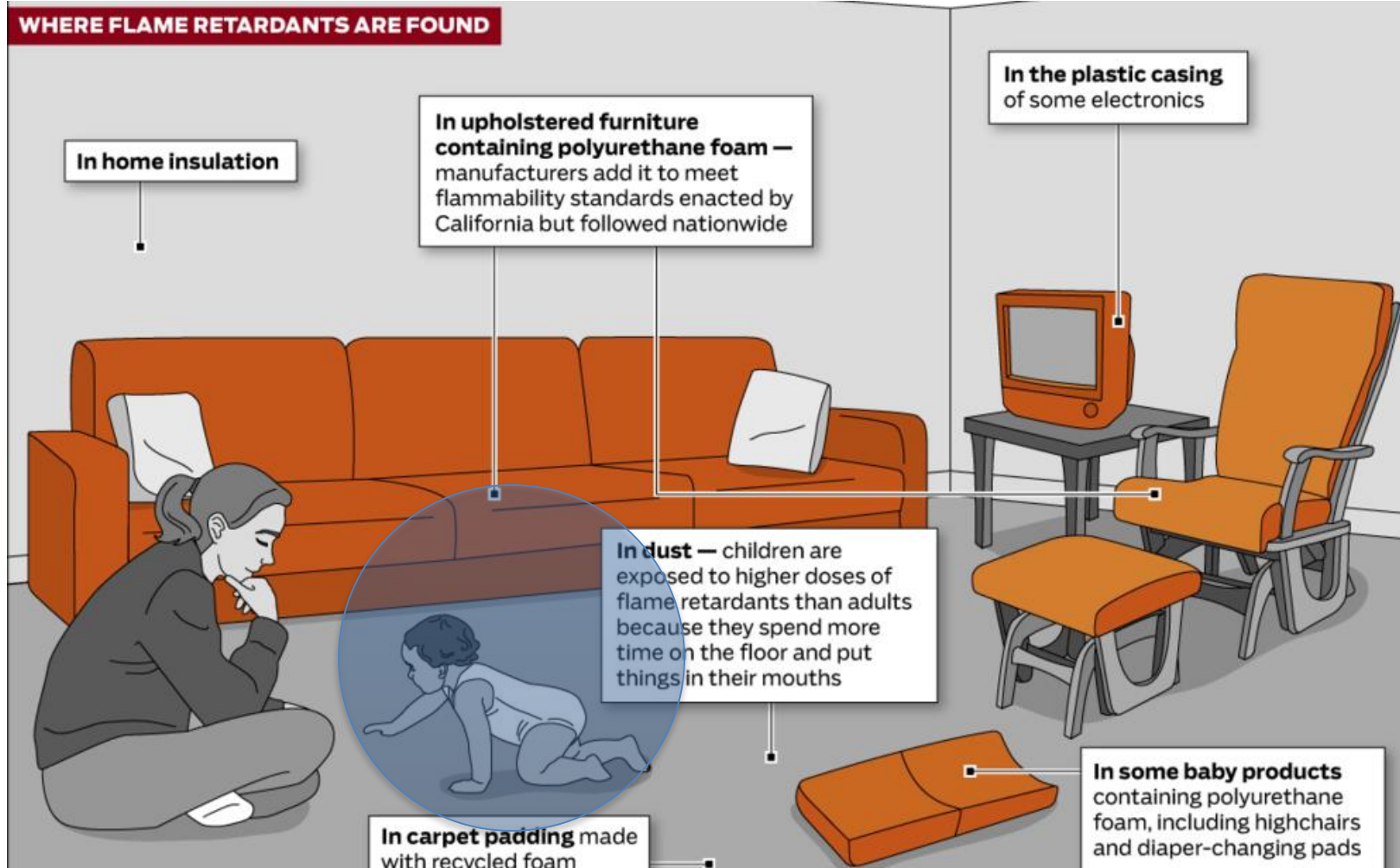
I have a small set of compounds, some with in vivo data and others without. I don't have the resources to test all compounds in vivo,

How do I decide which ones to test?





# Traditional Flame Retardants and their replacements (OPFRs)



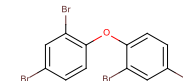




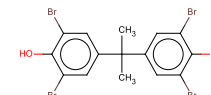
CAS	Chemical Name	Chemical.ID	Structure
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#### Representative Brominated FRs (BFRs)

5436-43-1 2,2',4,4'-Tetrabromodiphenyl ether BDE-47



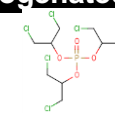
79-94-7 3,3',5,5'-Tetrabromobisphenol A TBBPA



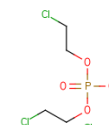
← e.g. phased-out BDE

#### Organophosphorous FRs (OPFRs)- aliphatic, halogenated

13674-87-8 Tris(1,3-dichloro-2-propyl)phosphate TDCIPP



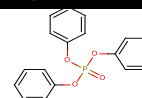
115-96-8 Tris(2-chloroethyl) phosphate TCEP



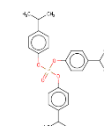
e.g. banned organohalogens

#### Organophosphorous FRs (OPFRs)- Aromatic

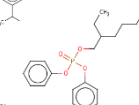
115-86-6 Triphenyl phosphate TPHP



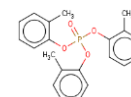
68937-41-7 Phenol, isopropylated, phosphate (3:1) IPP\*



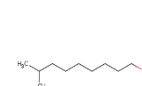
1241-94-7 2-Ethylhexyl diphenyl phosphate EHDP\*



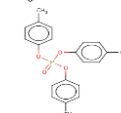
1330-78-5 Tricresyl phosphate TMPP\*



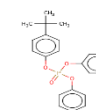
29761-21-5 Isodecyl diphenyl phosphate IDDP



56803-37-3 tert-Butylphenyl diphenyl phosphate BPDP\*



78-30-8 Tri-o-cresyl phosphate TOCP

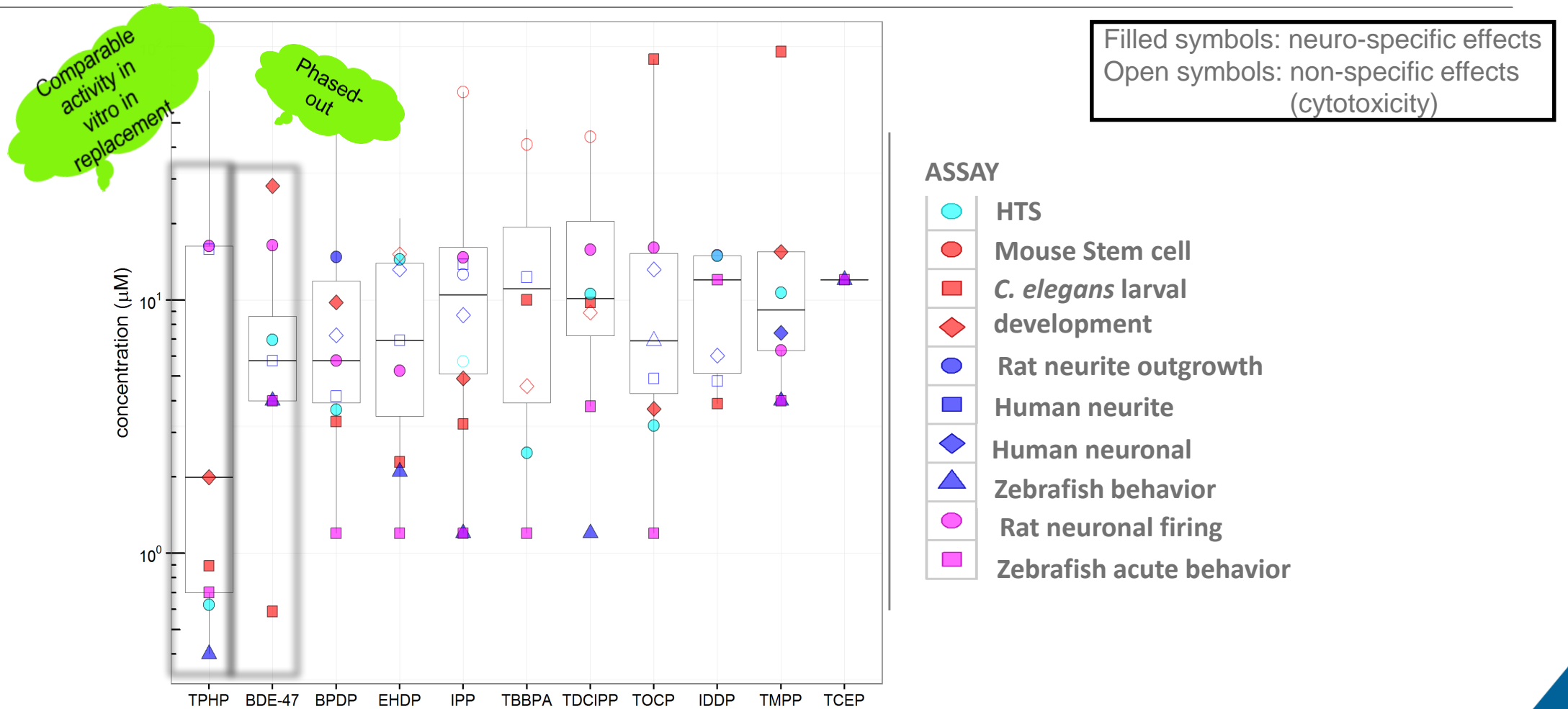


Proposed Replacements

\*representative isomer in mixture is shown as structure



# Comparison of OPFRs with phased-out compounds






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How might data from in vitro assays be used for DNT testing?

Scenario #2:

I have a Guideline DNT (and/or a literature reports) for a compound that shows a weak/no effect or contradictory results.

Can in vitro assays help increase the confidence in these data OR can they increase the confidence that the in vivo data are spurious?





# BPA *In Vivo* DNT

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Studies are divided on potential BPA effects on DNT in rodents

## **Negative study examples:**

**Stump et al (2010):** OECD 426 in Crl:CD(SD) rats (n = 24/dose)

**Ryan et al. (2010):** Hormonally mediated neuro endpoints

## **Positive study examples:**

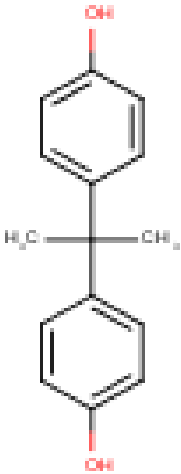
**Johnson et al. (2016)** Spatial navigational learning and memory





# Cheminformatics Model Results for BPA

- BPA: Cheminformatic flags for mitochondria and protonophore

CAS	Name	SMILES	Structure
80-05-7	Bisphenol A	<chem>CC(C)(C1=CC=C(O)C=C1)C1=CC=C(O)C=C1</chem>	

- Other targets, including neuro targets, were negative



# *In Vitro* Data: DNT Assays

- Tested in 8 alternative assays

casrn	name	dsstox_substance_id	hN2_NOG_hitcall	hNP1_hitcall	ratcort_NOG_hitcall	synap_hitcall	MEA_NFA_hitcall	MEA_hitcall	UKN2 (cMINC)	UNK 4 (NOG)	UNK5	NPC1	NPC2-5	NPC6	#Assays Tested in
80-05-7	Bisphenol A	DTXSID7020182	0	1	0	0	0	1		0	0				8

## Positive Assays:

- hNP1 Neuroprogenitor Proliferation** (Harrill et al., 2018):
  - Apoptosis hNP1:  $EC_{30} = 30.9 \mu M$
- BPA MEA**: Altered MEA in rat pup cortical cell cultures (Frank et al., 2017)
  - Tested BPA 0.03-30  $\mu M$  with 15-min MEA Recordings: 2, 5, 7, 9, 12 DIV
  - Cell viability: CellTiter-Blue and Total LDH (LDH Release)
  - BMD from NTP's DNT DIVER:  $\downarrow$  No. network spikes  $0.39 \mu M$ 
    - $\downarrow$  No. actively bursting electrodes at  $1.53 \mu M$



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How might data from in vitro assays be used for DNT testing?

Scenario #3:

I have a negative Guideline DNT (or a literature report) for a compound that undergoes revaluation.

Can in vitro assays help increase the confidence in these data?





# Problem formulation

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## Systematic scientific assessment

1. Assessment question A1: How certain are we that deltamethrin/flufenacet is **developmental neurotoxicant in humans, based on the data collected, appraised, synthesised and integrated** in line with the IATA framework?
2. Assessment question A2: To what extent does the additional evidence provided by **the vitro testing battery on deltamethrin change the uncertainty on deltamethrin/flufenacet DNT** as assessed in point 1?



Problem formulation

Definition of the methods

**I A T A**

In vivo

OECD 426

In vitro

In vitro  
battery

Human  
observ.

Systematic review

Data collection

Evidence appraisal

Evidence synthesis  
and integration

AOP?



Characterization  
of a  
DNT Hazard

**STEP 2**

Deltamethrin

Flufenacet

Uncertainty analysis





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How might data from in vitro assays be used for DNT testing?

Scenario #4:

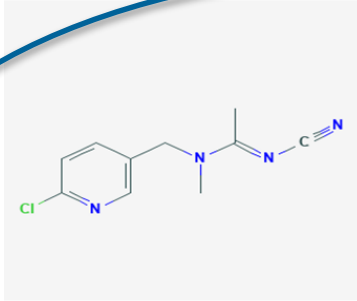
I have a non conclusive Guideline DNT study but reports from in vitro studies for a compound that shows an effect.

Can in vitro assays help increase the confidence in these data and inform decision-making?

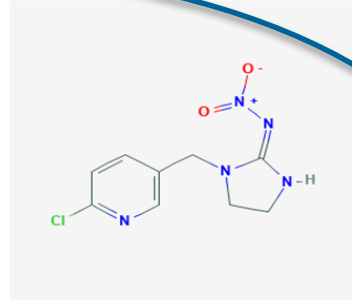




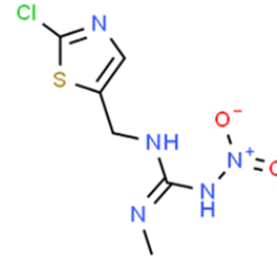
# Selected neonicotinoids



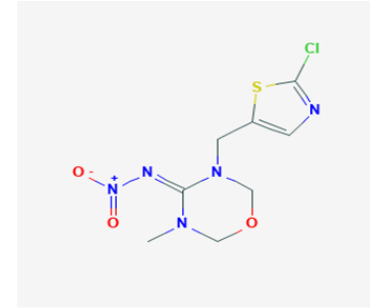
**Acetamiprid**  
(Log P 0.8; neutral)



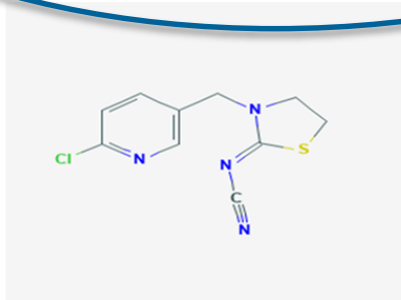
**Imidacloprid**  
(Log P 0.4; neutral)



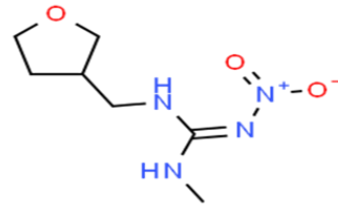
**Clothianidin**  
(Log P 0.7; neutral)



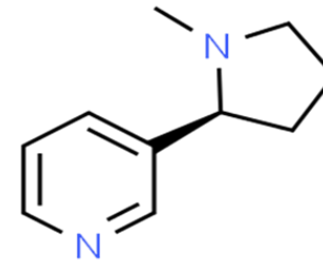
**Thiamethoxam**  
(Log P -0.1; neutral)



**Thiacloprid**  
(Log P 1.3; neutral)



**Dinotefuran**  
(Log P -0.6; Acid)



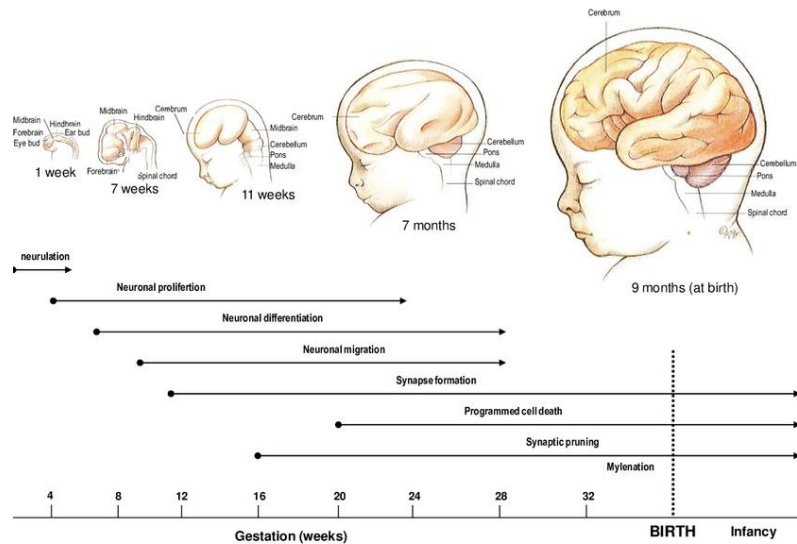
**Nicotine**  
(Log P 1.2; basic)



## What's next.....

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- Results are expected for the **1<sup>st</sup> Q 2020** to further inform the CSs.
- Outcome of the testing will be BMC-response curves for **121 compounds** across the assays.
- **Database** and **prediction models** for the individual assays and the whole battery.
- Implementation of **kinetics** in data interpretation.
- Development of a draft guidance for in vitro DNT testing for processing of the data and interpretation of results.



# Thanks For Listening



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