Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2022)24/ANN1

Unclassified

English - Or. English

2 September 2022

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Appendix A. Protocol for the Scientific Opinion of the PPR Panel on Developing Integrated Approaches to Testing and Assessment (IATA) on developmental neurotoxicity: deltamethrin and flufenacet case studies

Series on Testing and Assessment
No. 362

JT03501733

OECD Environment, Health and Safety Publications

SERIES ON TESTING AND ASSESSMENT

NO. 362

Appendix A. Protocol for the Scientific Opinion of the PPR Panel on Developing Integrated Approaches to Testing and Assessment (IATA) on developmental neurotoxicity: deltamethrin and flufenacet case studies

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Paris 2022

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Glossary [and/or] Abbreviations

Glossary: an alphabetical list of words relating to a specific subject, text or dialect, with explanations; a brief dictionary.

Abbreviation: a shortened form of a word or phrase (such as Mr, Prof.). It also includes acronyms (a group of initial letters used as an abbreviation for a name or expression, each letter being pronounced separately – such as DVD, FDA – or as a single word – such as EFSA, NATO).

5-HT	5-hydroxytryptamine
ADHD	Attention-deficit/hyperactivity disorder
ADME	Absorption, distribution, metabolism and excretion
AO	Adverse Outcome
AOP	Adverse Outcome Pathway
BASC	Behavioural Assessment System for Children
BDNF	Brain Derived Neurotrophic Factor
BKCI-S	Book Citation Index– Science
BR	Burst Rate
BrdU	Bromodeoxyuridine
BrdU NeuN	Bromodeoxyuridine Neuronal Nuclear Marker
BRIEF	Behaviour Rating Inventory of Executive Function
Bw	Body Weight
Camk	Calcium/Calmodulin Dependent Protein Kinase
CAT	Critical Appraisal Tool
CCR	Current Chemical Reactions
CREB	cAMP Response Element-Binding Protein
CWM	Cincinnati Water Maze
DA	Dopamine
DART	Digital Access to Research Theses
DM	Deltamethrin
DNT	Developmental neurotoxicity
DNT-IVB	DNT in vitro battery
DOPAC	3,4-Dihydroxyphenylacetic acid
DQ	Development Quotient
drd1	dopamine receptor d1
drd2a	dopamine receptor d2a
drd3	dopamine receptor d3
EBSCO	Elton B. Stephens Company
EKE	Expert Knowledge Elicitation
ESCI	Emerging Sources Citation Index

F	Flufenacet
GABA	Gamma-Aminobutyric Acid
Gap-43	Growth-associated protein-43
GD	Guidance Document
GluN1	Glutamate Receptor Ionotropic, NMDA 1
GlunN2A	Glutamate Receptor Ionotropic, NMDA 2A
GluN2B	Glutamate Receptor Ionotropic, NMDA 2B
hiPSC	Human Induced Pluripotent Stem Cells
HVA	Homovallinic acid
IATA	Integrated Approaches for Testing and Assessment
IC	Index Chemicus
IQ	Intelligence quotient
ISI	Interspike Interval
IVB	In Vitro Battery
IVIVE	in vitro to in vivo extrapolation'
KE	Key Event
KER	Key Event Relationship
LDH	Lactate Dehydrogenase
LOD	Limit of Detection
LUHMES	Lund Human Mesencephalic Cells
MAP2	Microtubule Associated Protein
MBD	Mean Burst Duration
MEA	Microelectrode arrays
MeSH	Medical Subject Headings
MFIB	Mean firing rate in burst
MFR	Mean Firing Rate
MI	Mental development Index
MIE	Molecular Initiating Events
MISIB	Mean Interspike Interval In Burst
MoA	Mode of Action
MRI	Magnetic Resonance Imaging
mRNA	messenger RNA
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MWM	Morris Water Maze
NE	Norepinephrine
NMDA	N-methyl-D-aspartate
OECD	Organisation for Economic Co-operation and Development
OHAT/NTP	The Office of Health Assessment and Translation/National Toxicology Programme
pCREB	cAMP response element-binding protein phosphorylated
PFAS	Perfluoroalkyl substances
PFOS	Perfluorooctane sulfonate, perfluorooctane sulfonic acid
PPR	Pesticide Peer Review
PQDT	Proquest Dissertations and Theses
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTrkB	Tropomyosin Receptor Kinase B Phosphorylated
r/HNNf	Rat/Human Neuronal Network Formation
RoB	Risk of Bias
RoB DH	Risk of Bias Definitely High
RoB DL	Risk of Bias Definitely Low
RoB PH	Risk of Bias Probably High
RoB PL	Risk of Bias Probably Low
ROS	Reactive oxygen species
Ry	Ryanodine

RyR	Ryanodine Receptors
SCI	Science Citation Index Expanded
SD	Spike Duration
SD	Spectrum Disorder
SDQ	Strengths and Difficulties Questionnaire
sEPSC	Spontaneous Excitatory Post-Synaptic Currents
SQ	Scientific Question?
SVZ	Subventricular zone
TG 426	OECD Test Guideline 426
TH	Tyrosine Hydroxylase
TNF	Tumour Necrosis Factor alpha
ToR	Terms of References
TrkB	Tropomyosin Receptor Kinase B
TTX	Tetrodotoxin
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labelling
UA	Uncertainty Analysis
US EPA	United States Environmental Protection Agency
VCI	Verbal Comprehension Index
VZ	Ventricular Zone
WG	Working Group
WISC	Wechsler Intelligence Scale for Children
WM	Water Maze
WMI	Working Memory Index
WoE	Weight of evidence
ZF	Zebrafish

Summary

This protocol describes the strategy for the assessment that EFSA will carry out on developing integrated approached to testing and assessment (IATA) on developmental neurotoxicity (DNT) using deltamethrin and flufenacet as case studies. The protocol was developed with the aim of defining as much as possible beforehand the strategy that will be applied for collecting data, appraising and integrating the evidence from different sources of data, i.e. public literature, regulatory studies and an outsourced high-throughput battery of *in vitro* data, and different evidence streams, i.e. *in vivo*, *in vitro* and human data. The problem formulation and the plan for the methods to address it are both covered in this document. The questions and the strategy for a systematic literature review are described in detail including the definition of the DNT endpoints and test methods for *in vivo*, *in vitro* and human streams of evidence, the search string and eligibility criteria. The methods for appraising the risk of bias of all the evidence are also anticipated in the protocol, including the Critical Appraisal Tools, adapted from the OHAT framework, the predefined rational for appraising the evidence and the definition of the key domains and questions for each line of evidence. The methods for integration and uncertainty analysis applying the Adverse Outcome Pathway (AOP) conceptual framework are also presented in the protocol up to the methods used to screen the evidence to be considered in the putative AOP. The methods for analysing and expressing the uncertainty in the key event relationships could not be planned upfront.

1 Introduction

1.1. Background and Terms of Reference

The Working Group (WG) of the PPR Panel will consider developing IATA case studies using a DNT risk assessment-based problem formulation using all available information on defined pesticide active substances. The PPR Panel will specifically:

- Propose pesticide active substances for which an IATA case study should be developed. In selecting those active substances, it will consider: the completeness of the available database, the concern for DNT based on the qualitative toxicological and epidemiological profile and the chemical class of each active substance.
- Propose a DNT risk assessment problem formulation that could address the regulatory needs under Regulation 1107/2009 (European Commission, 2009) on the approval of active substances.
- Develop an iterative AOP informed IATA case study for each selected pesticide active substance taking into account the level of uncertainties associated with the available database.
- Integrate the outcome of the in vitro testing battery for DNT in the AOP informed IATA case study and provide a new uncertainties analysis to guide on the use and interpretation of the in vitro DNT testing battery.
- Discuss and contextualise in the conclusion of the Scientific Opinion whether the proposed testing battery is fit for purpose and its limitations.

1.2. Choice of the methodological approach

To address this mandate, a Working Group (WG) of EFSA's PPR Panel was tasked to conduct a hazard assessment on defined pesticide active substances (deltamethrin and flufenacet) and DNT, using an extensive evidence base and applying an IATA approach.

1.3. Selection of the case studies

To execute the Terms of Reference the WG have selected two substances, i.e. pesticides active substances for which an OECD 426 is available will be used. One substance – deltamethrin – is a known acute neurotoxic substance and therefore a potential concern for DNT exists. This substance will be used for the evaluation of the DNT-IVB and the impact of mechanistic understanding in an AOP informed IATA for DNT hazard characterisation. The second substance – flufenacet – is expected to be not of concern for DNT and will therefore be used for the evaluation of the DNT-IVB to support lack of DNT hazard also from the mechanistic perspective.

1.4. Application of an Adverse Outcome Pathway conceptual framework

To develop a biological plausible, dose and time concordant integrated framework including *in vitro*, *in vivo* (i.e. experimental animal) and epidemiological evidence, the WG decided to apply a theoretical AOP conceptual framework (OECD, 2017) with the intention of classifying the available evidence (coming from multiple evidence streams, test systems, test methods, endpoints and measures of them) in evidence informing molecular initiating events (MIEs), evidence informing the neuro developmental processes where key events (KEs) are most likely to occur in an AOP, and evidence informing the adverse outcome (AO).

1.5. Sources of evidence and approach to dealing with it and to analysing uncertainty

The evidence for this scientific assessment would initially be collected from the scientific literature and application dossier(s) submitted to EFSA and subsequently integrated with further evidence generated by an *in vitro* battery commissioned by EFSA to an external contractor. The procurement of the DNT *in vitro* testing battery was assembled and challenged with 121 compounds including known human or animal DNT compounds or are known to not disturb brain development *in vivo*. The testing battery covers basic neurodevelopmental key events, which, when disturbed, lead to an adverse outcome, e.g. functional and/or morphological alterations in brain (e.g. proliferation, synaptogenesis, oligodendrocytes, apoptosis).

The approach to collecting, appraising, synthesising/integrating evidence and analysing uncertainty would follow EFSA's 'Principles and process for dealing with data and evidence' (EFSA, 2015; EFSA and EBTC, 2018a)[1] and guidance on uncertainty analysis (EFSA Scientific Committee, 2018b, 2018c) and would be in line with the IATA framework (OECD, 2016).

This implied planning the methods for conducting the assessment upfront, in a protocol.

1.6. Scope of this protocol

This protocol was drafted with the aim to define *a priori*, i.e. before starting any formal data collection, the methods for conducting the scientific assessment required by this mandate. It follows the recommendations for protocol development defined by a WG of EFSA's Scientific Committee, which were under development at the time when the plan for this assessment started[2].

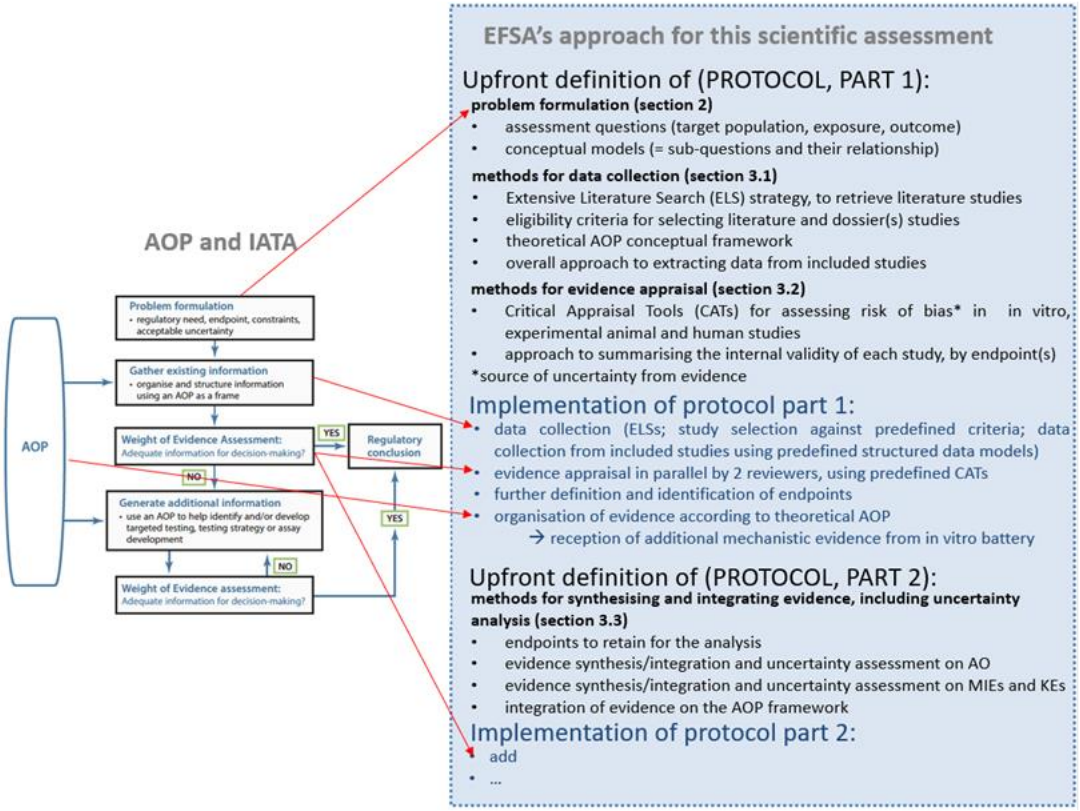
The document illustrates the problem formulation and the planned methods for collecting relevant data, appraising/synthesising/integrating evidence and analysing uncertainty.

The relationship between EFSA's approach for planning and carrying out this scientific assessment and the IATA framework is illustrated in Figure 1.

[1] http://www.efsa.europa.eu/en/methodology/evidence

[2] https://www.efsa.europa.eu/en/supporting/pub/en-1843

Figure 1. Relationship between EFSA's approach for conduct scientific assessment process (EFSA, 2015; EFSA Scientific Committee, 2018a, 2018b, 2018c) planned in this protocol and the IATA framework



Source: OECD GD 260, OECD, 2016.

1.7. Process for developing this protocol

The process for developing this protocol was iterative and required multiple Working Group exchanges and literature scoping. Overall, it can be summarised into two main phases:

1) First planning phase (Sections 2, 3.1 and 3.2 of this protocol), which involved problem formulation and, based on that, the definition of the methods for collecting data and appraising evidence (Sections 3.1. and 3.2 in this protocol). Problem formulation implied a preliminary definition of the relevant endpoints based on the knowledge and expertise the Working Group at the start of the assessment, when the available evidence was not yet known, and of the tools that would be used to critically appraise the evidence. The latter were also piloted tested and finetuned accordingly. This step was followed by an implementation phase, during which data were collected from the scientific literature and application dossiers and the identified evidence was appraised according to the plan. This allowed the Working Group to develop a clear understanding of the available evidence and further identify and define relevant DNT endpoints. At the end of this process, the *in vitro* battery was made available by the contractor.
2) Second planning phase. Based on the above, the Working Group was able to prioritise the relevant endpoints based on the internal validity of the available studies and plan the methods for the second part of the assessment accordingly, i.e. (i) the methods for synthesis/integration and uncertainty analysis of mechanistic evidence on MIE and KEs and of evidence on AO; and (ii) the approach for overall evidence integration using the AOP framework. These methods are described in Section 3.3 of this protocol.

PROTOCOL: PART 1

2 Problem formulation

This section of the protocol illustrates the assessment questions (Section 2.1) defined by the Working Group based on the interpretation of the mandate and the related conceptual models, i.e. all the sub-questions and their relationship (Section 2.2).

2.1. Assessment questions resulting from the translation of the mandate

This mandate was translated into the following four assessment questions:

1. Assessment question A1: How certain are we that **deltamethrin** is developmental neurotoxicant in humans, based on the data collected, appraised, synthesised and integrated using the methods described in this protocol and in line with the IATA framework?
2. Assessment question A2: To what extent does the additional evidence provided by the mechanistic evidence (including the outsourced *in vitro* testing battery) on **deltamethrin** change the uncertainty on deltamethrin DNT as assessed in point 1?
3. Assessment question B1: same question as A1, but on **flufenacet**;
4. Assessment question B2: same question as A2, but on **flufenacet**.

2.2. Target population

Humans, all ages.

2.3. Exposure

In humans the assessment would focus on prenatal (fetal exposure) and early post-natal (i.e. through breast feeding only) exposure to deltamethrin and flufenacet. For rodents (fetus and new-born) the exposure routes will include oral intake through the diet (dam) or through the milk (pups), direct treatment through oral gavage (pups). *In vitro* exposure is intended as nominal concentration used in the essay.

Deltamethrin is a pyrethroid ester insecticide approved under Regulation (EC) No 1107/2009. It is rapidly absorbed after oral administration. Deltamethrin is a synthetic insecticide based structurally on natural pyrethrins, which rapidly paralyse the insect nervous system giving a quick knockdown effect. It is rapidly absorbed after oral administration with Tmax values of 1 to 6 hours and urinary excretion of around 30–60%, it is distributed to most tissues. Deltamethrin acts as a Type II pyrethroid: acute clinical signs include such as sedation, salivation, dyspnoea, ataxia, clonic spasm, convulsion, gait alteration, impaired righting reflex, reduced or absent forelimb/hindlimb grasp, writhing and walking with splayed hindlimbs, repetitive jaw movement, lacrimation, weakness, chromodacryorrhoea, anorexia, staining of the fur and vocalisation. Repeated toxicity effects after oral administration are death and neurological effects clinical signs too.

Flufenacet is a synthetic aromatic amide, approved under Regulation (EC) No 1107/2009 as an herbicide. It is rapidly absorbed after oral administration (around 75–94%) and excreted by urine 90%. It is widely distributed and extensively metabolised. Acute clinical signs are non-specific and include ataxia, laboured breathing, decreased activity and, lacrimal, nasal and perianal staining. The main target organs after repeated exposure are liver, thyroid, kidney and eye and in dogs also nervous system.

2.4. Developmental neurotoxicity and related endpoints

Developmental neurotoxicity refers to any adverse effect on the normal development of nervous system structures and/or functions, due to the exposure to a toxic substance (US EPA, 1998; quoted in NAFTA guidance). For these assessments on deltamethrin and flufenacet and DNT, at the time when this protocol started to be developed, the endpoints reported in Table 1, Table 2 and Table 3 were deemed relevant.

Further endpoints were expected to be identified as the assessment would progress and the classification and definitions refined. In addition, it was decided that, once all endpoints were identified, only some would be prioritised for e.g. a concentration (or dose)–response assessment and/or any other more structured/quantitative approach to evidence synthesis/integration.

The endpoints were clustered by evidence stream in endpoints of *in vitro* mechanistic studies, *in vivo* experimental animal studies and human observational studies.

For *in vitro* mechanistic studies, they were classified according to the key neuro developmental processes that they could inform in the relevant AOP, (Table 1).

For *in vivo* experimental animal studies, endpoints are classified according to three categories representing, but not limited to, adverse outcomes (Table 2).

For human observational studies, endpoints are classified according to some major adverse outcomes (Table 3).

The endpoints were uploaded on DistillerSR® (Evidence Partners, Ottawa, Canada) and during the appraisal all relevant endpoints were identified and included, in the cases when were needed, in the software from the evidence.

Table 1. Preliminary list of relevant endpoints for *IN VITRO* studies defined at the start of the assessment. *In vitro* studies

Endpoint category	Specific endpoints
Proliferation endpoints	To add (if needed)
Apoptosis endpoints	To add (if needed)
Differentiation	Neurogenesis Gliogenesis Oligodendrocyte differentiation Astrocytic differentiation
Migration endpoints	Neuronal migration Radial migration. Glial migration To add (if needed)
Growth/maturation	Neurite outgrowth Neuronal morphology Synaptogenesis Neuronal cell types To add (if needed)
Network formation/function	MEA MEA (MFR) MEA (Number of burst) MEA (Burst duration)

	MEA (Intervals between bursts) Other to add if needed
Cytotoxicity/viability	MTT assay LDH level Neutral red accumulation Other to add (if needed)
Channels/transporters	Sodium Calcium Chloride Potassium Other to add (if needed)
Proteins	Synaptophysin SNAP25 Synaptobrevin MAP2 Other to add (if needed)
Receptors	GABA NMDA DA 5-HT Other to add (if needed)
Neurotransmitters	Other to add (if needed)
Enzymatic activity	Calcineurin Calmodulin Dephosphorylation Other to add (if needed)
Microglia activation	TNF alpha Other to add (if needed)
Oxidative stress	ROS production Nrf2 expression/translocation Mitochondrial membrane integrity Other to add (if needed)
Cell organelles integrity	Nuclear integrity Lysosomal integrity Mitochondrial membrane integrity Other to add (if needed)
Neurophysiology/ patch clamp	Membrane excitability Other to add (if needed)
Genomic	Other to add (if needed)
Behavioural endpoints (in zebrafish)	Thigmotaxis Locomotor activity Spasms Swimming activity Other to add (if needed)
Pathology (in zebrafish)	Cranio-morphological effects Curvature of the body axis Quantitative morphometric examination (body area, head area, head-body angle) Qualitative morphometric evaluation Other to add (if needed)
Genes (in zebrafish)	Other to add (if needed)
Other	

Table 2. Preliminary list of relevant endpoints for experimental ANIMAL studies defined at the start of the assessment

Preliminary list of endpoints for experimental animal studies	
Endpoint category	**Specific endpoints**
Physical and developmental landmarks	Sexual maturation (age) Clinical observation (visual observation, period)
Neuropathology endpoints [see OECD TG 426 as a standard reference]	Brain weight (measure unit, absolute and relative to body weight, period) Quantitative morphometric evaluation (linear measurement, areal measurements, brain morphometric landmarks, brain regions measured, stereology. Period) Qualitative neuropathology examination (diagnostic criteria, severity score criteria, standard and special stain used, period) Neuroimaging (quantitative, e.g. MRI)
Behavioural endpoints See OECD TG 426 and	**Behavioural ontogeny**: functional observation batterye.g. righting reflex, negative geotaxis, motor activity… (period, test system)

NAFTA guidance as a standard reference	**Motor activity**: horizontal direction; other direction, additional fine movements (test system, detection system (computer), period, increase or decrease in: ambulatory counts, ambulatory distance, ambulatory time, vertical movements (rearing), discrete non-ambulatory movements, total activity) **Auditory startle response** (integrity of a sensory- evoked motor response) **with or without habituation** (learning endpoint):effects on overall startle amplitude, effects on habituation in startle magnitude, comparison between ages of testing, sex differences (period, test method, decrease in response amplitude after repeated startle stimulus presentation during the test session and measurement of latency). **Learning and memory** (learning is defined as a relatively permanent change in behaviour that is the result of experience. Memory is defined as the retention and use of acquired information about locations, events or temporal order to modify subsequent behaviour. Learning and memory can be inferred from changes in behaviour). The endpoints assessed are test system related: Letter Mazes M, Y, E (Position, Discrimination: latency to escape, errors, trials to criterion). Morris Water Maze (Spatial Learning: latency to escape over trials, path length to locate hidden platform, search parameters during retention probe trials). Passive Avoidance (Associative Learning: latency, trials to criterion). Biel Water Maze (Sequential learning: latency, errors) Cincinnati Maze (Sequential/Egocentric Learning: latency, errors) **Social behaviour (qualitative or quantitative, tbc test method)** **Elevated maze** **Swim test** **Tactile startle** **Conditioned freezing** **Amphetamine challenge** **MK801 challenge**
Clinical chemistry endpoints	**Hormones** **Cholinesterase activity** **Brain deltamethrin analysis**
Endpoint category 'other'	**Ophthalmological evaluation** **Neurotransmitters** **Long term potentiation**

Table 3. Preliminary list of relevant endpoints for HUMAN studies defined at the start of the assessment. (based on DSM-5 NDV classification)

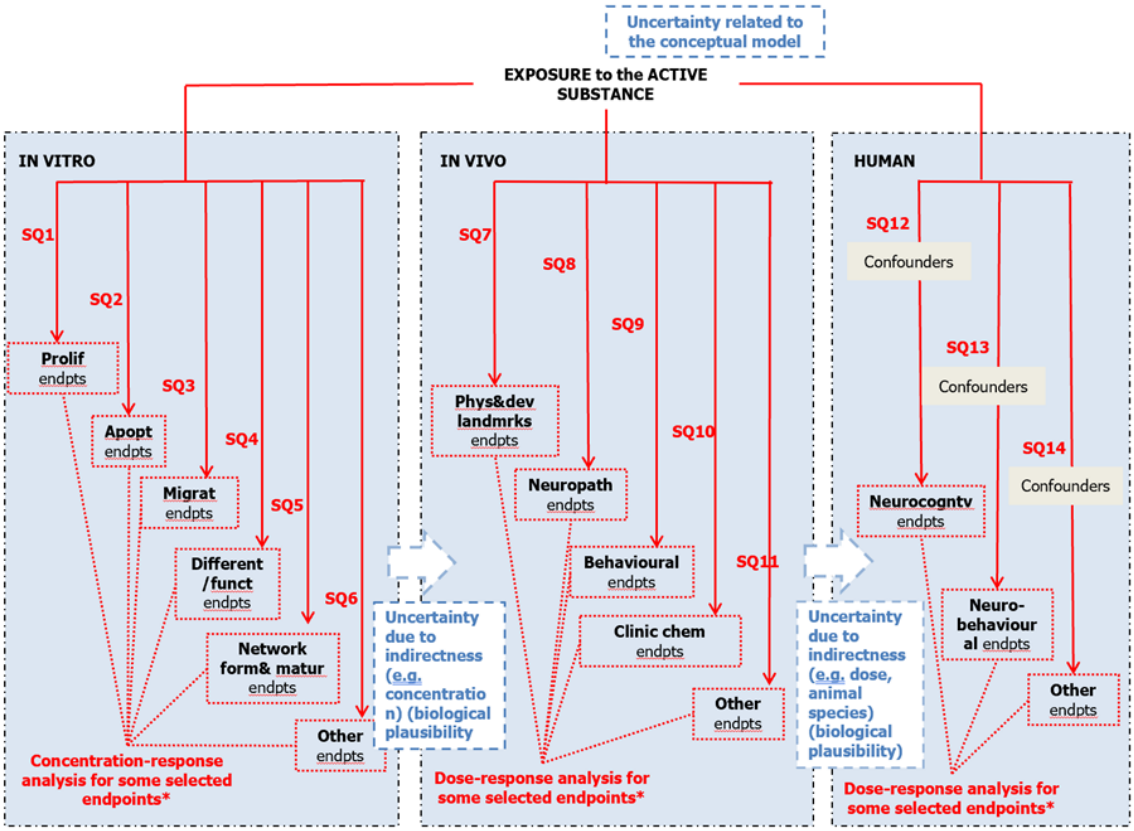
Preliminary list of endpoints for human observational studies	
Endpoint category	**Specific endpoints**
Intellectual disability	To add (if needed)
Communication disorders	To add (if needed)
Autism SD	To add (if needed)
ADHD	To add (if needed)
Motor disorders	To add (if needed)
Specific learning disorders	To add (if needed)
Other	

2.5. Conceptual model(s)

The conceptual model is the description of all the sub-questions resulting from 'unpacking' the assessment question(s) arising from the mandate, along with an explanation of their relationship.

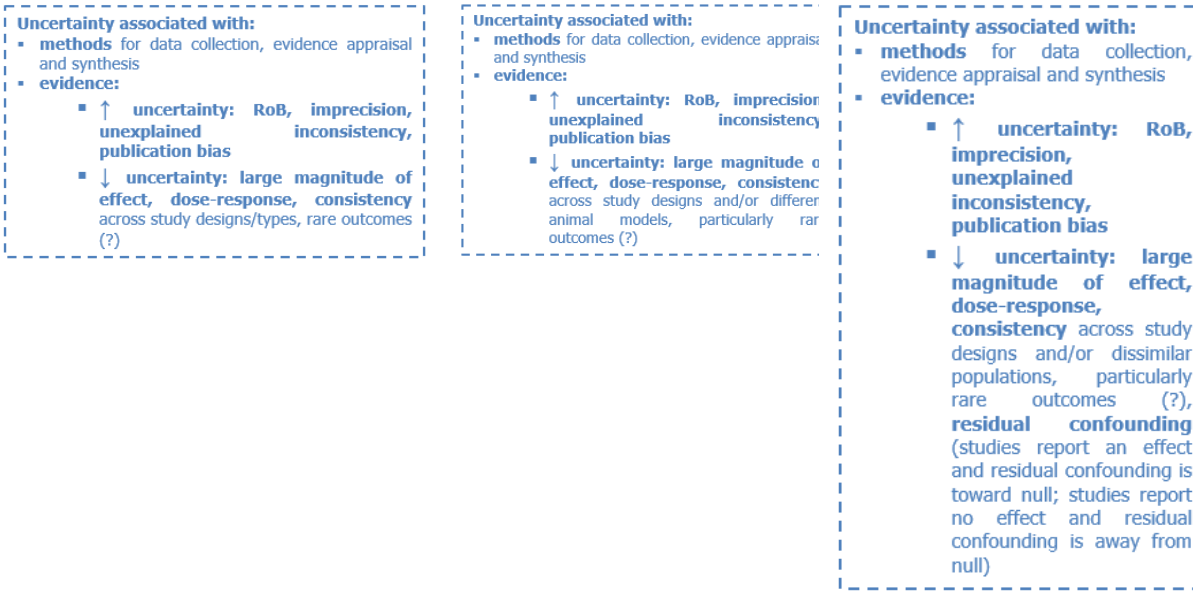
For simplicity, the conceptual model of the two assessments on deltamethrin and flufenacet is depicted in one individual figure (Figure 2). The figure also shows the possible sources of uncertainty associated with the model and the methods applied for data collection, appraisal and synthesis and the evidence.

Figure 2. Conceptual model for the two assessments on the two active substances and related sources of uncertainty



* The Working Group decided that only some endpoints would possibly be selected for this type of analysis, based on:

- if possible, the neurobiological meaning of the endpoints
- the accuracy/objectivity of the measurement method
- the amount, heterogeneity and validity of the available data.



	SQs 1–6: Does exposure to deltamethrin/flufenacet triggers the specific endpoint/KE as measured in acute and developmental protocol (wash-out yes/no) (assuming a monotonic concentration-response relationship) in *in vitro* studies **(EFSA DNT-IVB and literature)** carried out in human and/or rat and/or mouse neuro cells in development?	**SQs 7–11**: Does exposure to deltamethrin/flufenacet affec this specific endpoint/endpoint category/adverse outcome in a dose-response relationship ir experimental animal studies exposed during pregnancy and/or post-natal until weaning [maximum up to 21 days post-natal for rats and mice]?

Legend:

Main relationship of **interest within evidence stream**

Evidence that further informs the main relationship of interest **within evidence stream**

Potential confounders

Potential confounders identified *a priory* to be considered in the appraisal of individual observational studie

Uncertainty due to **indirectness** of evidence **across evidence streams (biological plausibility)**

Other sources of uncertainty

3 Methods for conducting the assessment

This section of the protocol details the methods that it was planned to apply for answering the assessment questions formulated above. Uncertainty analysis is inherent throughout all steps of this process. Therefore it was decided that, for each step of the process, possible sources of uncertainty would be anticipated along with their expected relative impact on the conclusions and the methods to analyse them, individually and combined. Overall, a quantitative uncertainty assessment would be the preferred option.

3.1. Methods for collecting data on the relationship between Deltamethrin and flufenacet and DNT endpoints

The pre-defined methods for identifying and selecting evidence on the relationship between the two active substances and DNT endpoints are illustrated in this section ('gathering existing evidence' in the IATA framework).

It was planned that, once selected for inclusion in the assessment, the relevant evidence would be organised according to a theoretical AOP conceptual framework as described at the beginning of this protocol (Section 1.3.2).

3.2. Eligibility criteria for study selection

The criteria for selecting *in vitro*, *in vivo* and human studies on the relationship between exposure to deltamethrin and flufenacet and DNT are reported in the tables below. The studies matching these criteria might also provide evidence on adsorption, distribution, metabolism and excretion (ADME) and mode of action (MoA).

Table 4. Criteria for selecting IN VITRO studies on the relationship between deltamethrin and flufenacet and DNT

Study design	**IN**	*In vitro* i.e. test systems and methods obtained from primary cells or cell lines or pluripotent stem cells (including zebra fish at the embryonal stage, i.e. up to 120 hours post fertilisation)
Population	**IN**	*In vitro* test system from all species. Only cells of neuronal origin or complex system replicating the developmental brain processes or human pluripotent stem cell differentiated in nervous cells
	OUT	Studies assessing efficacy on e.g. insects' cells lines etc. or on herbs target species for flufenacet Frog cells PC12 cells NOT differentiated toward neuron-like phenotype (if differentiated, the decision to include depends on the endpoint)
Exposure	**IN**	Deltamethrin (assessment question A1) Flufenacet (assessment question B1) The exposure has to occur during the developmental process of the nervous system I.e. primary cells from developing animals e.g. post-natal day 1–2 or *in vivo* up to post-natal day 21. For zebrafish studies: exposure and assessment of endpoint BEFORE 120 hours post fertilisation

	OUT	Mixtures For zebrafish studies: exposure after 120 hours post fertilisation
Endpoints	**IN**	Developmental neurotoxicity as defined above

Table 5. Criteria for selecting experimental ANIMAL studies on the relationship between deltamethrin and flufenacet and DNT

Study design	**IN**	*In vivo* studies in animals
Population	**IN**	Mammals
	OUT	Studies assessing efficacy on e.g. insects etc. or on herbs target species for flufenacet
Exposure	**IN**	Deltamethrin (assessment question A1) Flufenacet (assessment question B1) All routes of exposure Only studies where the mixture is represented by protocols that are including multiple substances but administered individually to the test system Prenatal OR (prenatal AND post-natal) before weaning (e.g. post-natal day 21 in rats) For zebrafish studies: exposure and assessment of endpoint AFTER 120 hours before fertilisation
	OUT	Post-natal only exposure For zebrafish studies: exposure after 120 hours post fertilisation –
Endpoints	**IN**	Developmental neurotoxicity as defined above

Table 6. Criteria for selecting HUMAN studies for the sub-questions on deltamethrin and flufenacet and DNT

Study design	**IN**	Cohort Case control Cross sectional
	OUT	Ecological studies (studies comparing different populations) Studies with no biomarker
Population	**IN**	All population groups and ages
Exposure	**IN**	Deltamethrin (assessment 1) Flufenacet (assessment 2) All types of exposure [please specify] When exposure is measured by specific and not specific biomarkers for deltamethrin or flufenacet Prenatal and/or early post-natal exposure (exposure due to breastfeeding)
	OUT	Exposure after breastfeeding
Endpoints	**IN**	Developmental neurotoxicity as defined above

Table 7. Criteria for selecting studies on the relationship between deltamethrin and flufenacet and DNT related to report characteristics and relevant to all evidence streams

Time	**IN**	No time limits
Language	**IN**	All languages with abstract in English. If, based on the abstract, the study looks relevant, the full-text will be translated
Publication type	**IN**	Primary research studies (i.e. studies generating new data) PhD theses Reviews will be used as sources of further references and to assess the appropriateness of the search strategy applied
	OUT	Expert opinions, editorials, letters to the editor, conference proceedings and posters

3.3. Extensive literature searches

It was planned that publicly available studies matching the eligibility criteria defined above would be searched for, using tailored search strategies that were designed by an information specialist with input from the Working Group.

Search strings use text word searching in key fields combined with the databases' Subject Heading (e.g. MeSH) when possible. The bibliographic databases that will be searched to identify possible relevant studies are detailed in Table 9. The searches were adapted to the search capabilities of each database and platform searched.

Table 8. Bibliographic databases that will be searched for relevant studies

Source of information	Coverage date	Platform
PubMed	Inception–present	PubMed (NLM)
Science Citation Index Expanded (SCI-EXPANDED)	1975–present	Web of Science
Book Citation Index– Science (BKCI-S)	2005–present	
Emerging Sources Citation Index (ESCI)	2005–present	
Current Chemical Reactions (CCR-EXPANDED)	1985–present	
Index Chemicus (IC)	1993–present	
Toxline	Inception–present	TOXNET (NLM)
DART-Europe E-theses Portal	Inception–present	DART
EBSCO Open Dissertations	Inception–present	EBSCOhost
PQDT Open	Inception–present	ProQuest

Due to the limited number of publications on flufenacet, only terms related to the exposure concept would be used to interrogate the sources of information. Whereas, for deltamethrin, terms for the exposure were combined with relevant terms for DNT outcomes (human and *in vivo* studies) or methods (*in vitro* studies). A list of possible relevant studies previously identified by the experts of the WG, as well as other publications (Fritsche et al., 2015; National Academies of Sciences, Engineering and Medicine, 2017) were used to finetune the search terms provided by the experts in the WG. No limits of date of language have been applied to the searches.

A specific search string was designed to identify studies applying high-throughput methods to evaluate potential developmental neurotoxicity. The search strategy combines terms related to developmental neurotoxicity and high-through methods. Terms related to the compounds have not been added since this type of studies do not report all the compounds screened in title, abstract or keywords. Theses and dissertations databases will not be used for this search.

The complete search strings as run in the databases are available in **Annex A.**

The output of the searches i.e. records retrieved from bibliographic databases would be exported into one EndNote library (Clarivate Analytics) relevant for flufenacet and other one relevant to deltamethrin. Duplicate references would be removed by a combination of automatic and manual detection of duplicates. Data submitted to EFSA through application dossiers would be added to the EndNote libraries.

3.4. Dossier data

The plan implied that data submitted to EFSA through application dossiers for renewal of the pesticide active substance would be screened against the eligibility criteria illustrated above and, if relevant, included in the assessments. The selection process would be the same as for studies retrieved from the open literature (see next section) as well as the following steps.

3.5. Study selection process

All studies retrieved by the literature searches and submitted to EFSA by application dossiers would be uploaded on DistillerSR® (Evidence Partners, Ottawa, Canada) and screened against the eligibility criteria defined above.

It was planned that the study selection process would be carried out in two steps:

1) Step 1: titles and abstracts screening, to exclude obviously irrelevant studies. All other apparently relevant or of unclear relevance studies would be moved to the following step. During Step 1, the studies would also be clustered according to the relevant evidence stream (i.e. *in vitro*, *in vivo*, human studies).
2) Step 2: full-text screening, to select studies for inclusion/exclusion and cluster them according to the publication type (e.g. primary research study, review, PhD thesis).

Each study would be screened by two independent reviewers (Working Group expert or EFSA staff), to minimise the risk of error. Between-reviewer conflicts not solvable by discussion will be discussed at WG level.

During the study selection process, studies published in multiple publications might be identified and duplicates would be removed.

Eligibility criteria would be pilot tested on a subset of records and refined if prone to misinterpretation. It was agreed that the results of the different phases of the study selection process would be reported in a flowchart as recommended in the PRISMA statement on preferred reporting items for systematic reviews and meta-analyses (Moher et al., 2009).

Studies with abstract in English and full-text document in another language that seem relevant based on the abstract would be translated or screened by knowledgeable EFSA staff not necessarily part of the WG.

3.6. Data extraction from the included studies

It was planned that data would be extracted from the studies using predefined forms that comprise data on the characteristics of the studies (e.g. study design, funding source, test system, species, ethnicity), the concentration/dose/exposure characteristics, the endpoints and methods for measuring them, and the results. Data would be extracted in the original units of measurement, which would be subsequently harmonised to allow data analysis.

Instructions for extracting data would be developed and data extraction forms created in DistillerSR® and pilot tested on a subset of studies. The forms and instructions would be refined if needed.

If a full-text document reports on more than one study unit (e.g. multiple experiments reported in the same publication), the individual studies would be identified at this step (and at evidence appraisal- see next step) to allow for data extraction at individual study level.

Studies for which the information provided in the publication(s) does not allow a full scientific evaluation (e.g. studies with missing or ambiguous information) would be flagged and not taken forward in the assessment. If a study is reported in more than one publication, different publications reporting on the same study (e.g. on different outcomes (endpoints), at different time points) would be identified at this step.

Data would be extracted from each individual study by one EFSA staff and validated by another.

3.7. Sources of uncertainty arising from the methods for data collection

It was not possible to plan it at the time of the protocol development.

3.8. Methods for appraising evidence on the relationship between deltamethrin and dlufenacet and DNT endpoints

This section illustrates the planned methods for appraising evidence (part of 'weight of evidence' in the IATA framework).

The risk of bias (RoB) of a given study in relation to a specific outcome (and related endpoints) refers to the risk of systematic errors in the design, conduct or analysis that result in a mistaken estimation of the true effect of the exposure on the outcome.

It was planned that the internal validity or RoB of each study on the relation between the two active substances and DNT included in the assessment (including the *in vitro* battery outsourced by EFSA, Masjosthusmann et al., 2020) would be appraised using a customised version of the OHAT/NTP RoB assessment tool (ref 2015).[3] This tool provides a parallel approach to the evaluation of RoB in the context of hazard identification for human risk assessment of chemicals, and to facilitate consideration of RoB across evidence streams (i.e. human, animal and mechanistic studies), based on common terms and categories for RoB rating. For *in vitro* studies, the version of the NTP tool used in the monograph on PFOS and PFAS (NTP, 2016) would be used for this assessment on deltamethrin and flufenacet and DNT, as it represented the only case-example available at the time when this protocol was developed.[5]

To help identify the practice that may introduce bias in a study, the OHAT/NTP RoB tool outlines 11 RoB questions, grouped in six bias domains (selection, confounding, performance, attrition/exclusion, detection and selective reporting) and one 'other sources of bias' (Figure 3). Each RoB question addresses aspects relevant to specific study designs.

Figure 3. Overview of the appraisal questions in the OHAT/NTP tool and applicability by study design

Table 3. OHAT Risk-of-Bias Questions and Applicability by Study Design

Risk-of-Bias Questions	Experimental Animal*	*In Vitro* Experimental Studies	Human Controlled Trials**	Cohort	Case-Control	Cross-Sectional***	Case Series
1. Was administered dose or exposure level adequately randomized?	X	X	X				
2. Was allocation to study groups adequately concealed?	X	X	X				
3. Did selection of study participants result in the appropriate comparison groups?				X	X	X	
4. Did study design or analysis account for important confounding and modifying variables?				X	X	X	X
5. Were experimental conditions identical across study groups?	X	X					
6. Were research personnel blinded to the study group during the study?	X	X	X				
7. Were outcome data complete without attrition or exclusion from analysis?	X	X	X	X	X	X	
8. Can we be confident in the exposure characterization?	X	X	X	X	X	X	X
9. Can we be confident in the outcome assessment (including blinding of outcome assessors)?	X	X	X	X	X	X	X
10. Were all measured outcomes reported?	X	X	X	X	X	X	X
11. Were there no other potential threats to internal validity?	X	X	X	X	X	X	X

Source: PFAS monograph 2016 – NTP 2016.

[3] The OHAT/NTP tool was developed based on guidance from the Agency for Healthcare Research and Quality (Viswanathan et al., 2012), the Cochrane risk-of-bias tool for non-randomised studies of interventions (Sterne et al., 2014), the Cochrane Handbook (Higgins and Green, 2011), CLARITY Group at McMaster University (CLARITY, 2013) and other sources.

Reviewers are required to answer RoB questions by applying a 4-level rating scale (Figure 4). The OHAT/NTP RoB tool encourages judging the direction of bias, when possible. In the tool, empirical evidence about the direction of bias is discussed for each of the RoB questions. If there is no clear rationale for judging the likely direction of bias, reviewers are invited to simply outline the evidence and not to attempt a guess. It was planned to follow this approach.

Figure 4. Rating instructions for the RoB questions

++	***Definitely Low** risk of bias*: There is direct evidence of low risk of bias practices (May include specific examples of relevant low risk of bias practices)
+	***Probably Low** risk of bias*: There is indirect evidence of low risk of bias practices **OR** it is deemed that deviations from low risk of bias practices for these criteria during the study would not appreciably bias results, including consideration of direction and magnitude of bias
– NR	***Probably High** risk of bias*: There is indirect evidence of high risk of bias practices **OR** there is insufficient information (e.g., not reported or "NR") provided about relevant risk of bias practices
––	***Definitely High** risk of bias*: There is direct evidence of high risk of bias practices (May include specific examples of relevant high risk of bias practices)

Source: OHAT/NTP RoB tool. [4]

The RoB questions and rating instructions provided in the tool were tailored to the specific subquestions illustrated in this protocol and are reported in the following subsections, for each evidence stream. The customised version of the tool would be uploaded onto the review management software DistillerSR® to allow web-based appraisal of the studies.

For each study, **the appraisal would be performed at endpoint or group of endpoints level** because, for the same study, the design and conduct may affect the RoB differently depending on the endpoints measured.

It was agreed that each study would be appraised by two independent reviewers from the Working Group and EFSA staff for *in vitro* studies only. Possible discrepancies not solvable by discussion would be taken to the attention of the whole WG. If, upon further discussion, the WG cannot reach an agreement on a RoB rating for a domain, the more conservative judgement (the highest RoB) would be selected.

3.9. In vitro studies

The RoB assessment tool that it was planned to use for *in vitro* studies on the relationship between the two active substances and DNT endpoints is reported in the table below. The tool is based on the OHAT/NTP tool developed for the Monograph on PFAS (NTP, 2016) and integrated with some items of the SciRAP tool (http://scirap.org/). This tool would be used to appraise all *in vitro* studies selected for this assessment, included the *in vitro* battery outsourced by EFSA. The question originally provided on 'allocation concealment' has been deleted after piloting the tool since the Working Group experts judged it was too difficult to apply it to the *in vitro* studies.

[4] https://ntp.niehs.nih.gov/whatwestudy/assessments/noncancer/handbook/index.html

Table 9. RoB assessment tool for IN VITRO studies on the relationship between the two active substances and DNT endpoints

RoB question (*IN VITRO*)		Endpoint-dependent (Y/N)	Rating	Rationale for judgement (based on OHAT/NTP tool and, partially, the SciRAP tool)
1	**Was administered dose or exposure level adequately randomised?** (domain: selection bias) Note: this a bout generating the random sequence	**N**	/	CRITERIA FOR DEFINING an experimental study unit, e.g.: • Test system CRITERIA FOR DEFINING an endpoint for appraisal within an experimental study unit: • Single concentration, if it is the only information available = endpoint • Multiple concentrations = 1 endpoint • Concentration-response, multiple time points = 1 endpoint
			DL RoB	**Direct evidence** that: • the cells/embryos or any test system other than *in vivo* were allocated to any study group including controls using a method with a **random component** • **OR** [for cells only] all cells in culture come from a **homogenous** cell suspension recently collected from cell culture vessels following appropriate cell culture techniques **ANDdirect evidence** that the study used a concurrent control group as an indication that randomisation covered all study groups,. Note: Acceptable methods of randomisation include: referring to a random number table, using a computer random number generator, coin tossing, or shuffling cards (Higgins and Green, 2011) Note: this has been adapted from the OHAT tool
			PL RoB	**Indirect evidence** that the cells/embryos or any test system other than *in vivo* were allocated to any study group including controls using a method with a random component (i.e. authors state random allocation, without description of the method), **AND** evidence that the study used a concurrent control group as an indication that randomisation covered all study groups, **OR it is deemed that allocation without a clearly random component would not appreciably bias results**
			PH RoB/NR	**Indirect evidence** that • cells/embryos or any test system other than *in vivo* were allocated to study groups using a method with a **non-random component**, • **OR** that there was a lack of a concurrent control group **OR** there is **insufficient information** provided about how cells were allocated to study groups (record 'NR' as basis for answer) nor it can be inferred
2	**Were experimental conditions identical across study groups?**	**N**	DL RoB	**Direct evidence** that: • **culture conditions** (e.g. the stage of differentiation of the cells, cell passage,

	(domain: performance bias)			temperature, humidity, CO_2 concentration) and the concentrations of any solvents (e.g. DMSO) used in getting the treatment compound into solution were **identical** across groups • **ANDthe same media** were used for control and experimental cells/embryos or any test system other than *in vivo* particularly for biological materials such as serum which must be from the same lot • **ANDappropriate adjustments** were made such as normalisation to blank/media controls, cell numbers in culture, use of positive (if present) and negative control responses in acceptance criteria, or others • **AND** non-treatment-related experimental conditions were identical across study groups (i.e. the study report explicitly provides this level of detail).
			PL RoB	**Indirect evidence** that: • culture conditions (e.g. the stage of differentiation of the cells, cell passage, temperature, humidity, CO_2 concentration) and the concentrations of any solvents (e.g. DMSO) used in getting the treatment compound into solution were **identical** across groups • **AND** the **same media** were used for control and experimental cells/embryos or any test system other than *in vivo***ORit is deemed that the media used would not appreciably bias results** • **AND appropriate adjustments** were made such as normalisation to blank/media controls, cell numbers in culture, use of positive (if present) and negative control responses in acceptance criteria, or others, **OR** it is **deemed that not considering or only considering a partial list of covariates in the final analyses would not appreciably bias results** • **AND** as described above, identical non-treatment-related experimental conditions are assumed if authors did not report differences in culture conditions or handling.
			PH RoB/NR	**Indirect evidence** that: • **culture conditions** [same as above] **differed** across groups • **OR that the media differed across groups** • **OR that appropriate adjustments were not made such as failing to normalise to blank/media controls, adjust for** cell numbers in culture, use positive and negative control responses in acceptance criteria, or others • **OR** there **that non-treatment-related experimental conditions were not comparable** between study groups **OR** there is **insufficient information** provided on maintaining identical concentrations of solvents (record 'NR' as basis for answer) **OR** there is **insufficient information** provided about analysis of relevant covariates (record 'NR' as basis for answer).
			DH RoB	**Direct evidence** from the study report that • **culture conditions** [same as above] **differed** across groups • **ORthat the media differed across groups** • **OR** that **appropriate adjustments** were **NOT made** such as failing to normalise to

				blank/media controls, adjust for cell numbers in culture, use positive and negative control responses in acceptance criteria, or other relevant covariates • **OR** that **non-treatment-related experimental conditions** were **NOT comparable** between study groups.
3	**Were the <u>research personnel (cell maintenance and cell dosing)</u> * blinded to the study group during the study?** (domain: performance bias) *not same as outcome assessors – blinding of outcome assessors is assessed in another question	**N**	DL RoB	**Direct evidence** that: • the research personnel were **adequately blinded** to study group, **AND** it is **unlikely that they could have broken the blinding** during the study. Methods used to ensure blinding include central allocation, sequentially numbered treatment containers of identical appearance; sequentially numbered culture plates, or equivalent • **OR** the use of **robotic testing systems** during the study that are deemed to eliminate the opportunity for performance bias to influence results.
			PL RoB	**Indirect evidence** that the research personnel were adequately **blinded** to study group, **AND** it is unlikely that they could have broken the blinding during the study **ORit is deemed that lack of adequate blinding during the study would not appreciably bias results** (e.g. minimal possibility of researchers to handle cells or plates after treatment due to primarily automated procedures)
			PH RoB/NR	**Indirect evidence** that the research personnel were **not adequately** blinded to study group **OR** there is **insufficient information** provided about blinding to study group during the study (record 'NR' as basis for answer).
			DH RoB	**Direct evidence** that the research personnel were **NOT adequately blinded** to study group.
4	**Were outcome data complete without attrition or exclusion from analysis?** (domain: attrition/exclusion bias) (Cells/embryos or measurements missing within an endpoint)	**Y**	DL RoB	There is **direct evidence** that the rate of loss/exclusion of cells/embryos or measurements was limited **in a way thatthe proportion lost would not appreciably bias results*** [Note: when assessing what 'limited' means, consider the initial number of cells/embryos and the reasons for the loss e.g. accident] ***e.g. the reasons for missing cells/embryos unlikely to be related to outcome (or for survival data, censoring unlikely to be introducing bias); missing outcome data balanced in numbers across study groups, with similar reasons for missing data across groups and reasons were documented when cells/embryos were removed from a study.
			PL RoB	There is **indirect (inferred) evidence** that the rate of loss/exclusion of cells/embryos or measurements was limited **in a way thatthe proportion lost would not appreciably bias results*** [Note: when assessing what 'limited' means, consider the initial number of cells/embryos and the reasons for the loss e.g. accident]
			PH RoB/NR	There is **indirect (inferred) evidence** that loss/exclusion of cells/embryos or measurements was **unacceptably large OR not adequately addressed** **OR** there **is insufficient information** provided about loss/exclusion of cells/embryos (record 'NR' as basis for answer).
			DH RoB	There is **direct evidence** that loss of loss of cells/embryos or measurements was **unacceptably large OR not adequately addressed**. Unacceptable handling of attrition or exclusion includes: reason for loss is likely to be related to true outcome, with either imbalance in numbers or reasons for loss across study groups
5	**Can we be confident in the exposure**	**Y**	/	Note: **purity** defined by the WG as follows:

	characterisation? (domain: detection bias)			• deltamethrin: if technical material is not used, at least 98% • flufenacet: if technical material is not used, at least 98% 3 situations provided by the WG • All analytical work performed (gold standard) • 98% purity, but no analytical method actually used (supplier provides it) • Purity unknown AND no analytical methods
			DL RoB	**Direct evidence** that: • the exposure to deltamethrin or flufenacet or their salts (including purity and stability) was independently characterised (method of analysis declared and limit of detection reported)and purity confirmed generally as =98% (including commercial material) • **AND** the **duration of exposure** was suitable for the test system and investigated endpoints, • **AND** exposure was **consistently administered** (i.e. with the same method and time frame) across treatment groups • **ANDsolubility** in the vehicle is declared and**/or considered** appropriate at the concentrations used • **AND** the **number of concentrations** tested are suitable for the evaluation of a concentration-response (at least four concentrations) [this makes it endpoint-dependent] ○ **AND control solutions were checked for contamination** ○ **AND** if assay media were examined for actual exposure concentrations, there is direct evidence that most of the exposure data measurements are above the limit of quantitation for the assay such that different exposure groups can be distinguished
			PL RoB	**Indirect evidence** that: the exposure to deltamethrin or flufenacet or their salts was **98% purity, but no analytical method actually used** (supplier provides it) **OR** purity **was independently confirmed as =98%** and **it is deemed that impurities of up to 2% would not appreciably bias results** **AND** solubility in the vehicle is declared and**/or considered** appropriate at the concentrations used **AND** the **duration of exposure** was suitable for the test system and investigated endpoints, **AND** the **number of concentrations** tested are suitable for the evaluation of a concentration-response (at least four concentrations)
			PH RoB/NR	There is **insufficient information** provided about the validity of the exposure assessment method, but no direct evidence for concern (record '**NR**' as basis for answer). PH if they used only one concentration
			DH RoB	**Direct evidence** that: • the exposure to deltamethrin or flufenacet, or their salts (including purity) was **not pure**

				• **OR** solubility of the test substance was not appropriately controlled.
6	**Can we be confident in the outcome assessment?** (domain: detection bias)	Y	/	**Classification of endpoints** made *a priori* by the WG and examples of 'best methods' for measuring the endpoints: 1 **Proliferation KE endpoints** (measurement methods: e.g. BrdU staining, KI67); 2 **Apoptosis KE endpoints** (measurement methods: e.g. caspase-3/7 activation); • **Migration KE endpoints** (measurement methods: e.g. High Content Analysis – HCA; radial migration: migration distance measure) 3 **Differentiation/function KE endpoints** (measurement methods: e.g. HCA) • Neurite outgrowth • Neuronal morphology • Neurite outgrowth • Oligodendrocyte differentiation 4 **Network formation and maturation KE endpoints**: (measurement methods: e.g. Micro Electrodes Assay) 5 **Synaptogenesis KE endpoints** (measurement methods: e.g. HCA) 6 **Other endpoints** (to be specified)
			DL RoB	**Direct evidence** that: • the outcome was assessed using **well established methods** • **AND** assessed at time points suitable to generate sensitive, valid and reliable data • **AND** assessed at the same time point after exposure in all study groups • **AND** the outcome assessors were adequately blinded to the study group, and it is unlikely that they could have broken the blinding before reporting outcomes • **AND** conditions for cultivation and/or maintenance of the cells/embryo or any test system other than *in vivo* were appropriate for the endpoint (the stage of differentiation of the cells, incubation temperature, humidity, CO_2 concentration, media used, number of cell passages, control of contamination)
			PL RoB	**Indirect evidence** that: • the outcome was assessed using **acceptable methods** (i.e. deemed valid and reliable but not the gold standard) **AND** assessed **at time points suitable** to generate sensitive, valid and reliable data **AND** assessed **at the same time point after exposure** in all study groups **ANDconditions for cultivation and/or maintenance** of the cells/embryo or any test system other than *in vivo***were appropriate for the endpoint** (the stage of differentiation of the cells, incubation temperature, humidity, CO_2 concentration, media used, number of cell passages,

				control of contamination) ○ **OR it is deemed that the outcome assessment methods used would not appreciably bias results** • **AND** there is **indirect evidence** that the outcome assessors were adequately blinded to the study group, and it is unlikely that they could have broken the blinding before reporting outcomes ○ **OR it is deemed that lack of adequate blinding of outcome assessors would not appreciably bias results**, which is more likely to apply to objective outcome measures.
			PH RoB/NR	**Indirect evidence** that: • the outcome assessment method is **inappropriate** • OR assessed at time points not suitable for the endpoint • OR the time point after initial exposure differed by study group, • OR conditions for cultivation and/or maintenance of the cells/embryo or any test system other than *in vivo* were NOT appropriate for the endpoint (the stage of differentiation of the cells, incubation temperature, humidity, CO_2 concentration, media used, number of cell passages, control of contamination) • OR there is indirect evidence that it was possible for outcome assessors to infer the study group before reporting outcomes without sufficient quality control measures **OR** there is insufficient information provided about blinding of outcome assessors (record 'NR' as basis for answer).
			DH RoB	**Direct evidence** that: • the outcome assessment method is inappropriate • OR assessed at time points not suitable for the endpoint • OR the time point after initial exposure differed by study group, • OR conditions for cultivation and/or maintenance of the cells/embryo or any test system other than *in vivo* were NOT appropriate for the endpoint (the stage of differentiation of the cells, incubation temperature, humidity, CO_2 concentration, media used, number of cell passages, control of contamination) • OR there is direct evidence for lack of adequate blinding of outcome assessors, including no blinding or incomplete blinding without quality control measures.
7	**Were all measured outcomes reported?** (domain: selective reporting bias)	**N**	/	***NOTE:*** *It is recognised that selective reporting is difficult to assess with confidence for most studies unless the study protocol is available. Selective reporting bias can be assessed by comparing the 'methods' and 'results' section of the paper, and by considering outcomes measured in the context of knowledge in the field. Selective reporting bias may be suspected if the study does not report outcomes in the results section that would have been expected based on the methods, or if a composite score is present without the individual component outcomes*
			DL RoB	**Direct evidence** that all of the study's measured outcomes (primary and secondary) outlined

				in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported. This would include outcomes reported with sufficient detail to be included in meta-analysis or fully tabulated during data extraction and analyses had been planned in advance.
			PL RoB	**Indirect evidence** that: • all of the study's measured outcomes (primary and secondary) outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported • **OR** analyses that had not been planned in advance (i.e. retrospective unplanned subgroup analyses) are clearly indicated as such and deemed that unplanned analyses were appropriate and selective reporting would not appreciably bias results (e.g. appropriate analyses of an unexpected effect). This would include outcomes reported with insufficient detail such as only reporting that results were statistically significant (or not).
			PH RoB/NR	**Indirect evidence** that • all of the study's measured outcomes (primary and secondary) outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have not been reported • **OR unplanned analyses were included that may appreciably bias results**, **OR** there is insufficient information provided about selective outcome reporting (record 'NR' as basis for answer).
			DH RoB	• **Direct evidence** that all of the study's measured outcomes (primary and secondary) outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have not been reported. In addition to not reporting outcomes, this would include reporting outcomes based on composite score without individual outcome components or outcomes reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified or reporting outcomes not pre-specified, or that unplanned analyses were included that would appreciably bias results.
8a	**Were there other potential threats to internal validity? – CYTOTOXICITY** (domain: other bias)	**N**	/	WG decisions: • Assessment of appropriateness of statistical analysis pending depending on the approach to synthesising the evidence. If the effect measurements of each study are not used, the appropriateness of the statistical analysis will not be assessed • Adherence with study protocol will not be assessed as it is unlikely that there will be protocols in published studies • For 'other bias': cytotoxicity and replicates/repetitions
			DL RoB	**Direct evidence** that cytotoxicity was **measured andthe test compound did not cause cytotoxicity that confounded the overall results of the study**
			PL RoB	**Indirect evidence** (i.e. it can be inferred) that cytotoxicity was measured and the test compound did not cause cytotoxicity that confounded the overall results of the study
			PH RoB/NR	**Indirect** evidence that effects **occurred at cytotoxicity levels** Insufficient information to assess cytotoxicity (NR)

			DH RoB	**Direct evidence** that effects occurred at cytotoxicity levels
8b	**Were there other potential threats to internal validity? – REPLICATES/REPETITIONS** (domain: other bias)	Y	DL RoB	**Direct evidence** that sufficient numbers of replicates or repetitions (to assess what 'sufficient' means, consider the variability; at least three for low variability) of the experiment were used to generate reliable and valid results
			PL RoB	**Indirect evidence** that sufficient numbers of replicates or repetitions (to assess what 'sufficient' means, consider the variability; at least 3 for low variability) of the experiment were used to generate reliable and valid results
			PH RoB/NR	Indirect evidence that the numbers of replicates or repetitions of the experiment were not sufficient Insufficient information the number of replicates or repetitions
			DH RoB	Direct evidence that the numbers of replicates or repetitions of the experiment were not sufficient

3.9.1. Summarising the internal validity of each in vitro study, by endpoint(s)

Appraisal questions for *in vitro* studies (key questions highlighted in yellow)	**Key (Y/N)**
1. Was administered dose or exposure level adequately randomised?	N
2. Were experimental conditions identical across study groups?	N
3. Were the research personnel blinded to the study group during the study?	N
4. Were outcome data complete without attrition or exclusion from analysis?	N
5. Can we be confident in the exposure characterisation?	Y
6. Can we be confident in the outcome assessment?	Y
7. Were all measured outcomes reported?	N
8a. Were there other potential threats to internal validity? – Cytotoxicity	Y
8.b Were there other potential threats to internal validity? – Replicates/repetitions	N

Algorithm to combine the answers to the appraisal questions and allocate studies to tiers of RoB	**Tier**

TIER 1: All key questions are scored +/++ AND maximum 1 non-key question is scored - or –	1 (low RoB)
TIER 2: study does not meet criteria for TIER 1 or TIER 3	2
TIER 3: one (or more) key question is scored -/– OR -/– for the majority of the non-key questions	3 (high RoB)

3.10. Experimental animal studies

The RoB assessment tool that it was agreed to use for experimental animal studies on the relationship between the two active substances and DNT endpoints is reported in the table below. The tool is based on the OHAT/NTP (NTP, 2015).

Table 10. RoB assessment tool for experimental animal studies on the relationship between the two active substances and DNT endpoints

RoB question (*IN VIVO*)		Endpoint-dependent (Y/N)	Rating	Rationale for judgement
1	**Was administered dose or exposure level adequately randomised?** (domain: selection bias) This is about generating the random sequence	**N**	DL RoB	**Direct evidence** that: • animals were allocated to any study group including controls using a method with a **random component**, both parents AND pups • **AND** there is direct evidence that the study used a **concurrent control group** as an indication that randomisation covered all study groups. Note: the method for randomisation must be specified for both parents AND pups Note: Acceptable methods of randomisation include: referring to a random number table, using a computer random number generator, coin tossing, shuffling cards or envelopes, throwing dice, or drawing of lots (Higgins and Green, 2011). Restricted randomisation (e.g. blocked randomisation) to ensure particular allocation ratios will be considered low risk of bias. Similarly, stratified randomisation and minimisation approaches that attempt to minimise imbalance between groups on important prognostic factors (e.g. body weight) will be considered acceptable. This type of approach is used by NTP, i.e. random number generator with body weight as a covariate. Note: Investigator-selection of animals from a cage is not considered random allocation because animals may not have an equal chance of being selected, e.g. investigator selecting animals with this method may inadvertently choose healthier, easier to catch or less aggressive animals.
			PL RoB	• **Indirect evidence** that animals were allocated to any study group including controls using a method with a **random component** (i.e. authors state that allocation was random (both for parents AND pups), without description of the method used), **AND** there is **direct or indirect evidence** that the study used a **concurrent control group** as an indication that randomisation covered all study groups • **OR it is deemed that allocation without a clearly random component during the study would not appreciably bias results**. For example, approaches such as biased coin or urn randomisation, replacement randomisation, mixed randomisation, and maximal randomisation may require consultation with a statistician to determine risk-of-bias rating (Higgins and Green, 2011).
			PH RoB/NR	There is **indirect evidence** that: • animals were allocated to study groups using a method with a **non-random** component • **OR** there was a **lack of a concurrent control group** **OR** there is **insufficient information provided about how subjects were allocated to study groups** (record 'NR' as basis for answer). Note: Non-random allocation methods may be systematic, but have the potential to allow researchers to

				anticipate the allocation of animals to study groups (Higgins and Green, 2011). Such 'quasi-random' methods include investigator-selection of animals from a cage, alternation, assignment based on shipment receipt date, date of birth or animal number.
			DH RoB	**Direct evidence** that: • animals were allocated to study groups using a **non-random** method including judgement of the investigator, the results of a laboratory test or a series of tests (Higgins and Green, 2011) • **OR** there is **direct evidence** that there was a **lack of a concurrent control group**, indicating that randomisation did not cover all study groups.
2	**Was allocation [note: of animals] to study groups adequately concealed?** (domain: selection bias) This is about assigning the sequence	**N**	/	From Cochrane RoB 2 (version 2016: https://www.bristol.ac.uk/media-library/sites/social-community-medicine/images/centres/cresyda/RoB2–0_indiv_main_guidance.pdf): Some review authors confuse allocation concealment with blinding of assigned interventions. **Allocation concealment** seeks to prevent bias in intervention assignment by protecting the allocation sequence **before and until assignment**, and can always be successfully implemented regardless of the study topic. In contrast, **blinding** seeks to prevent bias by protecting the sequence **after assignment**, and cannot always be implemented. This is often the situation, for example, in trials comparing surgical with non-surgical interventions. Therefore, allocation concealment up to the point of assignment of the intervention and blinding after that point address different sources of bias and differ in their feasibility. Among the different methods used to conceal allocation, central randomisation by a third party is perhaps the most desirable. Methods using envelopes are more susceptible to manipulation than other approaches. If investigators use envelopes, they should develop and monitor the allocation process to preserve concealment. In addition to use of sequentially numbered, opaque, sealed envelopes, they should ensure that the envelopes are opened sequentially, and only after the envelope has been irreversibly assigned to the participant. The question in the Cochrane tool reads as follows: 'Was the allocation sequence concealed until participants were recruited and assigned to interventions?'
			DL RoB	**Direct evidence** that at the time of assigning study groups the **research personnel did not know** what group animals were allocated to, and it is **unlikely that they could have broken the blinding of allocation** until after assignment was complete and irrevocable. Acceptable methods used to ensure allocation concealment include sequentially numbered treatment containers of identical appearance or equivalent methods.
			PL RoB	**Indirect evidence** that at the time of assigning study groups the research personnel **did not know** what group animals were allocated to and it is **unlikely that they could have broken the blinding of allocation** until after assignment was complete and irrevocable **OR it is deemed that lack of adequate allocation concealment would not appreciably bias results – these are all rodents and zebrafish studies – the WG agreed to score this as PLRoB as never reported but not having an impact.**
			PH RoB/NR	**Indirect evidence** that at the time of assigning study groups it was **possible for the research personnel to know** what group animals were allocated to, **or it is likely that they could have broken the blinding of allocation** before assignment was complete and irrevocable **OR** there is **insufficient information** provided about allocation to study groups (record 'NR' as basis for answer).
			DH RoB	**Direct evidence** that at the time of assigning study groups it was **possible for the research personnel to know** what group animals were allocated to, **or it is likely that they could have broken the blinding**

				of allocation before assignment was complete and irrevocable.
3	**Were experimental conditions identical across study groups?** (domain: performance bias)	**N**	DL RoB	**Direct evidence** that: • **same vehicle was used** in control and experimental animals • **AND** non-treatment-related experimental conditions were identical across study groups (e.g. housing conditions).
			PL RoB	**Indirect evidence** that the **same vehicle was used** in control and experimental animals **OR it is deemed that the vehicle used would not appreciably bias results** **AND** non-treatment-related experimental conditions were identical. I**dentical non-treatment-related experimental conditions are assumed if authors did not report differences** in housing or husbandry.
			PH RoB/NR	**Indirect evidence** that: • the vehicle differed between control and experimental animals • **OR** there **is indirect evidence that non-treatment-related experimental conditions were not comparable** between study groups **OR** authors **did not report the vehicle used** (record 'NR' as basis for answer),
			DH RoB	**Direct evidence** from the study report that: • **control animals were untreated, or treated with a different vehicle** than experimental animals, • **OR non-treatment-related experimental conditions were not comparable** between study groups.
4	**Were the <u>research personnel (='animal' caring, animal dosing. and, for behavioural endpoints, the personnel executing the test when the test is automated)</u>* blinded to the study group during the study?** (domain: performance bias) *not same as outcome assessors	**N** *(it is deemed that it will not affect the endpoints differently)*	DL RoB	**Direct evidence** that the research personnel were adequately blinded to study group, and it is **unlikely that they could have broken the blinding** during the study. Methods used to ensure blinding include central allocation; sequentially numbered treatment containers of identical appearance; sequentially numbered animal cages; or equivalent methods.
			PL RoB	• **Indirect evidence** that the research personnel were **adequately blinded to study group**, and it is **unlikely that they could have broken the blinding during the study**, • **OR it is deemed that lack of adequate blinding during the study would not appreciably bias results**. This would include cases where blinding was not possible, but research personnel took steps to minimise potential bias, such as restricting the knowledge of study group to veterinary or supervisory personnel monitoring for overt toxicity, or randomised husbandry or handling practices (e.g. placement in the animal room, necropsy order, etc.).
			PH RoB/NR	**Indirect evidence** that the research personnel were **not adequately blinded to study group** **OR** there is **insufficient information** provided about blinding to study group during the study (record 'NR' as basis for answer).
			DH RoB	**Direct evidence** that the research personnel were **not adequately blinded** to study group.
5	**Were outcome data complete without attrition or exclusion from analysis?** (domain: attrition/exclusion bias) (animals or measurements missing within an endpoint)	**Y**	DL RoB	**Direct evidence** that the rate of loss/exclusion of animals or measurements was limited **in a way thatthe proportion lost would not appreciably bias results*** [Note: when assessing what 'limited' means, consider the initial number of animals and the reasons for the loss e.g. accident] * the reasons for the loss/exclusion [add also for *in vitro*] of animals or measurements unlikely to be related to outcome (or for survival data, censoring unlikely to be introducing bias); missing outcome data balanced in numbers across study groups, with similar reasons for missing data across groups and reasons were

				documented when animals were removed from a study**.**
			PL RoB	There is **indirect (inferred) evidence** that […] [same as above].
			PH RoB/NR	There is **indirect (inferred) evidence** that loss/exclusion of animals or measurements was **unacceptably large OR not adequately addressed** **OR** there **is insufficient information** provided about loss of animals (record 'NR' as basis for answer).
			DH RoB	There is **direct evidence** that loss/exclusion of animals or measurements was **unacceptably large OR not adequately addressed**. Unacceptable handling of attrition or exclusion includes: reason for loss is likely to be related to true outcome, with either imbalance in numbers or reasons for loss across study groups.
6	**Can we be confident in the exposure characterisation?** (domain: detection bias)	**Y**	/	Note: **Purity** defined by the WG as follows: • Deltamethrin: if technical material is not used, at least 95% • Flufenacet: if technical material is not used, at least 95% **Stability of the solution** should be declared (alternatively the analysis of the test concentration should be provided)
			DL RoB	**Direct evidence** that: • the exposure to deltamethrin or flufenacet or their salts (including purity and stability) was independently characterised (method of analysis declared and limit of detection reported) and **purity** confirmed generally as =95% (including commercial material) • **AND** the **duration of exposure** was suitable for investigated endpoints • **AND** that exposure was consistently administered **in damswith concurrent exposure to the fetus and pups** (i.e. with the same method and time frame) **across treatment groups** • **AND** solubility in the vehicle is declared and appropriate at the concentrations used • **AND** the number of concentrations tested are suitable for the evaluation of a concentration–response (at least 4 doses , 3 doses + control) [this makes it endpoint-dependent] • **AND** control solutions were checked for contamination • **AND** if assay media were examined for actual exposure concentrations, there is direct evidence that most of the exposure data measurements are above the limit of quantitation for the assay such that different exposure groups can be distinguished
			PL RoB	**Indirect evidence** that: • the exposure to deltamethrin or flufenacet or their salts was **90% purity, but no analytical method actually used** (supplier provides it) **OR** purity **was independently confirmed as =90%(e.g. freshly prepared solutions)** and **it is deemed that impurities of up to 10% would not appreciably bias results** • **AND** solubility in the vehicle is declared and appropriate at the concentrations used • **AND** the **duration of exposure** was suitable for the test system and investigated endpoints, • **AND** the **number of concentrations** tested are suitable for the evaluation of a concentration-response (at least 4 doses (3 doses + control)).
			PH RoB/NR	There is **insufficient information** provided about the validity of the exposure assessment method, but no direct evidence for concern (record '**NR**' as basis for answer).
			DH RoB	**Direct evidence** that:

				• the exposure to deltamethrin or flufenacet, or their salts (including purity) was **not pure** • **OR** solubility of the test substance was not appropriately controlled.
7	**Can we be confident in the outcome assessment?** (domain: detection bias)	Y	/	**For endpoints see relevant section above under 'problem formulation'**
			DL RoB	**Direct evidence** that: • the outcome was assessed using **well established** methods (including training of the outcome assessors), • **AND assessed at the same time point after exposure in all study groups** • **AND the outcome assessors were adequately blinded** to the study group, **and it is unlikely that they could have broken the blinding** before reporting outcomes.
			PL RoB	**Indirect evidence** that: • the outcome was assessed using **acceptable methods** • **AND** assessed **at the same time point after initial exposure in all study groups** ○ **OR** it is deemed that the **outcome assessment methods used would not appreciably bias results** • **AND** there is **indirect evidence that the outcome assessors were adequately blinded** to the study group, and it is **unlikely that they could have broken the blinding before reporting outcomes** ○ **OR it is deemed that lack of adequate blinding of outcome assessors would not appreciably bias results, which is more likely to apply to objective outcome measures**. For some outcomes, particularly histopathology assessment, outcome assessors are not blind to study group as they require comparison to the control to appropriately judge the outcome, but additional measures such as multiple levels of independent review by trained pathologists can minimise this potential bias.
			PH RoB/NR	**Indirect evidence** that: • the outcome assessment method is an **insensitive instrument** • **OR the time point after initial exposure differed by study group** • **OR** it was **possible for outcome assessors to infer the study group before reporting outcomes without sufficient quality control measures** **OR** there is **insufficient information** provided about blinding of outcome assessors (record 'NR' as basis for answer).
			DH RoB	**Direct evidence** that: • the outcome assessment method is **an insensitive instrument** • **OR the time point after initial exposure differed by study group**, • **OR direct evidence** for **lack of adequate blinding of outcome assessors, including no blinding or incomplete blinding without quality control measures.**
8	**Were all measured outcomes reported?*** (domain: selective reporting bias) *these are the outcomes objective of the study (Note: set(s) completely omitted)	**N**	/	**NOTE**: It is recognised that selective reporting is difficult to assess with confidence for most studies unless the study protocol is available. Selective reporting bias can be assessed by comparing the 'methods' and 'results' section of the paper, and by considering outcomes measured in the context of knowledge in the field. Selective reporting bias may be suspected if the study does not report outcomes in the results section that would have been expected based on the methods, or if a composite score is present without the individual component outcomes

				When results are not given, this will be considered as risk of bias – high or low risk of bias will be based on experts judgement on a case by case depending on the number and type of endpoints not reported.
			DL RoB	**Direct** evidence that **all of the study's measured outcomes** (primary and secondary) outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been **reported**. This would include outcomes reported with sufficient detail to be included in meta-analysis or fully tabulated during data extraction and analyses had been planned in advance.
			PL RoB	• **Indirect evidence** that all of the study's measured outcomes (primary and secondary) outlined in methods, abstract, and/or introduction (that are relevant for the evaluation) have been **reported** • **OR analyses that had not been planned in advance** (i.e. retrospective unplanned subgroup analyses) **are clearly indicated as such** and **it is deemed that the unplanned analyses were appropriate and selective reporting would not appreciably bias results**(e.g. appropriate analyses of an unexpected effect). This would include outcomes reported with insufficient detail such as only reporting that results were statistically significant (or not).
			PH RoB/NR	**Indirect evidence** that: • all of the study's measured outcomes (primary and secondary) outlined in the methods, abstract, and/or introduction (that are relevant for the evaluation) have **not** been **reported** • **OR** unplanned analyses were included that may appreciably bias results **OR** there is **insufficient information** provided about selective outcome reporting (record 'NR' as basis for answer).
			DH RoB	**Direct evidence** that **all of the study's measured outcomes** (primary and secondary) outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) **have not been reported**. In addition to not reporting outcomes, this would include reporting outcomes based on composite score without individual outcome components or outcomes reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified or reporting outcomes not pre-specified, or that unplanned analyses were included that would appreciably bias results.
9	**Were there other potential threats to internal validity?** (domain: other bias)	**Y**	/	*For appraisal of statistical analysis and adherence to study protocol same comment as for in vitro studies appraisal tool* **Toxicity* (e.g. maternal or pups sign of overt toxicity)** is deemed to be a possible source of bias and should be measured in a study where e.g. dams or pups are exposed to the substance. As a minimum, the following parameters should be considered: survival OR body weight and body weight gain OR food/water consumption OR clinical signs.
			DL RoB	**Direct evidence** that **toxicity was measured and accounted for at all doses**
			PL RoB	**Indirect evidence** (i.e. it can be inferred) that maternal or pups overt toxicity* was measured and accounted for at all doses
			PH RoB/NR	**Indirect evidence** that effects occurred at e.g. maternal or pups overt toxicity **Insufficient information** to assess maternal toxicity (NR)

			DH RoB	**Direct evidence** that effects occurred at maternal or pups overt toxicity or this was not measured

3.10.1. Summarising the internal validity of each experimental animal study, by endpoint(s)

Appraisal questions for *IN VIVO* studies (key questions highlighted in yellow)	**Key (Y/N)**
1. Was administered dose or exposure level adequately randomised?	Y
2. Was allocation to study groups adequately concealed?	
3. Were experimental conditions identical across study groups?	
4. Were the research personnel* blinded to the study group during the study?	
5. Were outcome data complete without attrition or exclusion from analysis?	
6. Can we be confident in the exposure characterisation?	Y
7. Can we be confident in the outcome assessment?	Y
8. Were all measured outcomes reported?	
9. Were there other potential threats to internal validity? – systemic toxicity	

Algorithm to combine the answers to the appraisal questions and allocate studies to tiers of RoB	**Tier**
TIER 1: All key questions are scored +/++ AND maximum 1 non-key question is scored - or –	1 (low RoB)
TIER 2: study does not meet criteria for TIER 1 or TIER 3	2
TIER 3: one (or more) key question is scored -/– OR -/– for the majority of the non-key questions	3 (high RoB)

3.11. Human observational studies

The RoB assessment tool that it was planned to use for human observational studies on the relationship between the two active substances and DNT endpoints is reported in the table below. Specific items identified *a priori* and that would be considered in the assessment of confounding and biases related to the exposure and outcome characterisation are discussed in the tool.

The appraisal question on 'other potential threats to internal validity such as adherence to the protocol' has been dropped after the piloting. This decision was made based on: (1) all the studies retrieved were human observational cohorts published in peer reviewed journals and without a published protocol; (2) although it was considered that adherence to a strict study protocol that includes measures to assure or assess compliance can reduce the RoB, it was considered that would not appreciably bias results for this assessment.

Table 11. RoB assessment tool for HUMAN OBSERVATIONAL studies on the relationship between the two active substances and DNT endpoints

	RoB question (HUMAN OBS)	Endpoint-dependent (Y/N)	Rating	Rationale for judgement
1	**Did selection of study participants result in appropriate comparison groups?** (domain: selection bias)	N	/	
			DL RoB	**Cohort**: There is **direct evidence** that subjects (both exposed and non-exposed) were similar (e.g. recruited from the same eligible population, recruited with the same method of ascertainment using the same inclusion and exclusion criteria), recruited within the same time frame, and had the similar participation/response rates. **Note**: A study will be considered low risk of bias if baseline characteristics of groups differed but these differences were considered as potential confounding or stratification variables (see question #2).
			PL RoB	**Co**: There is **indirect evidence** that subjects (both exposed and non-exposed) were similar (e.g. recruited from the same eligible population, recruited with the same method of ascertainment using the same inclusion and exclusion criteria), recruited within the same time frame, and had the similar participation/response rates, **OR differences between groups would not appreciably bias results**.
			PH RoB/NR	**Co**: There is **indirect evidence** that subjects (both exposed and non-exposed) were not similar, recruited within very different time frames, or had the very different participation/response rates **OR** there is insufficient information provided about the comparison group including a different rate of non-response without an explanation (record 'NR' as basis for answer).
			DH RoB	**Co:** There is **direct evidence** that subjects (both exposed and non-exposed) were not similar, recruited within very different time frames, or had the very different participation/response rates.
2	**Did the study <u>design or analysis</u> account for important confounding and modifying variables?** (confounding bias)	**N**	/	Note: in the current OHAT tool, assessment of confounding requires consideration of whether or not (1) the design or analysis accounted for confounding and modifying variables, (2) the confounding variables were measured reliably and consistently, and (3) there were other exposures anticipated to bias results in reaching a single risk-of-bias rating on confounding. • modifying variables: variables that change the effect size without being associated with the exposure or the outcome (stratification) • confounder: variable associated with both the outcome and exposure and it is not on the pathway between exposure and outcome (control for) Possible confounders: **maternal age**, **maternal IQ,** sex, ethnicity, **<u>socio-economic factors</u>** (education level, incomes, occupation), lifestyle (smoking, alcohol consumption, illicit drugs, stress, diet), and **<u>co-exposure to other chemicals</u>** (either individually or in combination). Co-exposures: those that are associated with DNT endpoints e.g. OPs, lead…
			DL RoB	**Co**: There is **direct evidence** that appropriate adjustments or explicit considerations were made for modifying variables and confounders in the final analyses through the use of statistical models to reduce research-specific bias including standardisation, matching, adjustment in multivariate model, stratification, propensity scoring or other methods that were appropriately justified. Acceptable consideration of appropriate adjustment factors includes cases when the factor is not included in the final adjustment model because the author conducted analyses that indicated it did not need to be included, **AND** there is d**irect evidence** that modifying variables and confounders were assessed using valid and reliable measurements **AND** there is direct evidence that **other exposures** anticipated to bias results were not present or were appropriately measured and adjusted for. In occupational studies or studies of contaminated sites, other chemical exposures known to be associated with those settings were appropriately considered.
			PL RoB	**Co:** There is **indirect evidence** that appropriate adjustments were made, **OR it is deemed that not considering or only considering a partial list of** modifying variables **or confounders in the final analyses would not appreciably bias results**. **AND** there is evidence **(direct or indirect)** that primary covariates and confounders were assessed using valid and reliable measurements, **OR it is deemed that the measures used would not appreciably bias results (i.e. the authors justified the validity of the measures from previously published research**), **AND** there is evidence (direct or indirect) that other co-exposures

				anticipated to bias results were not present or were appropriately adjusted for, **OR it is deemed that co-exposures present would not appreciably bias results** Note: As discussed above, this includes insufficient information provided on co-exposures in general population studies.
			PH RoB/NR	**Co**: There is **indirect evidence** that the distribution of modifying variables and known confounders differed between the groups and was not appropriately adjusted for in the final analyses, OR there **is insufficient information** provided about the distribution of known confounders (record 'NR' as basis for answer), OR there is indirect evidence that modifying variables and confounders were assessed using measurements of unknown validity, OR there is insufficient information provided about the measurement techniques used to assess modifying variables and confounders (record 'NR' as basis for answer), OR there is **indirect evidence** that there was an unbalanced provision of additional co-exposures across the primary study groups, which were not appropriately adjusted for **OR there is insufficient** information provided about co-exposures in occupational studies or studies of contaminated sites where high exposures to other chemical exposures would have been reasonably anticipated (record 'NR' as basis for answer).
			DH RoB	**Co**: There is **direct evidence** that the distribution of modifying variables and known confounders differed between the groups, confounding was demonstrated and was not appropriately adjusted for in the final analyses, **OR** there is direct evidence that modifying variables and confounders were assessed using non-valid measurements, **OR** there is direct evidence that there was an unbalanced provision of additional co-exposures across the primary study groups, which were not appropriately adjusted for.
3	**Were outcome data complete without attrition or exclusion from analysis?** (domain: attrition/exclusion bias)	Y	DL RoB	**Co**: There is direct evidence that loss of subjects or measurements (i.e. incomplete outcome data) was adequately addressed and reasons were documented when human subjects or measurements were removed from a study. Acceptable handling of attrition includes: very little missing outcome data; reasons for missing subjects unlikely to be related to outcome (for survival data, censoring unlikely to be introducing bias); missing outcome data balanced in numbers across study groups, with similar reasons for missing data across groups, **OR** missing data have been imputed using appropriate methods and characteristics of subjects lost to follow up or with unavailable records are described in identical way and are not significantly different from those of the study participants.
			PL RoB	**Co**: There is **indirect evidence** that loss/exclusion of subjects or measurements (i.e. incomplete outcome data) was adequately addressed and reasons were documented when human subjects were removed from a study, OR it **is deemed that the proportion lost to follow-up would not appreciably bias results**. This would include reports of no statistical differences in characteristics of subjects lost to follow up or with unavailable records from those of the study participants. Generally, the higher the ratio of participants with missing data to participants with events, the greater potential there is for bias. For studies with a long duration of follow-up, some withdrawals for such reasons are inevitable.
			PH RoB/NR	**Co**: There is **indirect evidence** that loss/exclusion of subjects or measurements (i.e. incomplete outcome data) was unacceptably large and not adequately addressed OR there is insufficient information provided about numbers of subjects lost to follow-up (record 'NR' as basis for answer).
			DH RoB	**Co**: There is **direct evidence** that loss/exclusion of subjects or measurements (i.e. incomplete outcome data) was unacceptably large and not adequately addressed. Unacceptable handling of subject attrition includes: reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across study groups; or potentially inappropriate application of imputation.
4	**Can we be confident in the exposure characterisation?** (domain: detection bias)	**N**	/	**Gold standard (multiple measurements of deltamethrin in blood)**: The direction of the bias (towards or away from the null) will differ based on the nature of differences between comparison groups and may be difficult to predict. Non-differential misclassification of exposure will generally bias results towards the null, but differential misclassification can bias towards or away from the null, making it difficult to predict the direction of effect (Szklo and Nieto 2007). For controlled exposure studies, noncompliance with the allocated treatment could introduce differential misclassification if compliance was unequal across study groups. Adherence to a strict study protocol that includes measures to assure or assess compliance can reduce the risk of bias.
			DL RoB	**Co**: There is **direct evidence** that exposure was consistently assessed (i.e. under the same method and time frame) using **reliable estimates of deltamethrin exposure** (i.e. 'assessment of deltamethrin in blood') **Note**: measurement of specific metabolites in urine does not allow an accurate estimate of exposure to deltamethrin based on the uncertainty in deltamethrin kinetics, the uncertainty on the correct timing of biomarker collection and the presence of the metabolites in environmental media.
			PL RoB	**Co**: There is **indirect evidence** that the exposure was consistently assessed [same as above] using reliable methods that directly measure exposure [same as above]

			PH RoB/NR	**Co**: There is **direct evidence** that the exposure was assessed measuring specific metabolites in urine (1 or more measurements) (multiple assessments would not count to decrease risk of bias) NR not applicable to this appraisal question.
			DH RoB	**Co**: There is **direct evidence** that the exposure was assessed measuring non-specific metabolites in urine (multiple assessments would not count to decrease risk of bias).
5	**Can we be confident in the outcome assessment?** (domain: detection bias)	Y	/	Well established methods: validated test methods + a neuropsychologist is administering the test OR it is a clinical diagnosis If the paper states that it is a validated test it is considered PL RoB. Deviation from protocol introduced as the appraisal progressed: Questionnaires not designed to be administered by neuropsychologists are considered reliable if they are validated methods.
			DL RoB	**Co**: There is direct evidence • that the outcome was assessed using well established methods AND • subjects had been followed for the same length of time in all study groups AND • that the outcome assessors (including study subjects, if outcomes were self-reported) were adequately **blinded to the study group**, and it is unlikely that they could have broken the blinding before reporting outcomes.
			PL RoB	**Co**: There is **indirect evidence** that • the outcome was assessed using acceptable methods (i.e. deemed valid and reliable but not the gold standard) • AND subjects had been followed for the same length of time in all study groups [Acceptable, but not ideal assessment methods will depend on the outcome, but examples of such methods may include proxy reporting of outcomes and mining of data collected for other purposes] ○ **OR it is deemed that the outcome assessment methods used would not appreciably bias results** • AND there is indirect evidence that the outcome assessors (including study subjects, if outcomes were self-reported) were adequately blinded to the study group, and it is unlikely that they could have broken the blinding before reporting outcomes ○ **OR it is deemed that lack of adequate blinding of outcome assessors would not appreciably bias results, which is more likely to apply to objective outcome measures**.
			PH RoB/NR	**Co**: There is indirect evidence that: • the outcome assessment method is an insensitive instrument (e.g. a questionnaire used to assess outcomes with no information on validation) • OR the length of follow-up differed by study group • OR there is indirect evidence that it was possible for outcome assessors (including study subjects if outcomes were self-reported) to infer the study group before reporting outcomes OR there is insufficient information provided about blinding of outcome assessors (record 'NR' as basis for answer).
			DH RoB	**Co**: There is direct evidence that the outcome assessment method is an insensitive instrument, OR the length of follow-up differed by study group, OR there is direct evidence for lack of adequate blinding of outcome assessors (including study subjects if outcomes were self-reported), including no blinding or incomplete blinding.
6	**Were all measured outcomes reported?** (domain: selective reporting bias)	N	DL RoB	There is **direct evidence that all of the study's measured outcomes** (primary and secondary) outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been **reported**. This would include outcomes reported with sufficient detail to be included in meta-analysis or fully tabulated during data extraction and analyses had been planned in advance.
			PL RoB	There is **indirect evidence** that all of the study's measured outcomes (primary and secondary) outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been **reported**, **OR** **analyses that had not been planned in advance** (i.e. retrospective unplanned subgroup analyses) **are clearly indicated as such** and

				it is deemed that the unplanned analyses were appropriate and selective reporting would not appreciably bias results(e.g. appropriate analyses of an unexpected effect). This would include outcomes reported with insufficient detail such as only reporting that results were statistically significant (or not).
			PH RoB/NR	There is **indirect evidence** that all of the study's measured outcomes (primary and secondary) outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have not been **reported**, **OR** and there is indirect evidence that unplanned analyses were included that may appreciably bias results, **OR** there is **insufficient information** provided about selective outcome reporting (record 'NR' as basis for answer).
			DH RoB	There is **direct evidence** that **all of the study's measured outcomes** (primary and secondary) outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) **have not been reported**. In addition to not reporting outcomes, **this would include reporting outcomes based on composite score without individual outcome components** or **outcomes reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified or reporting outcomes not pre-specified, or that unplanned analyses were included that would appreciably bias results.**
7	**Were there other potential threats to internal validity? – statistics** (domain: other bias)	**Y**	/	**Were statistical methods appropriate**? [This will be checked by statisticians at EFSA]. From OHAT/NTP: The OHAT risk-of-bias tool suggests consideration of statistical methods with the other potential threats to internal validity. One of the common statistical issues identified has been reporting of statistical tests that require normally distributed data (e.g. t-test or ANOVA) without reporting that the homogeneity of variance was tested or confirmed. It is recommended that experts with some knowledge of statistical methods used in the literature participate in drafting the risk-of-bias criteria for identifying inappropriate statistical methods when a review protocol is developed. Even with early expert consultation and planning, statistical methods questions may arise when the actual studies are assessed. Additional consultation and modifications to the statistical methods risk-of-bias criteria may be necessary. When changes are made, they should be documented along with the date on which modifications were made and the logic for the changes.
			DL RoB	Direct evidence of appropriate statistical methods not related to control for confounding as this is already assessed in question 2
			PL RoB	Indirect evidence of appropriate statistical methods not related to confounding as confounding is already assessed in question 2
			PH RoB/NR	Indirect evidence of inappropriate statistical methods not related to control for confounding as this is already assessed in question 2 NR not applicable here (Not reported here would lead to DH RoB)
			DH RoB	Direct evidence of inappropriate statistical methods or not related to control for confounding as this is already assessed in question 2 Not reported

3.11.1. Summarising the internal validity of each human observational animal study, by endpoint(s)

Appraisal questions for HUMAN OBS studies (key questions highlighted in yellow)	Key (Y/N)
1. Did selection of study participants result in appropriate comparison groups?	
2. Did the study design or analysis account for important confounding and modifying variables?	Y
3. Were outcome data complete without attrition or exclusion from analysis?	
4. Can we be confident in the exposure characterisation?	Y
5. Can we be confident in the outcome assessment?	Y
6. Were all measured outcomes reported?	
7. Were there other potential threats to internal validity? – statistics	

Algorithm to combine the answers to the appraisal questions and allocate studies to tiers of RoB	Tier
TIER 1: All key questions are scored +/++ AND maximum 1 non-key question is scored - or –	1 (low RoB)
TIER 2: study does not meet criteria for TIER 1 or TIER 3	2
TIER 3: one (or more) key question is scored -/– OR -/– for the majority of the non-key questions	3 (high RoB)

3.12. Sources of uncertainty arising from the methods for evidence appraisal

Not addressed in the protocol.

PROTOCOL: PART 2

This second part of the protocol was drafted after collecting and appraising the evidence and receiving the *in vitro* battery from the external contractor. It describes the methods for synthesising and integrating evidence and analysing uncertainty (part of the 'weight of evidence' in the IATA framework).

3.13. Methods for synthesising and integrating evidence, including uncertainty analysis

Based on the outcomes of data collection and evidence appraisal, it was agreed to undertake evidence synthesis/integration and uncertainty analysis as follows:

1) First, synthesis/integration/uncertainty analysis of evidence on AO from human and in experimental in vivo studies, including zebrafish.
2) Second, synthesis/integration/uncertainty analysis of mechanistic evidence on MIE and KEs from *in vitro* and *in vivo* mechanistic data.
3) Third, integration of evidence from *in vitro* mechanistic evidence and from evidence on AO from in vivo applying the AOP conceptual framework in a top-down approach (OECD, 2018). An AOP will be postulated and the relationships between Key Events (KERs, also including the MIE and AO) in terms of biological plausibility, Empirical support (dose and temporal concordance) would be assessed. Essentiality of the KEs in the AOP would be also considered and the overall assessment of the AOP undertaken.

In line with the ToRs, this will allow us to see if and how the uncertainty in the hazard characterisation of DM and F changes after the inclusion of mechanistic evidence using the AOP framework for organising and integrating the knowledge.

To conduct fit for purpose hazard identification and characterisation in that includes a rational use of resources, endpoints were prioritised for being retained in the analysis as follows (see the relevant tables in the following sections):

- For the *in vivo* and *in vitro* streams of evidence, only endpoints falling in Tiers 1 or 2 of internal validity will be synthesised, in a graphical quantitative manner, and used for hazard identification and characterisation. Tier 3 endpoints will not be used for drawing conclusions and integration of the evidence in the AOP conceptual framework.
- For human studies, all endpoints (including those falling in Tier 3) will be synthesised in a qualitative manner and included in the uncertainty analysis, their risk of bias would be accounted and their contextualisation in the AOP will be consider after the UA.

3.14. Evidence synthesis/integration and uncertainty assessment on AO

3.14.1. Evidence on AO

Tables 12–14 list by evidence streams (humans, *in vivo*, zebrafish) the adverse outcome categories and the related specific endpoints that were identified in the literature and dossier data. Information is provided in the tables on whether they will be retained or excluded from the synthesis.

Table 12. Endpoints on adverse outcomes from HUMAN studies retained for or excluded from the synthesis

HUMAN STUDIES: ADVERSE OUTCOME CATEGORIES and SPECIFIC ENDPOINTS			
Apical adverse outcome category		**Specific endpoint**	
		Retained for the synthesis	**Excluded from the synthesis**
1.	**Intellectual disability**	• Mental development index • Development index • Psychomotor development index • Development Quotient • Motor development score • WISC – Verbal Comprehension Index (WISC-VCI), • WISC – Working Memory Index (WISC-WMI)	/
2.	**Communication disorders**		/
3.	**Autism SD**		/
4.	**ADHD**	• **ADHD**	/
5.	**Motor disorders**		/
6.	**Specific learning disorders**	• Verbal comprehension • Working memory	/
7.	**Behaviour**	▪ SDQ Internalising score ▪ SDQ Externalising score ▪ SDQ Reverse-scored prosocial behaviour • Cognitive • Receptive Communication • Expressive Communication • Fine motor • Gross motor • Language Composite • Motor Composite • Social Emotional • BASC scales/Internalising Composite • BASC scales/Externalising Composite • BASC scales/Adaptive Skills Composite • BASC scales/Behavioural Symptoms Index • BASC scales/Atypicality • BASC scales/Withdrawal • BRIEF scales/Metacognition Index • BRIEF scales/Behavioural Regulation Index • BRIEF scales/Global Executive Composite	/

Table 13. Endpoints on adverse outcomes from IN VIVO studies retained for or excluded from the synthesis

IN VIVO studies – ADVERSE OUTCOME CATEGORIES and SPECIFIC ENDPOINTS		
Apical adverse outcome category	**Specific endpoints**	
	Retained for the synthesis	**Excluded from the synthesis**
1. Neuropathology endpoints	**Brain weight** • Brain weight – Absolute • Brain weight – Relative • Brain weight – Absolute (not perfused) • Brain weight – Relative (not perfused) **Quantitative morphometric evaluation** • Quantitative morphometric evaluation – Cerebrum length (gross measurement) • Quantitative morphometric evaluation – Cerebellum (gross measurement) • Quantitative morphometric evaluation – Cerebellum • Quantitative morphometric evaluation – Frontal Cortex • Quantitative morphometric evaluation – Parietal Cortex • Quantitative morphometric evaluation – Caudate Putamen • Quantitative morphometric evaluation – Hippocampal Gyrus	**Brain weight:** • Cerebellum weight – Absolute • Cerebellum weight – Relative **Quantitative morphometric evaluation** • Quantitative morphometric evaluation – EGL • Quantitative morphometric evaluation – ML • Quantitative morphometric evaluation – IGL • Quantitative morphometric evaluation – PCL • Quantitative morphometric evaluation – CBL (total cerebellar cortex) • Quantitative morphometric evaluation – CP • Quantitative morphometric evaluation – IZ • Quantitative morphometric evaluation – VZ/SVZ • Quantitative morphometric evaluation – Reelin expression • Quantitative morphometric evaluation – Width of dendritic arbour • Quantitative morphometric evaluation – Length of dendritic arbour • Quantitativemorphometric evaluation – spine • density/μm of dendrite **Qualitative neuropathology examination**
2. Behaviour	**Behavioural ontogeny**: - Swimming behaviour – (straight channel swimming) **Motor activity**: • Motor activity – MWM (acquisition path efficiency) • Motor activity – MWM (cued) • Motor activity – MWM (reversal) • Motor activity – MWM (shift) • Motor activity – MWM acquisition speed • Motor activity – MWM acquisition latency • Motor activity (open field) – Total activity • Motor activity (figure-eight maze) – total activity • Locomotor activity (figure-eight maze) – total activity **Startle** • Startle acoustic and tactile (peak amplitude) • Auditory startle reflex (peak amplitude) • Auditory startle reflex (Latency to peak)	**Behavioural ontogeny**: • Impulsive behaviour – FR resets • Impulsive behaviour – Mean Long wait **Motor activity**: • Motor activity – Marble burying • Motor activity – Elevated zero maze • Motor activity – Rearing mean (0–20 min) • Motor activity – Rearing mean (20–40 min) • Motor activity – Rearing mean (40–60 min) • Motor activity – Locomotor mean (0–20 min) • Motor activity – Locomotor mean (20–40 min) • Motor activity – Locomotor mean (40–60 min) • Motor activity – Total activity (0 -20 min) • Motor activity – Total activity (20–40 min) • Motor activity – Total activity (40–60 min) • Motor activity – Qualitative • Locomotor activity (Y maze) – Total distance travelled

	Learning and memory • Learning and memory – WM passive avoidance performance • Learning and memory – WM performance (errors) • Learning and memory – WM performance (duration) • Learning and memory (CWM) – Errors • Learning and memory (CWM) – Latency • Learning and memory – freezing behaviour (pre-conditioned stimulus) • Learning and memory – freezing behaviour (post-conditioned stimulus) • Learning and memory – freezing behaviour – cued (pre-conditioned stimulus) • Learning and memory – freezing behaviour – cued (post-conditioned stimulus) • Learning and memory (freezing behaviour) – Contextual	• Locomotor activity (open field) – Total distance travelled • Locomotor activity – Total distance travelled (persistence) • Locomotor activity (open field) – Ambulatory count • Locomotor activity (open field) – Ambulatory count (day 1) • Locomotor activity (open field) – Ambulatory count (day 2) • Locomotor activity (open field) – Ambulatory count (day 3) • Motor activity (Open field) – Resting time • Motor activity (Open field) – Ambulatory time • Motor activity (Open field) – Stereotypic time • Motor activity (Open field) – distance travelled • Motor coordination (Latency to fall from rotarod) **Learning and memory** • Learning and memory (MWM) – swimming speed • Learning and memory (MWM) – swimming distance • Learning and memory (MWM) – time spent in the target quadrant • Learning and memory (MWM) – escape latency • Learning and memory (Y maze) – same arm entries • Learning and memory (Y maze) – % of alternation • Learning and memory (Y maze) – Errors

Table 14. Endpoints on adverse outcomes from in vitro (zebrafish embryos) studies retained for or excluded from the synthesis

***INVITRO* (zebrafish embryos) – ADVERSE OUTCOME CATEGORIES and SPECIFIC ENDPOINTS:**		
Apical adverse outcome category	**Specific endpoints**	
	Retained for the synthesis	**Excluded from the synthesis**
1. Neuropathology endpoints		**Quantitative morphometric evaluation** Quantitative morphometric evaluation (Craniofacial morphology – CCL) Quantitative morphometric evaluation (Craniofacial morphology – ID) Quantitative morphometric evaluation (Craniofacial morphology – LJL) **Qualitative morphometric evaluation**
2. Behaviour	Locomotor activity Locomotor activity – total distance travelled Locomotor activity – average velocity	**Thigmotaxic** **Spasms** **Locomotor activity** Locomotor activity – total activity Locomotor activity – rest total Locomotor activity – rest bouts Locomotor activity – rest bouts length Locomotor activity – waking activity

3.14.2. Evidence synthesis/integration and uncertainty analysis by specific endpoint and across endpoints, by apical AO category

The specific endpoints retained for the synthesis (Tables 12, 13, 14) will be summarised in a qualitative manner. Due to the sparse and heterogenous nature of the available data, no meta-analysis will be performed. Available evidence will be clustered hierarchically by evidence streams first and then by apical adverse outcomes categories and related subcategories and/or specific endpoints. The studies' results will be graphically displayed together with the main study characteristics as follows:

For HUMAN studies, characteristics to be displayed in the graphs:

- Metabolite (specific/unspecific) and LOD
- 'concentration' of metabolite
- Endpoint and method for assessment
- Statistical model (Linear regression; Logistic regression; Negative binomial; Bayesian Kernel Machine Regression; Linear mixed model; Generalised model; Reverse scale Cox regression; Difference between exposure OUT?)
- Adjustment and for what
- Tier
- Q2 (confounding) (key question in appraisal)
- Q4 (confidence in exposure characterisation) (key question in appraisal)
- Note: question on the confidence in outcome assessment would not be displayed as it was assessed as low RoB for all studies

For *IN VIVO* studies:

- SPECIES
- SEX
- DOSE
- TIER of VALIDITY
- MATERNAL OR SYSTEM TOXICITY (Q9 in CAT)
- Group sample size
- Exposure duration
- Exposure stage

For *IN VITRO ZF (behavioural)* studies:

- Species
- Test system and origin of the test system
- Stage of development of the primary cells
- Concentration
- Tier of internal validity
- System toxicity (Q9a in CAT)
- Number of biological replicates
- Exposure duration

Then it is planned to draw conclusions on the apical AO category through the step-wise process illustrated in **Table 15**, in short:

Step 1: assessment of each specific endpoint pertaining to an apical AO category:

- Step 1.1: For all the evidence streams: Assessment of the specific endpoint for being associated/affected by the exposure to DM (and flufenacet).
    - Outcome provided as Yes (probability of being associated/affected above 0.60)/No (probability of being associated/affected above 0.60) + list of uncertainties.
- Step 1.2 For in vivo and ZF evidence streams: Assessment of the minimum dose/concentration at which the endpoint is affected by the exposure to DM (it includes uncertainty analysis).

- Outcome provided as single dose/concentration or range and related uncertainties (see below).

Steps 1.1. and 1.2. will be performed by EFSA PREV Staff.

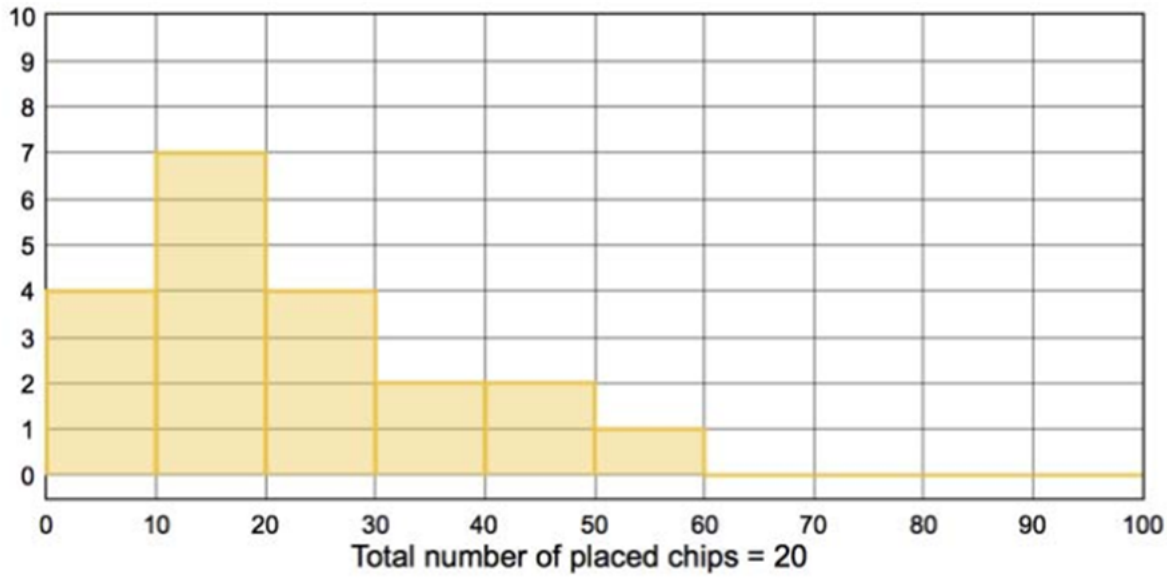
Step 2: Assessment of each apical AO category:

- Step 2.1a: For humans: Assessment of the probability that AO is associated to the exposure to DM.
  - Outcome provided as Uncertainty distribution of the probability that the AO is associated with the exposure to DM
- Step 2.1b: For *in vivo* and ZF: Assessment of the AO for being associated/caused by the exposure to DM (it includes listing uncertainty).
  - Outcome provided as Yes (probability of being associated/affected above 0.60)/No (probability of being associated/affected above 0.60) + list of uncertainties.
- Step 2.2 ONLY For in vivo and ZF evidence streams. Assessment of the minimum dose/concentration at which the AO is caused by the exposure to DM.
  - Outcome provided as Uncertainty distribution of the dose/concentration at which the AO is associated with the exposure to DM.

Steps 2.1.a and 2.2. will be performed by the Working Group experts through an Expert Knowledge Elicitation process (EFSA, 2014) with the purpose of expressing the uncertainty in the conclusions quantitatively in the form of an uncertainty probability distributions.

The roulette method (Gore, 1987; Johnson et al., 2010, O'Hagan, 2019.) will be used to elicit knowledge from the experts. This is a special case of the fixed interval approach since the range of values for the uncertainty distribution is agreed among the experts (or provided) before starting the elicitation process. In this method the expert is asked to distribute a number of chips into some bins, with the probability of a particular bin to include the true value interpreted as the proportion of chips allocated into that bin. The advantage of this method is that the experts can see the shape of their distribution building up as they allocate the chips. This facilitates the exercise also for people not familiar with the concept of probability distributions. The method is illustrated in the Figure 5 (text modified from EFSA, 2014; figure reproduced with permission from figure 24). Twenty chips are provided to the experts each corresponding to a probability of 0.05 (i.e. 1/20). The experts are requested to elicit a parameter whose true value is expected to be included in the range 0–100 (say 'maximum cm of water in Piazza San Marco in Venice during the spring 2020'). The distribution shown in Figure 5 describes the uncertainty on this parameter as elicited by a single expert. For instance, the seven chips in the bin [10,20] can be interpreted as probability of 7/20 (i.e. probability of 0.35 that the maximum water level will be between 10 and 20 cm next spring in Venice).

Figure 5. Expert Knowledge Elicitation – illustration of the roulette method



Source: EFSA, 2014.

Details on the questions to address, the role of the people involved in the process and the timelines are provided in Table 15.

Table 15. Evidence synthesis and uncertainty analysis for adverse outcome evidence by specific endpoint and across endpoints, by adverse outcome

Stream of evidence	Step 1. Endpoint assessment	Question	Answers	How/Who/by when	Step 2. AO assessment	Question	Answers	How/Who/By when
HUMAN	**Specific endpoint** **within a AO category**	Is it probable (i.e. more probable than not) that an association between DM exposure and the specific endpoint exists? By association it is meant the concurrence of DM exposure and any effect on the endpoint considered adverse, irrespective of its magnitude By probable it is meant that the association is considered possible with a probability of 0.60 or above By exposure it is meant the one occurring during prenatal and early post-natal. Effect can be expressed in different ways across studies (e.g. OR, RR) To answer this question, first list and assess the uncertainties on the association. Note: same sources of uncertainties that AO	Yes or No (Yes = more than **0.60**; No = less than 0.60) Provide a rationale for the answer and list related uncertainties	**Experts on human studies** provide their judgement individually Conflicts (if any) are solved by collegial discussion	**Apical** **AO category** (across specific endpoints assessed as 'YES' in the previous step and pertaining to the same AO)	What is the probability that DM exposure is associated with this apical AO? Note: same definition of association as in Step 1	Uncertainty distribution on the probability of the association	Expert Knowledge Elicitation (EKE roulette method) among **experts on human studies** 1. Evidence and related sources of uncertainty are discussed collectively including: • Study validity (RoB) • Exposure period • Age • Metabolite and LOD • 'concentration' of metabolite • Endpoint and method for assessment • Statistical model • Adjustment and for what • Q2 (confounding) • Q4 (confidence in exposure character) • Others (emerging from the discussion) 2. Experts provide their estimates of the probability of an association using the roulette method **individually** 3. Main inconsistencies in the individual uncertainty distributions are discussed and resolved 4. a mathematical summary is provided of the individual U distributions and revised if needed by collegial discussion -> consensus U distribution 5. Rational for the U distribution is provided
IN VIVO	**Specific endpoint**	Is it probable (more probable than not) that DM exposure affect this endpoint in a dose-response	Yes or No (Yes = more than **0.60**; No = less than 0.60)	**PREV staff provide** their judgement	**Apical** **AO category**	Is it probable (i.e. more probable than not) that DM exposure cause	Yes or No (Yes = more	***In vivo* experts** **April**

		relationship? By **'affect'** it is meant any effect considered adverse of the DM exposure to the endpoint, irrespective of its magnitude By **probable** it is meant that the effect is considered possible with a **probability of 0.60 or above** **By exposure** it is meant the one occurring during prenatal OR (prenatal AND post-natal) before weaning (Effect can be expressed in different ways across studies, e.g. intervention/control mean difference)	Provide a rationale for the answer and list related uncertainties. (for the 'No', add the doses at which the studies were tested and explain why)	individually **Conflicts** (if any) are solved by collegial discussion		this apical AO?	than **0.60**; No = less than 0.60)	
		<u>ONLY For the Yes:</u> What is the lowest dose at which exposure to DM is expected to affect the endpoint? To answer this question: Evidence and related sources of uncertainty are assessed including - Unexplained inconsistencies across: species, sex, tier of validity, exposure duration and exposure stage - difference in study precision (sample size) - maternal or system toxicity	1 dose or a dose range (depending on the level of uncertainty and number of studies) Provide a rationale for the answer and list related uncertainties.			What is the lowest dose at which exposure to DM is expected to cause the AO?	Uncertainty distribution using EKE (roulette method)	Expert Knowledge Elicitation (EKE roulette method) among experts on *in vivo* studies 1. Evidence and related sources of uncertainty are discussed collectively including:- Inconsistencies in dose/response relationship across studies not explained by • Species • Gender • Exposure period and duration • Measurement time - Intrinsic ADME characterisation- Lack of Biological plausibility of the combination of positive/negative specific endpoints- Others emerging from the discussion 2. Experts provide their estimates of the probability of an association using the roulette method **individually** 3. Main inconsistencies in the individual uncertainty distributions are discussed and resolved 4. a mathematical summary is provided of the individual U distributions and revised if needed by collegial discussion -> consensus U distribution

								5. Rational for the U distribution is provided
ZEBRA FISH (behavioural)	**Specific endpoint**	Is it probable (more probable than not) that DM exposure affect this endpoint in a concentration-response relationship? By **'affecting'** it is meant any effect considered adverse of the DM exposure to the endpoint, irrespective of its magnitude By **probable** it is meant that the effect is considered possible with a **probability of 0.60 or above** **By exposure** it is meant the one occurring by in vitro administration Effect can be expressed in different ways across studies (e.g. IC50, BMR30. Control might be baseline value for the same group or a different group f embryo)	Yes or No (Yes = more than **0.60**; No = less than 0.60)	**PREV staff** provide their judgement individually **Conflicts (if any)** are solved by collegial discussion	**Apical** **AO category**	Is it probable (i.e. more probable than not) that DM exposure cause this apical AO?	Yes or No (Yes = more than **0.60**; No = less than 0.60)	
		What is the lowest concentration at which exposure to DM is expected to affect the endpoint?	1 concentration or concentration range (depending on the level of uncertainty and number of studies) Provide a rationale for the answer and list related uncertainties.			What is the lowest concentration at which exposure to DM is expected to cause the AO?	Uncertainty distribution using EKE (roulette method)	Expert Knowledge Elicitation (EKE roulette method) among experts on *in vitro* studies 1. Evidence and related sources of uncertainty are discussed collectively including: - variability in the sensitivity of: o the methods o the exposure conditions o the test system - Others emerging from the discussion 2. Experts provide their estimates of the probability of an association using the roulette method **individually** 3. Main inconsistencies in the individual uncertainty distributions are discussed and resolved 4. a mathematical summary is provided of the individual U distributions and revised if needed by collegial discussion -> consensus U

								distribution 5. Rational for the U distribution is provided

3.15. Evidence synthesis/integration/uncertainty analysis of mechanistic evidence on MIEs and KEs

3.15.1. Evidence on MIEs and KEs

Tables 16–17 list by evidence streams (in vivo, in vitro including ZF) the MIEs and KEs and related specific endpoints that were identified in the literature and/or the outsourced battery. Information is provided in the tables on whether they will be retained or excluded from the synthesis based on the appraisal results.

Table 16. Endpoints on MIEs and KEs from IN VIVO MECHANISTIC studies retained for or excluded from the synthesis)

***IN VIVO* studies KEY EVENTS and SPECIFIC ENDPOINTS**		
Key event endpoint category	**Specific endpoints**	
	Retained for the synthesis	**Excluded from the synthesis**
1. Clinical chemistry	/	**Hormones** - Corticosterone
2. Neurochemistry	• **Neurotransmitters** Neurotransmitters (NE) • **Proteins** - Proteins – pCREB/CREB - Proteins – PTrkB/Trkb - Proteins – GluN1 - Proteins – GlunN2A - Proteins – GluN2B • **Growth factor** - Growth factor (BDNF) – Hippocampus Growth factor – BDNF (CA1 region of hippocampus)	• **Neurotransmitters** - Neurotransmitters - Dopamine uptake - Neurotransmitters (DOPAC) - Neurotransmitters (Homovanillic acid) - Neurotransmitters (dopamine) – nucleus accumbens - Neurotransmitters – Dopamine level - • **Growth factor** - Growth factor (BDNF) – Cortex - Growth factor (BDNF) – Striatum • **Quantitative Histochemistry (e.g. special stains)** - Quantitative Histochemistry (n° of Purkinje cells) - Quantitative Histochemistry (Pitx3) - Quantitative Histochemistry (PPH) - Quantitative Histochemistry (Pax6) - Quantitative Histochemistry (BrdU NeuN) - Quantitative Histochemistry (BrdU Ki67) - Quantitative Histochemistry (BrdU)

		- Quantitative Histochemistry (TUNEL stain) - Quantitative Histochemistry (Dat1) - Quantitative Histochemistry (Comt) - Quantitative Histochemistry (Vmat2) - Quantitative Histochemistry (Th) - Quantitative Histochemistry (Nurr1) - Quantitative Histochemistry (Tbr1) - Quantitative Histochemistry (Tbr2) Quantitative Histochemistry (Ki67)
3. Neurophysiology	/	• **LTP**

***IN VITRO* studies (including zebrafish embryos):KEY EVENTS and SPECIFIC ENDPOINTS**		
Key event endpoint category	**Specific endpoints**	
	Retained for the synthesis	**Excluded from the synthesis**
1. Genes (*in vitro* and zebrafish) /Genomic	**Transcriptional alteration (Genomic)** • Transcriptional alteration (Camk2a) • Transcriptional alteration (Camk2b) • Transcriptional alteration (nf-h) • Transcriptional alteration (tubulin-alpha) • Transcriptional alteration (tubulin-beta) • Transcriptional alteration (Gap-43) **Transcriptional alteration (Genes – Zebrafish)** • Transcriptional alteration – slc6a3 • Transcriptional alteration – drd1 • Transcriptional alteration – drd2a • Transcriptional alteration – drd3 • Transcriptional alteration – th1	**Transcriptional alteration (Genomic)** • Transcriptional alteration – Dbnf eIII-V mRNA • Transcriptional alteration – Dbnf eIII-V mRNA (with TTX) • Transcriptional alteration – Dbnf pIII activity • Transcriptional alteration – Dbnf eIII-V mRNA (without TTX) • Transcriptional alterations – Bdnf eIV-IX mRNA • Transcriptional alterations – Bdnf pIV activity **Transcriptional alteration (Genes – Zebrafish))** • Transcriptional alterations (adra2db) • Transcriptional alterations (Casp-9) • Transcriptional alterations (chrm5b) • Transcriptional alterations (GH1) • Transcriptional alterations (GST – glutathione-*S*-transferase) • Transcriptional alterations (hrh4) • Transcriptional alterations (Momo) • Transcriptional alterations (Nrf2a) • Transcriptional alterations (p2ry8) • Transcriptional alterations (p53) • Transcriptional alterations (tacr3l) • Transcriptional alterations (Ubo) • Transcriptional alterations (ucn31) • Transcriptional alterations (uts2r) • Transcriptional alterations (vipr1a)

		• Transcriptional alterations (Yot) • Transcriptional alterations (You) • Tyrosine hydroxylase (TH protein)
2. Neurophysiology/patch clamp	**Membrane excitability** • Membrane excitability (sEPSC) • Membrane excitability (Burst duration) • Membrane excitability (Events/Burst) • Membrane excitability (sEPSC) – Interevent interval	**Membrane excitability** • Membrane excitability (Interevent interval) • Membrane excitability • Membrane excitability (decrementing phase duration) • Membrane excitability (Interval between spikes) • Membrane excitability (mEPSC) • Membrane excitability (peak amplitude) • Membrane excitability (td) • Membrane excitability (ts) • Membrane excitability (tt)
3. Endpoints relevant to network formation and maturation/function (Micro Electrodes Assay)	**MEA** • MFIB (Mean firing rate in burst) • MFR (Mean Firing Rate) • BR (Burst Rate) • Number of Active Electrodes • Number of Actively Bursting Electrodes • Interspike Interval within a burst • % Spikes in Burst • MBD (Mean Burst Duration) • Mean interburst interval • Number of Network Spikes • Network Spike Peak • Network Spike Duration • SD of Network Spike Duration • ISI in Network Spike • Mean number of Spikes in Network Spikes • % Spikes in Network Spike • Mean Correlation • Normalised Mutual Information	/
4. Growth maturation	**Neuriteoutgrowth** • Neurite outgrowth – axonal length • Neurite outgrowth – n° of crossings (radius 30) • Neurite outgrowth – n° of crossings (radius 60)	**Neuriteoutgrowth** • Neurite outgrowth – Complexity • Neurite outgrowth – Complexity (100 μm distance from the body) • Neurite outgrowth – Complexity (120 μm distance from the body) • Neurite outgrowth – Complexity (140 μm distance from the body) • Neurite outgrowth – Complexity (160 μm distance from the body) • Neurite outgrowth – Complexity (180 μm distance from the body) • Neurite outgrowth – Complexity (20 μm distance from the body)

		• Neurite outgrowth – Complexity (200 μm distance from the body) • Neurite outgrowth – Complexity (40 μm distance from the body) • Neurite outgrowth – Complexity (60 μm distance from the body) • Neurite outgrowth – Complexity (80 μm distance from the body) **Synaptogenesis**
5. Proteins	**Phosphorylationproteins** Phosphorylation proteins (BDNF)	**Synaptic proteins** • Synaptic proteins (Kv1.1) • Synaptic proteins (Kv1.2) • Synaptic proteins (SNAP25) • Synaptic proteins (Synaptobrevin) • Synaptic proteins (Synaptophysin) **Apoptotic proteins** • Apoptotic proteins (Bax) • Apoptotic proteins (Bcl-2) • Apoptotic proteins (p53)
6. Neurotransmitters (*in vitro* and zebrafish)	**Neurotransmitters(ZB)** • Dopamine • DOPAC • HVA (homovallinic acid)	**Neurotransmitters(ZB)** • GABA • Glutamate
7. Microglia activation	/	**TNF alpha** **Visual inspection**
8. Endpoints relevant to apoptosis (caspase-3/7 activation)	/	**Apoptosis** • TUNEL staining method
9. Endpoints relevant to proliferation (BrdU staining)		/
10. Endpoints relevant to migration (High Content Analysis – HCA)	• Radial migration (migration distance measure)	/
11. Endpoints relevant to differentiation/function (HCA)	• Neurite outgrowth • Neuronal morphology • Oligodendrocyte differentiation	/

Table 17. Endpoints on MIEs from IN VITRO studies retained for or excluded from the synthesis)

IN VIVO studies: MOLECULAR INITIATING EVENTS and SPECIFIC ENDPOINTS		
Endpoint category (='molecular' initiating event category)	**Specific endpoints**	
	Retained for the synthesis	**Excluded from the synthesis**
Neurochemistry		**Sodiumchannel** • Sodium channel (Na alpha v.1.1) – Cortex • Sodium channel (Na alpha v.1.2) – Cortex • Sodium channel (Na alpha v.1.3) – Cortex • Sodium channel (Na alpha v.1.6) – Cortex • Sodium channel (Na alpha v.1.1) – Striatum • Sodium channel (Na alpha v.1.2) – Striatum • Sodium channel (Na alpha v.1.3) – Striatum • Sodium channel (Na alpha v.1.6) – Striatum • Sodium channel (Na alpha v.1.1) – Cortex vs Striatum • Sodium channel (Na alpha v.1.2) – Cortex vs Striatum • Sodium channel (Na alpha v.1.3) – Cortex vs Striatum • Sodium channel (Na alpha v.1.6) – Cortex vs Striatum • Sodium channel (Na beta 1) – Cortex • Sodium channel (Na beta 2) – Cortex • Sodium channel (Na beta 3) – Cortex • Sodium channel (Na beta 4) – Cortex • Sodium channel (Na beta 1) – Striatum • Sodium channel (Na beta 2) – Striatum • Sodium channel (Na beta 3) – Striatum • Sodium channel (Na beta 4) – Striatum • Sodium channel (Na beta 1) – Cortex vs Striatum • Sodium channel (Na beta 2) – Cortex vs Striatum • Sodium channel (Na beta 3) – Cortex vs Striatum • Sodium channel (Na beta 4) – Cortex vs Striatum **Receptor** Receptor – Muscarinic (cortex) • Receptor – Muscarinic (hippocampus) • Receptor – Muscarinic (striatum) • Receptor – Muscarinic (high affinity) • Receptor – Muscarinic (low affinity) • Receptor – Nicotinic (cortex) • Receptor – Nicotinic (hippocampus) **Transporters** • Transporter (dopamine) • Transporter (DAT) – Cortical • Transporter (DAT) – Striatum • Transporter (VMAT2) – Cortical • Transporter (VMAT2) – Striatum • Transporter (TH) – Striatum • Transporter (TH) – Cortical
IN VITRO studies: MOLECULAR INITIATING EVENTS and SPECIFIC ENDPOINTS		
Endpoint category (='molecular' initiating event category)	**Specific endpoints**	
	Retained for the synthesis	**Excluded from the synthesis**
Receptors	**Ryanodine** • Ryanodine – [3H]Ry binding to Ryrs in cortex	
Channels/transporters	**Calcium**	**Sodium**

	• Calcium [influx] • Calcium – frequency **Ryanodine** • Ryanodine – Mean close time (Tc) • Ryanodine – mean open time (T0) • Ryanodine – open probability (P0)	

3.15.2. Evidence synthesis/integration and uncertainty assessment by specific endpoint and across endpoints, by MIE and KE

The KE/MiE endpoints retained for the synthesis (Tables 15 and 16) will be summarised in a qualitative manner i.e. no meta-analysis will be performed since the evidence is too sparse and heterogeneous. The evidence will be clustered hierarchically by evidence streams first and then by adverse outcomes and related specific endpoints. The study results will be graphically displayed together with the main experiment characteristics, as follows:

For *IN VITRO* studies (including zebrafish non behavioural studies):

- For exposure: single/multiple and time of exposure and measurement
    - Test SYSTEM
    - Test METHOD (is defined by endpoint category and specific endpoint)
    - TIER of VALIDITY (studies in Tier 3 will not be included)
    - CYTOTOXICITY (Q9a in CAT)
    - Number of biological replicates (question 9b in CAT)

Then it is planned to draw conclusions on MIEs and KEs through the step-wise process illustrated in Table 18.

Step 1: assessment of each specific endpoints pertaining to a MIE/KE:

- Step 1.1: Assessment of the endpoint for being affected by the exposure to DM (flufenacet).
    - Outcome provided as Yes (probability of being affected above 0.60)/No (probability of being associated/affected above 0.60) + list of uncertainties..
- Step 1.2 Assessment of the minimum concentration at which the endpoint is affected by the exposure to DM.
    - Outcome provided as single dose/concentration or range and related uncertainties.

Step 2: Assessment of each MIE/KE

- Step 2.1: Assessment of the MIE/KE for being triggered/perturbated by the exposure to DM (it includes listing uncertainty).
    - Outcome provided as Yes (probability of being triggered/perturbated above 0.60)/No (probability of being triggered/perturbated above 0.60) + list of uncertainties.
- Step 2.2 Assessment of the minimum dose/concentration at which the MIE/KE is triggered/perturbated by the exposure to DM.
    - Outcome provided as Uncertainty distribution of the dose/concentration at which the MIE/KE is triggered/perturbated by the exposure to DM.

Step 2.2. will be performed by the Working Group experts through an Expert Knowledge Elicitation process (EFSA, 2014) with the purpose of expressing the uncertainty in the conclusions quantitatively in the form of an uncertainty probability distributions.

Table 18. Evidence synthesis and uncertainty analysis for MECHANISTIC EVIDENCE by specific endpoint and across endpoints, by MIE or KE

Stream of evidence	Step 1. Endpoint assessment	Questions	Answers	How/Who/by when	Step 2. MIE or KE assessment	Questions	Answers	Who/by when
***IN VIVO*/ *IN VITRO* mechanistic**	**Specific endpoint**	Is it probable (more probable than not) that DM exposure affects this endpoint in a concentration-response relationship? By **'affecting'** it is meant any effect considered adverse of the DM exposure to the endpoint, irrespective of its magnitude By **probable** it is meant that the effect is considered possible with a **probability of 0.60 or above** **By exposure** it is meant the one occurring by in vitro administration Effect can be expressed in different ways across studies (e.g. IC50, BMR30. Control might be baseline value for the same group or a different group f embryo)	Yes or No (Yes = more than **0.60**; No = less than 0.60) Provide a rationale for the answer and list related uncertainties. (for the 'No', add the doses at which the studies were tested and explain why)	**PREV staff** provide their judgement individually **Conflicts** (if any) are solved by collegial discussion	**Endpoint category** (across specific endpoints assessed as ?YES' in the previous step and pertaining to the same MIE or KE)	Is it probable (i.e. more probable than not) that exposure to deltamethrin triggering MIE/perturbates KE?	(Yes = more than **0.60**; No = less than 0.60)	**Experts on mechanistic studies**
		What is the lowest concentration at which exposure to DM is expected to affect the endpoint? List the uncertainties on the concentration/dose. Sources of uncertainty **within endpoint** For *in vivo* (see Table 16), for *in vitro*: Lack of concentration/response relationship Inconsistencies in concentration/response relationship not explained by sensitivity: • of the method (including data evaluation); • of Exposure conditions. • of Test system.	1 concentration or a concentration range (depending on the level of uncertainty and number of studies) Provide a rationale for the answer and list related uncertainties.			What is the lowest concentration at which exposure to DM is expected to trigger MIE/perturbate the KE? List related uncertainties to be discussed to reconcile the elicitation of the experts and to justify the final U distribution. To answer this question:	Uncertainty distribution using EKE (roulette method)	Expert Knowledge Elicitation (EKE roulette method) among experts on mechanistic studies list the For *in vitro* 1. Evidence and related sources of uncertainty are discussed collectively For *in vivo*: -uncertainties on the dose (See Table 16). For *in vitro*: - unexplained inconsistencies in concentration/response across studies/battery after considering sensitivity • of the method;

								• of exposure conditions; • of Test system. - Intrinsic ADME characterisation -Biological plausibility of the combination of positive/negative - Others emerging from the discussion 2. Experts provide their estimates of the probability of an association using the roulette method **individually** 3. Main inconsistencies in the individual uncertainty distributions are discussed and resolved 4. a mathematical summary is provided of the individual U distributions and revised if needed by collegial discussion -> consensus U distribution 5. Rational for the U distribution is provided

3.16. Integration of evidence by developing and assessing a putative AOP(s)

An AOP/AOPs (OECD, 2016, 2017) will be developed in a top-down approach, the apical adverse outcomes will be taken and mapped with the affected KE and MiE endpoints including the concentration and doses probabilistic distributions.

The AO evidence will be integrated with the KE/MiE evidence and one or more putative AOP will be postulated. For that purpose, using the IATA framework, all the evidence form the systematic literature review and the results of the *in vitro* testing battery for the stressor(s) in the problem formulation will be will integrated, KERs will be postulated based on the classification of the evidence as molecular, cellular, organ and organism responses and expert knowledge on the biological plausibility. The relationships between Key Events (KERs, also including the MIE and AO) in terms of biological plausibility, Empirical support (dose and temporal concordance) and essentiality of the KE in the AOP would be assessed in accordance with OECD AOP handbook for developing AOP (OECD, 2018).

3.16.1. Assessment of the contribution of the battery

The contribution of the mechanistic data (battery and public literature) will be assess when comparing the uncertainty on DNT hazard of the AO endpoints and of the overall AOP. Moreover, the experts will be asked to specifically assess the contribution of the battery as requested in the ToRs.

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ANNEX A: Search strings

A.1. Flufenacet

PubMed

Search	Query
#1	Search?('FOE 5043' [Supplementary Concept] OR Flufenacet[tiab] OR 142459–58–3[tiab] OR 142459583[tiab] OR '142459 58 3'[tiab] OR 'FOE 5043'[tiab] OR (5034[tiab] AND (BAYFOE[tiab] OR BAY-FOE[tiab])) OR FOE5034[tiab] OR Thiafluamide[tiab] OR Fluthiamide[tiab] OR Fluthiamid[tiab])

Web of Science Platform

Search	Query
# 1	TS=(Flufenacet OR 142459–58–3 OR 142459583 OR '142459 58 3' OR 'FOE 5043' OR 'BAY-FOE 5043' OR FOE5034 OR 'BAYFOE 5034' OR Thiafluamide OR Fluthiamide OR Fluthiamid) *Indexes=SCI-EXPANDED, BKCI-S, ESCI, CCR-EXPANDED, IC Timespan=All years*

Toxline

Search	Query
# 1	((flufenacet OR foe 5043 OR 142459583 OR '142459 58 3' OR 'foe 5043' OR (5034 AND (bayfoe OR bay foe)) OR foe5034 OR thiafluamide OR fluthiamide OR fluthiamid))

DART-Europe E-theses Portal

Search	Query
1	Keywords = Flufenacet OR 142459–58–3 OR 142459583 OR '142459 58 3' OR 'FOE 5043' OR 'BAYFOE 5034' OR ' BAY-FOE 5034' OR FOE5034 OR Thiafluamide OR Fluthiamide OR Fluthiamid

EBSCO Open Dissertations

Search	Query
S1	Flufenacet OR 142459–58–3 OR 142459583 OR '142459 58 3' OR 'FOE 5043' OR 'BAYFOE 5034' OR ' BAY-FOE 5034' OR FOE5034 OR Thiafluamide OR Fluthiamide OR Fluthiamid

PQDT Open

Search	Query
1	Flufenacet OR 142459–58–3 OR 142459583 OR '142459 58 3' OR 'FOE 5043' OR 'BAYFOE 5034' OR ' BAY-FOE 5034' OR FOE5034 OR Thiafluamide OR Fluthiamide OR Fluthiamid

A.2. Deltamethrin

A.2.1. Human studies

PubMed

Search	Query
#7	Search #5 NOT #6
#6	Search?(rat[ti] OR rats[ti] OR mouse[ti] OR mice[ti] OR anopheles[ti] OR mosquito[ti] OR mosquitoes[ti] OR mosquitos[ti] OR rodent[ti] OR rodents[ti] OR fish[tiab] OR zebrafish[ti]) NOT medline[sb])
#5	Search?#3 NOT #4
#4	Search?('animals'[MeSH Terms]) NOT ('animals'[mesh] AND 'humans'[Mesh])
#3	Search #1 AND #2
#2	Search 'Attention'[Mesh] OR 'Aptitude Tests'[Mesh] OR 'Behavior'[Mesh:NoExp] OR 'Behavioral Symptoms'[Mesh] OR 'Adolescent Behavior'[Mesh:NoExp] OR 'Child Behavior'[Mesh] OR 'Cognition'[Mesh] OR 'Cognition Disorders'[Mesh] OR 'Cognitive Dysfunction'[Mesh] OR 'Executive Function'[Mesh] OR 'Growth and Development'[Mesh:noExp] OR 'Human development'[Mesh:noExp] OR 'Intelligence'[Mesh:NoExp] OR 'Learning'[Mesh] OR 'Memory'[Mesh] OR 'Neurobehavioral Manifestations'[Mesh] OR 'Neurocognitive Disorders'[Mesh] OR 'Neurodevelopmental Disorders'[Mesh] OR 'Neurologic Manifestations'[Mesh] OR 'Neuropsychological Tests'[Mesh] OR 'Psychomotor Disorders'[Mesh] OR 'Psychomotor Performance'[Mesh] OR 'Motor Activity'[Mesh] OR Attention[tiab] OR Attentiv*[tiab] OR ADDH[tiab] OR ADHD[tiab] OR ADHS[tiab] OR AD/HD[tiab] OR Aptitude*[tiab] OR Hkd[tiab] OR Hyperactiv*[tiab] OR Hyper activ*[tiab] OR Hyperkin*[tiab] OR Hyper kin*[tiab] OR Distractib*[tiab] OR Inattention[tiab] OR Inattentiv*[tiab] OR Behavi*[tiab] OR brain disorder*[tiab] OR brain damage*[tiab] OR brain dysfunct*[tiab] OR Cognition[tiab] OR Cognitiv*[tiab] OR Metacognit*[tiab] OR Metamemory[tiab] OR Volition[tiab] OR Executive control[tiab] OR Executive function*[tiab] OR executive dysfunction*[tiab] OR executive impairment*[tiab] OR DNT[tiab] OR (Development*[tiab] AND (disabilit*[tiab] OR disorder*[tiab] OR deviation*[tiab] OR 'Neurotoxicity Syndromes'[Mesh] OR neurotoxic*[tiab] OR toxic*[tiab] OR abnormal*[tiab] OR activit*[tiab])) OR Defiance disorder* [tiab] OR Defiant disorder*[tiab] OR Disruptive disorder* [tiab] OR Disruption disorder*[tiab] OR Abnormal development*[tiab] OR Intelligence[tiab] OR Comprehension*[tiab] OR Intellectual*[tiab] OR IQ[tiab] OR Memory[tiab] OR Item recall[tiab] OR Remembering[tiab] OR Learning*[tiab] OR Neurobehav*[tiab] OR Neurocogniti*[tiab] OR Neurodevelopment*[tiab] OR Autism[tiab] OR Autistic[tiab] OR Neurologic*[tiab] OR Nervous disease*[tiab] OR Nervous disorder*[tiab] OR Nervous dysfunction*[tiab] OR Nervous manifestation*[tiab] OR Nervous system*[tiab] OR Neuropsychologic*[tiab] OR Psycholog*[tiab] OR Psychomot*[tiab] OR Motor*[tiab] OR Locomot*[tiab] OR Processing speed[tiab] OR Processing velocity[tiab] OR Maze test[tiab] OR Maze tests[tiab] OR Maze testing[tiab] OR reaction time[tiab] OR response inhibition[tiab] OR Stanford Binet[tiab] OR Binet Test*[tiab] OR Bender Gestalt Test[tiab] OR Aphasia Test*[tiab] OR Bayley* [tiab] OR Wechsler[tiab] OR WISC[tiab] OR McCarthy Scale* [tiab] OR Continuous Performance Test[tiab] OR Continuous Performance Tests[tiab] OR Continuous Performance Task[tiab] OR Continuous Performance Tasks[tiab] OR Conners*[tiab] OR CRS-T[tiab] OR CRS-P[tiab] OR academic achievement*[tiab] OR scholastic achievement*[tiab]
#1	Search?Deltamethrin[tiab] OR '52918 63 5'[tiab] OR 52918635[tiab] OR 52918–63–5[tiab] OR pyrethroid*[tiab] OR Decamethrin[tiab] OR Decamethrine[tiab] OR (IPO[tiab] AND 8831[tiab]) OR (FMC[tiab] AND 45498[tiab]) OR IPO8831[tiab] OR FMC45498[tiab]

Web of Science Platform

Articles assigned only to thematic categories no relevant to human studies such as, veterinary sciences or entomology will be removed.

Search	Query
# 7	#3 not #4 Refined by:?DOCUMENT TYPES:?(ARTICLE OR BOOK CHAPTER OR CORRECTION OR REVIEW OR NOTE OR LETTER)

	Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years
# 6	#3 not #4 Refined by:?DOCUMENT TYPES:?(ARTICLE OR BOOK CHAPTER OR CORRECTION OR REVIEW OR NOTE OR LETTER) Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years
# 5	#3 not #4 Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years
# 4	TI='(animal*' OR mosquito OR mosquitos OR mosquitoes OR aedes OR anopheles OR mouse OR mice OR rat OR rats OR rodent* OR fish OR zebrafish) Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years
# 3	#2 AND #1 Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years
# 2	TS='(Attention' OR Attentiven* OR ADDH OR ADHD OR ADHS OR 'AD/HD' OR Conners* OR Hkd OR Hyperactiv* OR 'Hyper activ*' OR Hyperkin* OR 'Hyper kin*' OR Distractib* OR Inattention OR Inattentiv* OR Aptitude* OR 'Stanford Binet' OR 'Binet Test*' OR 'Bender Gestalt Test' OR 'Aphasia Test*' OR Bayley* OR Wechsler OR WISC OR 'McCarthy Scale*' OR 'Continuous Performance Test' OR 'Continuous Performance Tests' OR 'Continuous Performance Task' OR 'Continuous Performance Tasks' OR CRS-T OR CRS-P OR 'Strengths and Difficulties Questionnaire' OR SDQ OR Behavi* OR 'Brain disorder*' OR 'Brain damage*' OR 'Brain dysfunct*' OR Cognition OR Cognitive OR Metacognit* OR Metamemory OR Volition OR (Executive NEAR/2 (control OR function* OR dysfunction* OR impairment*)) OR DNT OR (Development* NEAR/2 (disorder* OR disabilit* OR * deviation* OR neurotoxic* OR toxic* OR abnormal* OR syndrom*)) OR ((Defiance OR disruptive OR disruption) NEAR/2 (disorder*)) OR Intelligence OR Comprehension* OR Intellectual* OR IQ OR Memory OR Item recall OR Remembering OR Learning* OR Neurobehav* OR Neurocogniti* OR Neurodevelopment* OR Autism OR Autistic OR Neurologic* OR (Nervous NEAR/2 (disease* OR disorder* OR dysfunction* OR manifestation* OR system)) OR Neuropsychologic* OR Psycholog* OR Psychomot* OR Motor* OR Locomot* OR 'Processing speed*' OR 'Processing velocit*' OR 'Maze test' OR 'Maze tests' OR 'Maze testing' OR 'reaction time' OR 'response inhibition' OR 'academic achievement*' OR 'scholastic achievement*') Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years
# 1	TS='(Deltamethrin' OR 52918–63–5 OR 52918635 OR Pyrethroid* OR Decamethrin OR Decamethrine OR 'IPO 8831' OR 'FMC 45498' OR IPO8831 OR FMC45498) Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years

Toxline

Search	Query
# 3	(#5 NOT (animal [na] OR animals [na] OR mosquito [na] OR mosquito [na] OR mosquitoes [na] OR aedes [na] OR anopheles [na] OR mouse [na] OR mice [na] OR rat [na] OR rats [na] OR rodent* [na] OR fish [na] OR zebrafish [na])) AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR PUBDART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org] OR PubMed [org])
# 2	(#1 NOT (animals [mh] NOT (humans [mh] AND animals [mh])))
# 1	((deltamethrin OR 52918–63–5 [rn] OR pyrethroid* OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498) AND (attention OR attentive OR attentiveness OR addh OR adhd OR adhs OR 'ad hd' OR aptitude OR aptitudes OR hkd OR hyperactive OR hyperactivity OR 'hyper active' OR 'hyper activity' OR hyperkinesia OR 'hyper kinesia' OR distractible OR inattention OR inattentive OR behavior OR behaviour OR behaviors OR behaviours OR behavioural OR behavioral OR brain OR cognition OR cognitive OR cognitiveness OR metacognition OR metacognitive OR metamemory OR volition OR 'executive control' OR 'executive function' OR 'executive functions' OR 'executive dysfunction' OR 'executive dysfunctions' OR 'executive impairment' OR 'executive impairments' OR dnt OR 'human development' [mh] OR 'growth AND development' [mh] OR ((development [na] OR development [ab] OR developmental [na] OR developmental [ab] OR developmentally [na] OR developmentally [ab]) AND (disorder OR disorders OR disability OR disabilities OR deviation OR deviations OR neurotoxic OR neurotoxicity OR neurotoxics OR toxic OR toxics OR toxicity OR abnormal OR abnormality OR abnormalities OR syndrome OR syndromes)) OR 'defiance disorder' OR 'defiance disorders' OR 'defiant disorder' OR 'defiant disorders' OR 'disruptive disorder' OR 'disruptive disorders' OR 'disruption disorder' OR 'disruption disorders' OR intelligence OR comprehension OR comprehensiveness OR intellectual OR iq OR memory OR 'item recall' OR remembering OR learning OR neurobehavior OR neurobehaviour OR neurobehaviors OR neurobehaviours OR neurobehavioral OR neurobehavioural OR neurocognition OR neurocognitive OR neurodevelopment OR neurodevelopmental OR autism OR autistic OR neurologic OR neurological OR 'nervous disease' OR 'nervous diseases' OR 'nervous disorder' OR 'nervous disorders' OR 'nervous dysfunction' OR 'nervous dysfunctions' OR 'nervous manifestation' OR 'nervous manifestations' OR 'nervous system' OR neuropsychologic OR neuropshychological OR psychological OR psychomotor OR

	motor OR motoric OR locomotor OR 'processing speed' OR 'processing velocity' OR 'maze test' OR 'maze tests' OR 'maze testing' OR 'reaction time' OR 'response inhibition' OR 'stanford binet' OR 'binet test' OR 'binet tests' OR 'bender gestalt test' OR 'bender gestalt tests' OR 'aphasia test' OR 'aphasia tests' OR bayley OR baleys OR wechsler OR wisc OR 'mccarthy scale' OR 'mccarthy scales' OR 'continuous performance' test OR 'continuous performance tests' OR 'continuous performance task' OR 'continuous performance tasks' OR conners OR 'crs-t' OR 'crs-p' OR 'crs t' OR 'crs p' OR 'academic achievement' OR 'academic achievements' OR 'scholastic achievement' OR 'scholastic achievements'))

DART-Europe E-theses Portal

Search	Query
5	(deltamethrin OR 52918–63–5 OR pyrethroid* OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498) AND (development*)
4	Keywords = (deltamethrin OR 52918–63–5 OR pyrethroid* OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498) AND (ttention OR attentive OR attentiveness OR addh OR adhd OR adhs OR 'ad hd' OR aptitude OR aptitudes OR hkd OR hyperactive OR hyperactivity OR 'hyper active' OR 'hyper activity' OR hyperkinesia OR 'hyper kinesia' OR distractible OR inattention OR inattentive OR behavior OR behaviour OR behaviors OR behaviours OR behavioural OR behavioral OR brain OR cognition OR cognitive OR cognitiveness OR metacognition OR metacognitive OR metamemory OR volition OR 'executive control' OR 'executive function' OR 'executive functions' OR 'executive dysfunction' OR 'executive dysfunctions' OR 'executive impairment' OR 'executive impairments' OR dnt)
6	Keywords = (deltamethrin OR 52918–63–5 OR pyrethroid* OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498) AND ('defiance disorder' OR 'defiance disorders' OR 'defiant disorder' OR 'defiant disorders' OR 'disruptive disorder' OR 'disruptive disorders' OR 'disruption disorder' OR 'disruption disorders' OR intelligence OR comprehension OR comprehensiveness OR intellectual OR iq OR memory OR 'item recall' OR remembering OR learning OR neurobehavior OR neurobehaviour OR neurobehaviors OR neurobehaviours OR neurobehavioral OR neurobehavioural OR neurocognition OR neurocognitive OR neurodevelopment OR neurodevelopmental OR autism OR autistic OR neurologic OR neurological OR 'nervous disease' OR 'nervous diseases' OR 'nervous disorder' OR 'nervous disorders' OR 'nervous dysfunction' OR 'nervous dysfunctions' OR 'nervous manifestation' OR 'nervous manifestations' OR 'nervous system' OR neuropsychologic OR neuropshychological OR psychological)
7	Keywords = (deltamethrin OR 52918–63–5 OR pyrethroid* OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498) AND (psychomotor OR motor OR motoric OR locomotor OR 'processing speed' OR 'processing velocity' OR 'maze test' OR 'maze tests' OR 'maze testing' OR 'reaction time' OR 'response inhibition' OR 'stanford binet' OR 'binet test' OR 'binet tests' OR 'bender gestalt test' OR 'bender gestalt tests' OR 'aphasia test' OR 'aphasia tests' OR bayley OR baleys OR wechsler OR wisc OR 'mccarthy scale' OR 'mccarthy scales' OR 'continuous performance' test OR 'continuous performance tests' OR 'continuous performance task' OR 'continuous performance tasks' OR conners OR 'crs-t' OR 'crs-p' OR 'crs t' OR 'crs p' OR 'academic achievement' OR 'academic achievements' OR 'scholastic achievement' OR 'scholastic achievements')

EBSCO Open Dissertations

Search	Query
S7	(human* OR child* OR adult* OR boy* OR girl* OR baby* OR young OR youth*) AND (S5 AND S6)
S6	human* OR child* OR adult* OR boy* OR girl* OR baby* OR young OR youth*
S5	S3 not S4
S4	TI (animal* OR mosquito OR mosquitos OR mosquitoes OR aedes OR anopheles OR mouse OR mice OR rat OR rats OR rodent* OR fish OR zebrafish)
S3	S1 AND S2
S2	(deltamethrin OR 52918–63–5 OR pyrethroid* OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498)
S1	attention OR attentive OR attentiveness OR addh OR adhd OR adhs OR 'ad hd' OR aptitude OR aptitudes OR hkd OR hyperactive OR hyperactivity OR 'hyper active' OR 'hyper activity' OR hyperkinesia OR 'hyper kinesia' OR distractible OR inattention OR inattentive OR behavior OR behaviour OR behaviors OR behaviours OR behavioural OR behavioral OR brain OR cognition OR cognitive OR cognitiveness OR metacognition OR metacognitive OR metamemory OR volition OR 'executive control' OR 'executive function' OR 'executive functions' OR 'executive dysfunction' OR 'executive dysfunctions' OR 'executive impairment' OR 'executive impairments' OR dnt OR ((development OR development OR developmental OR developmental OR developmentally OR developmentally) AND (disorder OR disorders OR disability OR disabilities OR deviation OR

	deviations OR neurotoxic OR neurotoxicity OR neurotoxics OR toxic OR toxics OR toxicity OR abnormal OR abnormality OR abnormalities OR syndrome OR syndromes)) OR 'defiance disorder' OR 'defiance disorders' OR 'defiant disorder' OR 'defiant disorders' OR 'disruptive disorder' OR 'disruptive disorders' OR 'disruption disorder' OR 'disruption disorders' OR intelligence OR comprehension OR comprehensiveness OR intellectual OR iq OR memory OR 'item recall' OR remembering OR learning OR neurobehavior OR neurobehaviour OR neurobehaviors OR neurobehaviours OR neurobehavioral OR neurobehavioural OR neurocognition OR neurocognitive OR neurodevelopment OR neurodevelopmental OR autism OR autistic OR neurologic OR neurological OR 'nervous disease' OR 'nervous diseases' OR 'nervous disorder' OR 'nervous disorders' OR 'nervous dysfunction' OR 'nervous dysfunctions' OR 'nervous manifestation' OR 'nervous manifestations' OR 'nervous system' OR neuropsychologic OR neuropshychological OR psychological OR psychomotor OR motor OR motoric OR locomotor OR 'processing speed' OR 'processing velocity' OR 'maze test' OR 'maze tests' OR 'maze testing' OR 'reaction time' OR 'response inhibition' OR 'stanford binet' OR 'binet test' OR 'binet tests' OR 'bender gestalt test' OR 'bender gestalt tests' OR 'aphasia test' OR 'aphasia tests' OR bayley OR baleys OR wechsler OR wisc OR 'mccarthy scale' OR 'mccarthy scales' OR 'continuous performance' test OR 'continuous performance tests' OR 'continuous performance task' OR 'continuous performance tasks' OR conners OR 'crs-t' OR 'crs-p' OR 'crs t' OR 'crs p' OR 'academic achievement' OR 'academic achievements' OR 'scholastic achievement' OR 'scholastic achievements'

PQDT Open

Search	Query
2	(deltamethrin OR 52918–63–5 OR pyrethroid* OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498) and ti(attention OR attentive OR attentiveness OR addh OR adhd OR adhs OR 'ad hd' OR aptitude OR aptitudes OR hkd OR hyperactive OR hyperactivity OR 'hyper active' OR 'hyper activity' OR hyperkinesia OR 'hyper kinesia' OR distractible OR inattention OR inattentive OR behavior OR behaviour OR behaviors OR behaviours OR behavioural OR behavioral OR brain OR cognition OR cognitive OR cognitiveness OR metacognition OR metacognitive OR metamemory OR volition OR 'executive control' OR 'executive function' OR 'executive functions' OR 'executive dysfunction' OR 'executive dysfunctions' OR 'executive impairment' OR 'executive impairments' OR dnt OR ((development OR development OR developmental OR developmental OR developmentally OR developmentally) AND (disorder OR disorders OR disability OR disabilities OR deviation OR deviations OR neurotoxic OR neurotoxicity OR neurotoxics OR toxic OR toxics OR toxicity OR abnormal OR abnormality OR abnormalities OR syndrome OR syndromes)) OR 'defiance disorder' OR 'defiance disorders' OR 'defiant disorder' OR 'defiant disorders' OR 'disruptive disorder' OR 'disruptive disorders' OR 'disruption disorder' OR 'disruption disorders' OR intelligence OR comprehension OR comprehensiveness OR intellectual OR iq OR memory OR 'item recall' OR remembering OR learning OR neurobehavior OR neurobehaviour OR neurobehaviors OR neurobehaviours OR neurobehavioral OR neurobehavioural OR neurocognition OR neurocognitive OR neurodevelopment OR neurodevelopmental OR autism OR autistic OR neurologic OR neurological OR 'nervous disease' OR 'nervous diseases' OR 'nervous disorder' OR 'nervous disorders' OR 'nervous dysfunction' OR 'nervous dysfunctions' OR 'nervous manifestation' OR 'nervous manifestations' OR 'nervous system' OR neuropsychologic OR neuropshychological OR psychological OR psychomotor OR motor OR motoric OR locomotor OR 'processing speed' OR 'processing velocity' OR 'maze test' OR 'maze tests' OR 'maze testing' OR 'reaction time' OR 'response inhibition' OR 'stanford binet' OR 'binet test' OR 'binet tests' OR 'bender gestalt test' OR 'bender gestalt tests' OR 'aphasia test' OR 'aphasia tests' OR bayley OR baleys OR wechsler OR wisc OR 'mccarthy scale' OR 'mccarthy scales' OR 'continuous performance' test OR 'continuous performance tests' OR 'continuous performance task' OR 'continuous performance tasks' OR conners OR 'crs-t' OR 'crs-p' OR 'crs t' OR 'crs p' OR 'academic achievement' OR 'academic achievements' OR 'scholastic achievement' OR 'scholastic achievements')
1	(deltamethrin OR 52918–63–5 OR pyrethroid* OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498) AND (psychomotor OR motor OR motoric OR locomotor OR 'processing speed' OR 'processing velocity' OR 'maze test' OR 'maze tests' OR 'maze testing' OR 'reaction time' OR 'response inhibition' OR 'stanford binet' OR 'binet test' OR 'binet tests' OR 'bender gestalt test' OR 'bender gestalt tests' OR 'aphasia test' OR 'aphasia tests' OR bayley OR baleys OR wechsler OR wisc OR 'mccarthy scale' OR 'mccarthy scales' OR 'continuous performance' test OR 'continuous performance tests' OR 'continuous performance task' OR 'continuous performance tasks' OR conners OR 'crs-t' OR 'crs-p' OR 'crs t' OR 'crs p' OR 'academic achievement' OR 'academic achievements' OR 'scholastic achievement' OR 'scholastic achievements')

A.2.2. In vivo studies

PubMed

Search	Query
#3	Search #1 AND #2
#2	Search?Deltamethrin[tiab] OR 52918–63–5[tiab] OR 52918635[tiab] OR Decamethrin[tiab] OR Decamethrine[tiab] OR (IPO[tiab] AND 8831[tiab]) OR (FMC[tiab] AND 45498[tiab]) OR IPO8831[tiab] OR FMC45498[tiab]
#1	Search?'Attention'[Mesh] OR Attention[tiab] OR (('Behavior'[Mesh:noExp] OR 'Behavior, Animal'[Mesh] OR Behavi*[tiab]) AND ('Growth and Development'[Mesh:NoExp] OR development*[tiab] OR exposure*[tiab] OR ontogen*[tiab] OR neurotoxic*[tiab] OR 'Neurotoxicity Syndromes'[Mesh] OR toxic*[tiab])) OR 'Cognition'[Mesh] OR 'Cognition Disorders'[Mesh] OR 'Cognitive Dysfunction'[Mesh] OR cognition[tiab] OR cognitiv*[tiab] OR 'Learning'[Mesh] OR learning[tiab] OR 'Memory'[Mesh] OR memor*[tiab] OR 'Embryonic and Fetal Development'[Mesh] OR 'Prenatal Exposure Delayed Effects'[Mesh] OR (('Embryonic Structures'[Mesh] OR Embryo*[tiab] OR fetal*[tiab] OR foetal*[tiab] OR fetus*[tiab] OR foetus[tiab] OR gestational[tiab] OR 'neonatal'[tiab] OR 'neo natal'[tiab] OR postnatal[tiab] OR 'post natal'[tiab] OR prenatal[tiab] OR 'pre natal' OR perinatal[tiab] OR 'peri natal'[tiab] OR 'in utero'[tiab] OR immature[tiab]) AND ('Brain'[Mesh] OR brain*[tiab] OR 'cerebral cortex'[tiab] OR cerebellum[tiab] OR development*[tiab] OR exposure*[tiab] OR 'Motor Skills'[Mesh] OR locomot*[tiab] OR motor*[tiab] OR 'Nervous System'[Mesh] OR ' Nervous System Diseases'[Mesh] OR Nervous system*[tiab] OR 'Neuroanatomy'[Mesh] OR neuroanatom*[tiab] OR neurobehav*[tiab] OR neurocognit*[tiab] OR neurolog*[tiab] OR neuropath*[tiab] OR neurotoxic*[tiab] OR 'Neurotoxicity Syndromes'[Mesh] OR toxic*[tiab])) OR DNT[tiab] OR (('Growth and Development'[Mesh:NoExp] OR development*[tiab]) AND ('Brain'[Mesh] OR brain*[tiab] OR cerebellum[tiab] OR 'cerebral cortex'[tiab] OR exposure*[tiab] OR 'Motor Skills'[Mesh] OR locomot*[tiab] OR motor*[tiab] OR 'Morphogenesis'[Mesh] OR morphogen*[tiab] OR morphometr*[tiab] OR nervous system*[tiab] OR 'Nervous System'[Mesh] OR 'Nervous System Diseases'[Mesh] OR 'Neuroanatomy'[Mesh] OR neuroanatom*[tiab] OR neurobehav*[tiab] OR neurocognit*[tiab] OR neurolog*[tiab] OR neurotoxic*[tiab] OR 'Neurotoxicity Syndromes'[Mesh] OR ontogen*[tiab] OR startle*[tiab])) OR (('Growth'[Mesh] OR grow*[tiab]) AND ('Brain'[Mesh] OR brain*[tiab] OR 'Nervous System'[Mesh] OR nervous system*[tiab])) OR developmental activity[tiab] OR developmental activities[tiab] OR developmental toxic*[tiab] OR (development*[ti] AND (activit*[ti] AND toxic*[ti])) OR Neurodevelopment*[tiab] OR neurohistopatholog*[tiab] OR Neuropatholog*[tiab] OR 'Neuropathology'[Mesh] OR 'Neurobehavioral Manifestations'[Mesh] OR 'Neurocognitive Disorders'[Mesh] OR 'Neurodevelopmental Disorders'[Mesh] OR 'Neurologic Manifestations'[Mesh]

Web of Science Platform

Search	Query
#3	#1 AND #2 Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years
#2	TS='(Deltamethrin' OR 52918–63–5 OR '52918 63 5' OR 52918635 OR Decamethrin OR Decamethrine OR 'IPO 8831' OR 'FMC 45498' OR IPO8831 OR FMC45498) Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years
#1	TS='(Attention' OR ((Behavior* OR Behaviour*) AND (development* OR exposure* OR ontogen* OR neurotoxic* OR toxic*)) OR cognition* OR cognitiv* OR learning OR memory OR ((Embryo* OR fetal* OR foetal* OR fetus* OR foetus* OR gestational OR immature OR 'in utero' OR neonatal OR 'neo natal' OR perinatal OR 'peri natal' OR postnatal OR 'post natal' OR prenatal OR 'pre natal') AND (brain* OR cerebellum OR 'cerebral cortex' OR development* OR exposure* OR locomot* OR motor* OR neuro* OR 'nervous system*' OR toxic*)) OR (grow* AND (brain* OR 'nervous system*')) OR DNT OR (Development* AND (brain* OR cerebellum OR 'cerebral cortex' OR exposure* OR locomot* OR motor* OR morphogen* OR morphometr* OR neuro* OR 'nervous system*' OR ontogen* OR startle*)) OR (development* NEAR/3 (activ* OR toxic*)) OR Neurodevelopment* OR Neurohistopatholog* OR Neuropatholog*) Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years

Toxline

Search	Query
#1	(((deltamethrin OR deltamethrin OR '52918 63 5' OR 52918635 OR decamethrin OR decamethrine OR

	'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498) AND (attention OR ((behavior OR behaviors OR behavioral OR behaviour OR behaviours OR behavioral) AND ('growth AND development' [mh] OR development [na] OR development [ab] OR developmental [na] OR exposure OR exposures OR exposed OR ontogenesis OR ontogenetic OR neurotoxic OR neurotoxics OR neurotoxicity OR toxic OR toxicity OR toxics)) OR cognition OR cognitive OR learning OR memory OR ((embryo OR embryonic OR embryos OR fetal OR foetal OR fetus OR fetuses OR foetus OR foetuses OR gestational OR 'in utero' OR immature OR neonatal OR 'neo natal' OR perinatal OR 'peri natal' OR postnatal OR 'post natal' OR prenatal OR 'pre natal') AND (brain OR brains OR 'cerebral cortex' OR cerebellum OR 'growth AND development' [mh] OR development [na] OR development [ab] OR developmental [na] OR developmental [ab] OR developmentally [ab] OR developmentally [na] OR exposure OR exposures OR exposed OR locomotor OR motor OR motoric OR motors OR neuroanatomy OR neuroanatomic OR neurobehaviour OR neurobehavior OR neurobehaviours OR neurobehaviors OR neurobehavioral OR neurobehavioural OR neurocognition OR neurocognitive OR neurologic OR neurology OR neuropathy OR neuropathies OR neuropathic OR neurotoxic OR neurotoxics OR neurotoxicity OR 'nervous system' OR 'nervous systems' OR toxic OR toxicity OR toxics OR 'growth AND development' [mh] OR development [na] OR development [ab] OR developmental [na])) OR ((grow OR growing OR growth) AND (brain OR brains OR 'nervous system' OR 'nervous systems')) OR dnt OR (('growth AND development' [mh] OR development [na] OR development [ab] OR developmental [na]) AND (brain OR brains OR 'cerebral cortex' OR cerebellum OR exposure OR exposures OR exposed OR locomotor OR motor OR motoric OR motors OR morphogenesis OR morphogenetic OR morphometry OR morphometric OR neuroanatomy OR neuroanatomic OR neurobehaviour OR neurobehavior OR neurobehaviours OR neurobehaviors OR neurobehavioral OR neurobehavioural OR neurocognition OR neurocognitive OR neurologic OR neurology OR neuropathy OR neuropathies OR neuropathic OR neurotoxic OR neurotoxics OR neurotoxicity OR 'nervous system' OR 'nervous systems' OR ontogenetic OR ontogenesis OR startle OR startles)) OR 'developmental activity' OR 'developmental activities' OR 'developmental toxicity' OR 'developmental toxicities' OR neurodevelopment OR neurodevelopmental OR neurodevelopmentally OR neurohistopathology OR neurohistopathologic OR neuropathology OR neuropathologic OR 'embryonic AND fetal development' [mh] OR 'embryonic development' [mh] OR 'fetal development' [mh] OR 'prenatal exposure delayed effects' [mh] OR 'neurobehavioral manifestations' [mh] OR 'neurocognitive disorders' [mh] OR 'neurodevelopmental disorders' [mh] OR 'neurologic manifestations' [mh]))) AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR PUBDART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org] OR PubMed [org])

DART-Europe E-theses Portal

Search	Query
1	Keywords = (attention OR behav* OR cognit* OR learning OR memory OR embryo* OR fetal* OR foetal* OR fetus* OR gestational OR 'in utero' OR immature OR neonatal OR 'neo natal' OR perinatal OR 'peri natal' OR postnatal OR 'post natal' OR prenatal OR 'pre natal' OR brain OR brains OR 'nervous system' OR 'nervous systems' OR dnt OR development* OR neurodevelopment* OR neurohistopathol* OR neuropathol*) AND (deltamethrin OR deltamethrin OR '52918 63 5' OR 52918635 OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498)

EBSCO Open Dissertations

Search	Query
S3	((attention OR behav* OR cognit* OR learning OR memory OR embryo* OR fetal* OR foetal* OR fetus* OR gestational OR 'in utero' OR immature OR neonatal OR 'neo natal' OR perinatal OR 'peri natal' OR postnatal OR 'post natal' OR prenatal OR 'pre natal' OR brain OR brains OR 'nervous system' OR 'nervous systems' OR dnt OR development* OR neurodevelopment* OR neurohistopathol* OR neuropathol*))
S2	(attention OR behav* OR cognit* OR learning OR memory OR embryo* OR fetal* OR foetal* OR fetus* OR gestational OR 'in utero' OR immature OR neonatal OR 'neo natal' OR perinatal OR 'peri natal' OR postnatal OR 'post natal' OR prenatal OR 'pre natal' OR brain OR brains OR 'nervous system' OR 'nervous systems' OR dnt OR development* OR neurodevelopment* OR neurohistopathol* OR neuropathol*)?
S1	(deltamethrin OR deltamethrin OR '52918 63 5' OR 52918635 OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498)?

PQDT Open

Search	Query
1	(deltamethrin OR deltamethrin OR '52918 63 5' OR 52918635 OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498) and ti(attention OR behav* OR cognit* OR learning OR memory OR embryo* OR fetal* OR foetal* OR fetus* OR gestational OR 'in utero'

	OR immature OR neonatal OR 'neo natal' OR perinatal OR 'peri natal' OR postnatal OR 'post natal' OR prenatal OR 'pre natal' OR brain OR brains OR 'nervous system' OR 'nervous systems' OR dnt OR development* OR neurodevelopment* OR neurohistopathol* OR neuropathol*)?

A.2.3. In vitro studies

PubMed

Search	Query
#3	**Search** #1 AND #2
#2	**Search**?Deltamethrin[tiab] OR 52918–63–5[tiab] OR 52918635[tiab] OR Decamethrin[tiab] OR Decamethrine[tiab] OR (IPO[tiab] AND 8831[tiab]) OR (FMC[tiab] AND 45498[tiab]) OR IPO8831[tiab] OR FMC45498[tiab]
#1	**Search**?'Cells, Cultured'[MeSH] OR 'Cell Physiological Phenomena'[Mesh] OR '*In Vitro* Techniques'[MeSH] OR Astrocyte*[tiab] OR brain slice*[tiab] OR calcium channel*[tiab] OR calcium signal*[tiab] OR cell based[tiab] OR cell line*[tiab] OR cell migrat*[tiab] OR cell model*[tiab] OR cell proliferat*[tiab] OR cell system*[tiab] OR cellular assay*[tiab] OR cellular endpoint*[tiab] OR cellular exposure*[tiab] OR cellular migrat*[tiab] OR cellular method*[tiab] OR cellular model*[tiab] OR cellular proliferat*[tiab] OR cellular system*[tiab] OR cellular technique*[tiab] OR electrical activit*[tiab] OR electrode arra*[tiab] OR ESC[tiab] OR glial[tiab] OR immortalised[tiab] OR immortalized[tiab] OR '*in vitro*'[tiab] OR intercellular communicat*[tiab] OR IPS cell*[tiab] OR iPSC[tiab] OR LUHMES[tiab] OR microglia*[tiab] OR myelinogenes*[tiab] OR myelin formation*[tiab] OR network formation*[tiab] OR nerve cell*[tiab] OR neural cell*[tiab] OR neuronal cell*[tiab] OR neural connect*[tiab] OR neuronal connect*[tiab] OR neural crest[tiab] OR neural differentiat*[tiab] OR neuronal differentiat*[tiab] OR neural network*[tiab] OR neuronal network*[tiab] OR neural plastic*[tiab] OR neuronal plastic*[tiab] OR neural precursor*[tiab] OR neuronal precursor*[tiab] OR neural progenitor*[tiab] OR neural prun*[tiab] OR neuronal prun*[tiab] OR neuroblastoma*[tiab] OR neuroinflammation*[tiab] OR neurosphere[tiab] OR neuroprogenitor*[tiab] OR neurotransmitter release*[tiab] OR oligodendrocyte*[tiab] OR pheochromocytoma*[tiab] OR pluripotent cell*[tiab] OR primary cell*[tiab] OR schwann cell*[tiab] OR stem cell*[tiab] OR neurite outgrowth[tiab] OR synaptogenes*[tiab] OR synapse formation*[tiab] OR synapse plastic*[tiab] OR synaptic prun*[tiab]

Web of Science Platform

Search	Query
# 4	#2 AND #1 Refined by:?DOCUMENT TYPES:?(ARTICLE OR REVIEW OR EDITORIAL MATERIAL OR LETTER) Indexes='SCI-EXPANDED,' BKCI-S, CCR-EXPANDED, IC Timespan='All' years
# 3	#2 AND #1 Indexes='SCI-EXPANDED,' BKCI-S, CCR-EXPANDED, IC Timespan='All' years
# 2	TS='(Deltamethrin' OR 52918–63–5 OR '52918 63 5' OR 52918635 OR Decamethrin OR Decamethrine OR 'IPO 8831' OR 'FMC 45498' OR IPO8831 OR FMC45498) Indexes='SCI-EXPANDED,' BKCI-S, CCR-EXPANDED, IC Timespan='All' years
# 1	TS='(Astrocyte*' OR (brain NEAR/2 slice*) OR (calcium NEAR/2 (channel* OR signal*)) OR ((cell OR cells) NEAR/2 ('based' OR line* OR migrat* OR model* OR prolifer* OR system*)) OR (Cellular NEAR/2 (assay* OR endpoint* OR exposure* OR migration OR method* OR model* OR proliferat* OR system* OR technique*)) OR 'electrical activit*' OR 'electrode arra*' OR ESC OR glial OR immortalised OR immortalized OR '*in vitro*' OR (intercellular NEAR/2 communicat*) OR 'IPS cell*' OR iPSC OR LUHMES OR microglia* OR myelinogenes* OR 'myelin formation*' OR 'network formation*' OR 'neural cell*' OR 'neuronal cell*' OR 'nerve cell*' OR ((neural OR neural) NEAR/2 (connect* OR different* OR network OR plastic* OR precursor* OR prun*)) OR 'neural crest' OR neural progenitor* OR neuroprogenitor* OR neuroblastoma* OR neuroinflammation* OR neurosphere OR 'neurotransmitter release*' OR oligodendrocyte* OR pheochromocytoma* OR 'pluripotent cell*' OR 'primary cell*' OR 'schwann cell*' OR 'stem cell*' OR 'neurite outgrowth' OR synaptogenes* OR (synap* NEAR/2 (format* OR plastic* OR prun*))) Indexes='SCI-EXPANDED,' BKCI-S, CCR-EXPANDED, IC Timespan='All' years

Toxline

Search	Query
# 3	(#1 OR #2)

# 2	((deltamethrin OR deltamethrin OR '52918 63 5' OR 52918635 OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498) AND (astrocyte OR astrocytes OR 'brain slice' OR 'brain slices' OR 'calcium channel' OR 'calcium channels' OR 'calcium signal' OR 'calcium signals' OR 'calcium signalling' OR 'cell based' OR 'cell line' OR 'cell lines' OR 'cell migrate' OR 'cell migrated' OR 'cell migration' OR 'cell migrations' OR 'cell model' OR 'cell models' OR 'cell proliferates' OR 'cell proliferating' OR 'cell proliferation' OR 'cell proliferations' OR 'cell system' OR 'cell systems' OR 'cellular assay' OR 'cellular assays' OR 'cellular endpoint' OR 'cellular endpoints' OR 'cellular exposure' OR 'cellular exposures' OR 'cellular migration' OR 'cellular migrations' OR 'cellular method' OR 'cellular methods' OR 'cellular model' OR 'cellular models' OR 'cellular proliferation' OR 'cellular proliferations' OR 'cellular system' OR 'cellular systems' OR 'cellular technique' OR 'cellular techniques' OR 'electrical activities' OR 'electrical activity' OR 'electrode array' OR 'electrode arrays' OR esc OR glial OR immortalised OR immortalized OR '*in vitro*' OR 'intercellular communication' OR 'intercellular communications' OR 'ips cell' OR 'ips cells' OR ipsc OR luhmes OR microglia OR microglias OR myelinogenesis OR myelinogeneses OR 'myelin formation' OR 'myelin formations' OR 'network formation' OR 'network formations' OR 'nerve cell' OR 'nerve cells' OR 'neural cell' OR 'neural cells' OR 'neuronal cell' OR 'neuronal cells' OR 'neuronal connection' OR 'neuronal connections' OR 'neuronal connectivity' OR 'neural crest' OR 'neuronal differentiation' OR 'neuronal differentiations' OR 'neuronal differentiating' OR 'neuronal network' OR 'neuronal networks' OR 'neural network' OR 'neural networks' OR 'neural plasticity' OR 'neuronal plasticities' OR 'neuronal plasticity' OR 'neuronal plasticities' OR 'neural precursor' OR 'neural precursors' OR 'neuronal precursor' OR 'neuronal precursors' OR 'neural progenitor' OR 'neural progenitors' OR 'neural pruning' OR 'neuronal pruning' OR neuroblastoma OR neuroblastomas OR neuroinflammation OR neuroinflammations OR neurosphere OR neuroprogenitor OR neuroprogenitors OR 'neurotransmitter release' OR 'neurotransmitter releases' OR 'neurotransmitter released' OR oligodendrocyte OR oligodendrocytes OR pheochromocytoma OR pheochromocytomas OR 'pluripotent cell' OR 'pluripotent cells' OR 'primary cell' OR 'primary cells' OR 'schwann cell' OR 'schwann cells' OR 'stem cell' OR 'stem cells' OR 'neurite outgrowth' OR synaptogenes OR synaptogenesis OR synaptogeneses OR 'synapse formation' OR 'synapse formations' OR 'synapse plasticity' OR 'synapse plasticities' OR 'synaptic pruning' OR 'synaptic prunings')) AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR PUBDART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org] OR PubMed [org])
# 1	((deltamethrin OR deltamethrin OR '52918 63 5' OR 52918635 OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498) AND ('cells cultured' [mh] OR 'cell physiological phenomena' [mh] OR '*in vitro* techniques' [mh])) AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR PUBDART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org] OR PubMed [org])

DART-Europe E-theses Portal

Search	Query
1	Keywords = ((deltamethrin OR deltamethrin OR '52918 63 5' OR 52918635 OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498) AND (astrocyte OR astrocytes OR 'brain slice' OR 'brain slices' OR 'calcium channel' OR 'calcium channels' OR 'calcium signal' OR 'calcium signals' OR 'calcium signalling' OR 'cell based' OR 'cell line' OR 'cell lines' OR 'cell migrate' OR 'cell migrated' OR 'cell migration' OR 'cell migrations' OR 'cell model' OR 'cell models' OR 'cell proliferates' OR 'cell proliferating' OR 'cell proliferation' OR 'cell proliferations' OR 'cell system' OR 'cell systems' OR 'cellular assay' OR 'cellular assays' OR 'cellular endpoint' OR 'cellular endpoints' OR 'cellular exposure' OR 'cellular exposures' OR 'cellular migration' OR 'cellular migrations' OR 'cellular method' OR 'cellular methods' OR 'cellular model' OR 'cellular models' OR 'cellular proliferation' OR 'cellular proliferations' OR 'cellular system' OR 'cellular systems' OR 'cellular technique' OR 'cellular techniques' OR 'electrical activities' OR 'electrical activity' OR 'electrode array' OR 'electrode arrays' OR esc OR glial OR immortalised OR immortalized OR '*in vitro*' OR 'intercellular communication' OR 'intercellular communications' OR 'ips cell' OR 'ips cells' OR ipsc OR luhmes OR microglia OR microglias OR myelinogenesis OR myelinogeneses OR 'myelin formation' OR 'myelin formations' OR 'network formation' OR 'network formations' OR 'nerve cell' OR 'nerve cells' OR 'neural cell' OR 'neural cells' OR 'neuronal cell' OR 'neuronal cells' OR 'neuronal connection' OR 'neuronal connections' OR 'neuronal connectivity' OR 'neural crest' OR 'neuronal differentiation' OR 'neuronal differentiations' OR 'neuronal differentiating' OR 'neuronal network' OR 'neuronal networks' OR 'neural network' OR 'neural networks' OR 'neural plasticity' OR 'neuronal plasticities' OR 'neuronal plasticity' OR 'neuronal plasticities' OR 'neural precursor' OR 'neural precursors' OR 'neuronal precursor' OR 'neuronal precursors' OR 'neural progenitor' OR 'neural progenitors' OR 'neural pruning' OR 'neuronal pruning' OR neuroblastoma OR neuroblastomas OR neuroinflammation OR neuroinflammations OR neurosphere OR neuroprogenitor OR neuroprogenitors OR 'neurotransmitter release' OR 'neurotransmitter releases' OR 'neurotransmitter released' OR oligodendrocyte OR oligodendrocytes OR pheochromocytoma OR pheochromocytomas OR

	'pluripotent cell' OR 'pluripotent cells' OR 'primary cell' OR 'primary cells' OR 'schwann cell' OR 'schwann cells' OR 'stem cell' OR 'stem cells' OR 'neurite outgrowth' OR synaptogenes OR synaptogenesis OR synaptogeneses OR 'synapse formation' OR 'synapse formations' OR 'synapse plasticity' OR 'synapse plasticities' OR 'synaptic pruning' OR 'synaptic prunings' OR teratocarcinoma))

EBSCO Open Dissertations

Search	Query
S3	S1 AND S2
S2	(astrocyte OR astrocytes OR 'brain slice' OR 'brain slices' OR 'calcium channel' OR 'calcium channels' OR 'calcium signal' OR 'calcium signals' OR 'calcium signalling' OR 'cell based' OR 'cell line' OR 'cell lines' OR 'cell migrate' OR 'cell migrated' OR 'cell migration' OR 'cell migrations' OR 'cell model' OR 'cell models' OR 'cell proliferates' OR 'cell proliferating' OR 'cell proliferation' OR 'cell proliferations' OR 'cell system' OR 'cell systems' OR 'cellular assay' OR 'cellular assays' OR 'cellular endpoint' OR 'cellular endpoints' OR 'cellular exposure' OR 'cellular exposures' OR 'cellular migration' OR 'cellular migrations' OR 'cellular method' OR 'cellular methods' OR 'cellular model' OR 'cellular models' OR 'cellular proliferation' OR 'cellular proliferations' OR 'cellular system' OR 'cellular systems' OR 'cellular technique' OR 'cellular techniques' OR 'electrical activities' OR 'electrical activity' OR 'electrode array' OR 'electrode arrays' OR esc OR glial OR immortalised OR immortalized OR '*in vitro*' OR 'intercellular communication' OR 'intercellular communications' OR 'ips cell' OR 'ips cells' OR ipsc OR luhmes OR microglia OR microglias OR myelinogenesis OR myelinogeneses OR 'myelin formation' OR 'myelin formations' OR 'network formation' OR 'network formations' OR 'nerve cell' OR 'nerve cells' OR 'neural cell' OR 'neural cells' OR 'neuronal cell' OR 'neuronal cells' OR 'neuronal connection' OR 'neuronal connections' OR 'neuronal connectivity' OR 'neural crest' OR 'neuronal differentiation' OR 'neuronal differentiations' OR 'neuronal differentiating' OR 'neuronal network' OR 'neuronal networks' OR 'neural network' OR 'neural networks' OR 'neural plasticity' OR 'neuronal plasticities' OR 'neuronal plasticity' OR 'neuronal plasticities' OR 'neural precursor' OR 'neural precursors' OR 'neuronal precursor' OR 'neuronal precursors' OR 'neural progenitor' OR 'neural progenitors' OR 'neural pruning' OR 'neuronal pruning' OR neuroblastoma OR neuroblastomas OR neuroinflammation OR neuroinflammations OR neurosphere OR neuroprogenitor OR neuroprogenitors OR 'neurotransmitter release' OR 'neurotransmitter releases' OR 'neurotransmitter released' OR oligodendrocyte OR oligodendrocytes OR pheochromocytoma OR pheochromocytomas OR 'pluripotent cell' OR 'pluripotent cells' OR 'primary cell' OR 'primary cells' OR 'schwann cell' OR 'schwann cells' OR 'stem cell' OR 'stem cells' OR 'neurite outgrowth' OR synaptogenes OR synaptogenesis OR synaptogeneses OR 'synapse formation' OR 'synapse formations' OR 'synapse plasticity' OR 'synapse plasticities' OR 'synaptic pruning' OR 'synaptic prunings'))
S1	(deltamethrin OR deltamethrin OR '52918 63 5' OR 52918635 OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498)

PQDT Open

Search	Query
3	1 OR 2
2	deltamethrin OR deltamethrin OR '52918 63 5' OR 52918635 OR decamethrin OR decamethrine OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498 and ti('electrical activities' OR 'electrical activity' OR 'electrode array' OR 'electrode arrays' OR esc OR glial OR immortalised OR immortalized OR '*in vitro*' OR 'intercellular communication' OR 'intercellular communications' OR 'ips cell' OR 'ips cells' OR ipsc OR luhmes OR microglia OR microglias OR myelinogenesis OR myelinogeneses OR 'myelin formation' OR 'myelin formations' OR 'network formation' OR 'network formations' OR 'nerve cell' OR 'nerve cells' OR 'neural cell' OR 'neural cells' OR 'neuronal cell' OR 'neuronal cells' OR 'neuronal connection' OR 'neuronal connections' OR 'neuronal connectivity' OR 'neural crest' OR 'neuronal differentiation' OR 'neuronal differentiations' OR 'neuronal differentiating' OR 'neuronal network' OR 'neuronal networks' OR 'neural network' OR 'neural networks' OR 'neural plasticity' OR 'neuronal plasticities' OR 'neuronal plasticity' OR 'neuronal plasticities' OR 'neural precursor' OR 'neural precursors' OR 'neuronal precursor' OR 'neuronal precursors' OR 'neural progenitor' OR 'neural progenitors' OR 'neural pruning' OR 'neuronal pruning' OR neuroblastoma OR neuroblastomas OR neuroinflammation OR neuroinflammations OR neurosphere OR neuroprogenitor OR neuroprogenitors OR 'neurotransmitter release' OR 'neurotransmitter releases' OR 'neurotransmitter released' OR oligodendrocyte OR oligodendrocytes OR pheochromocytoma OR pheochromocytomas OR 'pluripotent cell' OR 'pluripotent cells' OR 'primary cell' OR 'primary cells' OR 'schwann cell' OR 'schwann cells' OR 'stem cell' OR 'stem cells' OR 'neurite outgrowth' OR synaptogenes OR synaptogenesis OR synaptogeneses OR 'synapse formation' OR 'synapse formations' OR 'synapse plasticity' OR 'synapse plasticities' OR 'synaptic pruning' OR 'synaptic prunings')
1	deltamethrin OR deltamethrin OR '52918 63 5' OR 52918635 OR decamethrin OR decamethrine

	OR 'ipo 8831' OR 'fmc 45498' OR ipo8831 OR fmc45498 and ti(astrocyte OR astrocytes OR 'brain slice' OR 'brain slices' OR 'calcium channel' OR 'calcium channels' OR 'calcium signal' OR 'calcium signals' OR 'calcium signalling' OR 'cell based' OR 'cell line' OR 'cell lines' OR 'cell migrate' OR 'cell migrated' OR 'cell migration' OR 'cell migrations' OR 'cell model' OR 'cell models' OR 'cell proliferates' OR 'cell proliferating' OR 'cell proliferation' OR 'cell proliferations' OR 'cell system' OR 'cell systems' OR 'cellular assay' OR 'cellular assays' OR 'cellular endpoint' OR 'cellular endpoints' OR 'cellular exposure' OR 'cellular exposures' OR 'cellular migration' OR 'cellular migrations' OR 'cellular method' OR 'cellular methods' OR 'cellular model' OR 'cellular models' OR 'cellular proliferation' OR 'cellular proliferations' OR 'cellular system' OR 'cellular systems' OR 'cellular technique' OR 'cellular techniques')

A.3. High-throughput methods

PubMed

Search	Query
#3	Search ((((((‘Nervous System’[Mesh] OR ‘Brain’[Mesh] OR nervous system*[tiab] OR brain*[tiab]) AND (development*[tiab] OR ‘Growth and Development’[Mesh:noexp])) OR ‘Neurodevelopmental Disorders’[Mesh] OR ‘Neurotoxicity Syndromes’[Mesh] OR neurodevelopmental endpoint*[tiab] OR neurodevelopmental disorder*[tiab]) AND (toxic[tiab] OR toxics[tiab] OR toxicity[tiab] OR toxicities[tiab] OR toxicant*[tiab] OR ‘Toxicity Tests’[Mesh])) OR DNT[tiab] OR (developmental[tiab] AND neurotoxi*[tiab]) OR (neurodevelopmental[tiab] AND toxic*[tiab]))) AND (‘High-Throughput Screening Assays’[Mesh] OR ‘Small Molecule Libraries’[Mesh] OR compound librar*[tiab] OR compounds librar*[tiab] OR chemical librar*[tiab] OR molecule librar*[tiab] OR molecules librar*[tiab] OR molecular librar*[tiab] OR ‘high-throughput’[tiab] OR highthroughput[tiab] OR laboratory automation*[tiab] OR large scale[tiab] OR New Alternative Method*[tiab] OR NAM[tiab] OR automated screen*[tiab] OR high content screeen*[tiab] OR ((‘*in vitro*’[tiab] OR invitro[tiab]) AND screen*[tiab]) OR Tox21[tiab] OR ‘Tox 21’[tiab] OR Tox21[tiab] OR ToxCast[tiab] OR ‘Tox cast’[tiab])
#2	Search ’High-Throughput Screening Assays’[Mesh] OR ‘Small Molecule Libraries’[Mesh] OR compound librar*[tiab] OR compounds librar*[tiab] OR chemical librar*[tiab] OR molecule librar*[tiab] OR molecules librar*[tiab] OR molecular librar*[tiab] OR ‘high- throughput’[tiab] OR highthroughput[tiab] OR laboratory automation*[tiab] OR large scale[tiab] OR New Alternative Method*[tiab] OR NAM[tiab] OR automated screen*[tiab] OR high content screeen*[tiab] OR ((‘*in vitro*’[tiab] OR invitro[tiab]) AND screen*[tiab]) OR Tox21[tiab] OR ‘Tox 21’[tiab] OR Tox21[tiab] OR ToxCast[tiab] OR ‘Tox cast’[tiab]
#1	Search ((((‘Nervous System’[Mesh] OR ‘Brain’[Mesh] OR nervous system*[tiab] OR brain*[tiab]) AND (development*[tiab] OR ‘Growth and Development’[Mesh:noexp])) OR ‘Neurodevelopmental Disorders’[Mesh] OR ‘Neurotoxicity Syndromes’[Mesh] OR neurodevelopmental endpoint*[tiab] neurodevelopmental effect*[tiab] OR neurodevelopmental disorder*[tiab]) AND (toxic[tiab] OR toxics[tiab] OR toxicity[tiab] OR toxicities[tiab] OR toxicant*[tiab] OR ‘Toxicity Tests’[Mesh])) OR DNT[tiab] OR (developmental[tiab] AND neurotoxi*[tiab]) OR (neurodevelopmental[tiab] AND toxic*[tiab])

Web of Science Platform

Search	Query
# 3	#2 AND #1 Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years
# 2	TS='(((‘Nervous' system*’ OR brain*) NEAR development* AND toxic*) OR ((neurodevelopmental NEAR/5 (endpoint* OR disorder* OR effect OR effects)) AND toxic*) OR DNT OR (neurodevelopmental AND toxic*) OR (neurodevelopment* NEAR/5 toxic*) OR (developmental AND neurotoxi*) OR (development* NEAR/5 neurotoxic*)) Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years
# 1	TS='(((compound' OR compounds OR chemical OR molecule OR molecules) NEAR/5 library*) OR ‘high throughput’ OR highthroughput OR (laboratory NEAR/5 automation*) OR ‘large scale’ OR ‘new alterative method*’ OR NAM OR ((automated OR ‘hight content’ OR ‘*in vitro*’ OR invitro) AND screen*) OR Toxcast OR ‘tox cast’ OR ‘Tox 21’ OR Tox21) Indexes='SCI-EXPANDED,' BKCI-S, ESCI, CCR-EXPANDED, IC Timespan='All' years