Scientific Review of AOP 442 : Binding to VGSC during development leads to cognitive function decrease

The scientific review has been independent, and conflicts of interest avoided; Reviewers have been transparently selected as part of EFSA PPR Panel and Working Group experts; Public disclosure on AOP-Wiki of (i) the names of the reviewers and of the review manager, (ii) the collective outcome of their individual declaration of interest analysis, (iii) reviewers' comments and the responses of the AOP authors to the reviewers' comments, (iv) the summary of the scientific review, as appropriate is transparently reported; The collective scientific expertise of the Review panel covers the full scope of the AOP.

Peer reviewers:

- Marina Marinovich, Member of EFSA Plant Protection Products Panel. Professor Toxicology. University of Milan.
- Tamara Coja, Chair of EFSA Plant Protection Products Panel. Senior Expert Toxicology at AGES Österreichische Agentur für Gesundheit und Ernährungssicherheit
- David Pamies, Head of Organoid Facility at the University of Lausanne. Lausanne, Switzerland.

Page	Text and revision as appropriate	EFSA PPR Panel Comment	EFSA Response
3	**AOP Developmental Strategy** This AOP was originally started as a case study for an evidence-based AOP informed IATA for a single chemical developmental neurotoxicity hazard characterization. This case study was developed to support human health ~~risk assessment~~hazard characterization of the pyrethroid pesticidal active substance, deltamethrin, and as a proof of concept on the applicability of the data provided in the Developmental Neurotoxicity In vitro Battery.	Risk assessment relies on hazard and exposure characterisation. AOPs are not at a phase where one could compare the results of most KE to a real exposure and identify whether a subsequent key event or adverse effect would be caused. Waiting for qAOP , it need to be stressed that currently AOPs are useful to identify plausibility of causation	Thank you for this comment. The technical report has been updated based on this.
4	Mapping of the literature landscape analysis culminated in identification of a new downstream KE. A chemically agnostic AOP was then developed by conducting both systematic	AOP should be agnostic. But does this still apply when the MIE target behaves like a	The agnostic concept in the AOP framework refers to the toxicodynamic dimension of the framework. This means that the sequence of KEs

	broad and focused literature searches (i.e., searching literature using systematic search terms and, most importantly, providing a transparent description of how the literature was searched and selected) in order to collect empirical evidence in support of proposed KERs.	receptor and thus can only identify one type of chemical structure?	from the activation of the MIE to the AO , when triggered, is qualitatively, and possibly quantitatively (e.g. throughout a response response mathematical equation) independent from the substance enabling the activation. Therefore, the MIE, in this case, which is dealing with a voltage gate Na channel inhibition, is a toxicodynamic event for which the selected class of stressor fits with natural and synthetic pyrethroids (Class I and class II) as well as with natural toxins of different chemical class (e.g. TTX). It is indeed expected that, independently of the chemical class, the inhibition of the channels will deliver the same cascade of events if sufficiently activated, even if the initial effect is the consequent of an artificial electrophysiological based inhibition. This is substantiated and here described, in the quantitative section, where is reported that a computer based electrophysiological simulation, can derive a change in the neuronal action potential.
6	Life Stages template	why the template is different from above?	why the template is different from above?
7	**MIEs Description: Binding to voltage-gated sodium channel (VGSC)** Due to their critical role in neuronal function, sodium channels are known molecular targets of neurotoxins and neurotoxicants (Caterall et al., 2012; Wakeling et al., 2012).	The phrase seems to allude to a wide range of substances, whereas in fact the reference is focused only on pyrethrins. Isn't there a better reference?	Thank you. A more general reference has been included. Catterall, W. A. (2012). Voltage-gated sodium channels at 60: structure, function and pathophysiology. The Journal of physiology, 590(11), 2577-2589. https://doi.org/10.1113/jphysiol.20
8	**Biological domains of applicability** VGSCs are present in many different cell types of the ~~central~~ nervous system (~~C~~NS), including neurons, oligodendrocytes,	The sentence says "VGSCs are present in many different cell types of the central nervous system (CNS)" but Schwann cells are the **glial cells that form the myelin sheath**	Thank you

	Schwann cells (Baker, 2002; Jessen and Mirsky, 2005; Ritche, 1992; Chiu, 1991) and microglia (Jung et al., 2013; Black and Waxman reviewed in Hossain et al., 2017; Paez et al., 2009; Berret et al., 2017).	**on axons outside the brain**. May be the term central can be omitted, also considering table 1	
9	Table 1 sodium channel alpha subunit nomenclature and effects of pyrethroids	what does TTX-sensitivity mean? Is it useful for classification? for potency?	TTX has been widely used as a chemical tool for blocking Na+ channels and are classified as TTX sensitive or resistant. This has been used to study the ontogenesis of VGSCs. We could include a sentence if it can help but the ontogenesis of VGSC is very complex and was outside our scope with the AOP
10	**Life Stages:** All life stages.	is there any coding of what the life stages are	https://aopwiki.org/handbooks/3. The structured ontology terms for life-stage are more comprehensive than those for taxa, but may still require further description/development and explanation in the free text section. Tbd in the panel
19	**Figure 1.** The three existing states of the VGSCs: Deactivated (closed), activated (open) and inactivated (closed). Figure extracted from Wakeling et al., 2012).	The figure would need the origin of source. I was also wondering if the details on VGSC gates (m, h, n) could be shown in a figure to better visualise it.	Origin included. Figure complemented
21-22	**Biological Context** - Level of Biological Organization: Cellular - **Cell term** ~~- Further information on Event Components and Biological Context may be viewed on the attached pdf. The biological context describes the location/biological environment in which the event takes place. For molecular/cellular events this would include the cellular~~	Thank you for this. This information is from the instructions. It has been deleted to avoid confusion.	

	~~context (if known), organ context, and species/life stage/sex for which the event is relevant. For tissue/organ events cellular context is not applicable. For individual/population events, the organ context is not applicable.~~ - **Organ term** - ~~Further information on Event Components and Biological Context may be viewed on the attached pdf. The biological context describes the location/biological environment in which the event takes place. For molecular/cellular events this would include the cellular context (if known), organ context, and species/life stage/sex for which the event is relevant. For tissue/organ events cellular context is not applicable. For individual/population events, the organ context is not applicable.~~		
22 and 28	**Other AOPs that use this KE** - **none**	part of an evidence based AOP informed IATA for deltamethrin developmental neurotoxicity? why NONE if part of an evidence-based AOP-informed IATA developmental neurotoxicity hazard characterization of deltamethrin.	This refers only to AOPs that are included in the Wiki
25	**Electrophysiological Techniques ~~For~~for Measurements of Action Potentials** There are a wide variety of electrophysiological techniques that allow for action potential measurement. At their core, all of them allow the recording of changes in either membrane potential or currents flowing across the membrane, and all ~~are capable of doing~~can do so with high temporal resolution	No references are included in this part of the text; I assume there is literature related to measurement techniques as well? If Khadria, 2022 and Ogden, 1994 are papers where the methods are described, I would suggest to include it at the beginning of the chapter, rather than at the end of optical measurements.	Thank you. Addressed

	(milliseconds) necessary to record APs. Different configurations each have inherent advantages and disadvantages and the selection of the appropriate technique depends on the specific questions to be addressed by an experiment. All these approaches make use of one or more electrodes to measure the electrical responses (changes in membrane voltage or current) in a cell or group of cells. The electrodes can be of various sizes and shapes, and may be placed inside the cell (intracellular recordings), on the cell (patch clamp recordings), or adjacent to the cell (extracellular recordings). Please, see Khadria, 2022 and Ogden, 1994 for further details.		
30	**How it is measured or detected** Neurotransmission can be measured by a wide variety of different approaches. The same technologies described in KE2 for AP generation can be used to measure neurotransmission by applying different protocols. These include patch clamp, intracellular and extracellular recordings, microelectrode array (MEA) recordings. KE 2005 can be measured using many methodologies that examine neural connectivity (i.e., neurotransmission), including the *in vitro* NNF assay. A standardized NNF test system to assess the potential impact of chemical exposure on neural network formation and function has been developed using rodent cortical neurons (Frank et al., 2017).	Please include references for measurement techniques.	Thank you
31	Components of the synapse include (a) a presynaptic module, in which calcium signals are transduced into chemical secretions (known as excitation–secretion coupling); (b) a postsynaptic module (postsynaptic density), which comprises the proteins that support the specialized postsynaptic membrane and the signalling that goes on there; and (c) a	This concept, present also below, may be deserve some line of explanation	Agree. Addressed in tracking.

	module that determines the specific wiring diagram of neurons during development (axonogenesis). Connections between neurons can be, in this way, mapped by acquiring and analyzing electron microscopic wiring diagrams.		
37	Figure 2. Trisynaptic circuit of hippocampal formation. For further details see Amaral and Lavenex, 2006.	Please include the title of Figure 2 and source of the picture.	We have created this one. Thank you
64	A variety of standardized learning and memory tests have been developed for human neuropsychological testing. These include episodic autobiographical memory, word pair recognition memory; object location recognition memory. Some components of these tests have been incorporated in general tests of adult intelligence (IQ) such as the Wechsler Adult Intelligence Scale (WAIS) which calculates four composite scores that examine various domains within an individual's overall cognitive ability: Verbal Comprehension Index (VCI), Perceptual Reasoning Index (PRI), Working Memory Index (WMI), and Processing Speed Index (PSI) (Climie and Rostad, 2011). Modifications have been made and norms developed for incorporating tests of learning and memory in children. Examples of some of these tests include:	tests used in the diagnosis of Alzheimer disease?	Alzheimer's disease falls outside the scope of this document, where the adverse outcome is "altered learning and memory" (i.e., cognition). This neurobehavioural outcome may occur not only in Alzheimer's disease but also in other neurodedegenerative disorders, neurological conditions and neurodevelopmental outcomes. The latter are the focus of this document. Anyhow, below some neuropsychological tests commonly used to assess cognitive decline in Alzheimer's disease are listed (Wang et al., 2023; https://eurjmedres.biomedcentral.com/articles/10.1186/s40001-023-01265-6). These tests offer convenience, affordability, and non-invasiveness in clinical settings. a) Montreal Cognitive Assessment (MoCA): A widely used screening tool that assesses various cognitive domains, including memory, attention, and executive function. b) Mini-Mental State Examination (MMSE): Another common test that evaluates cognitive

			function, including memory, orientation, and language. c) Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog): Specifically designed to assess cognitive decline in AD, this test covers memory, language, and other cognitive functions.
68	Vorhees, C and Williams M. **Tests for Learning and Memory in Rodent Regulatory Studies.** Current Research in Toxicology, **2024,** ~~**in press**~~ **Curr Res Toxicol. 2024; 6: 100151. doi: 10.1016/j.crtox.2024.100151**	Please check, seems to be already available.	Thank you
73	**Quantitative Understanding of the Linkage** There are currently no quantitative models that predict the relationship between these KEs.	Is there information to be added for quantitative understanding of the linkage? If not, maybe a sentence to explain.	Thank you
73	There are currently no quantitative models that predict the relationship between these KEs. However, it is possible to compute the population of VGSC that are ~~modified~~ affected by pyrethroid binding, and it has been estimated that less than 1% of the VGSC population (Narahashi et al., 1998) needs to be bound by pyrethroid to disrupt excitability in the neuron (KER2).	affected? impacted? involved?	addressed

85	**Figure 4. Sequence of Events from action potential generation to synaptic transmission.** **Self produced by EFSA WG.**	Please add origin of the figure, if not self produced.	Thank you
88	**Known Feedforward/Feedback loops influencing this KER** There are currently no known Feedforward/Feedback loops influencing this KER .	Is information to be added here?	Thank you
93	Spontaneous neurotransmitter release plays an important role in shaping neuronal morphology as well as modulating the properties of newly forming synaptic connections in the brain (Andreae and Burrone, 2018). Excessive or insufficient neurotransmission during critical windows of development can affect the complexity of the connectivity within pre- and post-synaptic neurons, leading to altered synaptic density and connectivity. The delicate balance between excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmission shapes brain circuitry, and when perturbed, it can lead to abnormal network activity (Cherubini et al., 2021). This has been widely studied in the hippocampus.	Throughout the paper, the importance of sodium channels in the proper development of many brain and non-brain functions is highlighted. So why do we only see a cognitive deficit after modification of this MiE? perhaps you can stress why you decided to look at this AO	The adverse consequences of a chemical interference at the VCSC will depend both on severity, duration, and developmental timing, indicating that exposure could produce different effects at different developmental windows of exposure. It is important to note that this could also occur in other areas of the brain as VGSC are foundational to the structure and function of all neurons. Here we focus on the hippocampus because of its well-known ties to cognition, and downstream outcome of concern for many chemical exposures, but there is less empirical evidence and biological knowledge on the adverse consequences in other brain areas,. Future work is required to develop an AOP and KERs for other brain areas.
95	**Dose and temporal concordance** Dose-response data is lacking for this KER. For future research, it is critical to generate data in which the upstream KE is modulated in a 'dose-response' manner to better support the causative relationship.	Is there information to be added?	Thank you

106 and 117	**Known modulating factors** There are currently no known modulating factors. **Known Feedforward/Feedback loops influencing this KER** There are currently no known Feedforward/Feedback loops influencing this KER .	Is there information to be added?	addressed
112	~~AhR activation in the thyroid leading to Subsequent Adverse Neurodevelopmental Outcomes in Mammals~~ | ~~adjacent~~ | ~~Moderate~~ | ~~Moderate~~ | ~~Prakash Patel (send email)~~ | ~~Under development: Not open for comment. Do not cite~~	why the same event by the same Author has different WoE and Quantitative understanding?	Good question, something is wrong in the Wiki. Maybe he included twice for a mistake. I would delete it from here to avoid misunderstandings.
125	**Life Stage Applicability** Life stage: during brain development (embryonic, fetal ~~infancy~~, childhood, adolescence developmental periods)	what does it mean? Is it a scientifically defined period?	Is it a scientifically defined period?

Specific questions

Reviewer 1.

1. **Scientific quality:**

o Does the AOP incorporate all appropriate scientific literature and evidence?

o Does the scientific content of the AOP reflect current scientific knowledge on this specific topic? yes as far as I can judge based on my knowledge

2. Weight of evidence (WoE):

o Is the WoE judgement/scoring well described and justified based on the evidence presented? If not, please explain. Yes, it is.

o Please consider WoE for each Key Event Relationship (KER) and for the AOP as a whole

The overall WoE for this AOP is strong, mainly for KER1-3 well supported by studies and general knowledge.

Reviewer 2.

1. Scientific quality:

o Does the AOP incorporate all appropriate scientific literature and evidence? According to Appendix A, a literature search was performed for event KE4 (altered neuronal network function) and the AO (impairment behavioural function), for which the knowledge was less well-established. It is assumed that this includes all appropriate scientific literature. The references for other parts of AOP were collected in previous exercises as a systematic literature search and they reflect current scientific knowledge.

o Does the scientific content of the AOP reflect current scientific knowledge on this specific topic? Yes, as far as I can judge with my knowledge.

2. Weight of evidence (WoE):

o Is the WoE judgement/scoring well described and justified based on the evidence presented? If not, please explain. Yes, the WoE judgement/scoring is considered very well described and justified.

o Please consider WoE for each Key Event Relationship (KER) and for the AOP as a whole

The expert knowledge elicitation was performed within an EFSA working group. The conclusions were achieved through discussions of individual judgements done by experts from different knowledge domains. The outcome is considered as very well explained and justified, for each KER and for the AOP as whole.

Reviewer 3.

1. **Scientific quality:**

o Does the AOP incorporate all appropriate scientific literature and evidence?

Yes. EFSA developed this AOP originally as an evidence stressor based AOP informed IATA (Available at ***OECD IATA CS 362. https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/cbc/mono(2022)24&doclanguage=en)***. For this IATA a systematic literature review was done for all deltamethrin Evidence and 3776 references were screened. For in vitro evidence 31 papers selected measuring 60 DNT endpoints, for HOS 8 publications selected measuring 11 DNT endpoints; for in vivo 17 publications selected measuring 52 DNT endpoints. The IATA culminated with strong empirical evidence that deltamethrin interacts with the biological target (MIE) and may subsequently cascade through a series of measurable KEs, ultimately resulting in an adverse health outcome (AO). In this IATA Key Event Relationships (KERs) provided evidence for causality using experimental data only for deltamethrin. Although the MIE and early event KEs had strong empirical support, the more downstream KEs and KERs did not. And downstream KERs were not adjacent. In order to further develop the non adjacent KERs several literature review tools were used in a step wise approach. Machine learning Tools (i.e., topic modeling) were employed to identify additional essential Key Events and to increase the scope of empirical evidence. Mapping of the literature landscape analysis culminated in identification of a new downstream KE. A chemically agnostic AOP was then developed by conducting both systematic broad and focused literature searches (i.e., searching literature using systematic search terms and, most importantly, providing a transparent description of how the literature was searched and selected) in order to collect empirical evidence in support of proposed KERs, all KERs of the AOP are now adjacent.

EFSA systematic methodology allowed to have a structured, evidence based and transparent approach that allows reproducibility of the work done. The implementation of a broad search to retrieve the available evidence, the exploration of the large corpus of papers with topic modelling, the use of tools such as Abstract Sifter to streamline the search string for retrieval of evidence of the additional KEs, the reconciliation of the final structure of the AOP with others uploaded in the AOP Wiki increase the quality of the methodological approach used.

o Does the scientific content of the AOP reflect current scientific knowledge on this specific topic?

Yes. The last update of the literature review was done in 2024 and in addition, in line with the AOP development handbook, a search was done in the AOP Wiki to find existing KEs and KERs that may be common to those in the newly developed AOP and the content adapted for KE757, KE758, KER749 and KER748.

The documentation provided allows traceability of the strategy and results.

2. Weight of evidence (WoE):

o Is the WoE judgement/scoring well described and justified based on the evidence presented? If not, please explain.

o Please consider WoE for each Key Event Relationship (KER) and for the AOP as a whole

EFSA applied a semi-formal Expert Knowledge Elicitation for the WoE assessment of the AOP. The uncertainty in the KERs was assessed using a structured expert knowledge approach weighing the evidence collected in the previous steps. In line with recommendations from the AOP handbook, only biological plausibility and empirical support were considered for rating certainty for KERs whereas essentially of the KEs was assessed separately. Biological plausibility and empirical support of the KERs along with essentiality of the KEs are described in line with the OECD AOP handbook (OECD, 2017) as crucial considerations for the assessment of the certainty in the causality of the AOP sequence.

The expert knowledge elicitation was performed with 8 WG members participating as domain experts. The experts were requested to answer the questions in the OECD Handbook. After discussion of the individual judgements, the experts achieved a collegial judgement for each of the KERs in the AOP and the two criteria biological plausibility and empirical support. The WoE was independently assessed by a group of 8 EFSA Working Group Experts with demonstrated independence and individual declaration of interest analysis. It is acknowledged that the EKE methodology is novel for AOP WoE assessment but clearly reported in the AOP documentation.

This is considered an added value since conflicts of interest avoided, e.g. experts have been transparent selected.