**Adverse Outcome Pathway (AOP) External Review Report**

**Title: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities (AOP 13)**

**OECD Project 1.22**

***AOP Reviewers:***

**Gemma Calamandrei, PhD**

Neuroscientist and Head of Neurotoxicology and Neuroendocrinology Unit

Italian National Health Institute, Italy

(Government/Experimental and Clinical Research)

**Didima M.G. de Groot, PhD, ERT**

Senior Scientist/Consultant Developmental Biology/Neurotoxicology and Safety Pharmacology

TNO and TNO Triskelion BV, The Netherlands

(Academic and Regulatory)

**April P. Neal Kluever, PhD, DABT**

Toxicologist

Center for Food Safety and Applied Nutrition, USFDA, USA

(Government/Regulatory)

**Merle G. Paule, PhD, ATS**

Senior Biomedical Research Scientist, and Director of Division of Neurotoxicology at NCTR, USFDA, USA

(Government/Basic and Clinical Research)

**Kaoru Sato, PhD**

Neuroscientist and Head of Neuropharmacological Laboratory,

Division of Pharmacology, National Institute of Health Sciences, Japan

(Government/Regulatory and Basic Neuroscience)

**Cristina Suñol, PhD**

Neurotoxicologist and Chief of the Neurotoxicity Group

Spanish National Research Council (CSIC), CIBER Epidemiologia y Salud Pública (CIBERESP), Spain

(Government/Basic Neurotoxicity)

***AOP Authors:***

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**Main Issues From AOP Reviewers and Responses From AOP Authors**

**Reviewers Comments (here onwards “comment”):**

*1a. The AOP is weak regarding electrophysiological aspects of NMDAR inhibition. An electrophysiologist should be included in the revision.*

*1b. The AOP is weak regarding epidemiological data in the context of exposure to NMDAR antagonists in human children. An epidemiologist should be included in the revision.*

**Authors Response (here onwards “response”):**

AOP Wiki is considered an interactive source for sharing knowledge in the field of toxicology and the idea is that people with different background and expertise can interact and contribute by editing the existing text. Furthermore, AOPs are not considered systematic reviews that will have to capture all the existing scientific knowledge and mostly relies in citing key papers and recent reviews. The authors believe that the key references and the reviews have been captured, and with reviewers help, more scientific literature will soon be cited in this AOP, improving the content significantly. The authors state that they cannot be an electrophysiologist, epidemiologist and molecular toxicologist at the same time and, most probably for this reason, some sections of the AOP present more weaknesses than others and the authors hope to improve these sections by inviting experts in the suggested fields.

Authors included a renowned electrophysiologist who has addressed electrophysiological inhibition of NMDARs and the authors have addressed these weaknesses. After discussion with the reviewer, it was agreed that an epidemiologist is not needed, and the authors have added all the suggested references related to epidemiological studies.

**Comment:**

1. *One reviewer suggests revision of the scheme and addition of the effect “Altered Ca2+-dependent gene transcription” after the effect “Reduced intracellular Ca2+ levels.”*

**Response:**

Regarding the recommendation of having in the AOP an additional effect entitled “Altered Ca2+-dependent gene transcription”, although that was the initial intention, when the authors were drawing this AOP, the need to have a key event (KE) that is measurable and essential to the progression of a defined biological perturbation leading to a specific adverse outcome made the authors decide to keep only brain derived neurotrophic factor (BDNF) as one of the most important Ca2+-dependent genes for neuronal differentiation during brain development. KE descriptions and weight of evidence (WoE) evaluation cannot be performed easily in so general KEs (Ca2+-dependent genes). Therefore, to be more specific the authors have selected BDNF so the assays for measurements of BDNF (at the mRNA and protein levels) can be applied for quantitative evaluation. However, new AOPs can be developed, describing other relevant genes that can be linked to the present AOP and an AOP Network can be created.

**Comment:**

1. *In the context of learning and memory, brain regions (cerebellum and basal ganglia) other than hippocampus and cortex need to be included.*

**Response:**

In the KE (Learning and memory, impairment) the authors indicate that "It is appropriate to state that while much emphasis has been given on the key role of the hippocampus in memory, it would probably be simplistic to attribute memory deficits solely to hippocampal damage (Barker and Warburton, 2011). There is substantial evidence that fundamental memory functions are not mediated by hippocampus alone but require a network that includes, in addition to the hippocampus, anterior thalamic nuclei, mammillary bodies and cortex (Aggleton and Brown, 1999; Mitchell et al., 2002). Cerebellum and basal ganglia, in addition to hippocampus and cortex, have been added as it was suggested by the reviewer.

**Charge Question 1: *Check if the AOP incorporates the critical scientific literature and if the scientific content of the AOP reflects the current scientific knowledge on this specific topic***

**Comment:**

1. *One reviewer suggests that this AOP can be improved by a more complete presentation of the complexity of the adverse outcome. Further inclusion of some suggested studies on the specific aspects of learning and memory in rodent models is needed.*

**Response:**

The KE "Learning and memory, impairment" has been amended incorporating information from the suggested review by Quirk GJ, Mueller D. (2008) Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology. 33: 56-72.

Information from this review (Mullally SL, Maguire EA., 2014. Learning to remember: the early ontogeny of episodic memory. Dev Cogn Neurosci. 9: 12-29) has been used to enrich the text of KE "Learning and memory, impairment" and to indicate the differences that exist between mature and developing organisms.

From the suggested studies on developmental neurotoxicity of NMDA receptor antagonism that focused on the specific aspect of learning and memory in rodent models, the studies of Billinger and Wormley have not been included as the first refers to ethanol and the second to dioxins. The ethanol has been taken out in the empirical support sections and the dioxins are not relevant to this AOP.

All other suggested studies on developmental neurotoxicity of NMDA receptor antagonism that focused on the specific aspect of learning and memory in rodent models have been added in the appropriate sections.

**Comment:**

1. *In the context of in silico studies on the prediction of NMDAR targeting, one reviewer has suggested citation of more relevant references.*

**Response:**

Authors provided relevant references in the revised AOP.

**Comment:**

1. *In addition to the effects of lead, social and parenting factors can cause cognitive deficits.*

**Response:**

Authors have addressed the social and parenting factors in the revised AOP.

**Comment:**

1. *One reviewer suggests that the scheme needs to be revised. That is after cellular effect “Reduced intracellular calcium levels,” add another event “Altered Ca2+-dependent gene transcription.”*

**Response:**

Authors incorporated suggested changes in the revised AOP.

**Comment:**

*5a. Under the heading “Calcium influx, decreased,” and subheading “How it is measured or detected,” one reviewer suggests an extended discussion on electrophysiological method (patch clamping), in addition to fluorescent methods.*

*5b. Under the heading “Neuronal network function in adult brain, decreased,” and subheading “How it is measured or detected,” one reviewer suggests additional discussion on electrophysiological methods, besides microelectrode array (MEA).*

**Response:**

Authors have added an electrophysiological method and additional discussion in the revised AOP.

**Comment:**

1. *Under the heading “Cell death, N/A indirectly leads to synaptogenesis, decreased,” subheading “Uncertainties or inconsistencies,” it is stated that Pb2+ caused a downregulation of Syn1 gene in the hippocampus of male PND 60 rats. Why is this statement an inconsistency?*

**Response:**

Authors have provided further explanation in the revised AOP and indicated that this is correct. It was explained in the conference call that N/A refers to the Action required (e.g. reduced, increased, impaired, upregulated, etc.) which is not applicable in the case of cell death.

**Comment:**

1. *One reviewer suggests that this AOP could be improved if greater details are provided regarding the type of BDNF (mRNA, protein) measured in each cited study.*

**Response:**

Authors decided to keep KE as it is, and explanation is provided for the type of BDNF. The reviewer was satisfied with the authors’ response to this comment during the teleconference (TC).

**Comment:**

1. *One reviewer suggests that the title of this AOP should be changed to “Chronic binding of antagonists to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities.” Further, the reviewer also suggests the development of another AOP on “Subchronic/acute binding of antagonists to N-methyl-D-aspartate receptors (NMDARs) during brain development.”*

**Response:**

The authors have changed the title to “**Chronic binding of antagonists to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities”**. However, the authors have been instructed not to define the KE as acute or chronic binding, but rather simply as binding and then utilize the appropriate key event relationship (KER) to determine which trajectory it heads down. Furthermore, a second AOP entitled “Subchronic/acute binding" can be developed in the future either by the current authors or somebody else.

**Comment:**

1. *One reviewer suggests revising the flow chart, as reduction in BDNF may not be the sole pathway.*

**Response:**

The authors do not claim that BDNF is the sole pathway, but they do claim that it is one of the most important pathways, that is why it is well studied. Other developers can suggest a different pathway by sharing some of the KEs and KERs upstream or downstream.

In the KER "Release of BDNF, Reduced" indirectly leads to "Dendritic morphology, Aberrant". Under the Uncertainties or Inconsistencies, the authors state: "Various molecular mechanisms have been identified as regulators of dendritic arborisation patterns and dendtitic spine formation (Jan and Jan, 2010). More specific, transcription factors, growth factors, receptor-ligand interactions, various signaling pathways, local translational machinery, cytoskeletal elements, Golgi outposts and endosomes have been identified as contributors to the organization of dendrites of individual neurons and the contribution of these dendrites in the neuronal circuitry (Jan and Jan, 2010)."

The inhibitory effect (efficacy) of antagonists on NMDA receptors has been found to be dependent on the type of subunits that form the NMDA receptors depending on the developmental stage, and the chemical structure of the antagonists. So, the authors acknowledge that the NMDAR subtypes can influence the outcome.

**Charge Question 2: *Verify the weight of evidence judgement/scoring provided by AOP developers for KEs, KERs and the overall AOP***

**Comment:**

1. *One reviewer suggests that the authors should provide more details on the role of BDNF increase/decrease in synaptogenesis, dendritic arbor, spine formation, and cell death (apoptosis).*

**Response:**

The authors improved this section and incorporated the suggested reference(s).

**Comment:**

1. *One reviewer suggests that the KE should be re-ordered in the text, since the text does not follow the flow chart or scheme.*

**Response:**

This is an IT issue and will be fixed soon.

**Comment:**

1. *In the present form, this AOP is focused and limited to “synaptogenesis.” One reviewer suggests that this AOP should be reorganized according to the developmental stages and should include neurogenesis, migration of neuroblasts, neurite elongation, and synaptogenesis.*

**Response:**

During the conference call it was agreed not to make any changes as all the suggested developmental process like neurogenesis, migration, neurite outgrowth can be altered and have an impact on synaptogenesis.

**Charge Question 3: *What would be the regulatory applicability of this AOP in your opinion?***

**Comment:**

1. *None of the reviewers identified any major issue in relation to regulatory applicability of this AOP.*

**Response:**

The authors thanked all reviewers for recognizing the potential regulatory context of this AOP.

**Charge Question 4: Overall assessment of the AOP-*Would you recommend this AOP to be submitted to the Working Group of the National Coordinators for the Test Guidelienes Programme (WNT) and the Task Force on Hazard Assessment (TFHA) for endorsement?***

**Comment:**

1. *All reviewers suggested recommendation of this AOP to WNT and TFHA, pending a satisfactory revision.*

**Response:**

The authors thank all reviewers for suggesting the potential recommendation of this AOP to WNT and TFHA. The authors have done their best to revise the AOP based on the suggested comments and corrections as thoroughly as possible.

**Conclusion**:

As review manager, I would like to applaud the authors of this AOP, which deals with a very timely topic. Also, I would like to commend the reviewers for their constructive comments and suggestions. This report is prepared based upon information that I gathered from the reviewers, authors, teleconferences and AOPWIKI. All reviewers are satisfied with authors’ responses and all key issues are resolved.

Respectfully submitted to OECD Secretariat by:

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