HHS Public Access

Author manuscript

Neurotoxicology. Author manuscript; available in PMC 2018 March 01.

Published in final edited form as:

Neurotoxicology. 2017 March ; 59: 240–255. doi:10.1016/j.neuro.2016.05.010.

Developing and applying the adverse outcome pathway concept for understanding and predicting neurotoxicity

Anna Bal-Price[1,*], **Pamela J. Lein**[2], **Kimberly P. Keil**[2], **Sunjay Sethi**[2], **Timothy Shafer**[3], **Marta Barenys**[4], **Ellen Fritsche**[4], **Magdalini Sachana**[1,#], and **M.E. (Bette) Meek**[5]

[1]European Commission Joint Research Centre, Institute for Health and Consumer Protection, Ispra, Italy

[2]Department of Molecular Biosciences, School of Veterinary Medicine, University of California Davis, Davis, California, 95616 USA

[3]Integrated Systems Toxicology Division, Office of Research and Development, U.S. Environmental Protection Agency, RTP, USA

[4]IUF - Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany

[5]McLaughlin Centre for Risk Science, University of Ottawa, Ottawa, Canada

Abstract

The Adverse Outcome Pathway (AOP) concept has recently been proposed to support a paradigm shift in regulatory toxicology testing and risk assessment. This concept is similar to the Mode of Action (MOA), in that it describes a sequence of measurable key events triggered by a molecular initiating event in which a stressor interacts with a biological target. The resulting cascade of key events includes molecular, cellular, structural and functional changes in biological systems, resulting in a measurable adverse outcome. Thereby, an AOP ideally provides information relevant to chemical structure-activity relationships as a basis for predicting effects of structurally similar compounds. AOPs could potentially also form the basis for qualitative and quantitative predictive modeling of the human adverse outcome resulting from molecular initiating or other key events for which higher-throughput testing methods are available or can be developed.

A variety of cellular and molecular processes are known to be critical for normal function of the central (CNS) and peripheral nervous systems (PNS). Because of the biological and functional complexity of the CNS and PNS, it has been challenging to establish causative links and quantitative relationships between key events that comprise the pathways leading from chemical exposure to an adverse outcome in the nervous system. Following introduction of the principles of MOA and AOPs, examples of potential or putative adverse outcome pathways specific for

[*]Corresponding author, Anna Bal-Price, (anna.price@ec.europa.eu).

[#]Environment Health and Safety Division, Environment Directorate, Organisation for Economic Co-operation and Development (OECD), Paris, France

developmental or adult neurotoxicity are summarized and aspects of their assessment considered. Their possible application in developing mechanistically informed Integrated Approaches to Testing and Assessment (IATA) is also discussed.

Keywords

Adverse Outcome Pathways; Mode of Action; neurotoxicity; risk assessment

INTRODUCTION

Modes of Action (MOA) and Adverse Outcome Pathways (AOPs) describe mechanistic knowledge at varying levels of biological organization to facilitate its assimilation, integration and evaluation for research and regulatory applications. While conceptually similar, MOA includes chemical related key events (KEs) such as metabolism, whereas AOPs are restricted to the biological cascade of KEs resulting from perturbation by a stressor. Thus, AOPs describe a sequence of measurable KEs originating from a molecular initiating event (MIE) resulting in cellular, structural and functional changes and ultimately measurable adverse outcomes (AOs) relevant to the human organism and the human population.

Recent international developments are anticipated to contribute to increasing collective confidence in applying AOPs for both regulatory risk assessment and research. These include an update of the World Health Organization/International Programme on Chemical Safety (IPCS) mode of action/human relevance (MOA/HR) framework. The modified framework is incorporated within an iterative roadmap, encouraging continuous refinement of problem formulation and MOA-based (integrated) testing and assessment strategies, with increasing reliance on *in vitro* methods at lower levels of biological organization in preliminary assessment and testing strategies. Weight of evidence (WoE) considerations for hypothesized MOAs/AOPs have been developed in this update and more recently evolved as a basis for contributing to the revision of guidance and electronic tools for an international knowledge base of AOPs, which was developed for an initiative of the Organization for Economic Cooperation and Development (OECD). These advances in considering weight of evidence as a basis to increase consistency and confidence in potential applications of AOPs are summarized and illustrated by examples for developmental or adult neurotoxicity. Possible application in developing mechanistically informed Integrated Approaches to Testing and Assessment (IATA) is also discussed.

1. The Need for Mechanistically Based Testing and Assessment

There is growing recognition of the need for more efficient methods and strategies to assess the hazards, exposures and risks of the wide array of chemicals to which humans are exposed. This has been reflected in, among others, progressive regulatory mandates in Canada, the European Union and, more recently, the Asian Pacific region, to systematically consider priorities for risk management for existing chemicals (see, for example, Council of Labor Affairs, Taiwan, 2012; Dellarco et al., 2010; European Commission, 2006; Hughes et al., 2009; Lowell Center for Sustainable Production, 2012; Meek and Armstrong, 2007).

This necessitates focus on rationally prioritized chemicals and endpoints, rather than the traditional time- and resource-intensive series of standard *in vivo* toxicology studies. It also requires the development and integration of information on KEs that will enable effective use of data collected from lower levels of biological organization and non-test methods, such as (quantitative) structure–activity relationships ((Q)SAR) and read-across relationships based on *in vitro* assays.

2. Evolution of MOA Analysis

MOA, as previously defined, is a biologically plausible series of KEs leading to an effect (Sonich-Mullin et al., 2001) which has traditionally, been considered in the context of specific chemicals and/or chemical groups. Formal MOA/HR (mode of action/human relevance) analysis is designed to increase transparency in the systematic consideration of the WoE of hypothesized MOA(s) for critical effects and their relevance to humans. An associated framework was developed in response to initiatives of the International Life Sciences Institute Risk Sciences Institute (ILSI RSI) and the IPCS, and derives from earlier work on MOA by the U.S. Environmental Protection Agency (USEPA, 2005) and IPCS (Sonich-Mullin et al., 2001).

The development and evolution of the IPCS ILSI RSI MOA/HR framework is described in several publications (Boobis et al., 2006; Boobis et al., 2008; Meek, 2008; Meek et al., 2003; Seed et al., 2005). Though developed principally to encourage the systematic application of mechanistic data in hazard characterization, risk assessment and identification of associated critical data gaps, more recently its use for a broader range of research and predictive contexts has also been considered (Carmichael et al., 2011; Meek and Klaunig, 2010). The framework is illustrated by an increasing number of case studies (n=30, currently), and is widely adopted in international and national guidance and assessments (Meek et al., 2008). Building on this experience, the framework has been updated recently to address uncertainty and to extend its utility to emerging areas in toxicity testing and non-testing methods by increasing understanding of its role in integrating information from different levels of biological organization. The update includes incorporation within a roadmap, encouraging continuous refinement of fit-for-purpose testing strategies and hazard characterization (Meek et al., 2014a).

3. Weight of Evidence (WoE) in MOA Analysis

The WoE for hypothesized MOAs in animals is addressed based on a specified subset of considerations modified from those proposed by Bradford Hill (B/H) for assessment of causality in epidemiological studies (Hill, 1965). To promote consistency in their application, defining questions and the nature of supporting data for each of the relevant considerations has been additionally delineated (Meek et al., 2014b). The modified considerations have also been rank ordered to reflect evolving experience concerning their relative contribution to WoE determinations (Meek et al., 2014b).

The approach provides for transparent and consistent framing of comparative WoE for contrasting hypotheses for overall data synthesis and evaluation of sufficiency, as a basis to support regulatory decision making. As such, it represents perhaps the only (or one of a very

few) frameworks designed to formally address transparency and consistency of higher level analysis involving assimilation and weighting of data from different lines of evidence (e.g., epidemiological, toxicological, mechanistic) based on defined *a priori* considerations to address critical aspects of options analysis for decision making.

In MOA analysis, human relevance or species concordance is also systematically considered, taking into account more generic information such as anatomical, physiological and biochemical variations. Application of the framework, involves: (1) delineation of KEs leading to the end (adverse) effect in a hypothesized MOA; (2) comparative systematic evaluation of the extent of the supporting WoE from toxicological and mechanistic studies for different hypotheses (Meek et al., 2014b); and (3) systematic consideration of the likely implications for hazard in humans (both qualitative and quantitative) based on hypothesized KEs. This analysis provides the foundation for subsequent considerations of chemical-specific dose-response and estimates of risk.

4. AOPs and Knowledge Base

More recently, the AOP concept has emerged from the field of ecotoxicology as a means to enhance the utility of QSAR modelling, biomarkers and other types of mechanistic data for both understanding and predicting potential adverse effects of chemical exposure in wildlife populations (Ankley et al., 2010). While experience in MoA analysis focused principally on later stages of cellular, biochemical and tissue events, the AOP focus of the eco-toxicological community (consistent with that in QSAR modelling) was on the chemically mediated MIE (equivalent to an early KE in a MOA) and AOs. They have evolved more recently to encompass only the non-chemical specific KEs (i.e., the biological process tripped by interaction of any stressor with an organism) to toxicodynamic KEs in a chemical specific MOA.

AOPs have been adopted within an international initiative to assimilate mechanistic information relevant to development of potentially predictive tools for adverse ecological and human health effects (OECD, 2013). The premise of the construct is that toxicity is the result of generalizable motifs of biological failure initiated by the interaction of a chemical with some biomolecule in the body. This molecular interaction elicits a perturbation in normal biology that ultimately impairs critical function of the organism leading to toxicity and eventually impacts on the population of concern (Edwards et al., 2016). Consequently, AOPs are described by identifying measurable KEs at varying levels of biological organization beginning with molecular interactions of the chemical with the biological system and proceeding through the organismal responses that impact populations (Villeneuve et al., 2014). They are anchored at one end by a MIE and at the other end, by an AO at the level of the organism (e.g. disease or overt toxicity) or population.

An essential component of the OECD program is a knowledge base (KB) to document AOPs, which will comprise four independent modules that are connected via an underlying data hub. The module that is currently most developed, the AOP-Wiki (http://aopwiki.org), was released in September 2014 to support formal AOP development by capturing supporting evidence in prose format. Additional envisaged components, Effectopedia (http://effectopedia.org/) and AOPXplorer (http://aopxplorer.org/), expected to be released by

February 2016, will capture related information such as quantitative response-response relationships between KEs, and relevant assays to measure KEs and collect AOP networks from automated processes. A fourth envisaged module, the Intermediate Effects Data Base, will connect the AOP-KB to chemical specific information in the OECD Chemical Screening Information Data Sets (SIDS) database (http://webnet.oecd.org/HPV/UI/Search.aspx) via the International Uniform ChemicaL Information Database (IUCLID) software (http://iuclid.eu). An important objective of the knowledge base is to provide a repository of information across different levels of biological organization in a construct that informs both research and regulatory objectives.

5. Interface of MOA Analysis and AOPs

The terms MOA and AOP are, then, conceptually similar, representing essentially the subdivision of the pathway between exposure and effect in either individuals or populations into a series of hypothesized KEs at different levels of biological organization (e.g., molecular, subcellular, cellular, tissue). Both represent pragmatic simplifications of complex biological pathways. The distinction between the two has been somewhat artificial, a result principally of experience with different types of data in the human health versus ecological communities.

However, the AOP concept has evolved recently to encompass only chemical agnostic KEs resulting from perturbations of normal biology between MIEs and adverse effects. As such, they identify KEs at various levels of biological organization for which associated assays or computational models may be helpful in predicting adverse effects. This is a welcome development in that it is anticipated to facilitate collective contribution to knowledge of networks in systems biology. Quantification of relationships between key events (KERs) is anticipated to contribute to prediction of the extent of biological response. As a result, while AOPs are often developed and documented based on supporting information that includes challenge by reference chemicals, they draw collectively upon such data solely as a basis to define biological pathways rather than the assessment of individual (e.g., chemical) stressors (Edwards et al., 2015).

While AOPs can be used for different purposes, one common application is their integration as the toxicodynamic KEs in a hypothesized MOA for a specific chemical and/or group. MOA includes, in addition to the AOP, considerations of metabolism. MOA analysis for species concordance additionally takes into account chemical-specific absorption, distribution, metabolism and elimination. It is anticipated, then, that increasingly, AOPs will be developed that contribute to chemical-specific MOA analysis (as one application) and vice versa.

6. Weight of evidence (WoE) for AOPs

Appropriately, given their conceptual similarity, consideration of WoE for hypothesized AOPs draws upon a subset of the Bradford Hill considerations as evolved from experience in MOA analysis (Meek et al., 2014b; OECD, 2014; Becker et al., 2015). The subset considered relevant to chemical agnostic AOPs include biological plausibility and empirical support of KERs and essentiality of KEs. These considerations, which are rank ordered in

relation to their relative contribution to weight of evidence/confidence in the data supporting a hypothesized AOP, are defined and distinguished as follows:

Biological Plausibility of KERs relates to how well we understand the mechanistic structural/functional relationships of the pathway. In essence, do we know enough to be able to "predict" what happens if we disturb the pathway (experimentally)?

Essentiality of KEs relates to experimental support for whether or not downstream KEs or the AO are prevented or modified if an upstream event is blocked. Experimental support includes, for example, testing in knockout models or investigations of reversibility.

Empirical Support relates to the nature of the expected quantitative impact on downstream KEs if earlier KERs are impacted and is normally tested by considering dose-response relationships for stressors which impact the pathway.

More consistent evaluation of the weight of supporting evidence for various components of the AOP based on these *a priori* defined considerations and associated examples of datasets supporting high, moderate and low confidence is anticipated to facilitate consideration of documented AOPs for different potential applications. It is also critical to explicitly identify critical data gaps as a basis for facilitating targeted research and regulatory uptake. Once evaluated, degrees of confidence and associated rationales are summarized as recommended by the OECD handbook (OECD, 2014). (See, also, for example, Yauk et al., 2015). The longer term objective is to increase transparency and consistency in organizing, linking and integrating information at different levels of biological organization into a more efficient, hypothesis-driven approach to chemical data generation and assessment and to better tailor development of mechanistic data to address regulatory needs. Based on initial experience in assessing the supporting information for hypothesized AOPs it is important to understand what type of information is required in the context of regulatory purposes.

7. Challenges for developing AOPs relevant to neurotoxicity evaluation

The brain is an extremely complex organ comprised of a variety of highly specialized neuronal and glial cell types with multiple and diverse cellular functions that differ between brain regions and different stages of brain development (Rice and Barone, 2000). Additionally, the function of molecules in the nervous system can change as a function of developmental window, e.g., acetylcholinesterase promotes axonal growth and synapse formation of some neuronal cell types in development but not in the adult brain, and the role of gamma-aminobutyric acid (GABA) receptors switches during development from excitatory to inhibitory (Jessell and Sanes, 2000).

There are a variety of neuronal subtypes in the central and peripheral nervous system that are characterized by the expression of specific neurotransmitters neuronal receptors. Furthermore, brain region-specific astrocytes and microglia play important roles in neurophysiology (including structural functions, regulation of metabolism and synapse formation) and the response to chemical stressors (e.g, neuroprotective or neurotoxic function). In addition, endothelial cells that form the blood-brain-barrier influence the access of substances to the brain. These different cell types with diverse functions represent a large

number of potential targets for neurotoxic chemicals, which implies the existence of a potentially large number of AOPs with a variety of MIEs that when triggered may lead to a range of different AOs.

The main hurdle for developing AOPs relevant to the nervous system is a general lack of understanding of molecular and cellular mechanisms of neurotoxicity, including mechanisms by which chemicals interact with molecular targets. This reflects in part the fact that many neurotoxicants can interact with multiple molecular targets. Therefore, it is unlikely that a single AO will be produced for all chemicals that trigger the same MIE. Moreover, even if the AOP is a simple linear progression from the MIE to AO, the incidence of later KEs is expected to be less than that for early KEs. Therefore, it is important to understand the key event relationships (KERs) and to provide relevant information or, even better, quantitative data supporting KERs, especially between the early KEs.

There is a general lack of knowledge of a threshold (chemical concentration and time of exposure) that triggers KE downstream to a level that overcomes adaptive changes and compensating mechanisms. Therefore, so far, the available AOPs relevant to adult and developmental neurotoxicity are mainly qualitative, not quantitative (Bal-Price et al., 2015b).

Evaluation of the scientific confidence of the existing data for development of the selected AOPs relevant for neurotoxicity and developmental neurotoxicity

I. The Acute Neurotoxicity induced by binding of Pyrethroid Insecticides to Voltage-Gated Sodium Channels

Synthetic pyrethroid insecticides have been utilized for nearly five decades, and their toxicity to both insects and mammals is well characterized. This class of compounds contains more than 20 different chemicals. Although all pyrethroids have common structural characteristics (alcohol and acid moieties separated by a central ester bond), they have a wide variety of different structural characteristics and also exist as stereoisomers (Soderlund et al., 2002). The acute toxicity of these compounds has been extensively studied and is well understood, which has allowed the development of the recently published Adverse Outcome Pathway for acute neurotoxicity through their binding to voltage-gated sodium channels (Bal-Price et al., 2015b).

Briefly, exposure to high doses of pyrethroid insecticides produce signs and symptoms of one of two generalized syndromes; Type I (or T) syndrome, which is characterized by tremor, hyperexcitability, and Type II (or CS) syndrome, which is characterized by choreoathetosis, excessive salivation, etc. The Molecular Initiating Event of the AOP leading to these syndromes begins with the pyrethroid binding to the alpha subunit of voltage-gated sodium channels (VGSC), and subsequent modification of the kinetics of channel function, which ultimately leads to the two toxicity syndromes described above.

To summarize, this AOP is comprised of the following KEs that are initiated by binding of pyrethroids to the alpha subunit of the VGSC (MIE): (1) changes in the kinetics of channel opening and closing (KE 1); (2) alterations in excitability of neuronal membranes (KE 2); (3) dysregulation of neural networks (KE 3); (4) Behavioral changes associated with Type I and II poisoning syndromes (Adverse Outcome).

A brief note about two unique aspects of this AOP. First, depending on the compound, the modifications of VGSC kinetics in KE2 can range from short-lived modifications (Type I compounds), to very long lasting modifications (Type II compounds; reviewed in: Soderlund et al., 2002). These modifications in KE1 influence the response in KE2; type I compounds produce repetitive firing of action potentials, whereas Type II compounds produce membrane depolarization, eventual depolarization-dependent block of action potentials, and nerve terminal depolarization leading to excessive neurotransmitter release. The MIE and KEs are causatively linked to the final adverse outcomes by structure-activity relationships wherein Type I compounds typically lack an alpha cyano group and produce short-term modifications of VGSC function and Type I syndrome, whereas Type II compounds have a cyano group, cause long-lasting modification of VGSC kinetics, and cause Type II syndrome. Second, although VGSCs are targets for modification by a number of different drugs, neurotoxicants and natural toxins, this AOP is relatively narrowly defined to apply to the pyrethroids and DDT (which behaves as a type I pyrethroid). Other compounds acting on VGSC bind to sites different than the pyrethroids, produce different alterations of VGSC function and/or have different clinical outcomes. Thus, separate AOPs need to be described for these other compounds.

Evaluation of weight of evidence for this AOP

Biological plausibility for the identified Key Events Relationships (KERs): The biological plausibility of the KERs is strong and well established, as VGSC are critical in the regulation of membrane voltage and excitability and are integral to the initiation of the neuronal action potential. The evidence for the binding to the alpha submit of the VGSC (Molecular Initiating Event) is very strong and supported by binding studies (Trainer et al., 1997), site-directed mutagenesis studies (Vais et al., 2000: Rinkevich et al., 2013) and x-ray crystallography (O'Reilly et al., 2006). However, despite this well-established relationship, there are some notable uncertainties. First, although two separate toxicity syndromes are described, only a single AOP was proposed. This is because, although many pyrethroid compounds fall neatly into one or the other categories, several compounds exhibit, at the whole animal level, signs of both the type I and II syndromes, and are referred to as "mixed" pyrethroids. At the cellular level, the time course of modification of VGSC kinetics is not a bimodal distribution, but rather a continuum from very short lasting modifications to very long lasting modifications. Thus, the proposed AOP has one MIE: binding to the alpha subunit of the VGSC. It does branch at the level of modification of VGSC kinetics, to account for the differences in response seen at the level of the adverse outcome in the whole animal.

Essentiality of the identified key events for AO (Type I and II Toxicity Syndromes): There are at least two clear indicators for the essentiality of the identified KEs

for the AO described above. First, as already mentioned, the structure-activity relationships between Type I and II compounds (presence or absence of the cyano group) and Type I and II syndromes provides a clear indication of the linkage between the KEs, KERs and the AO. Second, pyrethroids are chiral compounds with as many as three chiral centers, and differences in the toxicity of these compounds correlates with differences in the ability of different stereoisomers to alter VGSC function and their toxicity (Lund and Narahashi 1982). This supports further the essentiality of the KEs leading to the AO.

It should be noted that the AOP as described (Bal-Price et al., 2015b) uses the toxicity syndromes observed following pyrethroid exposure as the Adverse Outcome. These are really a collection of behaviors rather than a single adverse outcome, and not all pyrethroid effects result in a “bimodal” behavioral response. For example, both Type I and II compounds produce paresthesia following dermal exposure (McKillop et al., 1987; Hudson et al., 2014), and all pyrethroids inhibit motor activity following oral exposure (Wolansky et al., 2006). In addition, there are several uncertainties regarding the AOP, which have been hypothesized based on pyrethroid actions on VGSC. These uncertainties include the lack of an easily available biomarker for effect, the fact that there are 10 VGSC sub-units, and the relatively lesser amount of data at the whole animal level. Of those subunits readily expressed in the nervous system, it has been established that the rodent Nav 1.3, 1.6 and 1.8 isoforms of the alpha subunit of the VGSC are more sensitive to pyrethroids than Nav 1.2 (Meacham et al., 2008; Tan and Soderlund 2010). This information can be used to refine the AOP, along with considering more carefully which adverse outcome one is trying to describe. For example, the Nav1.3 subunit is highly (though not exclusively) expressed in the embryonic period, thus exploration of potential developmental effects of pyrethroids must consider the role of this channel during development (see Shafer et al., 2005). By contrast, the Nav1.8 subunit is restricted in its expression to the peripheral nervous system, including the small diameter neurons of the dorsal root ganglion. Thus, an AOP for paresthesia could be described wherein the MIE involves binding to the Nav1.8 VGSC in small diameter sensory neurons of the dorsal root, and disrupting firing properties of these neurons, leading to the paraesthesias observed following dermal exposure to pyrethroids. A similar approach to other aspects of pyrethroid neurotoxicity (e.g. startle responses, motor activity), will allow the definition of more specific AOPs and will increase their utility. This concept can and should be applied to other neurotoxic compounds. For example, “learning and memory” is a broad category of behaviors, and more specific description of the specific behavior altered (e.g. water maze learning) will allow for better description of the neural pathways involved in that behavior and clearer definition of the AOP.

II. Chronic antagonism of N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities

The N-methyl-D-aspartate glutamate receptor (NMDAR) regulates many critical neurodevelopmental processes, including synaptogenesis and synaptic plasticity, both of which are fundamental mechanisms of learning and memory (Traynelis et al., 2010). Based on the existing literature, it is well documented that binding of an antagonist to NMDAR during synaptogenesis triggers a cascade of key events at the cellular and tissue level that leads to impaired learning and memory. Therefore, in this AOP, antagonism of the NMDA

receptor has been identified as the MIE, and impaired learning and memory is defined as the AO. Antagonism of the NMDA receptor by a chemical (for instance by exposure to lead) triggers a cascade of causally linked cellular KEs that result in cognitive deficit. One of the initial KEs triggered by inhibition of NMDAR is decreased influx of calcium into the neuron that inhibits calcium-dependent signaling. One consequence of decreased intracellular calcium levels is reduced mRNA expression and synthesis of brain derived neurotrophic factor (BDNF) protein, which plays a fundamental role in neuronal survival, development and differentiation, including synaptogenesis. Decreased levels of BDNF are a fundamental cellular KE that triggers neuronal apoptosis, aberrant dendritic morphology, reduced presynaptic release of glutamate and decreased synaptogenesis, resulting in decreased neuronal network function.

In summary, this AOP identifies the following cascade of KEs: (1) Binding of antagonist to NMDARs (MIE); (2) Inhibition of NMDARs; (3) Reduced intracellular calcium levels; (4) Decreased transcription of BDNF mRNA leading to reduce cellular levels of BDNF; (5) Reduced presynaptic glutamate release, aberrant dendritic morphology and increased neuronal apoptosis; (6) Decreased synaptogenesis; (7) Decreased neuronal network function; (8) Impaired learning and memory (AO).

Here, we focus on the evaluation of two fundamental features of the AOP WoE evaluation: biological plausibility and essentiality of the described KERs.

Evaluation of weight of evidence for this AOP

Biological plausibility for the identified Key Events Relationships (KERs): The supporting evidence for the biological plausibility of the relationship between the *Inhibition of NMDARs* by binding of an antagonist (KE upstream) and *Decreased calcium influx* (KE downstream) is strong. There is structural and functional mechanistic understanding supporting this relationship. The inhibition of NMDARs leads to closure of the central ion channel pore and consequently reduction in Ca^{2+} influx. The function of NMDA receptors is commonly evaluated by measuring intracellular influx of Ca^{2+}, therefore, calcium imaging techniques have been extensively utilized to investigate the relationship between these two KEs (Higley and Sabatini, 2012). For example, the NMDA-mediated increase in Ca^{2+} was absent in brain slices from GluRε2 $^{-/-}$ mice that do not possess functional NMDA receptors in the developing neocortex (Okada et al., 2003), suggesting that NMDAR activation was responsible for Ca^{2+} influx.

Decreased Ca^{2+} influx (KE upstream) triggers *Reduced release of BDNF* (KE downstream). There is extensive scientific understanding of the mechanistic relationship between these two KEs. BDNF transcription is induced by Ca^{2+} entering in the neurons through either L-type voltage gated calcium channels (L-VGCC) (Tao et al., 1998) or the NMDAR (Tabuchi et al., 2000; Zheng et al., 2011). Ca^{2+} binds to and activates the transcription factor cAMP-response element binding protein (CREB). Additional transcription factors implicated in the transcription of BDNF include NFAT (nuclear factor of activated T cell), MEF2 (myocyte enhancer factor 2) and NFκB (nuclear factor κB) (reviewed in Zheng et al., 2012). The initial activation of CaM kinase, cAMP/PKA and Ras/ERK1/2 pathways mediated by the elevated intracellular Ca^{2+} triggers activation of other transcription factors and inhibition of

different elements of these pathways has been shown to decrease BDNF mRNA and protein levels (reviewed in Zheng et al., 2012).

Reduced release of BDNF (KE upstream) triggers the following downstream KEs: *Reduced presynaptic glutamate release, Aberrant dendritic morphology* and *Increased apoptosis*. There is extensive scientific understanding of the mechanistic relationship between these KEs. Stimulation of tyrosine kinase B (TrkB) receptors by BDNF leads to the activation of proteins such as Arc, Homer2, LIMK1 (Kang and Schuman, 1996; Schratt et al., 2004; Yin et al., 2002) that are known to promote actin polymerization and consequently the enlargement of dendritic spine heads (Sala et al., 2001). Furthermore, it has been shown that the inhibition of BDNF synthesis reduces the size of spine heads and impairs long term potentiation (LTP) (An et al., 2008; Waterhouse and Xu, 2009). Experimentally, it has been shown that activation of presynaptic TrkB receptors by BDNF also enhances glutamate release and increases the frequency of excitatory postsynaptic current (EPSCs) in hippocampal neurons derived from rat (Lessmann and Heumann, 1998; Takei et al., 1998; Minichiello, 2009). In addition, BDNF influences apoptosis of developing neurons through two distinct mechanisms (Bernd, 2008). mBDNF can trigger pro-survival signaling after binding to TrkB receptor, which subsequently inactivates specific components of the cell death machinery while also activating the transcription factor CREB, which drives expression of the pro-survival gene Bcl-2 (West et al., 2001). On the other hand, proBDNF binds to the p75 neurotrophin receptor (p75NTR) and activates RhoA that regulates actin cytoskeleton polymerization resulting in apoptosis (Lee et al., 2001; Miller and Kaplan, 2001; Murray and Holmes, 2011).

It is well documented that BDNF mRNA levels dramatically increase between embryonic days 11 to 13 of rat development, and this upregulation in BDNF is critical for neuronal survival and differentiation (reviewed in Murray and Holmes, 2011). Taking into consideration these important BDNF functions in the developing brain, a reduced level of BDNF (KE upstream) induced by binding of an antagonist to the NMDAR results in KE downstream, *Decreased synaptogenesis* leading to *Decreased neuronal network function* that are fundamental processes for learning and memory. Indeed, the ability of a neuron to communicate is based on neural network formation that relies on functional synapse establishment (Colón-Ramos, 2009). The main roles of synapses are the regulation of intercellular communication in the nervous system, and the information flow within neural networks. The connectivity and functionality of neural networks depends on where and when synapses are formed. Therefore, the decreased synapse formation during the process of synaptogenesis is critical and leads to decrease of neural network formation.

Decreased neuronal network function (KE upstream) leads to *Impairment of learning and memory* (KE downstream, AO). Learning and memory is dependent on neuronal network function. Learning-induced enhancement in neuronal excitability, a measurement of neural network function, has been shown in hippocampal neurons following classical conditioning in several experimental approaches (reviewed in Saar and Barkai, 2003). Furthermore, memory requires increased magnitude of EPSCs to be developed quickly and to be persistent for several weeks at least without disturbing already potentiated contacts (reviewed in Lynch, 2004).

Essentiality of the identified key events for AO (Impairment of learning and memory):

1. The evidence for the essentiality of the MIE (*Binding of antagonist to NMDAR* in neurons during synaptogenesis in hippocampus and cortex) for AO (*Impairment of learning and memory*) is evaluated as strong since it is well documented that learning and memory processes rely on physiological functioning of NMDA receptors. Their essential role has been demonstrated in both animal and human studies investigating NMDA itself, NMDA receptor antagonists and mutant mice lacking NMDA receptor subunits (reviewed in Haberny et al., 2002; Rezvani, 2006 and Granger et al., 2011).

2. Essentiality of the KE: *Inhibition of NMDA receptors*

   Evidence for the essentiality of this KE for the AO is suggested to be strong since the noncompetitive antagonist MK-801 has been shown to induce dose-dependent impairment of learning and memory (Wong et al., 1986) and data from rodent models confirmed these effects, as recently reviewed by van der Staay et al. 2011. Similarly, NMDA receptor blockage has been reported to impair learning in nonhuman primates (Ogura and Aigner, 1993). Moreover, human studies demonstrate that NMDA-receptor inhibition impairs learning and memory processes (reviewed in Rezvani, 2006).

3. Essentiality of the KE: *Decreased Calcium influx*

   Based on the existing data, evidence for the essentiality of *Decreased Calcium influx* for AO (*Impairment of learning and memory*) is determined to be strong. In the nervous system, many intracellular responses are mediated by calcium/calmodulin-regulated protein kinases (CaMKs), followed by protein phosphorylation (Wayman et al., 2008). Multifunctional CaMKs, such as CaMKII and members of the CaMK signaling cascade (CaMKK, CaMKI and CaMKIV), are highly abundant in the CNS where they regulate different protein substrates (Soderling, 1999). For instance, mice with a mutation in the alpha-CaMKII that is abundantly found in the hippocampus have shown spatial learning impairments, whereas some types of non-spatial learning processes have not been affected (Silva et al., 1992).

4. Essentiality of KE: *Decreased levels of BDNF*

   Evidence for the essentiality of this KE for the AO is determined to be strong. BDNF serves essential functions in brain development and, more specifically, in synaptic plasticity (Poo, 2001), and is crucial for learning and memory processes (Lu et al., 2008). The action of BDNF signaling on synapses via sustained TrkB activation happens within seconds of its release (Kovalchuk et al., 2004), and this action strengthens LTP processes, a cellular model for learning and memory (Kang and Schuman, 1996; Nagappan and Lu, 2005). Furthermore, there is experimental evidence showing that loss of BDNF via genetic deletion or pharmacological manipulation impairs LTP (Patterson et al., 1996; Monteggia et al., 2004) and decreases learning and memory (Lu et al., 2008).

5. Evidence for the essentiality of *Decreased presynaptic release of glutamate* (caused by reduced release of BDNF) for the AO is determined to be strong. The role of glutamate and different glutamatergic receptor subtypes in learning and memory processes is well understood based on the existing psychopharmacological *in vivo* studies conducted in rodents and primates (for example Riedel et al. 2003; Redini-Del Negro and Laroche, 1993). Similarly, support for the essentiality of the KE: *Aberrant dendritic morphology* caused indirectly by BDNF for the AO (*Impairment of learning and memory*) is determined to be strong. Spine morphology is considered to be an important morphological unit for establishing learning and memory (Sekino et al., 2007). As dendrites are the postsynaptic site of most synaptic contacts, dendritic development determines the number and pattern of synapses received by each neuron (McAllistair, 2000). Defects in dendritic growth are associated with severe neurodevelopmental disorders such as mental retardation (Purpura, 1975). Thus, the proper growth and arborization of dendrites is crucial for proper functioning of the nervous system, and changes in spine formation are implicated in impaired learning and memory (Yang et al. 2009b; Roberts et al. 2010).

6. Essentiality of the KE: *Decreased synaptogenesis*

   Support for the essentiality of the KE (*Decreased synaptogenesis*) for the AO (Impairment of learning *and memory*) is also determined to be strong. Learning and memory result from synaptic plasticity that modifies the way neurons communicate with each other (Bear, 1996). Plasticity is defined as changes in the structure, distribution and/or number of synapses, and it has been suggested that these changes underlie memory formation (Rusakov et al., 1997; Woolf, 1998; Klintsova and Greenough, 1999). In mutant mice lacking PSD-95 (post-synaptic protein), increased NMDA-dependent LTP has been recorded that severely impairs spatial learning (Migaud et al., 1998). Furthermore, recent genetic screening in human subjects as well as neurobehavioral studies in transgenic mice have suggested that loss of synaptophysin (a presynaptic vesicle protein) plays an important role in mental retardation and learning deficits (Schmitt et al., 2009; Tarpey et al., 2009).

7. Essentiality of the KE: *Decreased function of neuronal network*

   It is well understood and documented that the ability of neurons to communicate with each other is based on neural network formation that relies on functional synapse establishment (Colón-Ramos, 2009). The connectivity and functionality of neural networks depends on where and when synapses are formed. Therefore, decreased synapse formation during the process of synaptogenesis is detrimental and leads to decrease of neural network formation and function. Alterations in synaptic connectivity underlie the refinement of neuronal networks during development (Cline and Haas, 2008). Indeed, knockdown of PSD-95 (postsynaptic protein) blocks the functional and morphological development of glutamatergic synapses (Ehrlich et al., 2007).

This AOP is applicable for specific period of brain development that corresponds to the time of synaptogenesis, which occurs at different developmental stages across species. In humans, the period of synaptogenesis starts during the third trimester of pregnancy and continues until 2–3 years following birth (Bai et al., 2013). Furthermore, synaptogenesis does not happen at the same time across all brain regions and there are important differences in the ontogenetic expression profiles of excitatory *versus* inhibitory synapses (Erecinska et al., 2004).

Most of the evidence supporting the proposed AOP was generated from studies (*in vitro*, *in vivo* and epidemiological) following exposure to lead. Any chemicals that block NMDAR activity could trigger the described MIE and could potentially be chemical initiators for this AOP. However, there is a gap of knowledge in these type of studies as only very few chemicals have been evaluated for their effects on DNT effects (Grandjean and Landrigan, 2006; Bal-Price et al., 2015a) and an even smaller subset of these have been screened for effects on the NMDAR activity.

III. Sensitization of the ryanodine receptor (RyR) in the developing brain alters synaptic connectivity leading to neurobehavioral perturbations

RyRs are microsomal Ca^{2+}-induced Ca^{2+} ion channels that are expressed by neurons in multiple regions of the mammalian brain. RyRs associate with cytosolic, endoplasmic reticulum (ER) membrane-anchored and ER luminal proteins to form local Ca^{2+} release units that regulate Ca^{2+} release from the ER and modify gating responses of plasma membrane ion channels, including NMDA receptors and voltage-gated Ca^{2+} channels (Pessah et al., 2010). It has been demonstrated that diverse chemicals can interact with the RyR to enhance its sensitivity to activation by nanomolar levels of Ca^{2+} and decrease its sensitivity to inhibitory feedback by millimolar Ca^{2+} and Mg^{2+} (Pessah et al., 2010; Pessah and Wong, 2001). This sensitization of the RyR stabilizes the channel in its open conformation, which increases release of Ca^{2+} from the endoplasmic reticulum thereby increasing intracellular Ca^{2+} levels (Berridge, 2006; Pessah et al., 2010).

Changes in intracellular Ca^{2+} are a principal mechanism by which neuronal activity regulates diverse neurodevelopmental processes (Lohmann and Wong, 2005; Segal et al., 2000; Wayman et al., 2008) that are critical determinants of synaptic connectivity, including dendritic arborization (Libersat and Duch, 2004; Scott and Luo, 2001), dendritic spine formation and apoptosis (Barone et al., 2000; Martin, 2001; Sastry and Rao, 2000). The importance of neuronal activity in refining patterns of synaptic connectivity in the developing brain (Katz and Shatz, 1996; Yuste and Bonhoeffer, 2001) is evidenced by the marked effect of experience on the development and refinement of synaptic connections, which not only patterns neural circuitry during development but also underlies associative learning (Pittenger and Kandel, 2003). Altered patterns of synaptic connectivity are widely postulated to be the neurobiological substrate for many neurodevelopmental disorders, including autism spectrum disorders (ASD), attention deficit hyperactivity disorder (ADHD) and various intellectual disabilities (Bourgeron, 2009; Geschwind and Levitt, 2007; Judson et al., 2011; Penzes et al., 2011; Zoghbi and Bear, 2012).

In this AOP, sensitization of neuronal RyRs is identified as the molecular initiating event (MIE) and neurobehavioral perturbations as the adverse outcome (AO). This AOP is largely derived from mechanistic studies of the non-dioxin-like PCB congener PCB 95. PCB 95 potently sensitizes the RyR (Wong et al., 1997a; Wong and Pessah, 1996), which triggers a cascade of key cellular events that ultimately modulate neurodevelopmental processes that influence synaptic connectivity in the developing brain, specifically dendritic arborization, dendritic spine formation and neuronal apoptosis (Howard et al., 2003; Lesiak et al., 2014; Wayman et al., 2012a) thereby altering patterns of synaptic connectivity (Lein et al., 2007; Yang et al., 2009; Yang and Lein, 2010a). These altered patterns of synaptic connectivity underlie neurobehavioral perturbations.

In summary, the following sequence of KEs are proposed downstream of RyR sensitization (MIE): (1) Increased intracellular Ca^{2+} levels (KE1); (3) Activation of Ca^{2+}-dependent signaling pathways (KE2); (4) Increased dendritic arborization, increased dendritic spine formation and increased neuronal apoptosis (KE3); (4) Neurobehavioral deficits including social deficits and impaired learning and memory (AO).

Evaluation of the weight of evidence supporting this AOP

Biological plausibility for Key Events Relationships (KERs): Evidence for the biological plausibility of a mechanistic relationship between the *sensitization of neuronal RyRs* (KE upstream) and *increased intracellular Ca^{2+} levels* (KE downstream) is extremely strong. RyRs are functionally defined as Ca^{2+}-induced Ca^{2+} ion channels, and RyR activity is often assessed by measuring Ca^{2+} flux across membranes or lipid bilayers (Pessah et al., 2010; Van Petegem, 2015). Studies across multiple laboratories have shown that sensitization of neuronal RyRs, which increases their open probability, results in increased neuronal levels of intracellular Ca^{2+} levels [reviewed in (Pessah et al., 2010; Van Petegem, 2015)]. Consistent with this mechanistic relationship, PCB 95 has been shown to stabilize RyR1 in the open state by both cryo electron microscopy (Samso et al., 2009) and ^{3}[H]-ryanodine binding (Pessah et al., 2006), and exposure to PCB 95 results in increased intracellular Ca^{2+} levels in PC12 cells (Wong et al., 2001), primary hippocampal neurons (Wayman et al., 2012a) and cortical microsomes (Wong et al., 1997a).

Increased intracellular Ca^{2+} levels (KE upstream) results in *activation of Ca^{2+}-dependent signaling pathways* (KE downstream). There is extensive scientific understanding of the mechanistic relationship between these two KEs (Berridge, 1998; Konur and Ghosh, 2005; Redmond and Ghosh, 2005; Wayman et al., 2008). Particularly relevant to this AOP are studies demonstrating a causal link between increased intracellular Ca^{2+} levels and sequential activation of CaMKK, CaMKI and MEK/ERK to activate the transcription factor CREB, which then upregulates transcription of Wnt2 (Wayman et al., 2006) and miR132 (Impey et al., 2010). Elevated intracellular Ca^{2+} has also been causally linked to the activation of signaling pathways that trigger neuronal apoptosis (Berridge et al., 2000; Ermak and Davies, 2002; Ravagnan et al., 2002; Robertson et al., 2001). The biological plausibility between these two KEs is further supported by studies of the gain-of-function missense mutation in the L-type Ca^{2+} channel CaV1.2 that causes Timothy syndrome, which has a 60% rate of co-morbidity with autism (Splawski et al., 2004). Neurons differentiated

from induced pluripotent stem cells derived from Timothy syndrome patients revealed increased Ca^{2+} oscillations and upregulated expression of genes linked to Ca^{2+}-dependent regulation of CREB, including CaMK (Pasca et al., 2011).

There is extensive scientific evidence to support the biological plausibility of a mechanistic relationship between *activation of Ca^{2+}-dependent signaling pathways* (KE upstream) and *increased dendritic arborization, increased dendritic spine formation and increased neuronal apoptosis* (KEs downstream). Ca^{2+} imaging studies have demonstrated that increased intracellular Ca^{2+} in neurons coincides with increased growth of dendrites and dendritic spines (Lohmann and Wong, 2005), while mechanistic studies in primary neuronal cell cultures provide compelling evidence that the growth of dendrites and dendritic spines are mediated by Ca^{2+}-dependent signaling pathways [reviewed in (Konur and Ghosh, 2005; Lohmann, 2009; Redmond and Ghosh, 2005; Valnegri et al., 2015; Wayman et al., 2008)]. A specific Ca^{2+}-dependent signaling pathway has been linked to activity-dependent dendritic growth in cultured hippocampal neurons and slices: sequential activation of CaM-dependent protein kinase kinase (CaMKK), CaMKI, the Ras/MEK/ERK signaling pathway and the transcription factor CREB resulting in upregulation of Wnt-2, which acts in an autocrine manner to stimulate dendritic growth (Wayman et al., 2006). This Ca^{2+}-dependent signaling pathway was causally linked to dendritic growth by demonstrating that pharmacological blockade or genetic suppression of any single molecule in this signaling cascade blocked not only activation of downstream signaling molecules in the cascade, but also activity-dependent dendritic growth; conversely, expression of constitutively active forms of key molecules in the signaling pathway phenocopied the effects of activity on dendritic arborization.

Similar pharmacological blockade and siRNA knockdown approaches demonstrated that dendritic spine formation in primary hippocampal neurons is triggered by a Ca^{2+}-dependent signaling pathway involving CREB-mediated upregulation of miR132, which suppresses the translation of p250GAP, a negative regulator of synaptogenesis (Impey et al., 2010). Morphological characterization of dendritic spines in a doxycycline-regulated miR-132 transgenic mouse strain to drive varying levels of transgenic miR-132 expression, confirmed that miR132 function is required for activity-dependent spine formation in hippocampal neurons *in vivo* (Hansen et al., 2013). Different Ca^{2+}-dependent signaling pathways have been implicated in triggering neuronal apoptosis (Berridge, 2006; Colomer and Means, 2007; Li et al., 2014; Liu et al., 2013).

Further supporting the biological plausibility of a mechanistic link between *activation of Ca^{2+}-dependent signaling pathways* (KE upstream) and *increased dendritic arborization, increased dendritic spine formation and increased neuronal apoptosis* (KEs downstream) are data showing that miR132 represses expression of methyl-CpG-binding protein-2 (MeCP2) (Klein et al., 2007). MeCP2 is a transcriptional repressor, and disruption of its function results in significant dendritic and synaptic dysregulation (Zhou et al., 2006). MeCP2 knockout animals have significantly perturbed synthesis and release of brain-derived neurotrophic factor (BDNF), a neurotrophic factor that stimulates dendritic outgrowth and synaptogenesis (Jin et al., 2003; Rex et al., 2007; Wang et al., 2006). Dysfunction of MeCP2 has been implicated in significant cognitive impairment in experimental models and humans,

and both mutations and duplications of the gene have been associated with Rett syndrome and autism spectrum disorders (Cukier et al., 2012; LaSalle and Yasui, 2009; Percy, 2011). miR132 has also been shown to interact with fragile X mental retardation protein (FMRP) to regulate synapse formation in experimental models (Edbauer et al., 2010), and more recently, expression of miR132 has been shown to be dysregulated in schizophrenia (Kim et al., 2010; Miller et al., 2012), a disorder characterized by aberrant synaptic pruning (Woo, 2014).

Support for the biological plausibility of a mechanistic relationship between *increased dendritic arborization, dendritic spine formation and/or neuronal apoptosis* (KE upstream) and *neurobehavioral perturbations* (KE downstream) is strong. Altered patterns of dendritic growth and plasticity are associated with impaired behavior in experimental models (Berger-Sweeney and Hohmann, 1997) and are the most consistent pathological correlates of functional deficits in intellectual delay and neuropsychiatric disorders (Belmonte and Bourgeron, 2006; Bourgeron, 2009; Delorme et al., 2013; Penzes et al., 2011). The types of aberrations observed in post mortem samples of patients included abnormalities in the number, size and branching patterns of dendrites as well as changes in the density or shape of dendritic spines. Strong support for a mechanistic relationship between these KEs is provided by experimental studies using a doxycycline-regulated miR-132 transgenic mouse strain to drive varying levels of transgenic miR-132 expression. miR132 activity, which is required for activity-dependent spine formation *in vitro* and *in vivo*, also regulates cognitive behavior in rodent models (Hansen et al., 2013; Hansen et al., 2010). Interestingly, these *in vivo* studies indicate that while miR132 is required for cognitive function, overexpression of miR132 to supra-physiological levels compromises cognitive function coincident with significantly increased spine formation (Hansen et al., 2013). While dendritic arborization and dendritic spine density are often positively correlated with cognitive capacity, histological studies of brains from patients diagnosed with autism spectrum disorders (Hutsler and Zhang, 2010), or fragile X syndrome (Irwin et al., 2001) have revealed significantly increased spine densities in neurons relative to neurotypical controls. Similarly, functional MRI studies have shown an association between local functional hyperconnectivity and symptom severity in autism spectrum disorders (Keown et al., 2013). Such data suggest that hyperconnectivity may be as disruptive to normal cognitive function as hypoconnectivity. There is experimental evidence that increased neuronal apoptosis may also contribute to neurobehavioral deficits (Barone et al., 2000; Rice and Barone, 2000). Indeed, it is believed that removal of even a small number of postmitotic neurons during synaptogenesis can significantly alter patterns of connectivity, resulting in functional deficits in the absence of obvious pathology (Dikranian et al., 2001; Ikonomidou et al., 1999; Martin, 2001; Martin and Green, 1995).

Essentiality of the identified MIE for KE and AO: In the following sections, we discuss the evidence for the essentiality of the MIE, *RyR sensitization*, for each of the KEs and AO. This analysis is largely derived from experimental studies of PCB developmental neurotoxicity.

1. Essentiality of *RyR sensitization* (MIE) for *increased intracellular Ca^{2+} levels* (KE1): Strong evidence supports a causal relationship between the MIE and this

KE. A subset of non-dioxin-like (NDL) PCBs significantly increase RyR sensitivity to activation by nanomolar Ca^{2+} and attenuate their sensitivity to inhibitor feedback by millimolar Ca^{2+} and Mg^{2+} (Pessah et al., 2006; Pessah and Wong, 2001). Structure activity relationship studies reveal that non-coplanar NDL PCBs possessing 2–3 chlorine *ortho* substitutions are the most potent RyR sensitizers, with PCB 95 being the most potent and efficacious congener identified to date (Pessah et al., 2010; Pessah et al., 2006). Two lines of evidence confirm a causal link between *RyR sensitization* (MIE) and *increased intracellular Ca^{2+} levels* (KE): (*i*) the effect of PCB 95 is blocked by treatment with ryanodine at concentrations that inhibit RyR activity (Wayman et al., 2012a); and (*ii*) PCB 66, a congener with negligible effect on RyR activity, has no effect on neuronal Ca^{2+} fluxes.

2. Essentiality of *RyR sensitization* (MIE) for *activation of Ca^{2+}-dependent signaling pathways* (KE2): The evidence in support of a causal link between this MIE and KE is strong. Exposure of primary hippocampal neurons to the RyR active PCB 95, but not the RyR-inactive congener PCB 66, activates CaMKK, CaMKI, MEK/ERK and CREB and upregulates expression of *Wnt2* mRNA (Wayman et al., 2012a). PCB 95 activation of the CaMKI-CREB-Wnt2 signaling pathway is blocked by pharmacological antagonism of RyRs using FLA 365 or siRNA knockdown of RyR1 or RyR2 (Wayman et al., 2012a). In rat primary hippocampal cultures, the potent RyR sensitizer PCB 95 also stimulates miR132 upregulation via CREB-dependent mechanisms, and suppresses translation of p250GAP (Lesiak et al., 2014). PCB 95 effects on these signaling molecules are blocked by pharmacological antagonism of RyRs using FLA 365 or by siRNA knockdown of RyR1 or RyR2 (Lesiak et al., 2014).

3. Essentiality of *RyR sensitization* (MIE) for *increased dendritic arborization, increased dendritic spine formation and increased neuronal apoptosis* (KE3): Strong evidence supports a causal link between the MIE and these KEs. The potent RyR sensitizer PCB 95, but not the RyR-inactive congener PCB 66, enhances dendritic arborization in primary cortical (Yang et al., 2009) and hippocampal cell cultures (Wayman et al., 2012a), and the dendrite promoting activity of PCB 95 is blocked by pharmacological antagonism of RyRs using FLA 365 or siRNA knockdown of RyR1 or RyR2 (Wayman et al., 2012b; Yang et al., 2009). PCB 95 has also been shown to promote dendritic growth via RyR-dependent mechanisms in rat hippocampal slice cultures (Wayman et al., 2012b). Consistent with the proposed link between the MIE and this KE, gestational and lactational exposure to Aroclor 1254 increases dendritic arborization of cerebellar Purkinje cells (Lein et al., 2007; Yang et al., 2009) and pyramidal neurons in both the neocortex (Lein et al., 2007; Yang et al., 2009) and CA1 hippocampus (Wayman et al., 2012b) of juvenile rats.

   RyR activity has also been implicated in mediating dendritic spine formation. Local release of Ca^{2+} from intracellular stores, which is regulated in part by RyR activity, increases the size of dendritic spines (Korkotian and Segal, 1999), and RyR-inhibitory concentrations of ryanodine block BDNF-enhanced spine

formation in primary hippocampal neurons (Adasme et al., 2011). Consistent with these observations, proteomic studies have demonstrated that RyR-active PCBs increase expression of synaptic proteins in rat cerebellar neurons (Brunelli et al., 2012). Functional evidence is also consistent with a causal link between RyR sensitization and increased dendritic spine formation. Dendritic spines are the primary site of excitatory synaptic input in the brain (Segal, 2005), and PCB 95, but not PCB 66, increases excitability in hippocampal slice cultures (Wong et al., 1997b), and increases the ratio of excitatory to inhibitory neurotransmission in hippocampal slice cultures, an effect blocked by the RyR antagonist dantrolene (Kim et al., 2009). *In vivo* studies provide further support of this link: developmental exposure to PCB 95 increases the ratio of excitatory to inhibitory neurotransmission in the developing rat auditory cortex (Kenet et al., 2007).

Experimental evidence causally links RyR sensitization to neuronal apoptosis. Increased RyR activity (Andjelic et al., 1997; Danieli and Rampazzo, 2002; Hajnoczky et al., 2000; Mariot et al., 2000; Pan et al., 2000) and intracellular Ca^{2+} (Berridge et al., 2000; Ermak and Davies, 2002; Ravagnan et al., 2002) are critical components of apoptotic signaling pathways. Aroclor 1254, a mixture comprised of predominantly NDL PCBs (Kostyniak et al., 2005), as well as the RyR-active NDL congener PCB 47 significantly increase caspase-dependent apoptosis in primary hippocampal cultures (Howard et al., 2003). The pro-apoptotic activity of PCB 47 is inhibited by the RyR antagonist FLA 365 but not by antagonists of the IP_3 receptor (xestospongin C), L-type calcium channel (verapamil), or NMDA receptor (APV) (Howard et al., 2003). Further, PCB 77, a congener with little to no RyR activity, has no effect on apoptosis in primary hippocampal neurons (Howard et al., 2003). *In vivo* data are consistent with the proposed relationship between the MIE and increased neuronal apoptosis. Gestational and lactational exposure to Aroclor 1254 exposure at 1 mg/kg/day in the maternal diet increased caspase 3 activity in the hippocampus, cortex and cerebellum of newborn but not weanling rats (Yang and Lein, 2010), an observation confirmed by TUNEL staining (Yang and Lein, 2010). Collectively, these results provide strong evidence of causal links between RyR sensitization and neuronal apoptosis.

4. Essentiality of *RyR sensitization* (MIE) for *neurobehavioral perturbations* (AO): The evidence of the relationship between RyR sensitization and neurobehavioral perturbations do not confirm causality. As discussed above, experimental studies of the developmental neurotoxicity of PCBs have established causal links between RyR sensitization and effects on dendritic arborization, dendritic spine formation and neuronal apoptosis *in vitro* and shown associations between these endpoints *in vivo*. The most compelling data of a causal link between the MIE and AO are *in vivo* studies demonstrating that the effects of gestational and lactational exposure to Aroclor 1254 on RyR activation, as assessed by ^{3}[H]-ryanodine binding, dendritic arborization, as measured by Golgi staining, and deficits in spatial learning and memory as determined using the Morris water maze, demonstrated a similar non-monotonic dose-response relationship (Yang et

al., 2009). Independent studies have similarly shown that gestational and lactational exposure to Aroclor 1254 impairs radial-arm maze performance in adult male rats (Roegge et al., 2000) at doses shown to increase RyR activity in the cerebellum (Roegge et al., 2006). A major caveat of these studies using Aroclor 1254 with respect to this AOP is that while Aroclor 1254 is comprised predominantly of NDL PCBs with RyR activity, there are additional congeners in this technical mixture that have little to no effect on RyR activity (Kostyniak et al., 2005). However, developmental exposure to PCB 95 at low doses (0.1–1mg/kg/day in maternal diet) similarly increases dendritic arborization of CA1 pyramidal neurons in the hippocampus of juvenile rats (Wayman et al., 2012b). While this latter study did not assess behavioral function, independent studies have demonstrated that perinatal exposure to PCB 95 altered activity levels and behavior in the radial arm maze in adult rats (Schantz et al., 1997) and enhanced the ratio of excitatory to inhibitory currents within the primary auditory cortex of juvenile rats (Kenet et al., 2007). Also supportive of this AOP are data showing that perinatal exposure to RyR-active PCB 47, albeit in combination with PCB 77, a congener with no effect on RyR activity, alters social behavior in rats (Jolous-Jamshidi et al., 2010).

A number of *in vivo* studies have failed to show effects of developmental exposure to PCBs on dendritic arborization and/or behavior (Bushnell and Rice, 1999; Roegge et al., 2006; Roegge and Schantz, 2006). However, these seeming discrepancies may actually be consistent with the proposed AOP. For example, some negative studies [e.g., (Bushnell and Rice, 1999)] focused on PCB congeners, such as PCB 126, that have negligible effect on RyR activity (Pessah et al., 2006). Other studies that failed to find an effect of developmental Aroclor 1254 exposure on dendritic arborization (e.g., Roegge et al., 2006; Roegge and Schantz, 2006) may be explained by the fact that these studies tested doses at the high end or even higher than the dose range studied by Lein and colleagues (Wayman et al., 2012b; Yang et al., 2009; Yang and Lein, 2010) who reported that the dose-response relationship for Aroclor 1254 effects on RyR activity, dendritic growth and plasticity and spatial learning and memory exhibited a non-monotonic dose-response relationship (Yang et al., 2009).

Much of the evidence used to support the proposed AOP was generated from studies of Aroclor 1254, a technical mixture of both RyR-active and RyR-inactive PCB congeners (Kostyniak et al., 2005), or the potent RyR sensitizer, PCB 95. Recent studies have shown that another NDL congener, PCB 136, also sensitizes RyRs, and this molecular interaction has been causally linked to enhanced synchronized Ca^{2+} oscillations and increased dendritic arborization in primary hippocampal neurons (Yang et al., 2014).

RyR activity is modulated by a diverse set of chemicals (Xu et al., 1998), and there is growing evidence that chemicals other than PCBs, many of which also have non-coplanar structures, can sensitize and/or activate the RyR. Several examples include caffeine (Pessah et al., 1987), polybrominated diphenyl ethers (PBDEs) (Kim et al., 2011), triclosan (Ahn et al., 2008) and suramin (Papineni et

al., 2002). Whether these non-PCB RyR active compounds trigger the same downstream key events as RyR-active PCBs has not been directly or rigorously evaluated. However, the available published literature suggests that at least a subset of these structurally diverse RyR active compounds phenocopy key events triggered by RyR-active PCBs. For example, developmental exposure to caffeine increased dendritic length and branching in the rat prefrontal cortex (Juarez-Mendez et al., 2006), and has been shown to increase caspase-3 activation in multiple brain regions (Black et al., 2008). *In vitro* exposure to caffeine also activated CREB and CREB-dependent gene expression in mouse cortical neurons (Connolly and Kingsbury, 2010). PBDEs, a class of halogenated flame retardants, sensitize RyRs via interactions with the FKBP12/RyR complex and this molecular effect was causally linked to enhanced Ca^{2+} oscillations and increased neuronal network activity (Kim et al., 2011). Developmental exposure to PBDEs has also been shown to impair cognitive and motor behavior in rodent models (Dufault et al., 2005; Suvorov et al., 2009; Ta et al., 2011). Triclosan, an antimicrobial agent, and suramin, an antiparasitic drug, also sensitize RyRs (Ahn et al., 2008; Papineni et al., 2002). As predicted by the proposed AOP, triclosan induced apoptosis in cultured mouse cortical neurons via activation of Ca^{2+}-dependent caspases (Szychowski et al., 2015). Suramin decreased cell viability in neuronal cell lines and mouse primary neuronal cultures (Guo et al., 1990) and these neurotoxic effects were modulated by calcium influx (Sun and Windebank, 1996). Whether these RyR active chemicals interfere with neuronal connectivity in the developing brain to produce behavioral deficits, and whether these events are RyR-dependent remains to be determined. In conclusion, while limited, these data support the possibility that the proposed AOP may be relevant to diverse chemical structures.

IV. Description of the putative AOP: disrupted laminin-β1-integrin interaction leading to developmental neurotoxicity

The extracellular matrix (ECM) is a structural element that plays a prominent role in neurodevelopment and maturation of neural circuits (Lubbers et al. 2014) as it can influence cell adhesion, survival, proliferation, migration, and differentiation (Tzu and Marinkovich 2008). Interactions between the ECM protein laminin and integrin receptors represent a specific ECM-cell interaction that regulates many key aspects of neurodevelopment (Belvindrah et al. 2007; Graus-Porta et al. 2001; Lubbers et al. 2014; Warren et al. 2012). For example, selective loss of β1-integrin in excitatory neurons or in all brain cells leads to impaired hippocampus-dependent learning (Warren et al. 2012) or defective radial glia anchoring during cortical formation (Graus-Porta et al. 2001), respectively. Therefore, in this putative AOP, the disruption of laminin interactions with the β1-integrin has been identified as the MIE that triggers a series of KEs leading to impaired learning as the postulated AO. Chemicals that bind to the ECM protein laminin and thereby mask the laminin-β1-integrin binding site are suggested to disrupt important cellular processes necessary for brain development including cell adhesion, cell orientation and cell migration thereby resulting in improper cortical development. This putative AOP has been hypothesized based on the case study of Epigalloctechin gallate (EGCG), a flavonoid commercialized as a food supplement.

EGCG disrupts the cell-ECM binding of Neural Progenitor Cells (NPCs), thereby disturbs their adhesion, induces chaotic orientation of $GFAP^+$ (glial fibillary acidic protein) astrocytes processes and reduces migration as well as cellular density of NPCs in the neurosphere migration area (Barenys et al., submitted). Due to these properties, EGCG is studied as a model compound for this AOP.

In summary, this putative AOP is composed of the following KEs: (MIE) binding of compound to laminin; (KE1) interference of the laminin-β1-integrin binding; (KE2) disturbed adhesion; (KE3) chaotic cell orientation; (KE4) altered migration; (KE5) decreased cell density; (AO) impairment of learning based on studies with transgenic animals.

The generation of this putative AOP was initiated by basic research observations from screening studies applying the 'Neurosphere Assay' for developmental neurotoxicity testing (Baumann et al. 2014; Baumann et al. 2015). The experimental data supporting the early KE and their relationships of this AOP are detailed in our primary publication describing the effects of EGCG on cell migration in the neurosphere assay (Barenys et al., submitted). Based on these experimental data, here we aim to evaluate the biological plausibility and essentiality of the identified KEs to assess the general weight of evidence of this still very preliminary, putative AOP. This example illustrates the usefulness of implementing the AOP concept in basic toxicological research applications, as a basis to generate data of potential utility in regulatory application.

Evaluation of weight of evidence for this AOP

Biological plausibility for the identified Key Events Relationships (KERs): The MIE of this AOP is described by the binding of the model compound EGCG to the ECM protein laminin. Laminin is a major component of brain ECM and it is critical for normal brain function (Chen et al. 2009). That EGCG has the ability to bind to laminin is supported by two studies from two independent laboratories: an affinity chromatography study using columns (Suzuki and Isemura 2001) and a binding study (Lo et al. 2007). Our data provides additional evidence that binding of EGCG to the ECM is the MIE of this putative AOP since EGCG effects on NPCs are observed only under experimental conditions in which the ECM laminin is exposed to EGCG, but not under conditions in which the NPCs are treated with EGCG in the absence of laminin (Barenys et al., submitted).

This compound binding to laminin results in KE1: interference with the function of β1-integrin receptors of NPCs. The relationship between the MIE and KE1 is supported by the observation that in salivary gland adenocarcinoma cells and in the human monocyte cell line THP-1, EGCG prevents β1-integrin activation (Melgarejo et al. 2009; Park et al. 2010). Our own results also show that EGCG antagonizes soluble laminin binding to β1-integrin (Barenys et al., submitted).

The MIE and KE1 cause decreased adhesion of NPCs *in vitro* (KE2; Barenys et al., submitted). Faulty adhesion of radial glia not forming correct glia endfeet is also observed in developing brains of mice lacking the β1-integrin subunit specifically in NPCs *(CNS-(nestin-Cre)-fil-integrin-deficient* mice) supporting the role of β1-integrin for glia cell

adhesion during brain development (Graus-Porta et al. 2001). EGCG binding to laminin also disturbs cell adhesion in melanoma cells (Bracke et al. 1987; Suzuki and Isemura 2001) reinforcing this KER by studies in a different cell type. Decreased ECM adhesion of NPC causes chaotic process orientation of $GFAP^+$ cells differentiated from NPCs (KE3) (Barenys et al., submitted). That this de-alignment of radial glia processes is due to interference with β1-integrin function is also supported by *in vivo* studies with mice lacking the β1-integrin subunit in NPCs (CNS-(nestin-Cre)-β1-integrin-deficient mice). Besides faulty glial cell adhesion, these cells meander chaotically through parts of the developing brain (Graus-Porta et al. 2001). This phenotype of chaotic glia processes is still maintained *ex vivo* when nestin-Cre-β1-integrin-deficient cells are transferred into a culture dish (Belvindrah et al. 2007). These *in vivo* and *ex vivo* studies demonstrate the importance of β1-integrin for proper cell adhesion and thus support the KER that chemical interference with their function alter brain development.

Decreased adhesion to the ECM (KE2) and chaotic orientation of $GFAP^+$ cells (KE3) triggers an alteration in the migration pattern of NPCs (KE4) which leads to decreased cell density in the migration area (KE5). The alterations in the migration phenotype (KE4) are only observed when the laminin coated slides (laminin ECM) are exposed or pre-exposed to EGCG and not when the human NPCs are preexposed to the compound and plated in unexposed laminin (Barenys et al., submitted), supporting the relationship between the MIE and KE4. EGCG interference with migration of rat NPCs was observed earlier (Chen et al. 2003). Moreover, migration studies exposing human NPCs to a functionally blocking β1-integrin antibody reproduces the same phenotype than EGCG on migrating human NPCs (Barenys et al., submitted), adding more evidence to support the relationship between KE1 and KE4. A causal link between KE1 and KE4 is also supported by studies with smooth muscle cells in which migration was inhibited by EGCG in a concentration-dependent manner through interference with β1-integrin receptor binding to ECM proteins (Lo et al. 2007).

A concentration-dependent decrease in cell density in the migration area (KE5) is observed *in vitro* after exposure of NPCs to EGCG (Barenys et al., submitted). To evaluate the organ responses within this AOP, we performed neurohistological analyses for $BrdU^+$ cells in rats after developmental exposure to EGCG *in vivo*, to monitor neuronal migration into the cortex as previously described in Kakita et al., 2002 and Trentini et al., 2016. Offspring of exposed animals had a lower density of 5-bromo-2-deoxyuridine positive cells in cortical layers after high dose exposure during development (Barenys et al., unpublished data). Similarly, mice lacking the β1-integrin subunit in neural precursor cells (CNS-(nestin-Cre)-β1-integrin-deficient mice) present ‘less tightly packed’ cells in cortical layers (Graus-Porta et al. 2001). It is well documented that β1-integrin function during development is important to maintain the integrity of the glial scaffold (Manent et al. 2011).

There is currently no data on a compound-induced AO for this AOP available. However, β1-integrin deficient animals display behavioral abnormalities. When β1-integrin is lacking in excitatory neurons, hippocampus-dependent learning is impaired (Warren et al. 2012). These *Nex-Cre (itgb1$^{flox/flox}$ Nex-Cre+)* mice display a behavioral phenotype; they fail to

discriminate between novel and familiar objects in a hippocampus-dependent novel object recognition task (Warren et al. 2012).

Essentiality of the identified key events for AO (impairment of learning and memory): This AOP clearly displays the knowledge gap on compound exposure and AO. While the hypothetical AO explained above is based on experiments with transgenic animals that lack the β1-integrin protein, so far there has been no study performed with a compound that disrupts the binding of laminin to β1-integrins. Therefore, essentiality of the KE for the AO cannot be assessed. However, the *in vitro* data in combination with the existing data as described above suggest that there is concern for a potential developmental neurotoxicity hazard by the proposed MOA. Clearly, more data is needed to substantiate this concern.

The experimental data used to support the proposed AOP was obtained from studies on developmental exposure to EGCG or from studies with mice lacking the β1-integrin subunit in neural precursor cells or excitatory neurons. Other catechins containing galloyl/pyrogallol groups (Epigallocatechin: EGC, and epicatechin gallate: ECG) also inhibit human NPCs adhesion to laminin, suggesting that chemicals with similar structure can trigger the same key events (Barenys et al., submitted). This is in agreement with previous observations from Lo et al. (2007) demonstrating that both EGCG and ECG were able to inhibit cell adhesion on laminin. There is a need of more information about the ability of other compounds from different chemical families to trigger the same cascade of key events.

Potential relevance of the described, putative AOPs to current risk assessments

The potential of AOPs to support various regulatory and research applications is related to their completeness, and confidence in the underlying information (and extent of its documentation). The even incomplete AOPs provide an organizing construct for further incorporation of biological knowledge for potential applications. Early consideration of the extent of support and resulting confidence in various elements of hypothesized AOPs promotes better common understanding between the research and regulatory communities as a basis to facilitate application.

The extent of confidence required in supporting information for AOPs varies as a function of intended application as addressed in formal problem formulation (Meek et al., 2014a; OECD, 2013; OECD, 2015; Patlewicz et al., 2015; Perkins et al., 2015). Different applications to which AOPs can contribute, include: 1) supporting chemical category formation and “read-across”; 2) screening and priority setting for further testing; 3) hazard identification; 4) classification and labeling; 5) identifying research priorities and designing integrated testing strategies (ITS) or integrated approaches to testing and assessment (IATA); and 6) risk assessment. Necessarily, chemical specific information on exposure, metabolism and toxicokinetics (i.e., MOA analysis) and quantitation relevant to dose-response analysis is also taken into account to varying extents in these different applications. Within the context of IATA, AOPs have potential to increase confidence of decisions in any of these contexts (Meek et al., 2008: OECD, 2013; OECD, 2015; Patlewicz et al., 2015; Perkins et al., 2015).

Some examples of potential applications of the AOPs presented here are included below. The least developed AOP, which is applied for research gap and priority identification on the level of academic research is the AOP *Disrupted laminin-β1-integrin interaction leading to developmental neurotoxicity*. This AOP is an excellent example illustrating the value of the AOP organizing construct concept in basic toxicological research. Identification of critical KEs of this AOP has guided research design, which may facilitate consideration in any regulatory context

The AOP entitled *The Acute Neurotoxicity induced by binding to Voltage-Gated Sodium Channels* contributed to the cumulative chemical specific mode of action based assessment for pyrethroid insecticides that was conducted under the Food Quality Protection Act (FQPA), Briefly, the US Environmental Protection Agency had to determine whether or not to consider risks associated with exposure to all pyrethroid insecticides collectively, or to separately consider risks of Type I from Type II pyrethroids based on the different syndromes of toxicity (AOs). It was also proposed that some compounds (mostly Type II compounds) also had separate MOA based on modification of voltage-gated calcium channels (Shafer and Meyer 2004, Clark and Symington 2012) and a “maxi” chloride channel (Forshaw et al., 2000; Burr and Ray, 2004). However, the Agency determined that there was not enough evidence to warrant using these latter two potential MOA, and that although there are different syndromes of pyrethroid neurotoxicity, only one MOA underlies these syndromes (e.g. they have a common AOP).

More recently, the AOP concept has been utilized to make predictions about the sensitivity of different ecological species to the toxicity of pyrethroid insecticides. Lalone and colleagues (2013) compared the similarity of VGSC alpha subunits and the sensitivity (LC_{50}) of different ecological species; this could be used to predict the sensitivity of a given species (e.g. an endangered species) when information about that species is lacking and toxicity testing of that species is impractical or impossible.

The AOP entitled *Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities* is relevant to developmental neurotoxicity since it refers to a defined window of brain development when synaptogenesis takes place that is a key developmental process involved in learning and memory, defined in this AOP as an AO. Similarly, learning and memory deficit is also postulated as a possible AO of the putative AOP entitled *Disrupted laminin-β1-integrin interaction leading to developmental neurotoxicity* and an AOP on *Sensitization of the ryanodine receptor (RyR) in the developing brain alters synaptic connectivity leading to neurobehavioral perturbations*.

Throughout the years, a significant number of methods has been developed to assess neurobehaviorial change in laboratory animals, including impairment of learning and memory (OECD Guidance Document for Neurotoxicity Testing, 2004). This endpoint is important mainly for developmental neurotoxicity (DNT), for which there is a wide variety of tests to assess chemical effects on cognitive functions. Some of these tests are: habituation, ethologically based anxiety tests (elevated plus maze test, black and white box test, social interaction test), conditioned taste aversion (CTA), active avoidance, passive

avoidance, spatial mazes (Morris water maze, Biel water maze, T-maze), conditional discrimination (simple discrimination, matching to sample), delayed discrimination (delayed matching-to-sample, delayed alternation) and eye-blink conditioning. Learning and memory tests are required by the OECD Test Guideline for Developmental Neurotoxicity (426), the OECD Test Guideline for Combined Repeated Dose Toxicity Study with Reproduction/ Developmental Toxicity Screening Test (422) and the OECD Test Guideline for Extended One-Generation Reproductive Toxicity Study (443).

AOPs such as those described here can potentially contribute to the development of a mechanistically informed IATA for evaluation of chemicals with DNT potential, including those that cause impairment of learning and memory. Potentially, identification of common KEs and KERs that emerge among the available AOPs interconnected within a network can inform concerning assays that could potentially be informative in IATA to address the most critical pathways of toxicity involved in DNT.

Acknowledgments

The content of this paper is based on the INA15th session titled the *Application of Adverse Outcome Pathway (AOP) concept for Neurotoxicity Testing*. The speakers of this session are grateful for the financial travel support provided by the US EPA.

The project on AOP development titled "*Ryanodine receptor (RyR) sensitization alters neuronal connectivity leading to cognitive impairment"* was supported by the United States National Institute of Environmental Health (grants R01 ES014901 and P01 ES011269) and the United States Environmental Protection Agency (grant 83543201); Sunjay Sethi was supported by a Floyd and Mary Schwall Predoctoral Fellowship. The contents of this work are solely the responsibility of the grantee and do not necessarily represent the official views of the USEPA or NIEHS. Further, NIEHS does not endorse the purchase of any commercial products or services mentioned in the publication.

Preparation of this document has been funded in part by the U.S. Environmental Protection Agency. This document has been reviewed by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

Adasme T, Haeger P, Paula-Lima AC, et al. Involvement of ryanodine receptors in neurotrophin-induced hippocampal synaptic plasticity and spatial memory formation. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(7):3029–3034. [PubMed: 21282625]

Ahn KC, Zhao B, Chen J, et al. In vitro biologic activities of the antimicrobials triclocarban, its analogs, and triclosan in bioassay screens: receptor-based bioassay screens. Environmental health perspectives. 2008; 116(9):1203–1210. [PubMed: 18795164]

An JJ, Gharami K, Liao GY, et al. Distinct role of long 3' UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. Cell. 2008; 134(1):175–187. [PubMed: 18614020]

Andjelic S, Khanna A, Suthanthiran M, Nikolic-Zugic J. Intracellular Ca2+ elevation and cyclosporin A synergistically induce TGF-beta 1-mediated apoptosis in lymphocytes. Journal of immunology (Baltimore, Md : 1950). 1997; 158(6):2527–2534.

Ankley GT, Bennett RS, Erickson RJ, et al. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environmental toxicology and chemistry / SETAC. 2010; 29(3):730–741.

Bal-Price A, Crofton KM, Leist M, Allen S, et al. Internationsl Stakeholder NETwork (ISTNET): creating a developmental neurotoxicity (DNT) testing road map for regulatory purposes. Arch Toxicol. 2015a; 89:269–287. [PubMed: 25618548]

Bal-Price A, Crofton KM, Sachana M, et al. Putative adverse outcome pathways relevant to neurotoxicity. Critical reviews in toxicology. 2015b; 45(1):83–91. [PubMed: 25605028]

Bai X, Twaroski D, Bosnjak ZJ. Modeling anesthetic developmental neurotoxicity using human stem cells. Semin Cardiothorac Vasc Anesth. 2013; 17:276–287. [PubMed: 23859832]

Barenys M, Gassmann K, Baksmeier C, et al. Epigallocatechin Gallate (EGCG) inhibits adhesion and migration of neural progenitor cells in vitro. Archives of Toxicology. 2016 (submitted).

Barone S Jr, Das KP, Lassiter TL, White LD. Vulnerable processes of nervous system development: a review of markers and methods. Neurotoxicology. 2000; 21(1–2):15–36. [PubMed: 10794382]

Baumann J, Barenys M, Gassmann K, Fritsche E. Comparative Human and Rat "Neurosphere Assay" for Developmental Neurotoxicity Testing. Current Protocols in Toxicology. 2014:12.21. 1–12.21. 24.

Baumann, J., Dach, K., Barenys, M., et al. Methods in Pharmacology and Toxicology. 2015. Application of the Neurosphere Assay for DNT Hazard Assessment: Challenges and Limitations. © Springer Science+Business Media New York 2015 In press

Bear MF. A synaptic basis for memory storage in the cerebral cortex. Proc Natl Acad Sci U S A. 1996; 93(24):13453–13459. [PubMed: 8942956]

Becker RA, Ankley GT, Edwards SW, et al. Increasing Scientific Confidence in Adverse Outcome Pathways: Application of Tailored Bradford-Hill Considerations for Evaluating Weight of Evidence. Regulatory toxicology and pharmacology : RTP. 2015; 72(3):514–537. [PubMed: 25863193]

Belmonte MK, Bourgeron T. Fragile X syndrome and autism at the intersection of genetic and neural networks. Nature neuroscience. 2006; 9(10):1221–1225. [PubMed: 17001341]

Belvindrah R, Graus-Porta D, Goebbels S, Nave K-A, Müller U. β1 integrins in radial glia but not in migrating neurons are essential for the formation of cell layers in the cerebral cortex. Journal of Neuroscience. 2007; 27(50):13854–13865. [PubMed: 18077697]

Berger-Sweeney J, Hohmann CF. Behavioral consequences of abnormal cortical development: insights into developmental disabilities. Behavioural brain research. 1997; 86(2):121–142. [PubMed: 9134147]

Bernd P. The role of neurotrophins during early development. Gene Expr. 2008; 14:241–250. [PubMed: 19110723]

Berridge MJ. Neuronal calcium signaling. Neuron. 1998; 21(1):13–26. [PubMed: 9697848]

Berridge MJ. Calcium microdomains: organization and function. Cell calcium. 2006; 40(5–6):405–412. [PubMed: 17030366]

Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. Nature reviews Molecular cell biology. 2000; 1(1):11–21. [PubMed: 11413485]

Black AM, Pandya S, Clark D, Armstrong EA, Yager JY. Effect of caffeine and morphine on the developing pre-mature brain. Brain research. 2008; 1219:136–142. [PubMed: 18547548]

Boobis AR, Cohen SM, Dellarco V, et al. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Critical reviews in toxicology. 2006; 36(10):781–792. [PubMed: 17118728]

Boobis AR, Doe JE, Heinrich-Hirsch B, et al. IPCS framework for analyzing the relevance of a noncancer mode of action for humans. Critical reviews in toxicology. 2008; 38(2):87–96. [PubMed: 18259981]

Bourgeron T. A synaptic trek to autism. Current opinion in neurobiology. 2009; 19(2):231–234. [PubMed: 19545994]

Bracke ME, Castronovo V, Van Cauwenberge RM, et al. The anti-invasive flavonoid (+)-catechin binds to laminin and abrogates the effect of laminin on cell morphology and adhesion. Experimental cell research. 1987; 173(1):193–205. [PubMed: 3678379]

Brunelli L, Llansola M, Felipo V, et al. Insight into the neuroproteomics effects of the food-contaminant non-dioxin like polychlorinated biphenyls. Journal of proteomics. 2012; 75(8):2417–2430. [PubMed: 22387315]

Burr SA, Ray DE. Structure-activity and interaction effects of 14 different pyrethroids on voltage-gated chloride ion channels. Toxicological sciences : an official journal of the Society of Toxicology. 2004; 77(2):341–346. [PubMed: 14657519]

Bushnell PJ, Rice DC. Behavioral assessments of learning and attention in rats exposed perinatally to 3,3',4,4',5-pentachlorobiphenyl (PCB 126). Neurotoxicol Teratol. 1999; 21(4):381–392. [PubMed: 10440482]

Carmichael N, Bausen M, Boobis AR, et al. Using mode of action information to improve regulatory decision-making: an ECETOC/ILSI RF/HESI workshop overview. Critical reviews in toxicology. 2011; 41(3):175–186. [PubMed: 21401325]

Chen C-N, Liang C-M, Lai J-R, Tsai Y-J, Tsay J-S, Lin J-K. Capillary electrophoretic determination of theanine, caffeine, and catechins in fresh tea leaves and oolong tea and their effects on rat neurosphere adhesion and migration. Journal of agricultural and food chemistry. 2003; 51(25): 7495–7503. [PubMed: 14640605]

Chen Z-L, Haegeli V, Yu H, Strickland S. Cortical deficiency of laminin γ1 impairs the AKT/GSK-3β signaling pathway and leads to defects in neurite outgrowth and neuronal migration. Developmental Biology. 2009; 327(1):158–168. doi:http://dx.doi.org/10.1016/j.ydbio.2008.12.006. [PubMed: 19118544]

Clark JM, Symington SB. Advances in the mode of action of pyrethroids. Topics in current chemistry. 2012; 314:49–72. [PubMed: 22025067]

Cline H, Haas K. The regulation of dendritic arbor development and plasticity by glutamatergic synaptic input: a review of the synaptotrophic hypothesis. The Journal of physiology. 2008; 586(Pt 6):1509–1517. [PubMed: 18202093]

Colomer J, Means AR. Physiological roles of the Ca2+/CaM-dependent protein kinase cascade in health and disease. Subcell Biochem. 2007; 45:169–214. [PubMed: 18193638]

Colon-Ramos DA. Synapse formation in developing neural circuits. Current topics in developmental biology. 2009; 87:53–79. [PubMed: 19427516]

Connolly S, Kingsbury TJ. Caffeine modulates CREB-dependent gene expression in developing cortical neurons. Biochemical and biophysical research communications. 2010; 397(2):152–156. [PubMed: 20493822]

Council of Labor Affairs T. Existing Chemical Substance Inventory. Taiwan: Chemical Substance Nomination & Notification, Council of Labor Affairs, Executive Yuan; 2012.

Cukier HN, Lee JM, Ma D, et al. The expanding role of MBD genes in autism: identification of a MECP2 duplication and novel alterations in MBD5, MBD6, and SETDB1. Autism research : official journal of the International Society for Autism Research. 2012; 5(6):385–397. [PubMed: 23055267]

Danieli GA, Rampazzo A. Genetics of arrhythmogenic right ventricular cardiomyopathy. Curr Opin Cardiol. 2002; 17(3):218–221. [PubMed: 12015469]

Dellarco V, Henry T, Sayre P, Seed J, Bradbury S. Meeting the common needs of a more effective and efficient testing and assessment paradigm for chemical risk management. Journal of toxicology and environmental health Part B, Critical reviews. 2010; 13(2–4):347–360.

Delorme R, Ey E, Toro R, Leboyer M, Gillberg C, Bourgeron T. Progress toward treatments for synaptic defects in autism. Nature medicine. 2013; 19(6):685–694.

Dikranian K, Ishimaru MJ, Tenkova T, et al. Apoptosis in the in vivo mammalian forebrain. Neurobiol Dis. 2001; 8(3):359–379. [PubMed: 11447994]

Dufault C, Poles G, Driscoll LL. Brief postnatal PBDE exposure alters learning and the cholinergic modulation of attention in rats. Toxicol Sci. 2005; 88(1):172–180. [PubMed: 16107551]

Edbauer D, Neilson JR, Foster KA, et al. Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. Neuron. 2010; 65(3):373–384. [PubMed: 20159450]

Edwards SW, Tan YM, Villeneuve DL, Meek ME, McQueen CA. Adverse Outcome Pathways-Organizing Toxicological Information to Improve Decision Making. The Journal of pharmacology and experimental therapeutics. 2016; 356(1):170–181. [PubMed: 26537250]

Erecinska M, Cherian S, Silver IA. Energy metabolism in mammalian brain during development. Prog Neurobiol. 2004; 73:397–445. [PubMed: 15313334]

Ehrlich I, Klein M, Rumpel S, Malinow R. PSD-95 is required for activity-driven synapse stabilization. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(10): 4176–4181. [PubMed: 17360496]

EPA US. U.S. EPA, U.S. Environmental Protection Agency, Guidelines for Carcinogen Risk Assessment. US EPA, Washington, DC: 2005. http://www.epa.gov/cancerguidelines/

Ermak G, Davies KJ. Calcium and oxidative stress: from cell signaling to cell death. Mol Immunol. 2002; 38(10):713–721. [PubMed: 11841831]

European Commission EU. No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Off. J. Eur. Union L396. 2006:1–849. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:396:0001:0849:EN:PDF.

Forshaw PJ, Lister T, Ray DE. The role of voltage-gated chloride channels in type II pyrethroid insecticide poisoning. Toxicology and applied pharmacology. 2000; 163(1):1–8. [PubMed: 10662599]

Geschwind DH, Levitt P. Autism spectrum disorders: developmental disconnection syndromes. Curr Opin Neurobiol. 2007; 17(1):103–111. [PubMed: 17275283]

Granger AJ, Gray JA, Lu W, Nicoll RA. Genetic analysis of neuronal ionotropic glutamate receptor subunits. The Journal of physiology. 2011; 589(Pt 17):4095–4101. [PubMed: 21768264]

Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet. 2006; 368:2167–2178. [PubMed: 17174709]

Graus-Porta D, Blaess S, Senften M, et al. β1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. Neuron. 2001; 31(3):367–379. [PubMed: 11516395]

Guo XJ, Fantini J, Roubin R, Marvaldi J, Rougon G. Evaluation of the effect of suramin on neural cell growth and N-CAM expression. Cancer Res. 1990; 50(16):5164–5170. [PubMed: 2379176]

Haberny KA, Paule MG, Scallet AC, et al. Ontogeny of the N-methyl-D-aspartate (NMDA) receptor system and susceptibility to neurotoxicity. Toxicological sciences : an official journal of the Society of Toxicology. 2002; 68(1):9–17. [PubMed: 12075105]

Hajnoczky G, Csordas G, Madesh M, Pacher P. Control of apoptosis by IP(3) and ryanodine receptor driven calcium signals. Cell Calcium. 2000; 28(5–6):349–363. [PubMed: 11115374]

Hansen KF, Karelina K, Sakamoto K, Wayman GA, Impey S, Obrietan K. miRNA-132: a dynamic regulator of cognitive capacity. Brain structure & function. 2013; 218(3):817–831. [PubMed: 22706759]

Hansen KF, Sakamoto K, Wayman GA, Impey S, Obrietan K. Transgenic miR132 alters neuronal spine density and impairs novel object recognition memory. PloS one. 2010; 5(11):e15497. [PubMed: 21124738]

Higley MJ, Sabatini BL. Calcium signaling in dendritic spines. Cold Spring Harbor perspectives in biology. 2012; 4(4):a005686. [PubMed: 22338091]

Hill AB. THE ENVIRONMENT AND DISEASE: ASSOCIATION OR CAUSATION? Proceedings of the Royal Society of Medicine. 1965; 58:295–300. [PubMed: 14283879]

Howard AS, Fitzpatrick R, Pessah I, Kostyniak P, Lein PJ. Polychlorinated biphenyls induce caspase-dependent cell death in cultured embryonic rat hippocampal but not cortical neurons via activation of the ryanodine receptor. Toxicol Appl Pharmacol. 2003; 190(1):72–86. [PubMed: 12831785]

Hudson NL, Kasner EJ, Beckman J, et al. Characteristics and magnitude of acute pesticide-related illnesses and injuries associated with pyrethrin and pyrethroid exposures--11 states, 2000–2008. American journal of industrial medicine. 2014; 57(1):15–30. [PubMed: 23788228]

Hughes K, Paterson J, Meek ME. Tools for the prioritization of substances on the Domestic Substances List in Canada on the basis of hazard. Regulatory toxicology and pharmacology : RTP. 2009; 55(3):382–393. [PubMed: 19766685]

Hutsler JJ, Zhang H. Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. Brain Res. 2010; 1309:83–94. [PubMed: 19896929]

Ikonomidou C, Bosch F, Miksa M, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science. 1999; 283(5398):70–74. [PubMed: 9872743]

Impey S, Davare M, Lasiek A, et al. An activity-induced microRNA controls dendritic spine formation by regulating Rac1-PAK signaling. Mol Cell Neurosci. 2010; 43(1):146–156. [PubMed: 19850129]

Irwin SA, Patel B, Idupulapati M, et al. Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. Am J Med Genet. 2001; 98(2):161–167. [PubMed: 11223852]

Jessell TM, Sanes JR. Development. The decade of the developing brain. Curr Opin Neurobiol 2000. 2000; 10(5):599–611.

Jin X, Hu H, Mathers PH, Agmon A. Brain-derived neurotrophic factor mediates activity-dependent dendritic growth in nonpyramidal neocortical interneurons in developing organotypic cultures. J Neurosci. 2003; 23(13):5662–5673. [PubMed: 12843269]

Jolous-Jamshidi B, Cromwell HC, McFarland AM, Meserve LA. Perinatal exposure to polychlorinated biphenyls alters social behaviors in rats. Toxicol Lett. 2010; 199(2):136–143. [PubMed: 20813172]

Juarez-Mendez S, Carretero R, Martinez-Tellez R, Silva-Gomez AB, Flores G. Neonatal caffeine administration causes a permanent increase in the dendritic length of prefrontal cortical neurons of rats. Synapse. 2006; 60(6):450–455. [PubMed: 16892188]

Judson MC, Eagleson KL, Levitt P. A new synaptic player leading to autism risk: Met receptor tyrosine kinase. Journal of neurodevelopmental disorders. 2011; 3(3):282–292. [PubMed: 21509596]

Kakita A, Inenaga C, Sakamoto M, Takahashi H. Neuronal migration disturbance and consequent cytoarchitecture in the cerebral cortex following transplacental administration of methylmercury. Acta Neuropathol. 2002; 104:409–417. [PubMed: 12200629]

Kang H, Schuman EM. A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. Science (New York, NY). 1996; 273(5280):1402–1406.

Katz LC, Shatz CJ. Synaptic activity and the construction of cortical circuits. Science. 1996; 274(5290):1133–1138. [PubMed: 8895456]

Kenet T, Froemke RC, Schreiner CE, Pessah IN, Merzenich MM. Perinatal exposure to a noncoplanar polychlorinated biphenyl alters tonotopy, receptive fields, and plasticity in rat primary auditory cortex. Proc Natl Acad Sci U S A. 2007; 104(18):7646–7651. [PubMed: 17460041]

Keown CL, Shih P, Nair A, Peterson N, Mulvey ME, Muller RA. Local functional overconnectivity in posterior brain regions is associated with symptom severity in autism spectrum disorders. Cell Rep. 2013; 5(3):567–572. [PubMed: 24210815]

Kim AH, Reimers M, Maher B, et al. MicroRNA expression profiling in the prefrontal cortex of individuals affected with schizophrenia and bipolar disorders. Schizophrenia research. 2010; 124(1–3):183–191. [PubMed: 20675101]

Kim KH, Bose DD, Ghogha A, et al. Para- and ortho-substitutions are key determinants of polybrominated diphenyl ether activity toward ryanodine receptors and neurotoxicity. Environ Health Perspect. 2011; 119(4):519–526. [PubMed: 21106467]

Kim KH, Inan SY, Berman RF, Pessah IN. Excitatory and inhibitory synaptic transmission is differentially influenced by two ortho-substituted polychlorinated biphenyls in the hippocampal slice preparation. Toxicology and applied pharmacology. 2009; 237(2):168–177. [PubMed: 19289137]

Klein ME, Lioy DT, Ma L, Impey S, Mandel G, Goodman RH. Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. Nat Neurosci. 2007; 10(12):1513–1514. [PubMed: 17994015]

Klintsova AY, Greenough WT. Synaptic plasticity in cortical systems. Current opinion in neurobiology. 1999; 9(2):203–208. [PubMed: 10322189]

Konur S, Ghosh A. Calcium signaling and the control of dendritic development. Neuron. 2005; 46(3): 401–405. [PubMed: 15882639]

Korkotian E, Segal M. Release of calcium from stores alters the morphology of dendritic spines in cultured hippocampal neurons. Proc Natl Acad Sci U S A. 1999; 96(21):12068–12072. [PubMed: 10518577]

Kostyniak PJ, Hansen LG, Widholm JJ, et al. Formulation and characterization of an experimental PCB mixture designed to mimic human exposure from contaminated fish. Toxicol Sci. 2005; 88(2):400–411. [PubMed: 16177234]

Kovalchuk Y, Holthoff K, Konnerth A. Neurotrophin action on a rapid timescale. Current opinion in neurobiology. 2004; 14(5):558–563. [PubMed: 15464888]

Lalone CA, Villeneuve DL, Burgoon LD, et al. Molecular target sequence similarity as a basis for species extrapolation to assess the ecological risk of chemicals with known modes of action. Aquatic toxicology (Amsterdam, Netherlands). 2013; 144–145:141–154.

LaSalle JM, Yasui DH. Evolving role of MeCP2 in Rett syndrome and autism. Epigenomics. 2009; 1(1):119–130. [PubMed: 20473347]

Lee R, Kermani P, Teng KK, Hempstead BL. Regulation of cell survival by secreted proneurotrophins. Science. 2001; 294:1945–1948. [PubMed: 11729324]

Lein PJ, Yang D, Bachstetter AD, et al. Ontogenetic alterations in molecular and structural correlates of dendritic growth after developmental exposure to polychlorinated biphenyls. Environ Health Perspect. 2007; 115(4):556–563. [PubMed: 17450224]

Lesiak A, Zhu M, Chen H, et al. The environmental neurotoxicant PCB 95 promotes synaptogenesis via ryanodine receptor-dependent miR132 upregulation. J Neurosci. 2014; 34(3):717–725. [PubMed: 24431430]

Lessmann V, Heumann R. Modulation of unitary glutamatergic synapses by neurotrophin-4/5 or brain-derived neurotrophic factor in hippocampal microcultures: presynaptic enhancement depends on pre-established paired-pulse facilitation. Neuroscience. 1998; 86(2):399–413. [PubMed: 9881855]

Li Q, Chen M, Liu H, Yang L, Yang T, He G. The dual role of ERK signaling in the apoptosis of neurons. Front Biosci (Landmark Ed). 2014; 19:1411–1417. [PubMed: 24896360]

Libersat F, Duch C. Mechanisms of dendritic maturation. Mol Neurobiol. 2004; 29(3):303–320. [PubMed: 15181241]

Liu D, Zhang M, Yin H. Signaling pathways involved in endoplasmic reticulum stress-induced neuronal apoptosis. Int J Neurosci. 2013; 123(3):155–162. [PubMed: 23134425]

Lo HM, Hung CF, Huang YY, Wu WB. Tea polyphenols inhibit rat vascular smooth muscle cell adhesion and migration on collagen and laminin via interference with cell-ECM interaction. Journal of biomedical science. 2007; 14(5):637–645. [PubMed: 17436062]

Lohmann C. Calcium signaling and the development of specific neuronal connections. Progress in brain research. 2009; 175:443–452. [PubMed: 19660672]

Lohmann C, Wong RO. Regulation of dendritic growth and plasticity by local and global calcium dynamics. Cell Calcium. 2005; 37(5):403–409. [PubMed: 15820387]

Lowell Center for Sustainable Production. Chemicals Policy: Other Nations [Internet]. Lowell, Massachusetts: Chemicals Policy & Science Initiative, Lowell Center for Sustainable Production, University of Massachusetts Lowell; 2012.

Lu Y, Christian K, Lu B. BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory? Neurobiology of learning and memory. 2008; 89(3):312–323. [PubMed: 17942328]

Lubbers BR, Smit AB, Spijker S, van den Oever MC. Neural ECM in addiction, schizophrenia, and mood disorder. Progress in brain research. 2014; 214:263–284. [PubMed: 25410362]

Lund AE, Narahashi T. Dose-dependent interaction of the pyrethroid isomers with sodium channels of squid axon membranes. Neurotoxicology. 1982; 3(1):11–24. [PubMed: 6290951]

Lynch MA. Long-term potentiation and memory. Physiol Rev. 2004; 84:87–136. [PubMed: 14715912]

Manent JB, Beguin S, Ganay T, Represa A. Cell-autonomous and cell-to-cell signalling events in normal and altered neuronal migration. European Journal of Neuroscience. 2011; 34(10):1595–1608. [PubMed: 22103417]

Mariot P, Prevarskaya N, Roudbaraki MM, et al. Evidence of functional ryanodine receptor involved in apoptosis of prostate cancer (LNCaP) cells. Prostate. 2000; 43(3):205–214. [PubMed: 10797495]

Martin LJ. Neuronal cell death in nervous system development, disease, and injury (Review). Int J Mol Med. 2001; 7(5):455–478. [PubMed: 11295106]

Martin SJ, Green DR. Protease activation during apoptosis: death by a thousand cuts? Cell. 1995; 82(3):349–352. [PubMed: 7634323]

McAllister AK. Cellular and molecular mechanisms of dendrite growth. Cerebral cortex (New York, NY : 1991). 2000; 10(10):963–973.

McKillop CM, Brock JA, Oliver GJ, Rhodes C. A quantitative assessment of pyrethroid-induced paraesthesia in the guinea-pig flank model. Toxicology letters. 1987; 36(1):1–7. [PubMed: 2436357]

Meacham CA, Brodfuehrer PD, Watkins JA, Shafer TJ. Developmentally-regulated sodium channel subunits are differentially sensitive to alpha-cyano containing pyrethroids. Toxicology and applied pharmacology. 2008; 231(3):273–281. [PubMed: 18538810]

Meek, M., Armstrong, V. The assessment and management of industrial chemicals in Canada Risk assessment of chemicals. Springer; 2007. p. 591-621.

Meek ME. Recent developments in frameworks to consider human relevance of hypothesized modes of action for tumours in animals. Environmental and molecular mutagenesis. 2008; 49(2):110–116. [PubMed: 18213650]

Meek ME, Berry C, Boobis AR, et al. 2008Guyton, Kathryn Z., Barone, Stanley, Jr, Brown, Rebecca C., Euling, Susan Y., Jinot, Jennifer, Makris, Susan. Mode of action frameworks: a critical analysis. Journal of Toxicology and Environmental Health, Part B. 2008; 11(1):16–31.Journal of toxicology and environmental health Part B, Critical reviews. 11(8):681–683. author reply 684-5.

Meek ME, Boobis A, Cote I, et al. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. Journal of applied toxicology : JAT. 2014a; 34(1):1–18. [PubMed: 24166207]

Meek ME, Bucher JR, Cohen SM, et al. A framework for human relevance analysis of information on carcinogenic modes of action. Critical reviews in toxicology. 2003; 33(6):591–653. [PubMed: 14727733]

Meek ME, Klaunig JE. Proposed mode of action of benzene-induced leukemia: Interpreting available data and identifying critical data gaps for risk assessment. Chemico-biological interactions. 2010; 184(1–2):279–285. [PubMed: 20153303]

Meek ME, Palermo CM, Bachman AN, North CM, Jeffrey Lewis R. Mode of action human relevance (species concordance) framework: Evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. Journal of applied toxicology : JAT. 2014b; 34(6):595–606. [PubMed: 24777878]

Melgarejo E, Medina MA, Sanchez-Jimenez F, Urdiales JL. Epigallocatechin gallate reduces human monocyte mobility and adhesion in vitro. British journal of pharmacology. 2009; 158(7):1705–1712. [PubMed: 19912233]

Migaud M, Charlesworth P, Dempster M, et al. Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. Nature. 1998; 396(6710):433–439. [PubMed: 9853749]

Miller BH, Zeier Z, Xi L, et al. MicroRNA-132 dysregulation in schizophrenia has implications for both neurodevelopment and adult brain function. Proc Natl Acad Sci U S A. 2012; 109(8):3125–3130. [PubMed: 22315408]

Miller FD, Kaplan DR. Neurotrophin signalling pathways regulating neuronal apoptosis. Cell Mol Life Sci. 2001; 58:1045–1053. [PubMed: 11529497]

Minichiello L. TrkB signalling pathways in LTP and learning. Nature reviews Neuroscience. 2009; 10(12):850–860. [PubMed: 19927149]

Monteggia LM, Barrot M, Powell CM, et al. Essential role of brain-derived neurotrophic factor in adult hippocampal function. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101(29):10827–10832. [PubMed: 15249684]

Murray PS, Holmes PV. An Overview of Brain-Derived Neurotrophic Factor and Implications for Excitotoxic Vulnerability in the Hippocampus nternational. J Pept. 2011; 2011:12. Article ID 654085.

Nagappan G, Lu B. Activity-dependent modulation of the BDNF receptor TrkB: mechanisms and implications. Trends in neurosciences. 2005; 28(9):464–471. [PubMed: 16040136]

O'Reilly AO, Khambay BP, Williamson MS, Field LM, Wallace BA, Davies TG. Modelling insecticide-binding sites in the voltage-gated sodium channel. The Biochemical journal. 2006; 396(2):255–263. [PubMed: 16475981]

OECD. Guidance Document On Developing And Assessing Adverse Outcome Pathways. Environment Directorate; Joint Meeting Of The Chemicals Committee And The Working Party On Chemicals Pab Ed. 2013. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)6&doclanguage=en

OECD. Report of the Workshop on a Framework for the Development and Use Of Integrated Approaches to Testing and Assessment. Series on Testing and Assessment 215. 2015. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2015)22&doclanguage=en

OECD. Users' Handbook Supplement to the Guidance Document For Developing And Assessing Aops. 2014.

Ogura H, Aigner TG. MK-801 impairs recognition memory in rhesus monkeys: comparison with cholinergic drugs. The Journal of pharmacology and experimental therapeutics. 1993; 266(1):60–64. [PubMed: 8331575]

Okada H, Miyakawa N, Mori H, Mishina M, Miyamoto Y, Hisatsune T. NMDA receptors in cortical development are essential for the generation of coordinated increases in [Ca2+](i) in "neuronal domains". Cerebral cortex (New York, NY : 1991). 2003; 13(7):749–757.

Pan Z, Damron D, Nieminen AL, Bhat MB, Ma J. Depletion of intracellular Ca2+ by caffeine and ryanodine induces apoptosis of chinese hamster ovary cells transfected with ryanodine receptor. J Biol Chem. 2000; 275(26):19978–19984. [PubMed: 10764805]

Papineni RV, O'Connell KM, Zhang H, Dirksen RT, Hamilton SL. Suramin interacts with the calmodulin binding site on the ryanodine receptor, RYR1. J Biol Chem. 2002; 277(51):49167–49174. [PubMed: 12364321]

Park JH, Yoon JH, Kim SA, Ahn SG, Yoon JH. (-)-Epigallocatechin-3-gallate inhibits invasion and migration of salivary gland adenocarcinoma cells. Oncology reports. 2010; 23(2):585–590. [PubMed: 20043125]

Pasca SP, Portmann T, Voineagu I, et al. Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. Nature Med advance on-line pub. 2011

Patlewicz G, Simon TW, Rowlands JC, Budinsky RA, Becker RA. Proposing a scientific confidence framework to help support the application of adverse outcome pathways for regulatory purposes. Regul. Toxicol. Pharmacol. 2015; 71:463–477. [PubMed: 25707856]

Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER. Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron. 1996; 16(6):1137–1145. [PubMed: 8663990]

Penzes P, Cahill ME, Jones KA, VanLeeuwen JE, Woolfrey KM. Dendritic spine pathology in neuropsychiatric disorders. Nat Neurosci. 2011; 14(3):285–293. [PubMed: 21346746]

Perkins EJ, Antczak P, Burgoon L, Falciani F, Garcia-Reyero N, Gutsell S, Hodges G, Kienzler A, Knapen D, McBride M, Willett C. Adverse Outcome Pathways for Regulatory Applications: Examination of Four Case Studies With Different Degrees of Completeness and Scientific Confidence. Toxicol. Sci. 2015; 148:14–25. [PubMed: 26500288]

Percy AK. Rett syndrome: exploring the autism link. Archives of neurology. 2011; 68(8):985–989. [PubMed: 21825235]

Pessah IN, Cherednichenko G, Lein PJ. Minding the calcium store: Ryanodine receptor activation as a convergent mechanism of PCB toxicity. Pharmacology & therapeutics. 2010; 125(2):260–285. [PubMed: 19931307]

Pessah IN, Hansen LG, Albertson TE, et al. Structure-activity relationship for noncoplanar polychlorinated biphenyl congeners toward the ryanodine receptor-Ca2+ channel complex type 1 (RyR1). Chem Res Toxicol. 2006; 19(1):92–101. [PubMed: 16411661]

Pessah IN, Stambuk RA, Casida JE. Ca2+-activated ryanodine binding: mechanisms of sensitivity and intensity modulation by Mg2+, caffeine, and adenine nucleotides. Mol Pharmacol. 1987; 31(3): 232–238. [PubMed: 2436032]

Pessah, IN., Wong, PW. Etiology of PCB Neurotoxicity: From Molecules to Cellular Dysfunction. In: Robertson, L., Hansen, L., editors. Progress In Polychlorinated Biphenyl Toxicology. New York, NY: Academic Press; 2001. p. 179-184.

Pittenger C, Kandel ER. In search of general mechanisms for long-lasting plasticity: Aplysia and the hippocampus. Philos Trans R Soc Lond B Biol Sci. 2003; 358(1432):757–763. [PubMed: 12740123]

Poo MM. Neurotrophins as synaptic modulators. Nature reviews Neuroscience. 2001; 2(1):24–32. [PubMed: 11253356]

Production LCfS. Chemicals Policy: Other Nations [Internet]. Lowell, Massachusetts: Chemicals Policy & Science Initiative, Lowell Center for Sustainable Production, University of Massachusetts Lowell; 2012.

Purpura DP. Dendritic differentiation in human cerebral cortex: normal and aberrant developmental patterns. Advances in neurology. 1975; 12:91–134. [PubMed: 1155280]

Ravagnan L, Roumier T, Kroemer G. Mitochondria, the killer organelles and their weapons. J Cell Physiol. 2002; 192(2):131–137. [PubMed: 12115719]

Redini-del-Negro C, Laroche S. Learning-induced increase in glutamate release in the dentate gyrus is blocked by the NMDA receptor antagonist AP5. Neuroscience Research Communications. 1993; 13(3):157–165.

Redmond L, Ghosh A. Regulation of dendritic development by calcium signaling. Cell Calcium. 2005; 37(5):411–416. [PubMed: 15820388]

Rex CS, Lin CY, Kramar EA, Chen LY, Gall CM, Lynch G. Brain-derived neurotrophic factor promotes long-term potentiation-related cytoskeletal changes in adult hippocampus. J Neurosci. 2007; 27(11):3017–3029. [PubMed: 17360925]

Rezvani, AH. Involvement of the NMDA System in Learning and Memory. Levin, ED., editor. 2006.

Rice D, Barone S Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect. 2000; 108(Suppl 3):511–533. [PubMed: 10852851]

Riedel G, Platt B, Micheau J. Glutamate receptor function in learning and memory. Behavioural brain research. 2003; 140(1–2):1–47. [PubMed: 12644276]

Rinkevich FD, Du Y, Dong K. Diversity and Convergence of Sodium Channel Mutations Involved in Resistance to Pyrethroids. Pesticide biochemistry and physiology. 2013; 106(3):93–100. [PubMed: 24019556]

Roberts TF, Tschida KA, Klein ME, Mooney R. Rapid spine stabilization and synaptic enhancement at the onset of behavioural learning. Nature. 2010; 463(7283):948–952. [PubMed: 20164928]

Robertson JD, Chandra J, Gogvadze V, Orrenius S. Biological reactive intermediates and mechanisms of cell death. Adv Exp Med Biol. 2001; 500:1–10.

Roegge CS, Morris JR, Villareal S, et al. Purkinje cell and cerebellar effects following developmental exposure to PCBs and/or MeHg. Neurotoxicol Teratol. 2006; 28(1):74–85. [PubMed: 16309888]

Roegge CS, Schantz SL. Motor function following developmental exposure to PCBS and/or MEHG. Neurotoxicol Teratol. 2006; 28(2):260–277. [PubMed: 16487679]

Roegge CS, Seo BW, Crofton KM, Schantz SL. Gestational-lactational exposure to Aroclor 1254 impairs radial-arm maze performance in male rats. Toxicol Sci. 2000; 57(1):121–130. [PubMed: 10966518]

Rusakov DA, Davies HA, Harrison E, et al. Ultrastructural synaptic correlates of spatial learning in rat hippocampus. Neuroscience. 1997; 80(1):69–77. [PubMed: 9252221]

Saar D, Barkai E. Long-term modifications in intrinsic neuronal properties and rule learning in rats. Eur J Neurosci. 2003; 17:2727–2734. [PubMed: 12823479]

Sala C, Piech V, Wilson NR, Passafaro M, Liu G, Sheng M. Regulation of dendritic spine morphology and synaptic function by Shank and Homer. Neuron. 2001; 31(1):115–130. [PubMed: 11498055]

Samso M, Feng W, Pessah IN, Allen PD. Coordinated movement of cytoplasmic and transmembrane domains of RyR1 upon gating. PLoS biology. 2009; 7(4):e85. [PubMed: 19402748]

Sastry PS, Rao KS. Apoptosis and the nervous system. J Neurochem. 2000; 74(1):1–20. [PubMed: 10617101]

Schantz SL, Seo BW, Wong PW, Pessah IN. Long-term effects of developmental exposure to 2,2',3,5', 6-pentachlorobiphenyl (PCB 95) on locomotor activity, spatial learning and memory and brain ryanodine binding. Neurotoxicology. 1997; 18(2):457–467. [PubMed: 9291494]

Schmitt U, Tanimoto N, Seeliger M, Schaeffel F, Leube RE. Detection of behavioral alterations and learning deficits in mice lacking synaptophysin. Neuroscience. 2009; 162(2):234–243. [PubMed: 19393300]

Schratt GM, Nigh EA, Chen WG, Hu L, Greenberg ME. BDNF regulates the translation of a select group of mRNAs by a mammalian target of rapamycin-phosphatidylinositol 3-kinase-dependent pathway during neuronal development. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004; 24(33):7366–7377. [PubMed: 15317862]

Scott EK, Luo L. How do dendrites take their shape? Nat Neurosci. 2001; 4(4):359–365. [PubMed: 11276225]

Seed J, Carney EW, Corley RA, et al. Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. Critical reviews in toxicology. 2005; 35(8–9):664–672. [PubMed: 16417033]

Segal M, Korkotian E, Murphy DD. Dendritic spine formation and pruning: common cellular mechanisms? Trends Neurosci. 2000; 23(2):53–57. [PubMed: 10652540]

Sekino Y, Kojima N, Shirao T. Role of actin cytoskeleton in dendritic spine morphogenesis. Neurochemistry international. 2007; 51(2–4):92–104. [PubMed: 17590478]

Shafer TJ, Meyer DA. Effects of pyrethroids on voltage-sensitive calcium channels: a critical evaluation of strengths, weaknesses, data needs, and relationship to assessment of cumulative neurotoxicity. Toxicology and applied pharmacology. 2004; 196(2):303–318. [PubMed: 15081275]

Shafer TJ, Meyer DA, Crofton KM. Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs. Environmental health perspectives. 2005; 113(2):123–136. [PubMed: 15687048]

Silva AJ, Paylor R, Wehner JM, Tonegawa S. Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. Science (New York, NY). 1992; 257(5067):206–211.

Soderling TR. The Ca-calmodulin-dependent protein kinase cascade. Trends in biochemical sciences. 1999; 24(6):232–236. [PubMed: 10366852]

Soderlund DM, Clark JM, Sheets LP, et al. Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. Toxicology. 2002; 171(1):3–59. [PubMed: 11812616]

Sonich-Mullin C, Fielder R, Wiltse J, et al. IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regulatory toxicology and pharmacology : RTP. 2001; 34(2): 146–152. [PubMed: 11603957]

Splawski I, Timothy KW, Sharpe LM, et al. Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. Cell. 2004; 119(1):19–31. [PubMed: 15454078]

Sun X, Windebank AJ. Calcium in suramin-induced rat sensory neuron toxicity in vitro. Brain Res. 1996; 742(1–2):149–156. [PubMed: 9117388]

Suvorov A, Girard S, Lachapelle S, Abdelouahab N, Sebire G, Takser L. Perinatal exposure to low-dose BDE-47, an emergent environmental contaminant, causes hyperactivity in rat offspring. Neonatology. 2009; 95(3):203–209. [PubMed: 18799892]

Suzuki Y, Isemura M. Inhibitory effect of epigallocatechin gallate on adhesion of murine melanoma cells to laminin. Cancer letters. 2001; 173(1):15–20. [PubMed: 11578804]

Szychowski KA, Sitarz AM, Wojtowicz AK. Triclosan induces Fas receptor-dependent apoptosis in mouse neocortical neurons in vitro. Neuroscience. 2015; 284:192–201. [PubMed: 25313001]

Ta TA, Koenig CM, Golub MS, et al. Bioaccumulation and behavioral effects of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in perinatally exposed mice. Neurotoxicol Teratol. 2011; 33(3):393–404. [PubMed: 21334437]

Tabuchi A, Nakaoka R, Amano K, et al. Differential activation of brain-derived neurotrophic factor gene promoters I and III by Ca2+ signals evoked via L-type voltage-dependent and N-methyl-D-aspartate receptor Ca2+ channels. The Journal of biological chemistry. 2000; 275(23):17269–17275. [PubMed: 10748141]

Takei N, Numakawa T, Kozaki S, et al. Brain-derived neurotrophic factor induces rapid and transient release of glutamate through the non-exocytotic pathway from cortical neurons. The Journal of biological chemistry. 1998; 273(42):27620–27624. [PubMed: 9765296]

Tan J, Soderlund DM. Divergent actions of the pyrethroid insecticides S-bioallethrin, tefluthrin, and deltamethrin on rat Na(v)1.6 sodium channels. Toxicology and applied pharmacology. 2010; 247(3):229–237. [PubMed: 20624410]

Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME. Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron. 1998; 20(4): 709–726. [PubMed: 9581763]

Tarpey PS, Smith R, Pleasance E, et al. A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. Nature genetics. 2009; 41(5):535–543. [PubMed: 19377476]

Trainer VL, McPhee JC, Boutelet-Bochan H, et al. High affinity binding of pyrethroids to the alpha subunit of brain sodium channels. Molecular pharmacology. 1997; 51(4):651–657. [PubMed: 9106631]

Traynelis S, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev. 2010; 62:405–496. [PubMed: 20716669]

Trentini JF, O'Neill JT, Poluch S, Juliano SL. Prenatal carbon monoxide impairs migration of interneurons into the cerebral cortex. Neurotoxicology. 2016; 53:31–44. [PubMed: 26582457]

Tzu J, Marinkovich MP. Bridging structure with function: structural, regulatory, and developmental role of laminins. The international journal of biochemistry & cell biology. 2008; 40(2):199–214. [PubMed: 17855154]

USEPA. U.S. Environmental Protection Agency, Guidelines for Carcinogen Risk Assessment. US EPA, Washington, DC: 2005. http://www.epa.gov/cancerguidelines/

Vais H, Atkinson S, Eldursi N, Devonshire AL, Williamson MS, Usherwood PN. A single amino acid change makes a rat neuronal sodium channel highly sensitive to pyrethroid insecticides. FEBS letters. 2000; 470(2):135–138. [PubMed: 10734222]

Valnegri P, Puram SV, Bonni A. Regulation of dendrite morphogenesis by extrinsic cues. Trends Neurosci. 2015; 38(7):439–447. [PubMed: 26100142]

van der Staay FJ, Rutten K, Erb C, Blokland A. Effects of the cognition impairer MK-801 on learning and memory in mice and rats. Behavioural brain research. 2011; 220(1):215–229. [PubMed: 21310186]

Van Petegem F. Ryanodine receptors: allosteric ion channel giants. J Mol Biol. 2015; 427(1):31–53. [PubMed: 25134758]

Villeneuve DL, Crump D, Garcia-Reyero N, et al. Adverse outcome pathway (AOP) development I: strategies and principles. Toxicological sciences : an official journal of the Society of Toxicology. 2014; 142(2):312–320. [PubMed: 25466378]

Wang H, Chan SA, Ogier M, et al. Dysregulation of brain-derived neurotrophic factor expression and neurosecretory function in Mecp2 null mice. J Neurosci. 2006; 26(42):10911–10915. [PubMed: 17050729]

Warren MS, Bradley WD, Gourley SL, et al. Integrin β1 signals through Arg to regulate postnatal dendritic arborization, synapse density, and behavior. Journal of Neuroscience. 2012; 32(8): 2824–2834. [PubMed: 22357865]

Waterhouse EG, Xu B. New insights into the role of brain-derived neurotrophic factor in synaptic plasticity. Molecular and cellular neurosciences. 2009; 42(2):81–89. [PubMed: 19577647]

Wayman GA, Bose DD, Yang D, et al. PCB-95 modulates the calcium-dependent signaling pathway responsible for activity-dependent dendritic growth. Environ Health Perspect. 2012a; 120(7): 1003–1009. [PubMed: 22534176]

Wayman GA, Impey S, Marks D, et al. Activity-dependent dendritic arborization mediated by CaM-kinase I activation and enhanced CREB-dependent transcription of Wnt-2. Neuron. 2006; 50(6): 897–909. [PubMed: 16772171]

Wayman GA, Lee YS, Tokumitsu H, Silva AJ, Soderling TR. Calmodulin-kinases: modulators of neuronal development and plasticity. Neuron. 2008; 59(6):914–931. [PubMed: 18817731]

Wayman GA, Yang D, Bose DD, et al. PCB-95 promotes dendritic growth via ryanodine receptor-dependent mechanisms. Environ Health Perspect. 2012b; 120(7):997–1002. [PubMed: 22534141]

West AE, Chen WG, Dalva MB, Dolmetsch RE, Kornhauser JM, Shaywitz AJ, Takasu MA, Tao X, Greenberg ME. Calcium regulation of neuronal gene expression. Proceedings of the National Academy of Sciences of the United States of America. 2001; 98:11024–11031. [PubMed: 11572963]

Wolansky MJ, Gennings C, Crofton KM. Relative potencies for acute effects of pyrethroids on motor function in rats. Toxicological sciences : an official journal of the Society of Toxicology. 2006; 89(1):271–277. [PubMed: 16221961]

Wong EH, Kemp JA, Priestley T, Knight AR, Woodruff GN, Iversen LL. The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. Proceedings of the National Academy of Sciences of the United States of America. 1986; 83(18):7104–7108. [PubMed: 3529096]

Wong PW, Brackney WR, Pessah IN. Ortho-substituted polychlorinated biphenyls alter microsomal calcium transport by direct interaction with ryanodine receptors of mammalian brain. J Biol Chem. 1997a; 272(24):15145–15153. [PubMed: 9182535]

Wong PW, Garcia EF, Pessah IN. ortho-Substituted PCB95 alters intracellular calcium signaling and causes cellular acidification in PC12 cells by an immunophilin-dependent mechanism. J Neurochem. 2001; 76(2):450–463. [PubMed: 11208908]

Wong PW, Joy RM, Albertson TE, Schantz SL, Pessah IN. Ortho-substituted 2,2',3,5',6-pentachlorobiphenyl (PCB 95) alters rat hippocampal ryanodine receptors and neuroplasticity in vitro: evidence for altered hippocampal function. Neurotoxicology. 1997b; 18(2):443–456. [PubMed: 9291493]

Wong PW, Pessah IN. Ortho-substituted polychlorinated biphenyls alter calcium regulation by a ryanodine receptor-mediated mechanism: structural specificity toward skeletal-and cardiac-type microsomal calcium release channels. Mol Pharmacol. 1996; 49(4):740–751. [PubMed: 8609904]

Woo TU. Neurobiology of schizophrenia onset. Curr Top Behav Neurosci. 2014; 16:267–295. [PubMed: 23975845]

Woolf NJ. A structural basis for memory storage in mammals. Progress in neurobiology. 1998; 55(1): 59–77. [PubMed: 9602500]

Xu L, Tripathy A, Pasek DA, Meissner G. Potential for pharmacology of ryanodine receptor/calcium release channels. Ann N Y Acad Sci. 1998; 853:130–148. [PubMed: 10603942]

Yang D, Kania-Korwel I, Ghogha A, et al. PCB 136 atropselectively alters morphometric and functional parameters of neuronal connectivity in cultured rat hippocampal neurons via ryanodine receptor-dependent mechanisms. Toxicol Sci. 2014; 138(2):379–392. [PubMed: 24385416]

Yang D, Kim KH, Phimister A, et al. Developmental exposure to polychlorinated biphenyls interferes with experience-dependent dendritic plasticity and ryanodine receptor expression in weanling rats. Environ Health Perspect. 2009a; 117(3):426–435. [PubMed: 19337518]

Yang D, Lein PJ. Polychlorinated biphenyls increase apoptosis in the developing rat brain. Current neurobiology. 2010; 1(1):70–76. [PubMed: 24052688]

Yang J, Siao CJ, Nagappan G, et al. Neuronal release of proBDNF. Nature neuroscience. 2009b; 12(2): 113–115. [PubMed: 19136973]

Yauk CL, Lambert IB, Meek ME, Douglas GR, Marchetti F. Development of the adverse outcome pathway "alkylation of DNA in male premeiotic germ cells leading to heritable mutations" using the OECD's users' handbook supplement. Environmental and molecular mutagenesis. 2015; 56(9):724–750. [PubMed: 26010389]

Yin Y, Edelman GM, Vanderklish PW. The brain-derived neurotrophic factor enhances synthesis of Arc in synaptoneurosomes. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99(4):2368–2373. [PubMed: 11842217]

Yuste R, Bonhoeffer T. Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annu Rev Neurosci. 2001; 24:1071–1089. [PubMed: 11520928]

Zheng F, Zhou X, Luo Y, Xiao H, Wayman G, Wang H. Regulation of brain-derived neurotrophic factor exon IV transcription through calcium responsive elements in cortical neurons. PloS one. 2011; 6(12):e28441. [PubMed: 22174809]

Zheng F, Zhou X, Moon C, Wang H. Regulation of brain-derived neurotrophic factor expression in neurons. International journal of physiology, pathophysiology and pharmacology. 2012; 4(4): 188–200.

Zhou Z, Hong EJ, Cohen S, et al. Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. Neuron. 2006; 52(2):255–269. [PubMed: 17046689]

Zoghbi HY, Bear MF. Synaptic dysfunction in neurodevelopmental disorders associated with autism and intellectual disabilities. Cold Spring Harbor perspectives in biology. 2012; 4(3)

Highlights

The core findings of the article:

- Identification of similarities and dissimilarities between MoA and AOP and WOE considerations
- Discussion of challenges in developing AOPs relevant to neurotoxicity
- Evaluation of proposed AOPs for neurotoxicity and developmental neurotoxicity
- Relevance of the putative AOPs to current risk assessments