Adverse Outcome Pathways for Developmental Neurotoxicity

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Conflict of Interest Statement

- I declare no conflicts of interest.

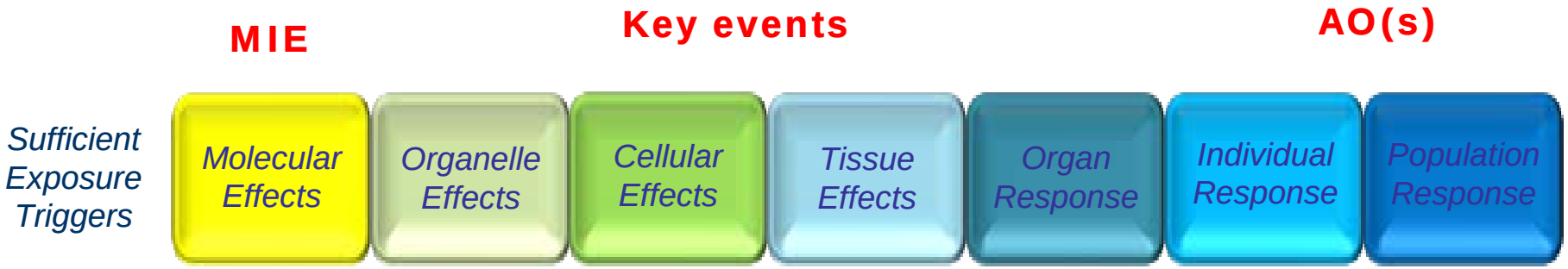
My Presentation Will Cover:

- Introduction to AOP concept
- Principles of AOP development
- Examples of AOPs relevant to developmental neurotoxicity
- Potential AOPs applications for regulatory purposes

Adverse Outcome Pathways (AOPs)

- Out of isolated Events a Pathway emerges: **Adverse Outcome Pathway** (AOP)
- An AOP is a conceptual framework that portrays **existing knowledge** between a **Molecular Initiating Event** and an **Adverse Outcome**
- An AOP is a **mechanistic explanation** of toxicity.
- AOPs underpin the ongoing paradigm shift
    - moving away from observational black-box thinking toward **predictive toxicology**
- A focal point for is: **collecting knowledge** and **assembling it into AOPs**

AOP Definition

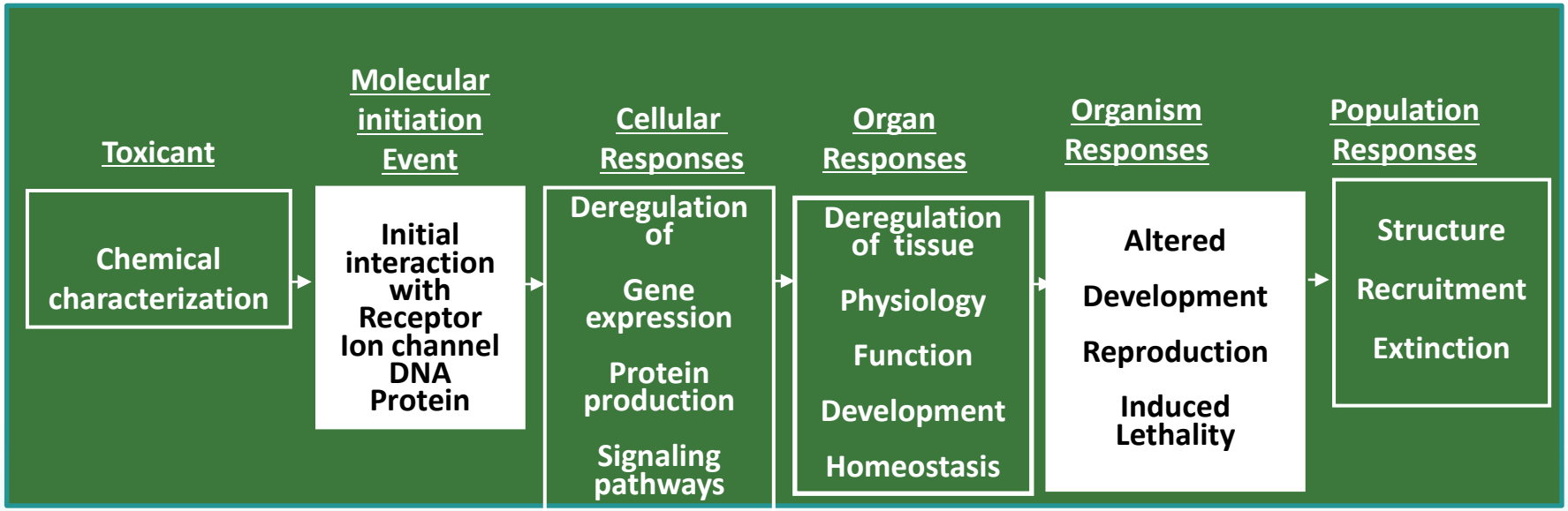


An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome, at a level of biological organization relevant to risk assessment.

(Ankley et al., 2010, Environ. Toxicol. Chem., 29(3): 730-741)

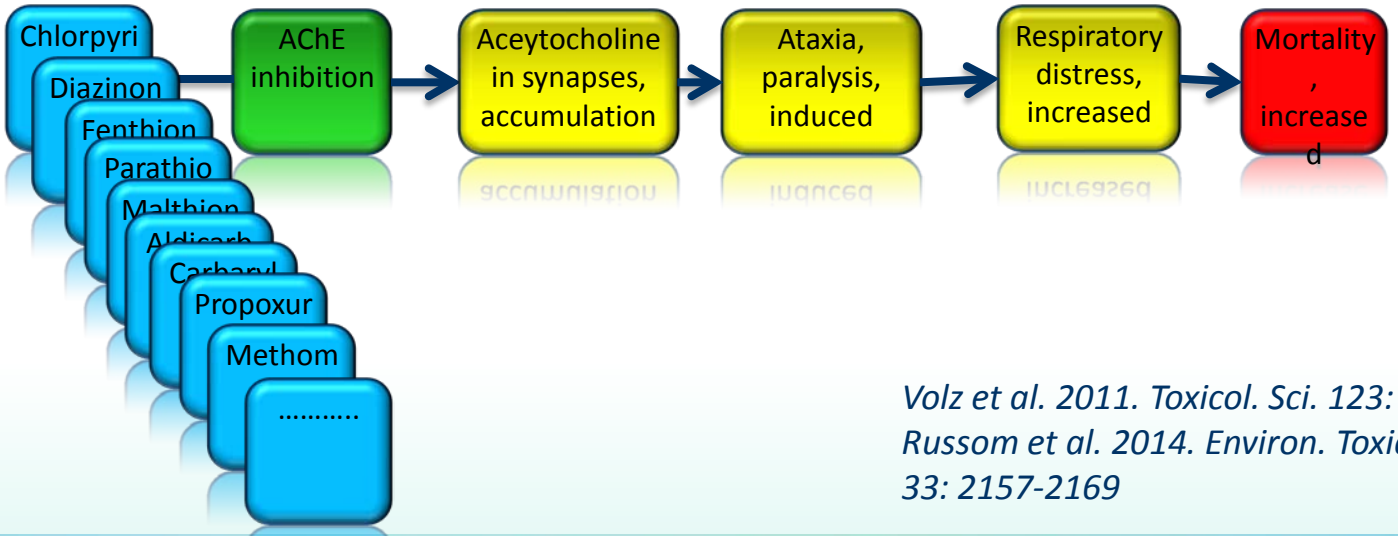
Key Events (KEs)

The AOP approach describes a sequence of measurable key events (KEs) that link chemical exposure to an adverse outcome (AO) at the organism or population level. Key events are connected to one another via causative or correlative relationships.



AOPs are Not Chemical-Specific

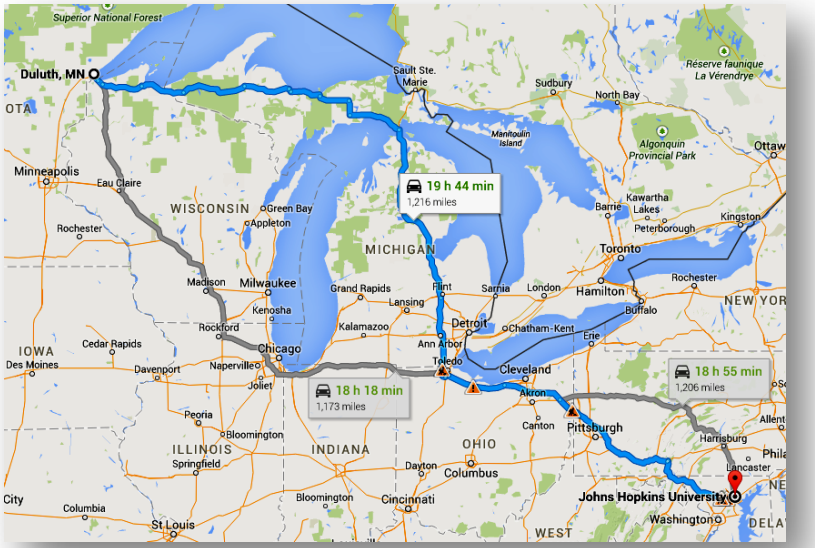
- Not trying to describe what a single chemical does
- Trying to describe what ANY chemical that perturbs the MIE with sufficient potency and duration is likely to do- Biological motifs of failure
- *Applying* those motifs in a predictive context requires understanding chemical-specific properties (e.g., potency, ADME) that dictate the magnitude and duration of perturbation at the MIE.



Volz et al. 2011. Toxicol. Sci. 123: 349-358
Russom et al. 2014. Environ. Toxicol. Chem. 33: 2157-2169

AOPs Characteristics

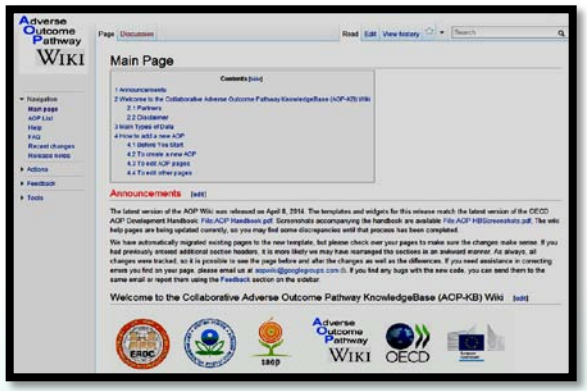
- AOP is a pragmatic simplification of complex biology
- By convention AOP consists of a single sequence of key events connecting MIE to AO (no branches)
- For a “pure ligand” – functional unit of prediction and evaluation



One set of directions from point: A to point B, not the map of all possible routes.

OECD AOP Development Program

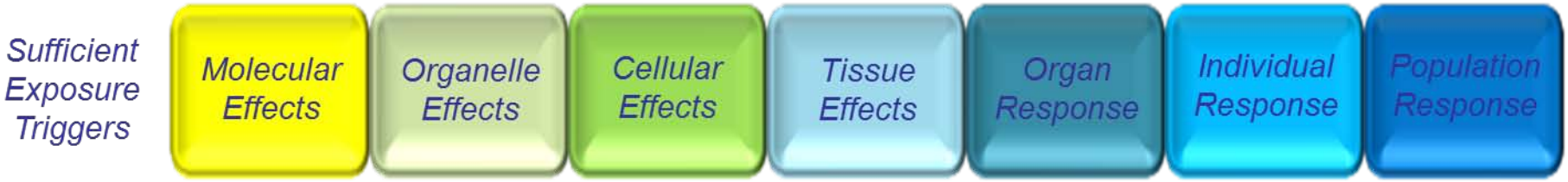
- 2013 OECD Guidance on Developing and Assessing AOPs
- Conventions and terminology
- Information content of an AOP description
- Weight of evidence evaluation
- Introduce standardization and rigor to AOP development



AOP Development and Description Case Studies

Users' handbook supplement to OECD guidance document for developing and assessing AOPs.

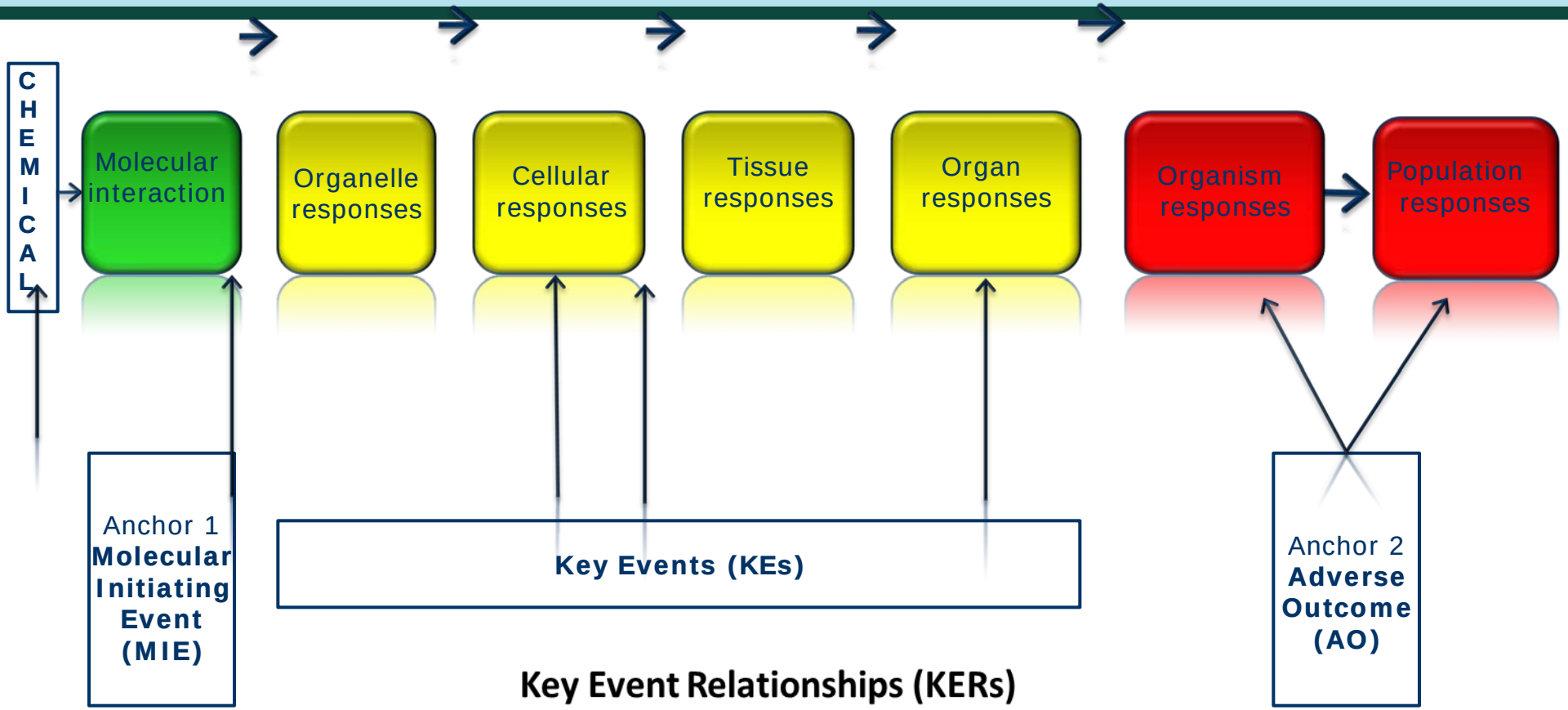
Key Event (KE)



Functional unit of observation/verification

- Observable Δ biological state (methods for measuring)
- Essential (but not necessarily sufficient)
- How this KE works (description)

Key Event Relationships (KERs)



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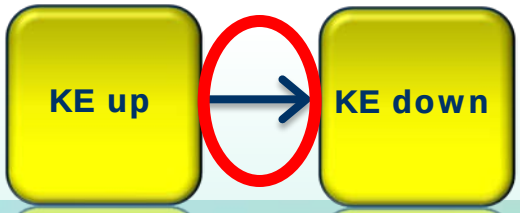
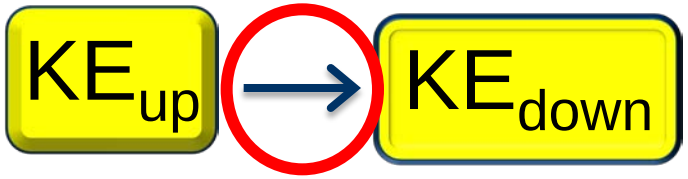


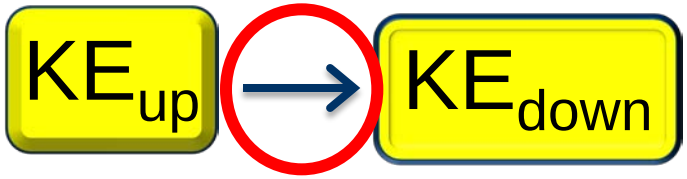
Key Event Relationship (KER)



Functional unit of inference/extrapolation

- Define a directed relationship (blocking KE_{up} will block KE_{down})
- State of KE_{up} provides some ability to predict of infer state of KE_{down}
- Supported by biological plausibility and evidence
- Quantitative understanding: empirical support

Empirical Support for KERs



- Evidence that a change in the upstream KE (KE_{up}) is associated with a subsequent change in the downstream KE (KE_{down})
  - For sufficient perturbation of Ke_{up} (i.e., a threshold of change in KE_{up} needed to elicit a change in KE_{down})
  - Generally tested by various stressors (e.g., specific chemicals)

- **Includes evidence that:**
  - stressors that perturb KE_{up} also perturb KE_{down} in expected fashion, with respect to dose, time and incidence
  - i.e., they are ***concordant***

- **Expected Pattern:**
  - KE_{up} occurs at lower doses than KE_{down}
  - KE_{up} precedes KE_{down}
  - for a given dose, the incidence of KE_{up} is greater than or equal to that of KE_{down}

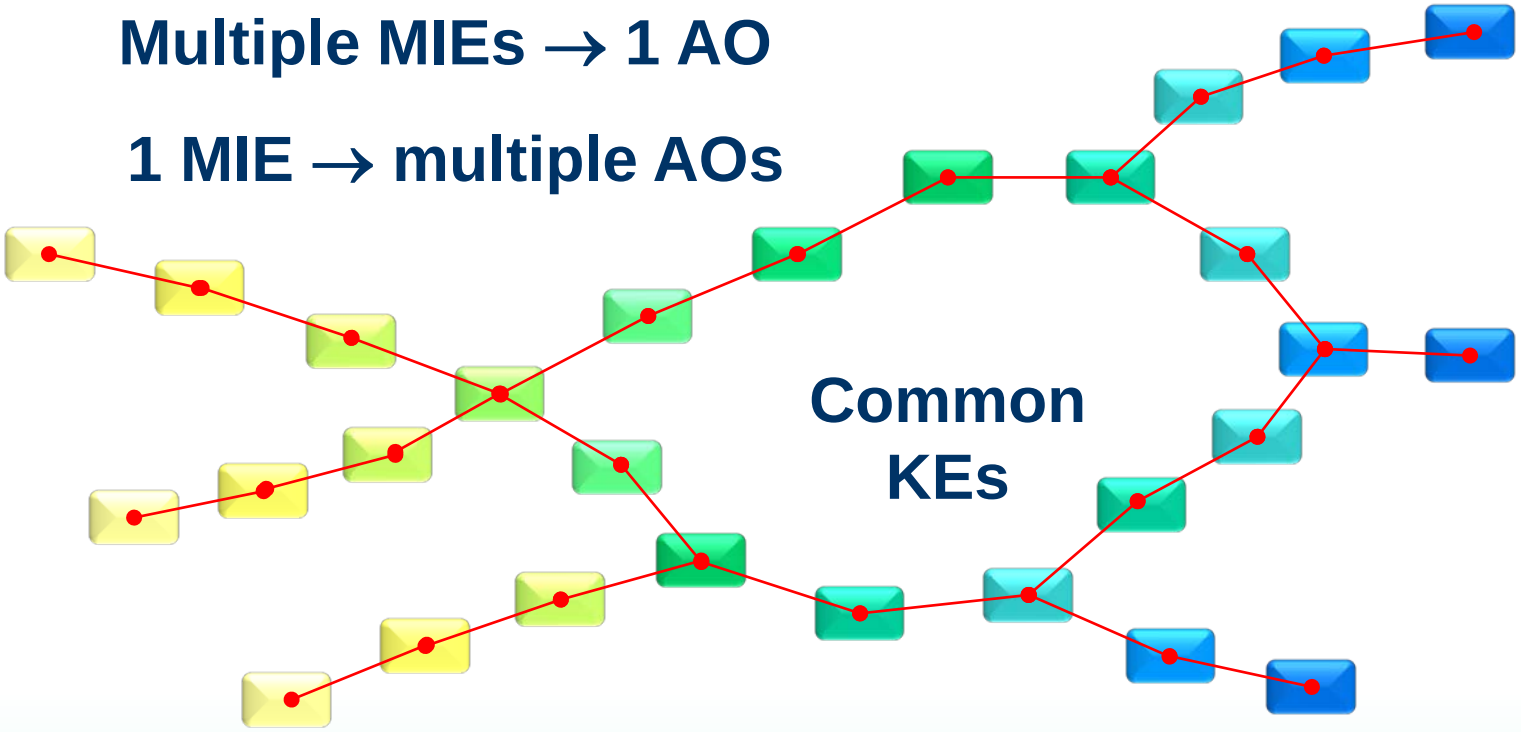
Assembling Weight of Evidence (WoE)

Modified Bradford-Hill Considerations	Conclusions
Biological Plausibility	KER is consistent with current biological understanding plausible.
Essentiality of Key events	Effects are reversible if the stressor is removed (e.g., Villeneuve et al. 2009; EHP 117: 624-631)
Concordance of Empirical Observations	**Dose response** – The key events observed at doses below or similar to those associated with the apical effect? **Temporality** – The key events are observed in hypothesized order? **Incidence** – The frequency of occurrence of the apical effect less than that for the key events?
Consistency	Same pattern of effects has been observed in several tested species (e.g., fathead minnow, zebrafish, medaka)
Analogy	Similar pattern of effects observed for known chemicals that belong to the same class

Inconsistencies/Uncertainties for Qualitative WoE for KERs

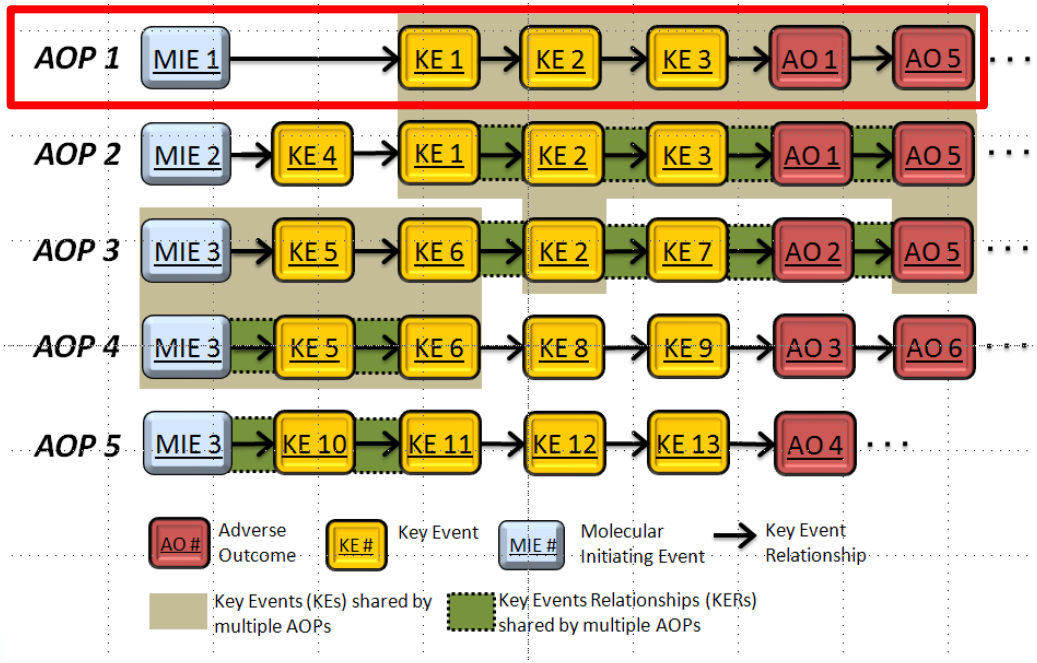
- **Biological Plausibility**
  - Biological understanding of this KER, **including the gaps of knowledge** that detract from understanding of the functional or structural relationships between the KEs
- **Empirical Support**
  - changes in KE_{up} that did elicit expected alterations in $KE_{down,}$ including change in KE_{up} that **did not** cause expected alterations in KE_{down}
  - Based on analysis of time, dose-response (incidence) relationships ***between*** KEs (i.e., for KERs)

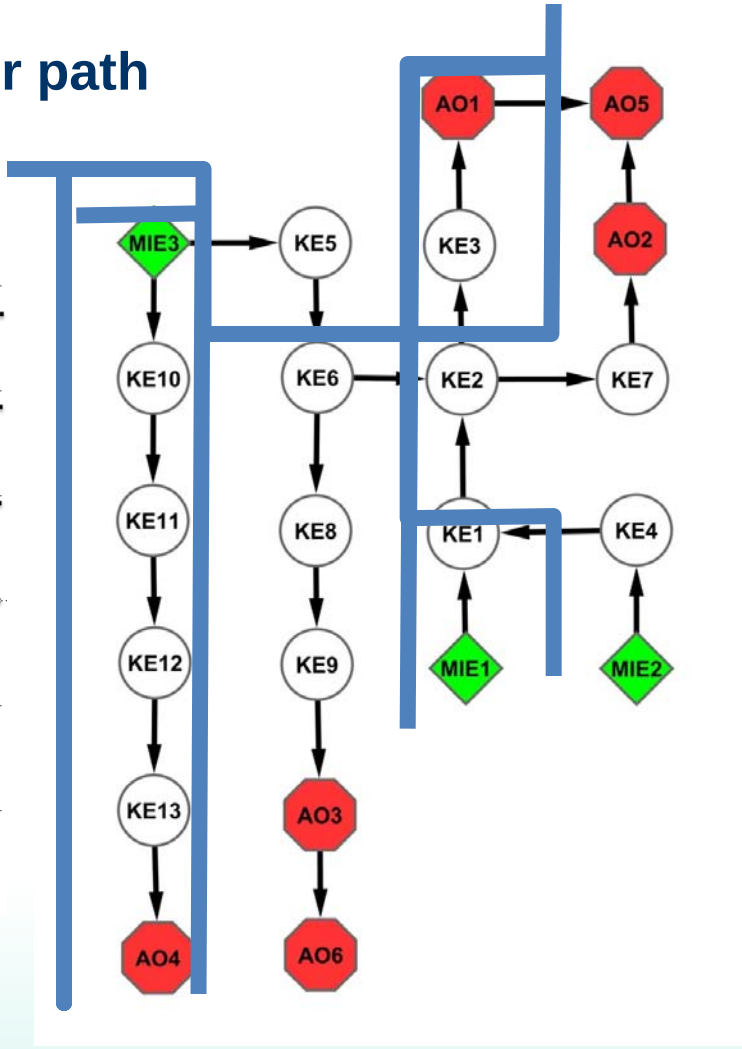
AOPs network



Network of AOPs

Each AOP is one sequential sequence or path through a broader network of AOPs.

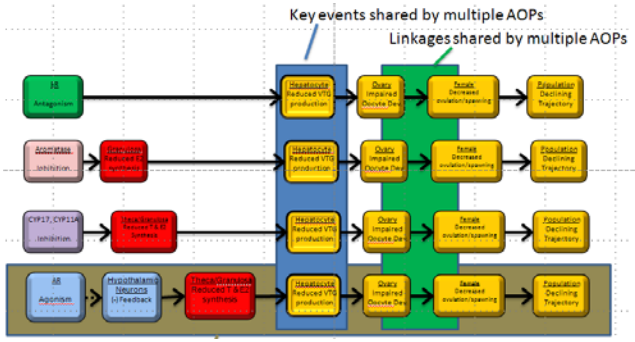




Summing Up

AOPs are modular

- KEs and KERs are shared by multiple AOPs
- No need to re-write the same descriptions over and over again
- Reusability (best practices)



AOP networks for prediction

- Entry of structured information in Knowledge Base (KB) allows for de-facto assembly of AOP networks.
- There is no objective “complete AOP”

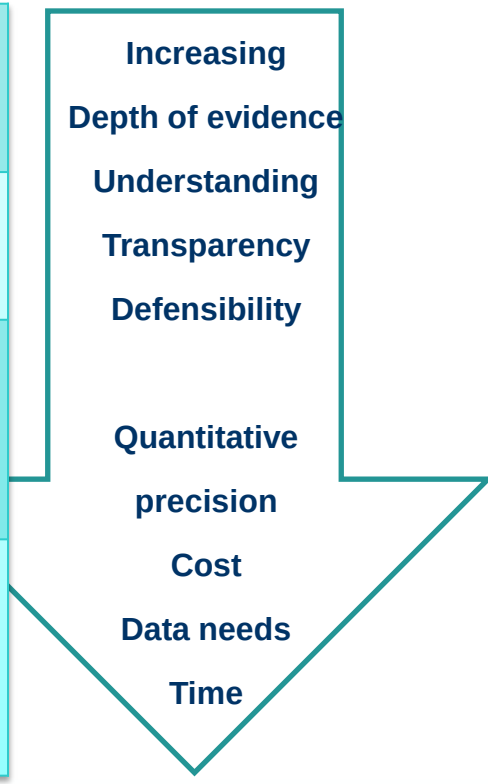
AOPs are living documents

- KE and KER descriptions can be expected to evolve over time
- As descriptions are updated and expanded – all AOP descriptions they link to update automatically

Stages of AOP Development

Operationally-defined "stages" of AOP development

Stages of AOP Development	Characteristics
Putative AOPs:	Hypothesized set of KEs and KERs primarily supported by biological plausibility and/or statistical inference
Formal AOPs (qualitative):	Include assembly and evaluation of the supporting weight of evidence – developed in AOP knowledgebase in accordance with internationally-harmonized OECD guidance
Quantitative AOPs:	Supported by quantitative relationships and/or computational models that allow quantitative translation of key event measurements into predicted probability or severity of adverse outcome



All stages have potential utility.
Level of development desired/required depends on the application.

Challenges for DNT AOPs Development

- Linking MIE and AO: general lack of understanding of the MIEs that are causally responsible for AOs (*e.g. methyl mercury exposure and adverse cognitive outcomes in children*).
- Diverse patho-physiology can underlie similar clinical phenotypes or conversely, similar patho-physiology can elicit diverse clinical outcomes (*e.g., autism*).
- Defining a threshold for KE up that triggers KE down (to define a transition from compensatory and defense processes to toxicity).

Challenges for DNT AOPs Development

- Lack of data to describe KERs in a quantitative manner, applying Bradford-Hill modified considerations (essentiality of KEs, concordance of empirical observations: dose response, temporality or incidence). The existing DNT AOPs are mainly qualitative.
- A broad variety of MIEs is acting on many different molecular targets and cellular structures (complexity of nervous system results in a network of AOPs).

AOPs are not singular – complex interactions occur: networking of AOPs

AOPs Relevant to DNT

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

Short name: Binding of antagonist to NMDARs impairs cognition

Authors: Magdalini Sachana, Sharon Munn, and Anna Price

Status: Accepted (May 2016). This AOP went through the process of internal and external OECD reviewing and is endorsed by WNT and TFHA (https://aopwiki.org/aops).

MIE: Binding of Antagonists to NMDARs During Synaptogenesis

- **Activation of the receptor N-methyl- D-aspartate (NMDAR):**
- results in sodium and calcium influx and the subsequent activation of calcium dependent cascade of intracellular events, important for strengthening of synaptic connections during development
- enhances BDNF release which promotes neuronal survival, differentiation and synaptogenesis (key neurodevelopment processes)
- long-term potentiation (LTP) and long term depression (LTD) affects synaptic strength, plasticity and memory formation

1st KE

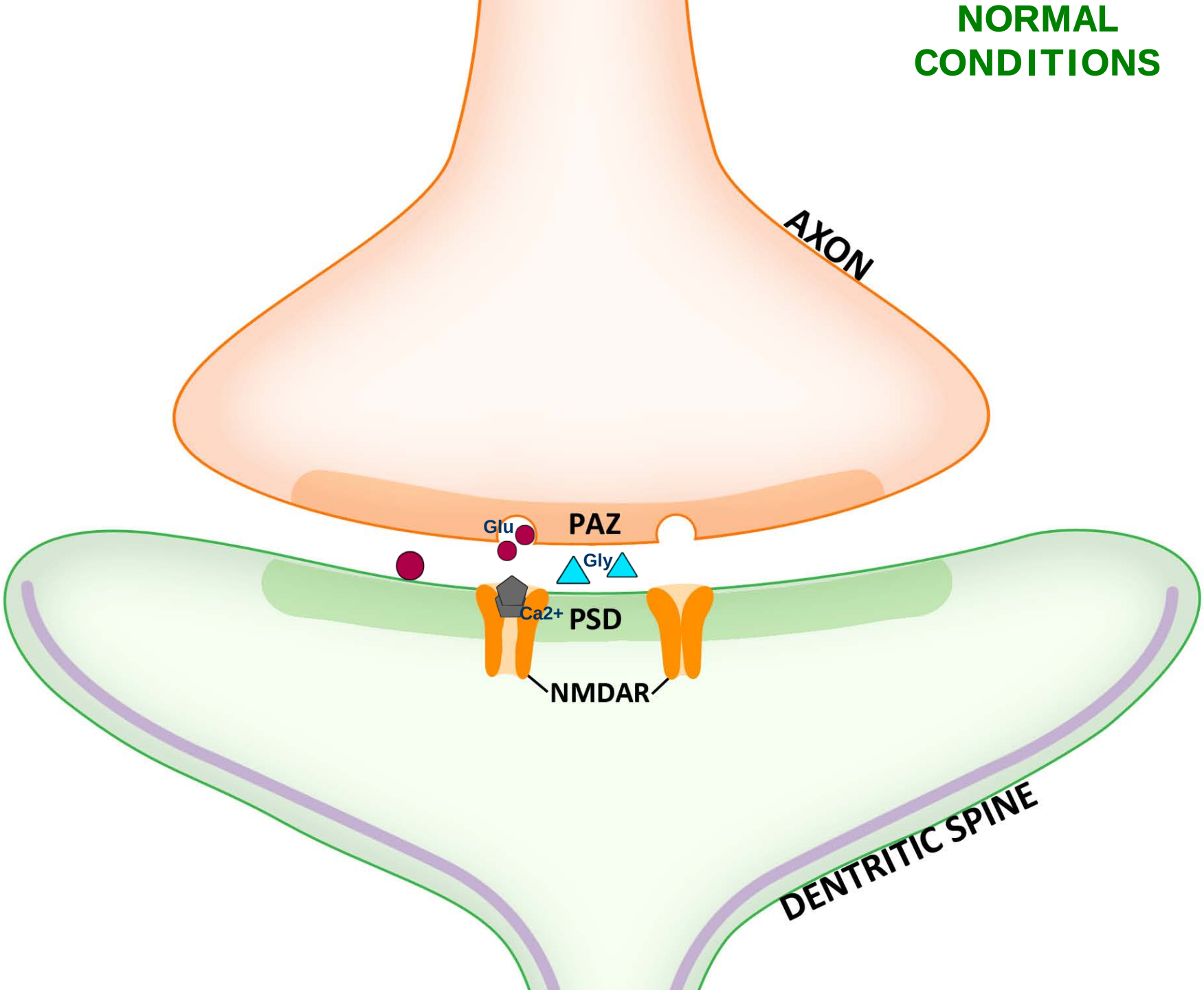
Inhibition of NMDARs function during brain development (synaptogenesis)

As an example lead (Pb^{2+}) literature was mainly reviewed:

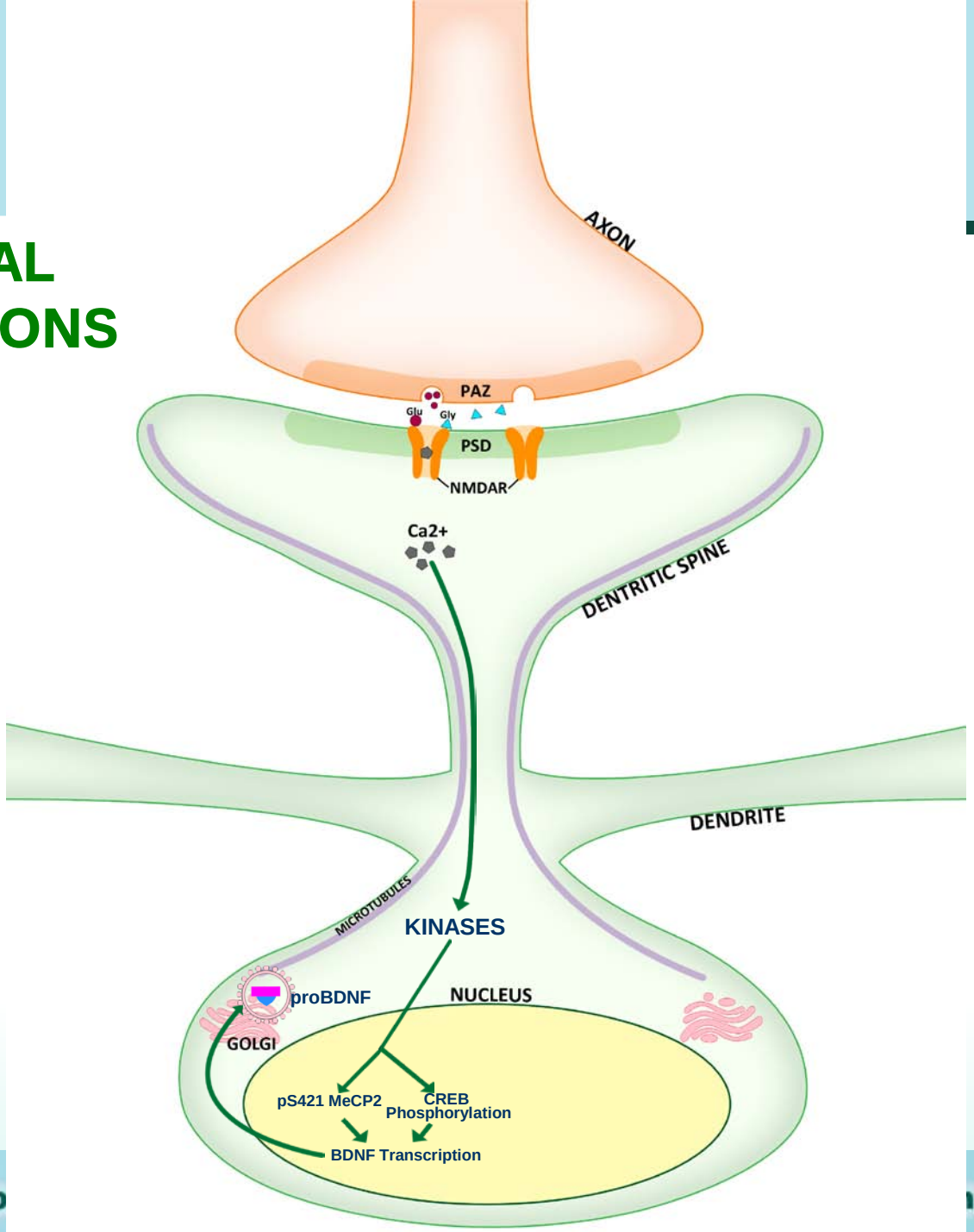
- Pb^{2+} acts as non-competitive, voltage-independent, NMDAR antagonist inhibiting NMDA-induced Ca^{2+} currents (IC_{50} around 1- 10 µM) (Alkondon et al., 1990).

- NMDAR sensitivity to Pb^{2+} binding is higher at the earlier developmental stages when compared to mature neurons (Guilarte and Miceli, 1992).

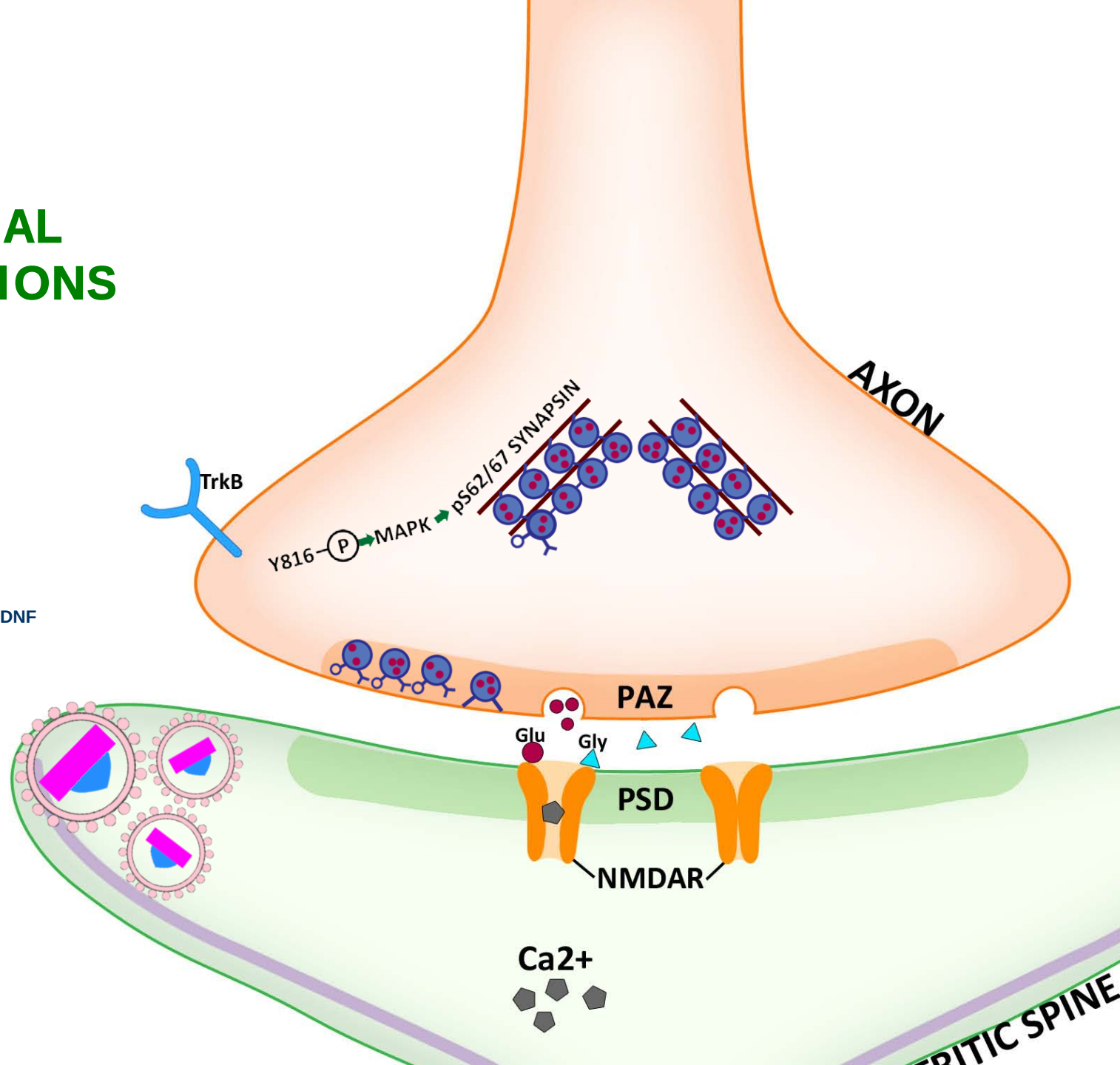
- It passes the human placenta and accumulates in fetal tissue during gestation (David et al., 1972). Even low levels of exposure to Pb^{2}causes significant functional damage to children's CNS (Lanphear et al., 2005).


NORMAL CONDITIONS
AXON
Glu
PAZ
Gly
Ca2+
PSD
NMDAR
DENTRITIC SPINE

NORMAL CONDITIONS



NORMAL CONDITIONS



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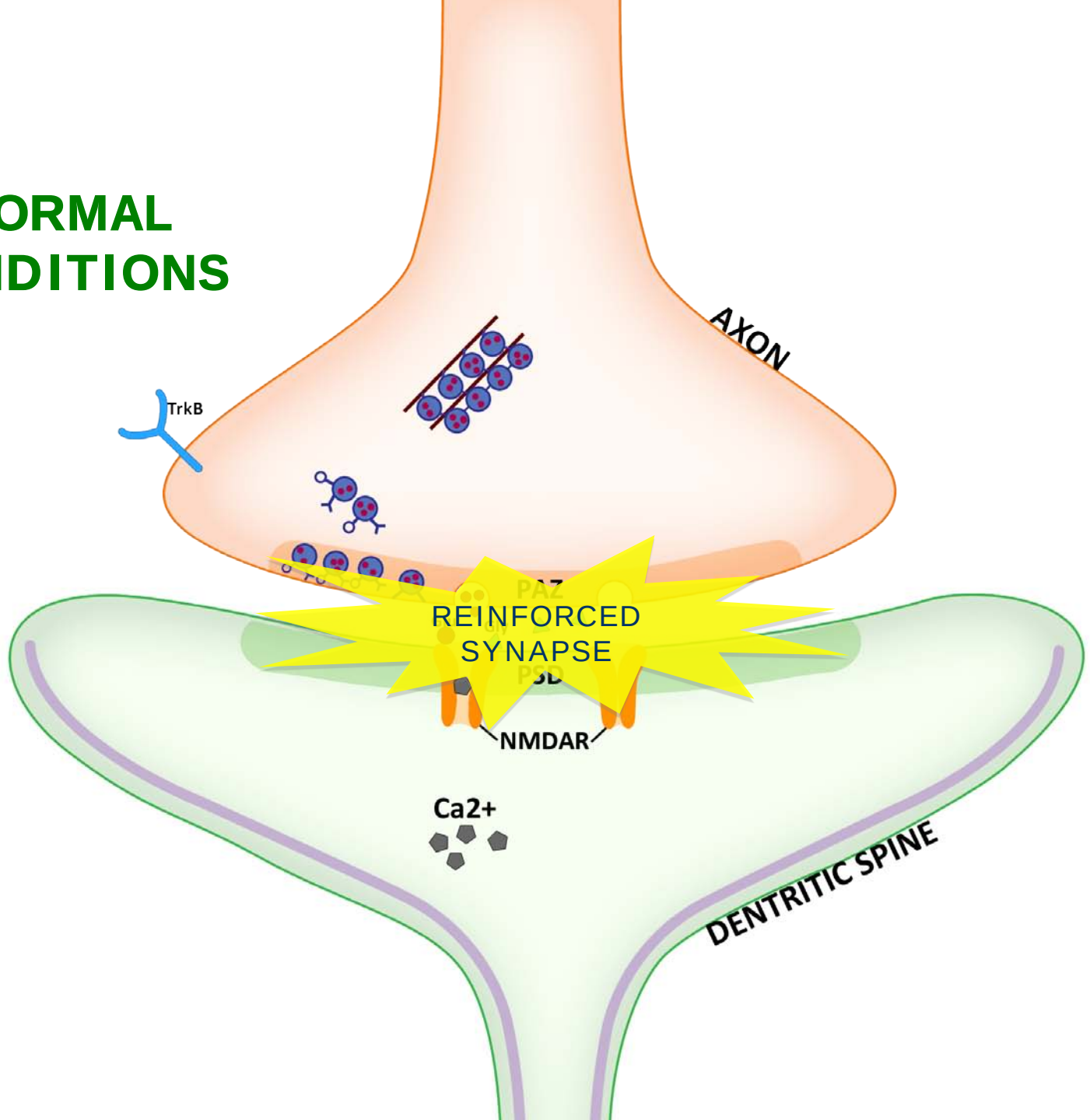

PRESENCE of Pb^{2+}

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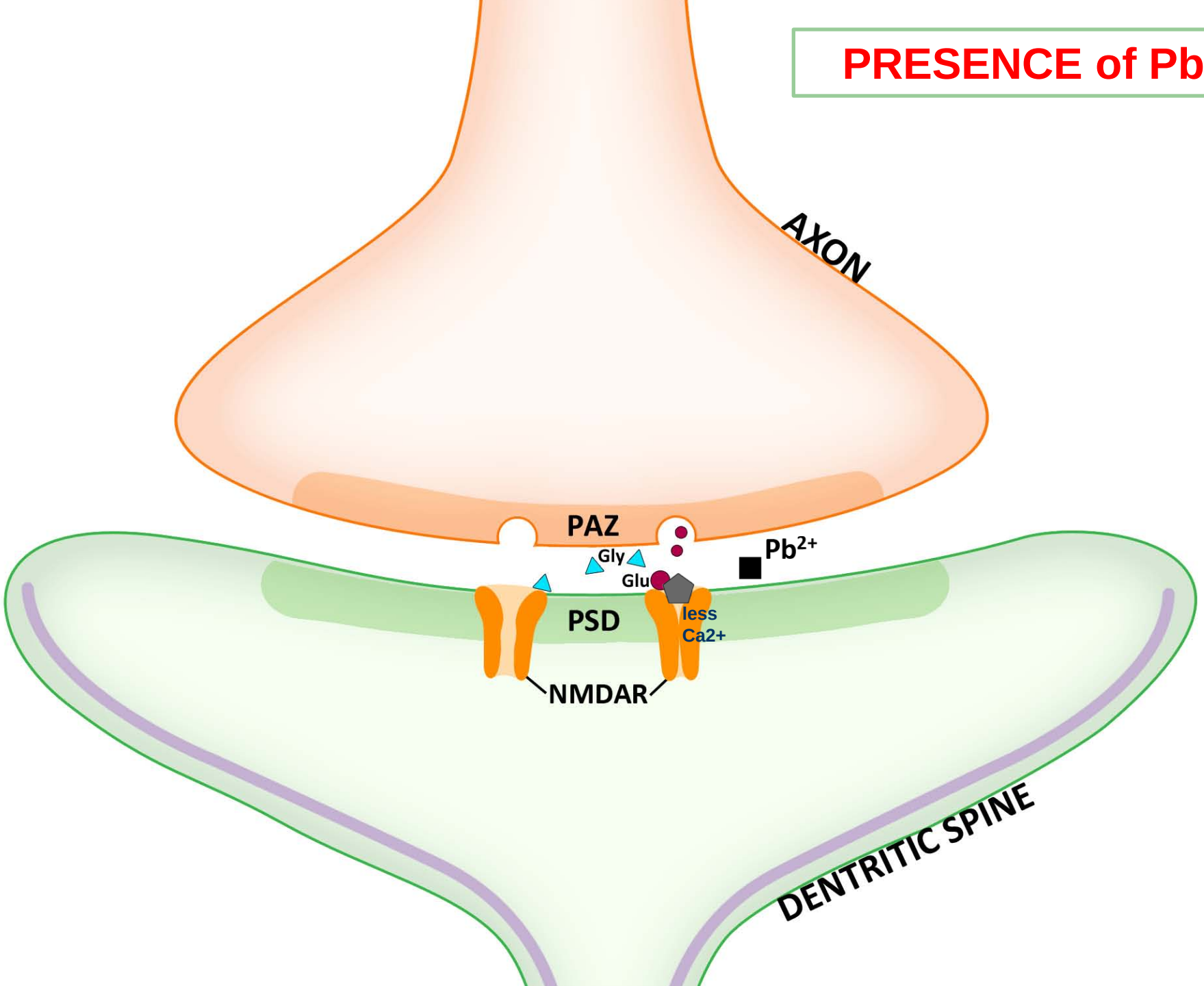

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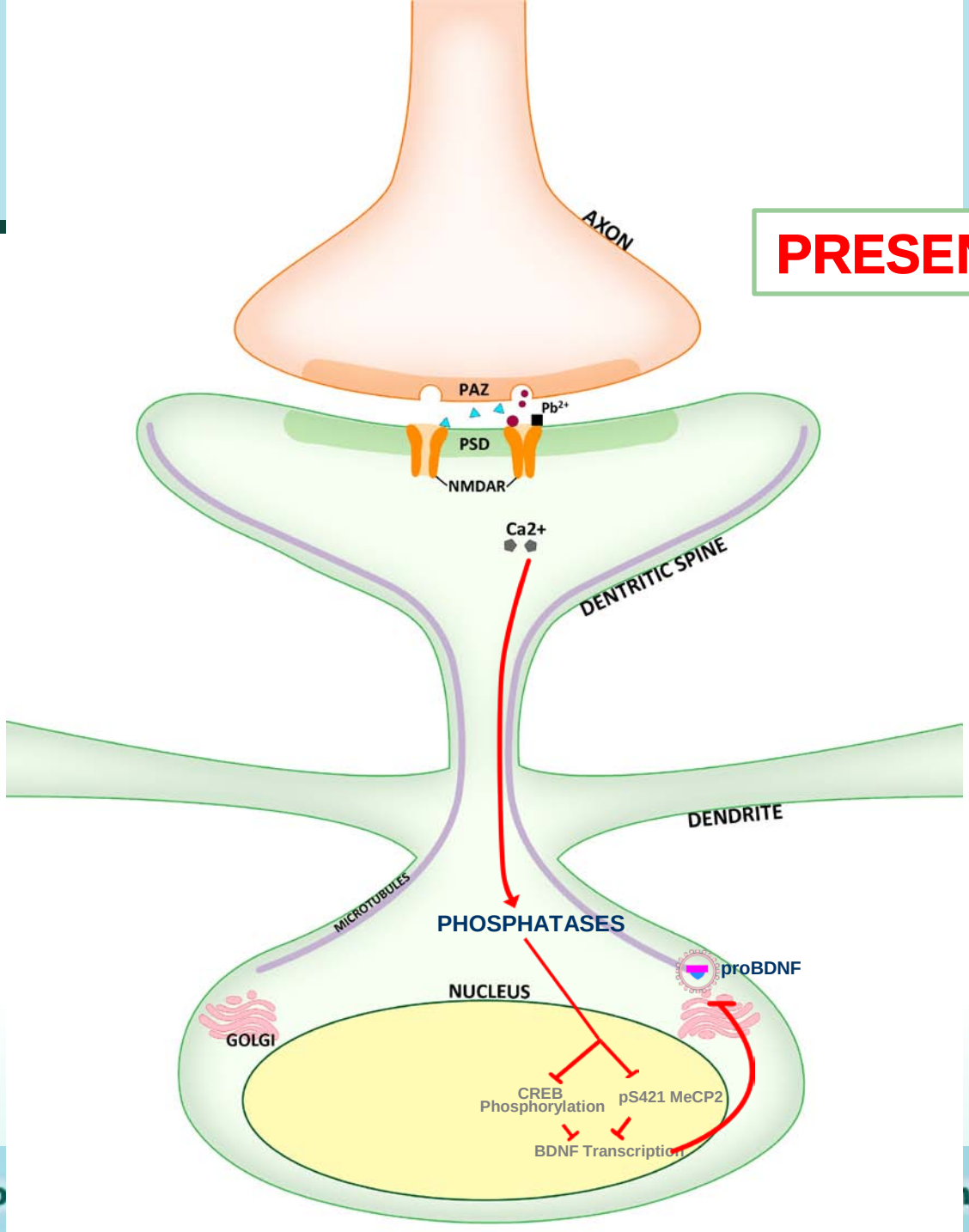

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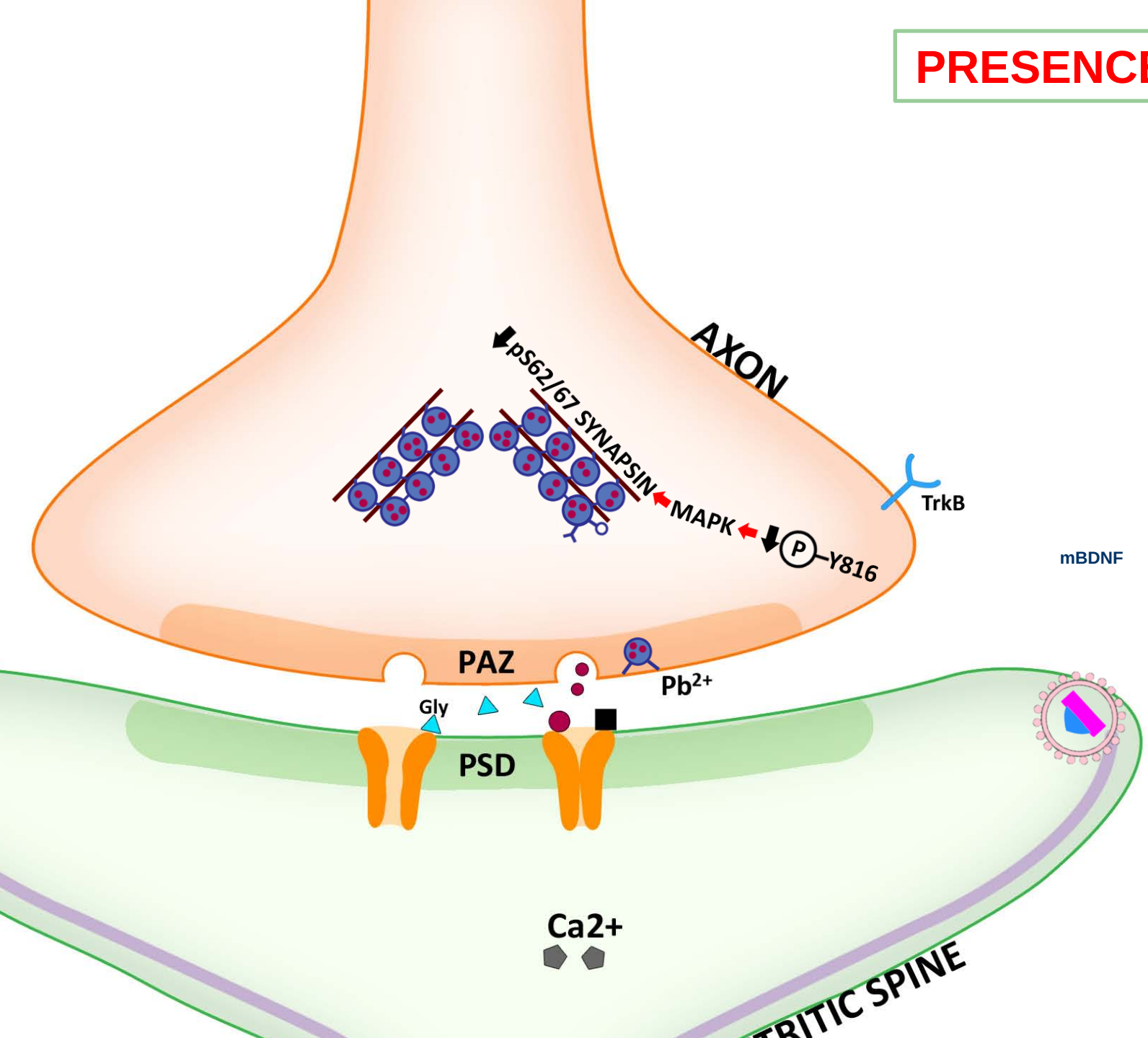

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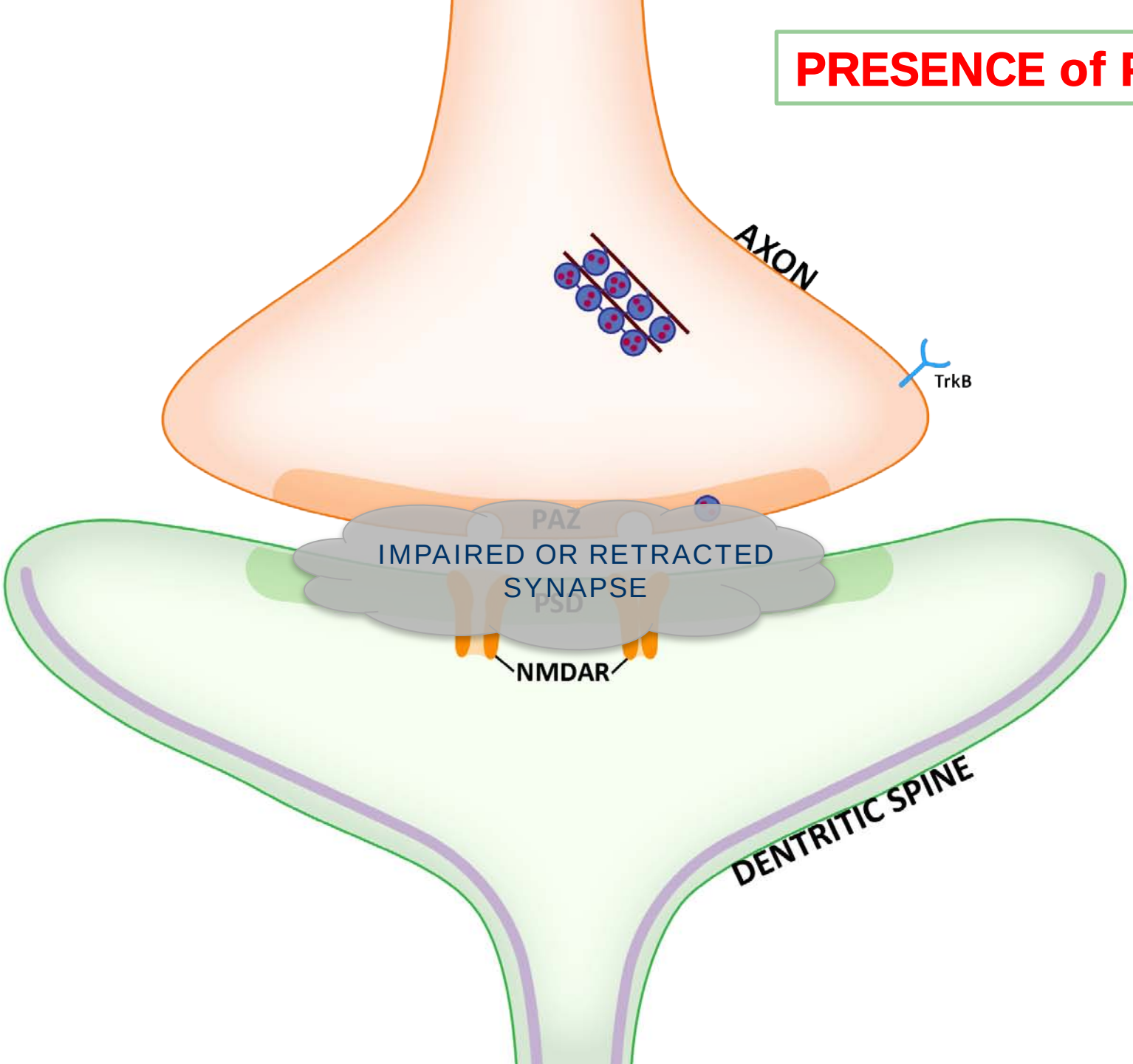
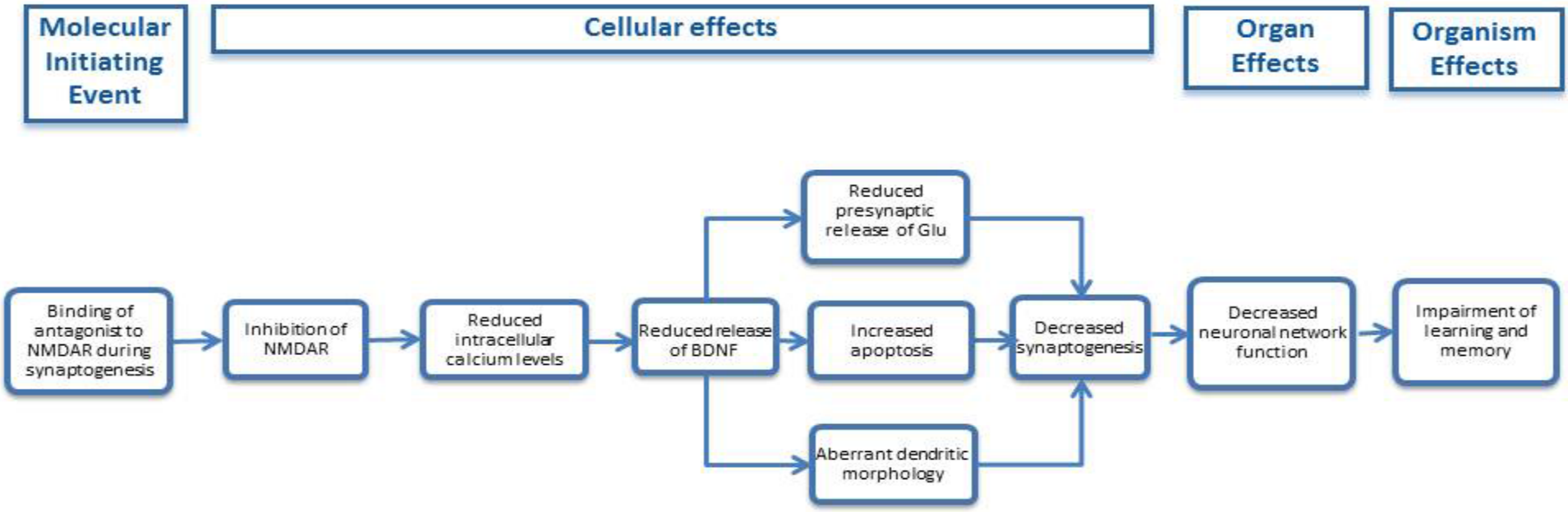
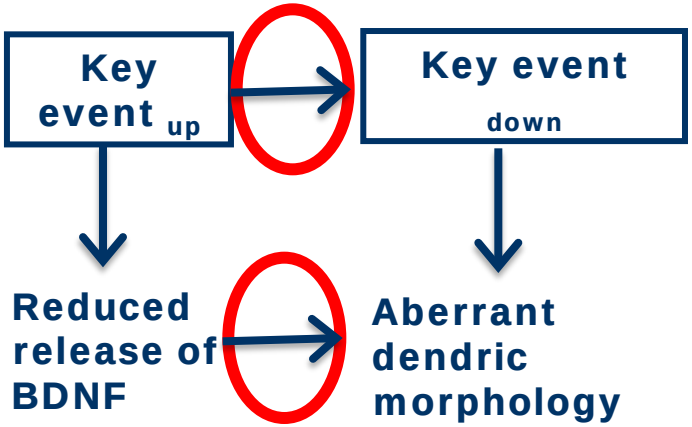


OECD Project 1.22

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



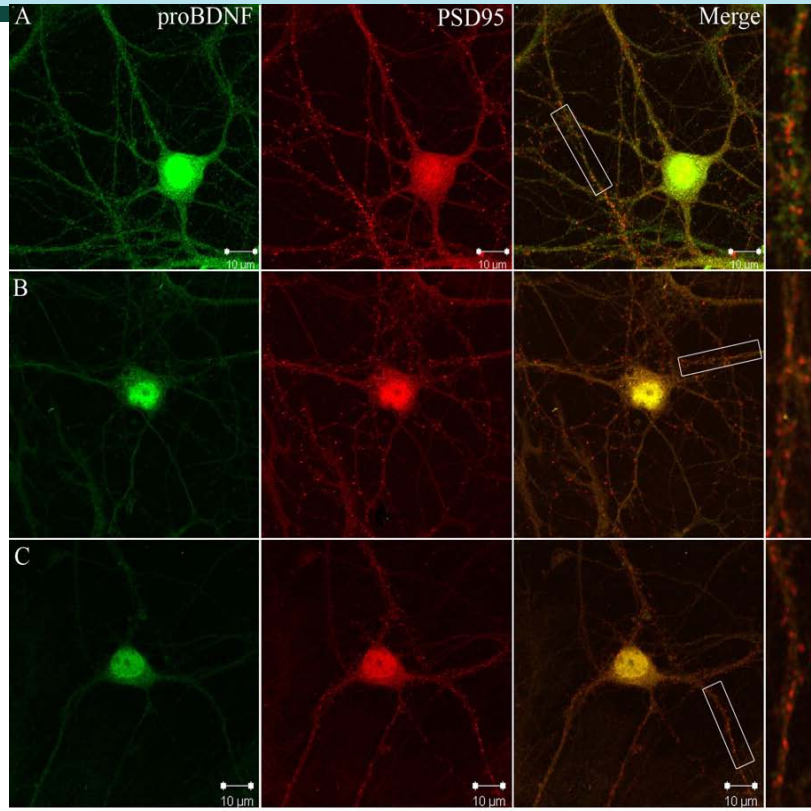
KER: Empirical Support for Linkage

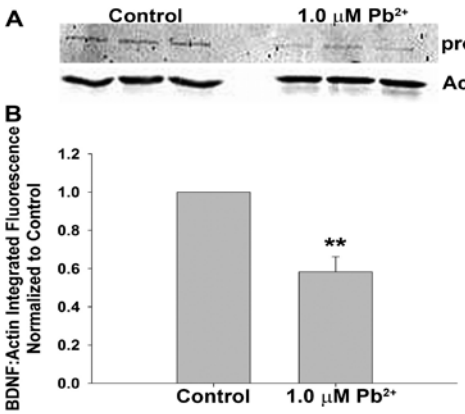


Control

1 μM Pb^{2+}

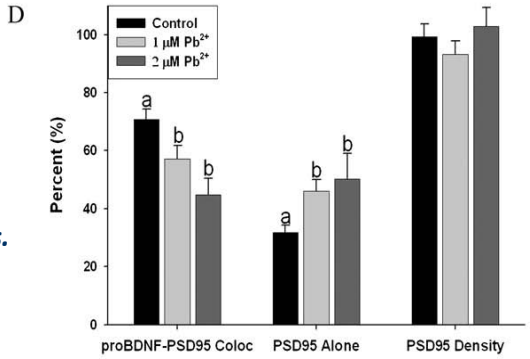
2 μM Pb^{2+}



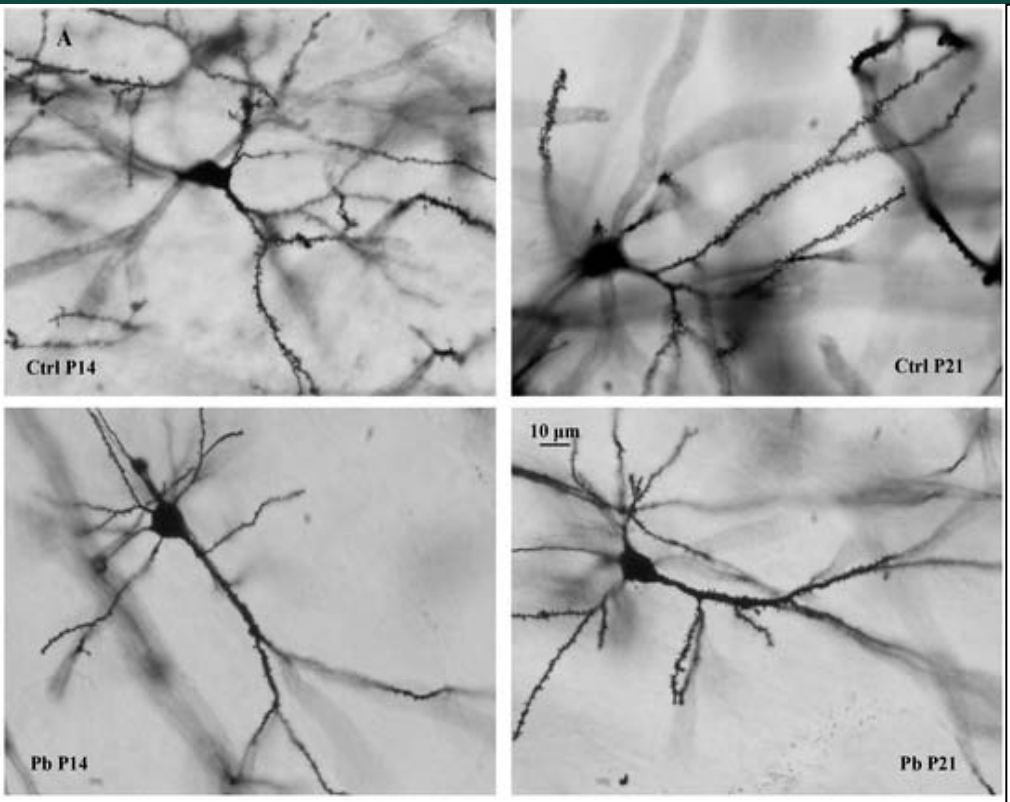


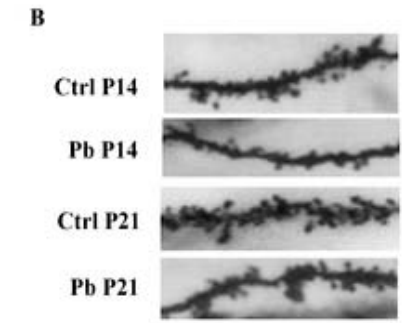
Lead exposure during synaptogenesis alters vesicular proteins and impairs vesicular release: Potential role of NMDA receptor-dependent BDNF signaling (*Neal AP et al., 2010; Toxicol Sci. 116: 249-263*).

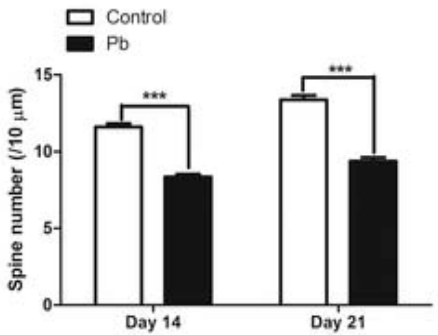
Dysregulation of BDNF-TrkB signalling in developing hippocampal neurons by Pb(2+): implications for an environmental basis of neurodevelopmental disorders. (Stansfield et al., 2012; Toxicol Sci. 127: 277-295).



Empirical Support for KEs Linkage: Decreased Dendritic Spine Density







The decreased dendritic spine density of pyramidal neurons in the hippocampus of rats 14 and 21 days old after exposure to lead water (250 ppm, 30 ml of water/day during lactation).

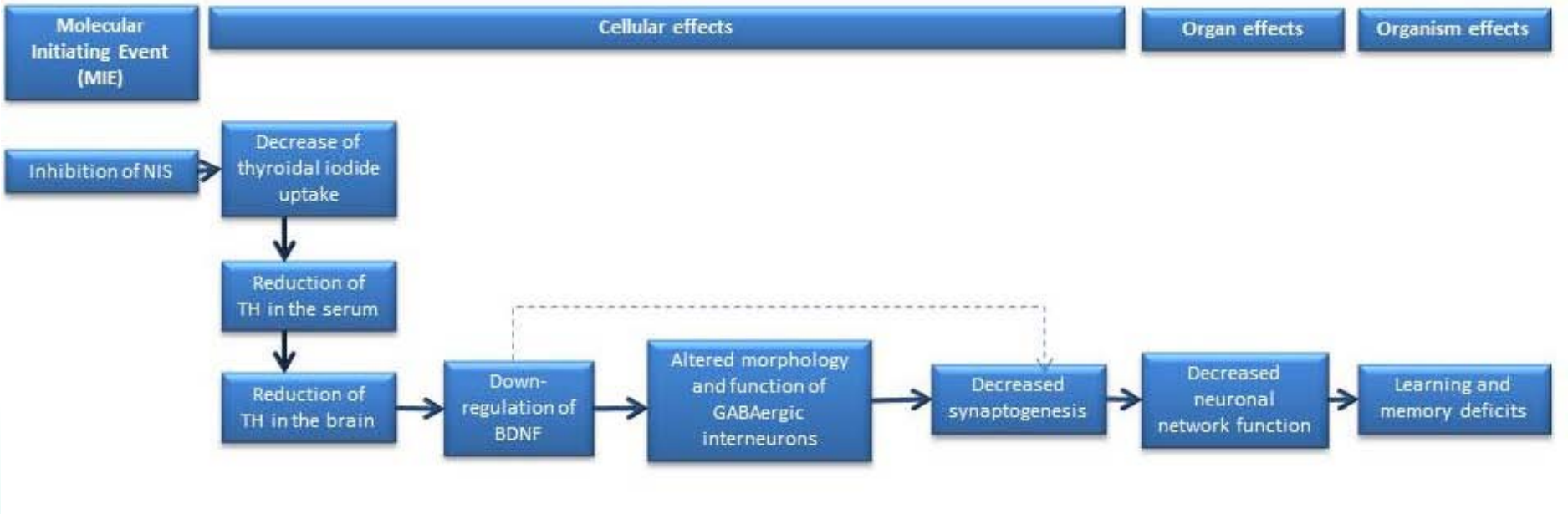
A and B) Golgi-Cox impregnated dendritic arborization and dendritic spines in CA1 area of hippocampus. Control (Ctrl) P14, lead (Pb) P14, Ctrl P21, lead (Pb) P21. Scale bar = 10 µm).

C) Histograms plot showing the quantitative evaluation of the dendritic spine density alternations (spines/10 µm) after lead exposure in P14 and P21 rats (***p<0.001)

(***Hu et al., 2014***: ***Developmental Lead Exposure Alters Synaptogenesis through Inhibiting Canonical Wnt Pathway In Vivo and In Vitro. PLoS ONE 9: e101894)***

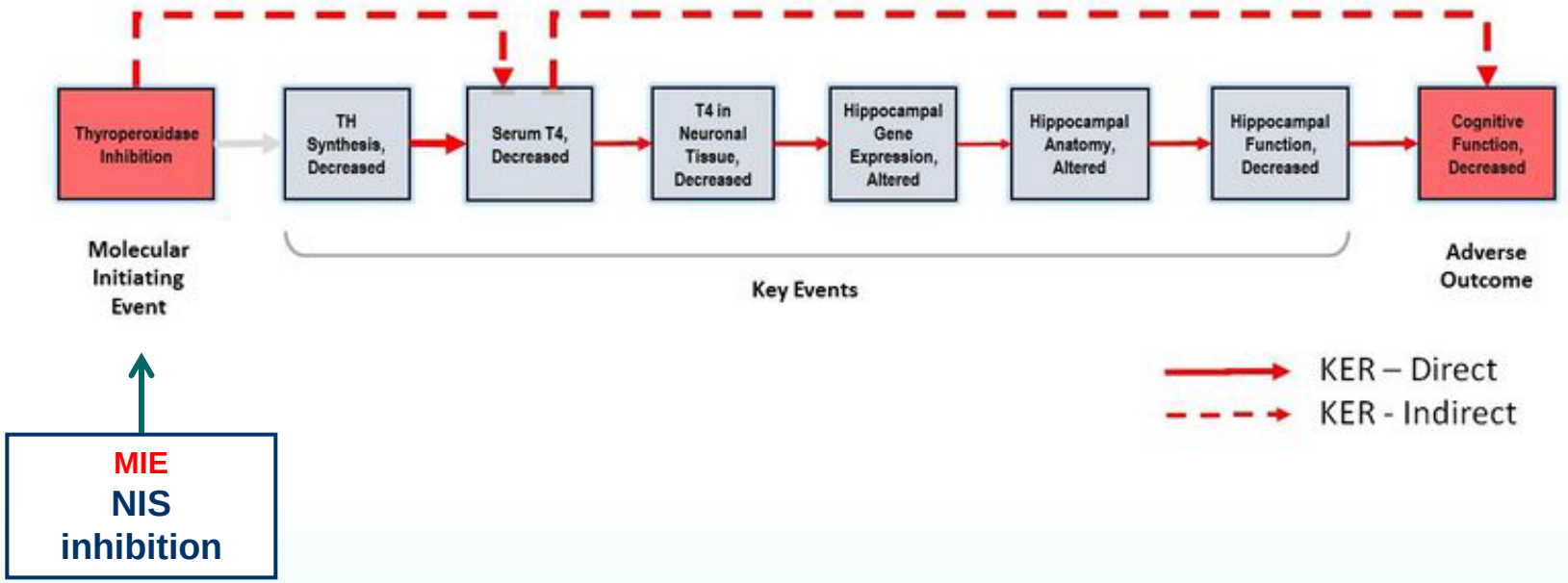
AOP Relevant to DNT

OECD AOP54: INHIBITION OF NA+/I- SYMPORTER (NIS) DECREASES THYRIOD HORMONE SYNTHESIS LEADING TO LEARNING AND MEMORY DEFICITS IN CHILDREN *(JRC, under development)*



AOP Relevant to DNT

AOP42: XENOBIOTIC INDUCED INHIBITION OF THYROPEROXIDASE AND SUBSEQUENT ADVERSE NEURODEVELOPMENTAL OUTCOMES IN MAMMALS *(US EPA, under the OECD internal reviewing process)*



Currently Available DNT AOPs at Different Stages of Development

1.	Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities. ***(AOP-Wiki; endorsed by WNT and TFHA)***
2.	Binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development can trigger neuro-inflammation and lead to neurodegeneration. ***(AOP-Wiki)***
3.	Inhibition of Na+/I- symporter (NIS) decreases TH synthesis leading to learning and memory deficits in children. ***(AOP-Wiki)***
4.	Xenobiotic Induced Inhibition of Thyroperoxidase (TPO) and Subsequent Adverse Neurodevelopmental Outcomes in Mammals. ***(AOP-Wiki)***
5.	Up-regulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, Subsequent Adverse Neurodevelopmental Outcomes in Mammals. ***(AOP-Wiki)***
6.	Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals. ***(AOP-Wiki)***
7.	Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity. ***(AOP-Wiki)***
8.	Impairment of learning and memory induced by binding of electrophilic chemicals to the SH(thiol)-group of protein and non-protein molecules in neuronal and glial cells during development. ***(Crit Rev Toxicol, 2015; 45(1):83-91)***
9.	The interaction of non-dioxin-like PCBs with ryanodine receptors causes their sensitization affecting neuronal connectivity that results in behavioural deficits (developmental neurotoxicity). ***(Crit Rev Toxicol**, 2015; 45(1):83-91)*
10.	Exposure to Mixtures of Metals and Neurodevelopmental Outcomes: A Multidisciplinary Review Using an Adverse Outcome Pathway Framework. ***(Risk Analysis,** 2015, 35(6):971-1016).*

AOP Wiki

Collaborative development of AOP descriptions & evidence

- **Qualitative, text-based descriptions of an AOP in a structured environment**
- Focus is on documenting the weight of evidence in support of the AOP
- **Synchronized with the OECD guidance and handbook documents**
- **Online only access to encourage crowd-sourcing of AOP development**
- Interfaces with the AOP Xplorer to provide AOP information in a network context

https://aopkb.org/aopwiki/index.php

AOP Applicability in Regulatory Context

Relies on the maturity of the AOP (putative – qualitative quantitative) and:

- the amount and type of supporting information
  - the confidence and precision of KEs measurements
  - the level of confidence in the KERs based on biological plausibility, empirical support and consistency of supporting data
  - the weight of evidence for the overall AOP
  - exposure and ADME data
  - prediction models

AOP Applicability in Regulatory Context

- **Facilitate purpose-driven design and validation of *in vitro* methods and screening assays based on identified MIEs and KEs**
  - Data from high-throughput methods can represent a true 'first-tier' screen for the thousands of chemicals currently lacking data
- **Large data set produced by HTS platforms will serve as a base for development of predictive computational models**
  - If the MIE predicts the Adverse Outcome – then you don't need to measure KEs or AO?

AOP Applicability in Regulatory Context

- **Chemical categorisation:** MIE as profiler, QSAR models development

- **Read-across**: predicting unknown properties of one chemical from known properties of similar chemicals
  - filling data gaps on the effects of chemicals by using mechanistic characteristics of the interaction between chemicals and the biological system (MIE) and biological responses (KEs).

AOP Applicability in Regulatory Context

- **Mechanistic support for epidemiological studies**
- **Hazard identification and hypothesis-driven testing:** identification of chemical's potential to cause an adverse effect
  - **Priority setting for further testing:** pre-testing for a positive result or an alert, to avoid further animal testing
- **Risk assessment,** if exposure and ADME data are available
- **Provide conceptual framework for formulating defined approaches (e.g., testing strategies).**

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Thank you for your attention.

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