Building a Network of Adverse Outcome Pathways (AOPs) Incorporating the Tau-Driven AOP Toward Memory Loss (AOP429)

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Abstract. The adverse outcome pathway (AOP) concept was first proposed as a tool for chemical hazard assessment facilitating the regulatory decision-making in toxicology and was more recently recommended during the BioMed21 workshops as a tool for the characterization of crucial endpoints in the human disease development. This AOP framework represents mechanistically based approaches using existing data, more realistic and relevant to human biological systems. In principle, AOPs are described by molecular initiating events (MIEs) which induce key events (KEs) leading to adverse outcomes (AOs). In addition to the individual AOPs, the network of AOPs has been also suggested to beneficially support the understanding and prediction of adverse effects in risk assessment. The AOP-based networks can capture the complexity of biological systems described by different AOPs, in which multiple AOs diverge from a single MIE or multiple MIEs trigger a cascade of KEs that converge to a single AO. Here, an AOP network incorporating a recently proposed tau-driven AOP toward memory loss (AOP429) related to sporadic (late-onset) Alzheimer’s disease is constructed. This proposed AOP network is an attempt to extract useful information for better comprehending the interactions among existing mechanistic data linked to memory loss as an early phase of sporadic Alzheimer’s disease pathology.

Keywords: Adverse outcome pathway, AOP-wiki, memory loss, network

INTRODUCTION

During the last decade, new approaches for assessing chemical toxicity have been developed by regulatory and academic bodies following the recent technological advances. These new developments represent more mechanistically based approaches, shifting to more realistic and efficient strategies, relevant to human biological systems. Considering the technological advances, within the field of chemical risk assessment, applying more predictive approaches can facilitate the determination of more accurate endpoints. Subsequently, more cost-effective, accurate, and human relevant testing strategies can be applied [1, 2]. The concept of adverse outcome pathway (AOP) has been proposed to improve the human relevance of chemical toxicity testing based on available mechanistically relevant approaches using existing human relevant data, resulting in a better understanding of the adverse effects of exposure to various chemicals [3].

During the BioMed21 workshop, the application of AOPs has been suggested for mapping the perturbation of normal human physiology during disease development [4, 5]. Notably, the AOP concept
has been already proposed for the characterization of crucial endpoints in the human pathogenesis of COVID-19 (https://ec.europa.eu/jrc/en/event/webinar/intro-webinar-ciao-project). In compliance with the Organisation for Economic Co-operation and Development (OECD), guidance on the development of the AOP concept is available, providing assistance on defining, constructing, and assessing the development of AOPs [6]. An AOP is characterized by a linear series of events, including a molecular initiating event (MIE) triggered by a stressor, which directly interacts with a biological target at molecular level, followed by intermediate key events (KEs) at cellular level, and ultimately leading to an adverse outcome (AO) at an organism or population level [3]. These KEs are linked to each other by key event relationships (KERs) which describe the causal links between a downstream event and an upstream event based on existing biological knowledge. Biological plausibility, essentiality and supporting evidence, among the reduced modified set of Bradford Hill criteria, are required for weighing the available evidence [6, 7].

In addition, the development of AOP networks has been proposed not only to improve understanding but also in predicting adverse effects used in risk assessment, research, and regulatory decision-making [8, 9]. The AOP networks are derived from existing individual AOPs, available from the AOP knowledgebase (AOP-wiki [6]), sharing common events. Hence, the AOP network concept is believed to improve the mechanistic understanding by providing insights into possible interactions among individual AOPs. The AOP-based networks can capture the complexity of biological systems described by different AOPs, in which multiple AOs diverge from a single MIE or multiple MIEs trigger a cascade of KEs that converge to a single AO [8].

Recently, a tau-driven AOP for the memory loss in sporadic (late-onset) Alzheimer’s disease (sAD) has been proposed [10]. In this AOP blueprint, several environmental neurotoxicants were also suggested as plausible MIE plug-ins, portraying possible chemical interference with early sAD pathology. In more detail, 27 tentative MIEs triggered by different stressors, including environmental chemicals and drugs, were plugged into the proposed processes of the tau-driven AOP under development (AOP429). It was hypothesized that neurotoxicity and sAD pathology may share common pathological processes [10]. Next, this hypothesis was further confirmed by identifying microRNA (miR)-gene interactions commonly regulated by the processes described in this tau-driven AOP [11].

In this present manuscript, individual AOPs listed in the AOP-wiki and sharing common processes/events with the plausible tau-driven AOP are assembled into networks. This AOP network concept is an attempt to better understand the interactions among existing mechanistic data linked to memory loss as an early phase of sAD pathology. In addition, this AOP network-based approach can also provide an effective tool for using the already available knowledge in order to predict same adverse effects of other stressors which could trigger common key events leading to memory loss.

**METHODS**

**Selection of AOPs**

We selected publicly available AOPs, endorsed by OECD, from the AOP-wiki [6], with commonly shared events, as those described by the proposed tau-driven AOP toward memory loss. An exemplified map of the tau-driven AOP was used for defining the different events of interest at molecular, cellular, and organism level (Fig. 1). Thus, seven known AOPs, namely AOP3 (inhibition of the mitochondrial complex I of nigro-striatal neurons leads to parkinsonian motor deficits), AOP10 (binding to the picrotoxin site of ionotropic gamma-aminobutyric acid (GABA) receptors leading to epileptic seizures in adult brain), AOP12 (chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development leads to neurodegeneration with impairment in learning and memory in aging), AOP13 (chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities), AOP42 (inhibition of thyroperoxidase and subsequent adverse neurodevelopmental outcomes in mammals), AOP48 (binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment), and AOP54 (inhibition of Na+/I-symporter (NIS) decreases thyroid hormone (TH) synthesis leading to learning and memory impairment), were included. In Table 1, the detailed data of the triggered MIEs, which can induce the KEs and ultimately lead to the AOs of these selected AOPs, are provided.
Fig. 1. Schematic representation of the proposed tau-driven AOP for memory loss, presenting a starting point (bidirectional relationship between glucose and cholesterol metabolism) which can trigger a cascade of key events (KEs) (mitochondrial dysfunction, oxidative stress, hyperphosphorylated tau, dysfunctional autophagy, toxic tau oligomers, dysfunctional axonal transport, dysfunctional synapses, neuroinflammation, and neuronal dysfunction), which eventually can lead to the adverse outcome (AO), memory loss (modified from [10]).

Besides the approved AOPs by OECD guidelines, in AOP-wiki, relevant AOPs, still under development, were also carefully looked up and found to commonly share some of the affected events represented by the tau-driven AOP: AOP16 (acetylcholinesterase inhibition leading to acute mortality), AOP17 (binding of electrophilic chemicals to SH (thiol)-group of proteins and/or to seleno-proteins involved in protection against oxidative stress during brain development leads to impairment of learning and memory), AOP26 (calcium-mediated neuronal ROS production and energy imbalance), AOP134 (Sodium iodide symporter (NIS) inhibition and subsequent adverse neurodevelopmental outcomes in mammals), AOP152 (interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity), AOP279 (microtubule interacting drugs lead to peripheral neuropathy), AOP281 (acetylcholinesterase inhibition leading to neurodegeneration), and AOP405 (organo-phosphate chemicals induced inhibition of acetylcholinesterase (AChE) leading to impaired cognitive function).

RESULTS AND DISCUSSION

Building the AOP network for memory loss

According to the proposed tau-driven AOP, the hypothetical starting point, glucose and cholesterol dysmetabolism, can induce a series of intermediate KEs such as mitochondrial dysfunction (KE1), oxidative stress (KE2), hyperphosphorylation of tau (KE3), or dysfunctional autophagy (KE4), converging to formation of toxic tau oligomers (KE5). Next, dysfunctional of axonal transport (KE6), synaptic dysfunction (KE7), or neuroinflammation, can be induced, resulting in neuronal dysfunction, which eventually leads to memory loss (AO). Several neurotoxicants/stressors [6, 10] can trigger MIEs, which may induce a cascade of KEs, leading to AOs, including memory loss or neurodegenerative-related disorders, as illustrated in Fig. 2. Many neurotoxicants can potentially bind to molecular targets (triggering MIEs), which in turn can induce cellular responses (intermediate KEs), and subsequently be linked to the presented AOs, which are part of different AOPs (either approved by OECD or under development). In Table 2, several stressors with their potentially targeted molecular events, induced effects, experimental evidence, involved in different events and/or AOPs, are given. By linking these potential stressors to MIEs which in turn can induce intermediate KEs and/or can be linked to the target AO, memory loss, possible interactions among the commonly affected events driven by different AOPs can be identified.

Further, a potential network of AOPs, including the seven approved AOPs (AOP3, AOP10, AOP12, AOP13, AOP42, AOP48, and AOP54) and the seven (still developing) AOPs (AOP17*, AOP26*, AOP134*, AOP152*, AOP279*, AOP281*, and AOP405*) is built, displaying the events commonly shared by these AOPs and the tau-driven AOP (Fig. 3). In this network, different MIEs, KEs, or AOs are shown to be shared by individual AOPs. The AOs
Table 1
Selected AOPs from AOP-wiki based on their shared common events with those of the tau-driven AOP. The AOP ID, AOP description, including potential stressors, molecular initiating events (MIEs), key events (KEs), and adverse outcome (AO), are provided.

| AOP ID: Description                                                                 | Stressor                                                                 | MIEs | KE1 | KE2 | KE3 | KE4 | KE5 | KE6 | KE7 | KE8 | AO               |
|------------------------------------------------------------------------------------|--------------------------------------------------------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|------------------|
| AOP3: Inhibition of the mitochondrial complex 1 of nigro-striatal neurons leads to | Perchlorate, Nitrouracil, Thiocyanate, Aminomethylbenzenesulfonates      |      |     |     |     |     |     |     |     |     | Parkinsonian motor deficits (AO:896) |
| AOP4: Binding of agonists to ionotropic glutamate receptors in adult brain causes  | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Impairment, Learning and memory (AO:341) |
| AOP5: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development leads to impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Cognitive Function, Decreased (AO:402) |
| AOP6: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Impairment, Learning and memory (AO:341) |
| AOP7: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Cognitive Function, Decreased (AO:402) |
| AOP8: Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Impairment, Learning and memory (AO:341) |
| AOP9: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Cognitive Function, Decreased (AO:402) |
| AOP10: Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Impairment, Learning and memory (AO:341) |
| AOP11: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Cognitive Function, Decreased (AO:402) |
| AOP12: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Impairment, Learning and memory (AO:341) |
| AOP13: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Cognitive Function, Decreased (AO:402) |
| AOP14: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Impairment, Learning and memory (AO:341) |
| AOP15: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Cognitive Function, Decreased (AO:402) |
| AOP16: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Impairment, Learning and memory (AO:341) |
| AOP17: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Cognitive Function, Decreased (AO:402) |
| AOP18: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Impairment, Learning and memory (AO:341) |
| AOP19: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Cognitive Function, Decreased (AO:402) |
| AOP20: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Impairment, Learning and memory (AO:341) |
| AOP21: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Cognitive Function, Decreased (AO:402) |
| AOP22: Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment in learning and memory abilities | Ethanol, Acrylamide, Allylisothiocyanate, Ethyl isocyanate, Allyl isocyanate |      |     |     |     |     |     |     |     |     | Impairment, Learning and memory (AO:341) |
Fig. 2. Potential stressors linked to plausible molecular initiating events (MIEs), which induce a cascade of key events (KEs), leading to adverse outcomes (AOs), including memory loss or neurodegenerative-related disorders, are provided. Stressors are indicated in yellow, molecular targets of the MIEs in orange, KEs in blue, and AO in red color. The hypothetical starting point, glucose and cholesterol dysmetabolism is shown in light blue color. The IDs of AOPs under development are shown in italics.

of these included under development AOPs, namely AOP134*, AOP152*, AOP281*, and AOP405*, are described by decreased cognitive function or neurodegeneration, which are indirectly linked to the AO of the tau-driven AOP429. Apparently, all shown individual AOPs seem to share a common event, either directly or indirectly.

In this proposed AOP network for neurodegenerative-related disorders or memory impairment, we integrated plausible MIEs which are potentially triggered by environmental neurotoxicants. Notably, only few of these stressors, including fipronil, 1,3,5-trinitro-1,3,5-triazine (RDX), endosulfan, and lead (Pb) have been elsewhere reported to trigger MIEs of the selected AOPs, confirming their potential involvement in neurodegenerative disorders. In more detail, fipronil, RDX, and endosulfan have been described as potential stressors for binding at picrotoxin site, iGABAR chloride channel (ID:667) leading to occurrence of epileptic seizures (ID:613) in the AOP10, while Pb for binding of antagonist, NMDA receptors (ID:201) leading to neurodegeneration (ID:352) and learning and memory impairment (ID:341) in the AOP12.

Among the presented AOPs, the AOP3 (AO: parkinsonian motor deficits (ID:896)) shares mitochondrial dysfunction 1 (KE:177) and neuroinflammation (KE:188), the AOP10 (AO: occurrence of epileptic seizure (ID:613)) shares the neuronal synaptic inhibition (KE:669), the AOP12 (AO: neurodegeneration (ID:352), AO: learning and memory impairment (ID:341)) shares neuroinflammation (KE:188), both AOP13 and AOP54 (AO: learning and memory impairment (ID:341)) share the decreased neuronal function (KE:386), the AOP48 (AO: learning and memory impairment (ID:341)) mitochondrial dysfunction 1 (KE:177), decreased neuronal network function (KE:618) and neuroinflammation (KE:188), with tau-driven AOP. Although the AOP42 does not seem to share any event with the tau-driven AOP, its AO (decreased cognitive function (ID:402)) is highly relevant to the memory loss, therefore, it is included in our network.

It is important to mention that some events of the tau-driven AOP, such as glucose and cholesterol dysmetabolism, dysfunctional autophagy, tau toxic oligomers, and hyperphosphorylation of tau, have not earlier been described in any of the existing AOPs in AOP-wiki. In this network of AOPs, we presented possible molecular targets for few of these not well-defined events in the context of the AOPs. Such an example, in the AOP3, its MIE, binding of inhibitor, NADH-ubiquinone oxidoreductase (complex I), has
Table 2
Included environmental neurotoxicants as stressors for triggering the plausible molecular initiating events (MIEs), linked to the proposed tau-driven AOP for memory loss

| Stressors                  | Category | Molecular Target | Effect                                                                 | Empirical Support                                                                 | Involved KEs | Involved AOPs                                                                 | Link to tau-driven AOP |
|----------------------------|----------|------------------|----------------------------------------------------------------------|-----------------------------------------------------------------------------------|--------------|-----------------------------------------------------------------------------|------------------------|
| 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) | Drug     | Complex I        | Inhibition of ETC, increased Ca\(^{2+}\) levels and reduced AKT phosphorylation, Mitochondrial complex I inhibitor | Mouse brain concentrations of MPP\(^+\) (approximately 12–49 μM) triggered approximately a 50% inhibition of Complex I. Even small (20 μM) amount of MPP\(^+\) exhibited pronounced deleterious effect on both the Ca\(^{2+}\)-induced changes in the membrane potential and the Ca\(^{2+}\)-accumulating capacity of mitochondria [12]. | Event: 177    | Mitochondrial dysfunction, Event: 887 NADH-ubiquinone oxidoreductase (complex I), Inhibition | Mitochondrial dysfunction |
| Acetaminophen              | Drug     | CYP46A1          | Increased 24S-OHC levels driving increased cholesterol turn-over and hypocholesterolemia | A CYP46A1 activator activated the enzyme in mouse brains at low concentrations (up to 30 μM) but inhibited the P450 at higher concentrations. It increased by > 30% the formation of cholesterol 24-hydroxylation in isolated mouse brain microsomes [13]. |              | Cholesterol dysmetabolism                                                   |                       |
| Alcohol                    | Solvent  | NMDAR, NR2B expression, NR1 expression, PSD-95 | Blocking NMDAR, NMDAR modulation resulting in excitotoxicity and neuronal damage reflect reduction in synaptic activity | 1. After a 7-day ethanol (50 mM) exposure of human embryonic stem cell (hESC)-derived cortical neurons followed by a 24-hour ethanol withdrawal treatment, four NMDA receptor subunit genes, including GRIN1, GRIN2A, GRIN2B, GRIN2D were highly upregulated [14]. 2. Inhibition of NMDA receptor function by ethanol (and interactions between ethanol and the noncompetitive NMDA receptor antagonist ifenprodil) in neocortical neurons from rat and human embryonic kidney (HEK) 293 cells expressing recombinant NMDA receptors [15]. 3. A significant association between high lifetime drinking and high daily alcohol intake with lower DNA methylation of NR2B in alcohol-dependent patients undergoing alcohol withdrawal [16]. 4. NR1 isoforms co-expressed in various combinations with one of the four NR2 subtypes in human embryonic kidney 293 cells, the sensitivity depended on the combination of NR1-3b/NR2C, NR1-3b/NR2D, and NR1-4b/NR2C pairs, and the NR1-2b/NR2C pair were inhibited by ethanol [17]. 5. In rat primary hippocampal cultures, chronic exposure to ethanol induced increase in PSD-95 expression and dendritic spine size [18]. An association between BDNF serum levels and the history of alcohol consumption in humans [19]. | MIE: Event: 201 | NMDA receptors, Binding of antagonist | Synaptic dysfunction, Neuronal inflammation, Neuregeneration, Impaired cognition |
|                           |          |                  | BDNF Presynaptic effects mediated by disruption of NMDAR-activity dependent BDNF signaling by inhibiting BDNF activity |                                                                     |              | AOP13: Binding of antagonist to NMDARs impairs cognition, AOP12: Binding of antagonist to NMDARs can lead to neuroinflammation and neurodegeneration |                       |
| Stressors | Category | KE | AOP | Link to tau-driven |
|-----------|----------|----|-----|-------------------|
| Atrazine (ATZ) | Herbicides | Complex I and III | Inhibition of ETC, increased Ca\(^{2+}\) levels and reduced AKT phosphorylation | Sprague-Dawley rats (n = 48) were treated for 5 months with low concentrations (30 or 300 µg/kg/day) of ATZ provided in drinking water. ATZ blocked the activities of oxidative phosphorylation complexes I and III, resulting in decreased oxygen consumption. It also suppressed the insulin-mediated phosphorylation of Akt [20]. |
| Benzalkonium chlorides | Antiseptics | DHCR7 | Impairment of cholesterol biosynthesis | Exposure to benzalkonium chlorides exhibited high potency in inhibiting DHCR7 when tested in mouse and human neuroblastoma cells by analyzing cholesterol and its precursor using gas chromatography-mass spectrometry [21]. |
| Benz(a)pyrene (B[a]P) | Polycyclic aromatic compounds (PACs) | CYPA1 | Oxidation of macromolecules before conjugation with glutathione, decreased cellular ATP production, MMP, oxidative phosphorylation and mitochondrial protein complexes I, II, and IV activity, and eventually apoptosis | 1. Intraperitoneal injection of B[a]P from embryonic day 7 at a dose of 250 mg kg\(^{-1}\) induced NTDs (13.3% frequency) in mice. B[a]P exposure significantly increased expression of genes associated with oxidative stress, Cyp1a1 [22]. 2. B[a]P-induced neurotoxicity occurred through mitochondria-mediated apoptosis (rat cerebral neurons). B[a]P-induced apoptosis was accompanied by loss of mitochondrial membrane potential, release of cytochrome c from mitochondria to the cytosol [23]. 3. Exposure of human neuroblastoma SK-N-SH cells to B[a]P at 0.5–40 µM for 24 h, increased in levels of ROS, significantly decreased mitochondrial membrane potential (MMP), up-regulated pro-apoptotic genes and down-regulated antioxidative genes expression, elevated cytochrome c protein levels and lipid peroxidation (LPO) in a concentration-related manner [24]. |
| Carbofuran | Pesticides | PP2A, GSK3β | Disturbed mitochondrial ETC, low ATP levels, increased oxidative stress, intracellular Ca\(^{2+}\) levels and tau hyperphosphorylation, Reduced dephosphorylation supporting tau hyperphosphorylation | Carbofuran exposure (rats) led to tau hyperphosphorylation at multiple AD-related phosphorylation sites with activation of GSK-3β and inhibition of PP2A [25]. |

(Continued)
| Stressors | Category | Molecular Target | Effect | Empirical Support | Involved KEs | Involved AOPs | Link to tau-driven AOP |
|-----------|----------|------------------|--------|-------------------|-------------|--------------|------------------------|
| Cadmium (Cd) | Heavy metals | PI3K/Beclin/ BCL2 signaling | p-tau induction and PI3K/Beclin/BCL2 signaling followed by excessive autophagy and apoptosis | 1. Exposure of human SH-SYSY cells to Cd increased intracellular ROS levels [26]. 2. Cd exposure to rat cerebral cortical neurons induced cytoprotective autophagy by activating the class III PI3K/beclin-1/Bcl-2 signaling pathway [27]. | PI3K-AKT | Oxidative stress, Dysfunctional autophagy |
| Chlorophenotane | Pesticides | CYP51, DHCR7? | Impairment of cholesterol biosynthesis | Acute sublethal and chronic administration of chlorophenotane (DDT) decreased brain lipid metabolism of rhesus monkeys [28]. |  | | Cholesterol dysmetabolism |
| Chlorpyrifos | Organophosphate pesticides | AChE | Irreversible binding between AChE and OP pesticides due to phosphorylation of enzyme, AChE inhibitors (pesticides) bind to the enzyme and interfere with the breakdown of AChE, leading to deposition of AChE in nerve synapses, causing disrupted neurotransmission. | 1. Estuarine fish (Oreochromis mossambicus) to a 24 h LC50 concentration of chlorpyrifos, after 6 h reached >40% AChE inhibition while after 24 h reached 90% AChE inhibition [29]. 2. An acute sublethal exposure of chlorpyrifos to Sprague-Dawley rat increased inhibition of AChE, increased levels of acetylcholine, and significantly impacted to motor activity [30]. | Chlorpyrifos exposure of human neural precursor cells (hNPCs) derived from human embryonic stem cells (hESCs) reduced the expression of AKT and ERK proteins involved in intracellular survival pathways [31]. | MIE: Event 12 AOP16: Acetylcholinesterase inhibition leading to acute mortality, AOP405: Organophosphate Chemicals induced inhibition of AChE leading to impaired cognitive function | Impaired cognitive function |
| P3K-AKT | Chronic cholinergic activity linked to defective P3K-AKT pathway activation | Chlorpyrifos exposure of human neural precursor cells (hNPCs) derived from human embryonic stem cells (hESCs) reduced the expression of AKT and ERK proteins involved in intracellular survival pathways [31]. | Glucose metabolism |
| NMDAR | Glutamate excitotoxicity leading to neuronal damage | Chlorpyrifos -induced neurotoxicity after CPF exposure with and without fipronil (FPN) on 4-week differentiated human neural progenitor stem cell culture model (ReNcell CX) [32]. | Synaptic dysfunction |
| NFkB | Release IL1β and TNFα? | Chlorpyrifos exposure of human neural precursor cells (hNPCs) derived from human embryonic stem cells (hESCs) induced nuclear accumulation of NFκB manner via ROS generation in a concentration-dependent [31]. | Neuroinflammation |
| LC3-II expression | Oxidative stress induced increased in numbers of autophagic vacuoles, autophagy, and apoptosis | 1. Chlorpyrifos-induced cytotoxicity in human SH-SYSY cells, and induced autophagic cell death by upregulating the expression of LC3-II and p62 [33]. 2. Chlorpyrifos generated oxidative stress and lipid peroxidation in different rat cell types causing neuronal damage by elevating the production of ROS, DNA damage, and lipid peroxidation in the CNS [34, 35]. | Oxidative stress, Dysfunctional autophagy |
| Stressor Category | Molecular Target | Effect | Empirical Support | Involved KEs | Involved AOPs | Link to tau-driven |
|------------------|------------------|--------|-------------------|--------------|---------------|-------------------|
| Conazole Fungicides | CYP51, DHCR7 | Impairment of cholesterol biosynthesis | Inhibits CYP51 by coordinating with the heme group, which halts substrate binding with a resulting increase of lanosterol in mouse Neuro2a cells [36]. | CYP51, DHCR7 | AOP200: Impaired cholesterol biosynthesis | Cholesterol dysmetabolism |
| Copper (Cu) Metals | LC3-II expression | Oxidative stress induced increased in numbers of autophagic vacuoles, autophagy, and apoptosis | CuCl2 induced a dose-dependent accumulation of the autophagosome marker, LC3-II, in human neuroblastoma cell line SK-N-SH [37]. | LC3-II | AOP010: Oxidative stress, Dysfunctional autophagy | Oxidative stress, Dysfunctional autophagy |
| Deltamethrin Pesticides | PP2A, GSK3β | Disturbed mitochondrial ETC, low ATP levels, increased oxidative stress, intracellular Ca2+ levels and tau hyperphosphorylation. Reduced dephosphorylation supporting tau hyperphosphorylation. | Exposure of deltamethrin (30 μM), PP2A/PP2B inhibitors, induced tau aggregation in human SH-SY5Y tau-BiFC cells and in HEK293 tau-BiFC cells. | PP2A, GSK3β | AOP017: Mitochondrial dysfunction, Oxidative stress, tau hyperphosphorylation | Mitochondrial dysfunction, Oxidative stress, tau hyperphosphorylation |
| Dibenzothiopene Alkyl-polycyclic aromatic compounds (PACs) | CYP1A1, CYP1B1 | Oxidation of macromolecules before conjugation with glutathione, decreased cellular ATP production, MMP, oxidative phosphorylation and mitochondrial protein complexes I, II, and IV activity, and eventually apoptosis | Exposure of human neuroblastoma SK-N-SH cells to dibenzothiophene (at 0.5–40 μM for 24 h) increased in levels of ROS, significantly decreased mitochondrial membrane potential, upregulated pro-apoptotic genes and down-regulated antioxidative genes expression, elevated cytochrome c protein levels and lipid peroxidation in a concentration-related manner [24]. | CYP1A1, CYP1B1 | AOP10: Mitochondrial dysfunction, Oxidative stress | Oxidative stress |
| Dichlorvos Organophosphate pesticides | AChE | Irreversible binding between AChE and OP pesticides is due to phosphorylation of enzyme. The AChE inhibitors (pesticides) bind to the enzyme and interfere with the breakdown of ACh, leading to the deposition of ACh in the nerve synapses and causing disrupted neurotransmission | Rats injected with a sublethal concentration of dichlorvos found a significant decrease in AChE activity, increased ACh concentrations, and enhanced contractile responses in jejunum muscle. At sublethal concentrations (56% of the LD50), researchers found a significant (18%) increase in the amount of ACh in brain tissue of rats exposed to disulfoton for 3 days and resulted in AChE inhibition of 68% with respect to controls [38]. | AChE | AOP405: Organophosphate Chemicals induced inhibition of AChE leading to impaired cognitive function | Impaired cognitive function |
| Dieldrin Organochlorine insecticides | iGABARs | Directly blocks chloride conductance through the ion channel | Long-term exposure of mouse primary cerebellar granule cell cultures to 3 μM dieldrin reduced the GABA<sub>A</sub> receptor function to 55% of control, as measured by the GABA<sub>A</sub>-induced 36Cl<sup>-</sup> uptake [39]. | iGABARs | AOP10: Blocking iGABA receptor ion channel leading to seizures | Seizures |

(Continued)
| Stressors | Category | Molecular Target | Effect | Empirical Support | Involved KEs | Involved AOPs | Link to tau-driven AOP |
|-----------|----------|------------------|--------|-------------------|--------------|--------------|----------------------|
| Domoic acid (DomA) Kainic acid-type neurotoxin (ASP) | | | | 1. DomA (4 mg/kg at 30, 60 and 240 min post-injection) promoted the expression of early inflammatory genes in mouse brain, such as COX2 and the development of neurodegeneration [40]. 2. DomA treatment (2 mg/kg per day for 3 weeks) in mice significantly stimulated the expression of inflammatory mediators, including IL-1β (1.7 fold increase), TNF-α (2 fold increase), GFAP (1.4 fold increase), Cox-2 (3 fold increase), and iNOS (1.6 fold increase) compared to controls [41]. 3. DomA (0.75 mg/kg body weight) when administered intravenously in adult rats induced neuronal degeneration followed by glial activation [42]. | MIE: Event 875 | AOP48: Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment. | Neuroinflammation |
| | iGlu, NMDRAs, KA & AMPARs | DomA induces excitotoxicity by an integrative action on ionotropic glutamate receptors at pre- and post-synaptic sides. DomA directly activates KA/AMPARs receptors followed by indirect activation of the NMDARs. | 1. In rat hippocampal neurons at 5 days after DomA administration, NMDAR1 immunoreactivity increased, and glutamate receptor genes were induced in response to DomA-induced neurotoxicity [43]. 2. DomA exposure of immature and mature primary cultures of neurons and glial cells from rat cerebellum induced neurotoxicity mediated by the AMPA/KA receptors [44]. | | MIE: Event 867 | I onotropic glutamate receptors, Binding of agonist | Synaptic dysfunction |
| Efavirenz Drug | CYP46A1 | | Increased 24S-OHC levels driving increased cholesterol turn-over and hypocholesterolemia | Efavirenz a CYP46A1 activator activated the enzyme in mouse brains at low concentrations (up to 20 μM) but inhibited the P450 at higher concentrations. It increased the formation of cholesterol 24-hydroxylation in isolated mouse brain microsomes [13]. | MIE: Event 667 | AOP10: Blocking iGABA receptor ion channel leading to seizures | Cholesterol dysmetabolism |
| Endosulfan Organochloride insecticide | iGABARs | Non-competitive ion channel blocker | Poisoning with endosulfan caused seizure, status epilepticus, or refractory status epilepticus in humans [45, 46], and eventually led to the death of a farmer [45] and a toddler [47]. | | | Seizures |
| | CYP51, DHCR7 | Impairment of cholesterol biosynthesis C | Exposure of mouse Neuro-2a cells to endosulfan (1.1 μM) induced elevation of lanosterol by inhibiting either CYP51 or DHCR7 [48]. | | | Cholesterol dysmetabolism |
| Felodipine Drugs | CYP27A1 | Reduced 27-OHC production, elevated cholesterol biosynthesis and reduced steroidal acid production | Felodipine administration to mice at a 1 mg/kg of body weight/day for 7 days induced reduction in 27-hydroxycholesterol levels in plasma, brain and liver, whereas tissue levels of total cholesterol were unchanged [49]. | | | Cholesterol dysmetabolism |
| Stressors Category | Molecular Target | Effect | Empirical Support | Involved KEs | Involved AOPs | Link to tau-driven function |
|------------------|-----------------|--------|------------------|--------------|--------------|-------------------------|
| Organophosphate pesticides | AChE | Irreversible binding between AChE and OP pesticides is due to phosphorylation of enzyme. The AChE inhibitors (pesticides) bind to the enzyme and interfere with the breakdown of ACh, leading to the deposition of ACh in the nerve synapses and causing disrupted neurotransmission. | Female mice exposed to either fenobucarb or propoxur, reported a major increase in AChE in brain tissue 10 minutes after injection, simultaneously major elevation in AChE inhibition [50]. | MIE: Event: 12 | Acetylcholinesterase inhibition (AChE), Inhibition | Impaired cognitive function |
| Organophosphate pesticides | CYP51, DHCR7 | Impairment of cholesterol biosynthesis | Exposure of human cell lines and human neuroprogenitor model derived from hiPSCs to fenpropimorph caused hypocholesterolemia, decreased absolute cholesterol levels, inhibited CYP51 and elevated 7-DHC levels [48]. | MIE: Event: 667 | iGABAR chloride channel, Binding at picrotoxin site, | Cholesterol dysmetabolism |
| Fungicides | iGABARs, Cl-channels regulated by GABA receptors | Directly blocks chloride conductance through the ion channel. | Acute human intoxication with fipronil revealed symptoms associated with the GABA transmission within the central nervous system, including seizure, agitation, and headache. In two patients, plasma fipronil concentrations were measured at 1600 and 3744 µg/L. In another patient, a peak measured plasma concentration at 1040 µg/L [51]. | MIE: Event: 64 | iGABAR chloride conductance, Reduction | Seizures |
| Drugs | CYP46A1 | Increased 24S-OHC levels driving increased cholesterol turnover and hypocholesterolemia | Galantine treatment in isolated heving brain microsomes stimulated CYP46A1 activity by 6–7-fold [13]. | AOP10: Blocking iGABAR ion channel leading to seizures | Tau hyperphosphorylation |

(Continued)
| Stressors | Category | Molecular Target | Effect | Empirical Support | Involved KEs | Involved AOPs | Link to tau-driven AOP |
|-----------|----------|------------------|--------|-------------------|--------------|--------------|-----------------------|
| Glufosinate | Phosphorous herbicides, Fungicides | NMDARs | Agonist action at the NMDAR is expected to occur through direct interaction with the glutamate binding site and requires binding of the glycine co-agonist as well as release of the magnesium block from the channel pore. | 1. In one human poisoning case (267 mg/kg dl-glufosinate, oral ingestion), the concentrations respectively of d- and l-GLF 1 h after exposure were 1050 and 1070 μM in plasma and after 27 h were 7.95 and 1.93 μM in plasma and 2.6 and 0.66 μM in cerebrospinal fluid, respectively. dl-GLufosinate concentrations in excess of 100 μM are needed to affect the NMDAR [54]. 2. Chronic exposure to glufosinate induced structural changes in the NMDAR-rich hippocampal region of the mouse brain, disrupting activation of NMDARs [55]. | MIE: Event: 875 | Ionotropic glutamate receptors, Binding of agonist | AOP19: Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory | Synaptic dysfunction |
| Iron (Fe) | Metals | LC3-II & Beclin expression | Oxidative stress induced increased in numbers of autophagic vacuoles, autophagy, and apoptosis | Fe given in the neonatal period in rats (single daily oral dose of vehicle or iron carbonyl (30 mg/kg) at postnatal days 12–14) impaired inhibitory avoidance memory and induced a decrease in proteins critically involved in the autophagy pathway, Beclin-1 and LC3, in hippocampus [56]. | | | Oxidative stress, Dysfunctional autophagy |
| Ketamine | Anaesthetics | NMDARs | Blocking NMDA receptor channel activity | In humans, ketamine exposure produced profound decrements in both attention and memory [57, 58]. | | | Synaptic dysfunction |
| Lindane | Insecticides | iGABARs | Non-competitive ion channel blocker | Toxic doses of lindane produced neuronal hyperexcitability in humans and other vertebrates by inhibiting both glycine and GABA-A receptors [59]. | | | Synthetic dysfunction |
| Lead (Pb) | Heavy metals | BDNF | Presynaptic effects mediated by disruption of NMDAR-activity dependent BDNF signaling by inhibiting BDNF activity | In primary rat hippocampal neurons exposed to 1 μM Pb2+ for 5 days during the period of synaptogenesis (DIV7–DIV12), decreased cellular pro-BDNF protein (40% compared to control) and extracellular levels of mBDNF (25% compared to control) [60]. | Event: 389 (Release of BDNF, Reduced) | AOP13: Binding of antagonist to NMDARs impairs cognition, AOP54: NIS inhibition and DNT effects, AOP12: Binding of antagonist to NMDARs can lead to neuroinflammation and neurodegeneration | Synthetic dysfunction |
| Event: 195 | NMDARs, Inhibition, Event: 201 NMDA receptors, Binding of antagonist | AOP12: Binding of antagonist to NMDARs can lead to neuroinflammation and neurodegeneration | Synaptic dysfunction, Neuroinflammation, Neurodegeneration |
| --- | --- | --- | --- |

1. In rat hippocampal neurons, Pb²⁺ (2.5–50 μM) inhibited NMDA-induced whole-cell and single-channel currents in a concentration-dependent manner, suggesting that Pb²⁺ can decrease the frequency of NMDA-induced channel activation. Pb²⁺ also affected the binding of [3H]MK-801 to the rat brain hippocampal membranes [61].
2. Studies in animal models of developmental Pb²⁺ exposure exhibit altered NMDAR subunit ontogeny and disruption of NMDAR-dependent intracellular signaling [60].

| Event: 52 | Ca²⁺ influx, decreased | Synaptic dysfunction? |
| --- | --- | --- |

- Pb²⁺ exposure decreased Ca²⁺ ion concentration and increased Ca²⁺ efflux by a calmodulin-dependent mechanism in embryonic rat hippocampal neurons [62].

| Event: 188 | Neuroinflammation |
| --- | --- |

- In vivo and in vitro models (rats), Pb exposure caused microglial activation, which upregulated the levels of pro-inflammatory cytokines IL-1β, TNF-α and of iNOS and caused neuronal injury and neuronal death in hippocampus [63].

| Tau | Phosphorylation of tau |
| --- | --- |

1. Monkeys exposed to Pb 1.5 mg/kg/day from birth to 360 days at 23 years of age show tau accumulation, overexpression of amyloid-beta protein precursor and of amyloid-beta enhanced pathologic neurodegeneration [64].
2. Mice exposed to Pb 0.2% in drinking water from PND 1–20 or from PND 1–20 and from 3–7 months at 700 days of age, elevated protein and mRNA for tau and aberrant site-specific tau [65].
3. Perinatal exposure to Pb leading to a blood concentration of 10 μg/dl promoted tau phosphorylation in rat forebrain, cerebellum and hippocampus [66].
4. Chronic exposure of rats to Pb via drinking water induced hyperphosphorylation of tau and excessive increase in autophagy, which might induce cell-programmed death and increase neurotoxicity [67].

(Continued)
Table 2
(Continued)

| Stressors     | Category               | Molecular Target                  | Effect                                                                 | Empirical Support                                                                                                                                                                                                 | Involved KEs                                                                                           | Involved AOPs                                                                                           | Link to tau-driven AOP                                                                 |
|---------------|------------------------|-----------------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|-------------------------------------------------|
| Beclin-1/LC3-II signaling, Akt/mTOR pathway, P38-Akt signaling | Beclin-1/LC3-II signaling followed by excessive autophagy | Pb exposure induced autophagy in astrocytes, by increased LC3II and Beclin 1 protein levels in both the rat hippocampus and astrocytes through blocking the downstream Akt/mTOR pathway in astrocytes [68]. Pb exposure induced a decrease in rat hippocampal glucose metabolism by reducing GLUT4 levels in the cell membrane through the P38-Akt pathway. In vivo and in vitro GLUT4 over-expression increased the membrane translocation of GLUT4 and glucose uptake, and reversed the Pb-induced impairment to synaptic plasticity and cognition [69]. | Oxidative stress, Dysfunctional autophagy                                                                                                        |                                                                                                       |                                                                                                       |                                                                                                           |
| Malathion     | Organophosphate pesticides | AChE                               | Irreversible binding between AChE and OP pesticides is due to phosphorylation of enzyme. The AChE inhibitors (pesticides) bind to the enzyme and interfere with the breakdown of AChE, leading to the deposition of AChE in the nerve synapses and causing disrupted neurotransmission | 1. In rats, administration of malathion induced reduction of plasma acetylcholinesterase activity [70]. 2. In silkworm exposed to malathion for 5 days increased mortality, decreased AChE, and increased in AChE as compared to controls [71]. | MIE: Event 12 Acetylcholinesterase (AChE), Inhibition                                                                                               | AOP405: Organophosphate Chemicals induced inhibition of AChE leading to impaired cognitive function (AOP under development) | Impaired cognitive function                                                                                          |
|               |                        | GSK3β, PP2A                          | Reduced dephosphorylation supporting tau hyperphosphorylation           | In rats, administration of malathion induced spatial learning and memory deficits with a simultaneous decrease of PSD93 and tau hyperphosphorylation at multiple AD-related phosphorylation sites with activation of GSK-3β and inhibition of PP2A [70]. |                                                                                                       |                                                                                                       | Tau hyperphosphorylation                                                                                     |
|               |                        | SH containing proteins               | Depletion of glutathione buffer, oxidation of macromolecules, and disturbed cellular redox homeostasis | 1. Subchronic exposure of rats to malathion increased malondialdehyde and 8-OHdG levels, whereas it decreased glutathione levels, also acetycholinesterase, superoxide dismutase, and catalase activities in the blood and brain tissues [72]. 2. In rats, administration of malathion induced amelioration of reduced GSH in hippocampus [70]. |                                                                                                       |                                                                                                       | Mitochondrial dysfunction, Oxidative stress                                                                 |
|               |                        | TNF-a, IL-6                          | Proinflammatory cytokines                                               | In rats, administration of malathion induced elevation of TNFα and IL-6 levels in the hippocampus [70].                                                                                                                  |                                                                                                       |                                                                                                       | Neuroinflammation                                                                               |
| Methylmercury (MeHg) | Metals                | Beclin-1 expression                 | Oxidative stress induced increased in numbers of autophagic vacuoles, autophagy, and apoptosis | 1. Exposure of human neural stem cells to MeHg upregulated autophagy by inhibiting mTOR levels and by enhancing Atg6/Beclin-1 protein levels [73]. 2. Oxidative stress has been suggested to enhance autophagy signaling pathway, as the underlying mechanism of MeHg-induced neurotoxicity [74]. |                                                                                                       |                                                                                                       | Oxidative stress, Dysfunctional autophagy                                                              |
| Stressors Category | Molecular Target | Effect | Empirical Support | Involved KEs | Involved AOPs | Link to tau-driven |
|--------------------|------------------|--------|-------------------|--------------|--------------|-----------------|
| Organophosphate pesticides | LC3-II expression | Irreversible binding between AChE and OP pesticides is due to phosphorylation of enzyme. The AChE inhibitors (pesticides) bind to the enzyme and interfere with the breakdown of ACh, leading to the deposition of ACh in the nerve synapses and causing disrupted neurotransmission. Sublethal exposure (12–48 h) to methyl parathion highly inhibited AChE levels in brain tissue in *Inh.* (Oreochromis mossambicus) with inhibition increasing from 36–62% as in comparison to controls over the time elapse [76]. |  |  | | |
| Drugs | CYP46A1 | Increased 24S-OHC levels driving increased cholesterol turnover and hypercholesterolemia | Exposure of bovine isolated brain microsomes to nilvadipine increased CYP46A1 activity [13]. |  |  | | |
| Organochlorine pesticides (OCPs) | CYP46A1 | Increased 24S-OHC levels driving increased cholesterol turnover and hypercholesterolemia | In rat astrocytes, MeHg decreased cell viability in a concentration- and time-dependent manner, with induction of both apoptosis and autophagy via increasing the LC3-II–LC3-I ratio, and of Beclin-1 protein [75]. |  |  | | |

MIE: Event: 12

AOP405: Organophosphate Chemicals induced inhibition of AChE leading to impaired cognitive function (AOP under development)

Impaired cognitive function

Cholesterol dysmetabolism

Mitochondrial dysfunction, Oxidative stress, tau hyperphosphorylation

Oxidative stress, Dysfunctional autophagy

Cholesterol dysmetabolism

Cholesterol dysmetabolism

(Continued)
| Stressors | Category                  | Molecular Target | Effect                                      | Empirical Support                                                                 | Involved KEs | Involved AOPs | Link to tau-driven AOP |
|----------|---------------------------|------------------|---------------------------------------------|-----------------------------------------------------------------------------------|--------------|---------------|------------------------|
| Okadaic acid (OKA) | Algal toxins | GSK3β, PP2A | Tau hyperphosphorylation | Administration of OKA in *rat* increased tau gene and protein expression, Ca^{2+}/CAMKII, calpain and GSK3β in the hippocampus and cerebral cortex, while decreased the PP2A gene and protein expression in these brain regions [81]. | Disturbed mitochondrial ETC, low ATP levels, increased oxidative stress, intracellular Ca^{2+} levels | Intracerebroventricular administration of OKA increased intracellular Ca^{2+}, impairing the mitochondrial ETC and generating intracellular ROS and RNS (reactive nitrogen species) in *rat* brain areas [82]. | Tau hyperphosphorylation |
| Paraaxon | Organophosphate pesticides | AChE | Irreversible binding between AChE and OP pesticides is due to phosphorylation of enzyme. The AChE inhibitors (pesticides) bind to the enzyme and interfere with the breakdown of AChE, leading to the deposition of AChE in the nerve synapses and causing disrupted neurotransmission. | Inhibition of striatal AChE activity and decreased extracellular AChE levels in *rat* acutely perfused after exposure to paraaxon (0.03; 0.1, 10 or 100 μM, 1.5 µl/min for 45 min) [83]. | NMDARs | Involvement of NMDA receptor in OKA intracerebroventricular-induced tau hyperphosphorylation in *rat* brain areas [81]. | Mitochondrial dysfunction, Oxidative stress |
| Paraoxon | Organophosphate pesticides | AChE | Irreversible binding between AChE and OP pesticides is due to phosphorylation of enzyme. The AChE inhibitors (pesticides) bind to the enzyme and interfere with the breakdown of AChE, leading to the deposition of AChE in the nerve synapses and causing disrupted neurotransmission. | An acute (48 h) sublethal exposure to methyl parathion found that AChE levels in brain tissue in *fish* (*Oreochromis mossambicus*) were significantly inhibited at all measured durations ranging from 12–48 h with inhibition increasing from 36–62% as compared to controls over the time span [76]. | | | Neuroinflammation |

MIE: Event 12: Acetylcholinesterase (AChE), Inhibition

AOP405: Organophosphate Chemicals induced inhibition of AChE leading to impaired cognitive function (AOP under development)

AOP16: Acetylcholinesterase inhibition leading to acute mortality
| Stressors Category | Molecular Target | Effect | Empirical Support | Involved KEs | Involved AOPs | Link to tau-driven | AOP |  |
|--------------------|-----------------|--------|------------------|--------------|--------------|------------------|-----|---|
| Polychlorinated Biphenyls (PCBs) | Persistent organic pollutants (POPs) | CYP450 | Oxidation of macromolecules before conjugation with GSH, decreased cellular ATP production, MMP, oxidative phosphorylation and mitochondrial protein complexes I, II, and IV activity, and eventually apoptosis. | Chronic exposure of human SH-SY5Y cells to PCBs decreased cellular ATP production, MMP, oxidative phosphorylation and the activity of mitochondrial protein complexes I, II, and IV, resulting in mitochondrial dysfunction [86]. | | | | |
| HMG-CoA reductase | Increased cholesterol serum levels result in impaired cholesterol biosynthesis | Reduction in these synaptic markers reflect reduction in synaptic activity | In human studies, exposure of PCBs induced elevated levels of the total cholesterol in blood [80]. | | | | | |
| Particle matter (PM) & Sulfur dioxide (SO₂) | Air pollutants | PSD-95, NR2B, BDNF, Synp, tau, expression | Reduction in these synaptic markers reflect reduction in synaptic activity | 1. PM₂₅ and SO₂ co-exposure led to neurodegeneration at low doses, reduced synaptic structural protein PSD-95 and NMDA receptor subunits (NR2B), and elevated tau phosphorylation, in vitro (mouse primary cortical neuron culture) and in vivo (mice) [87]. 2. Human cocultured neurons and astrocytes with PM₂₅ treatment exhibited reduction in the number of synapsin I by about 49.6% compared to the nontreated cocultured model [88]. 3. Human in utero exposure to PM₂₅ was inversely associated with placental BDNF expression [89]. | | | | |
| IL-1β | Proinflammatory cytokines | | Human astrocytes cocultured with neurons were activated by PM₂₅ treatment produced significant levels of proinflammatory chemokines (CCL1 and CCL2) and cytokines (IL-1β and IFN-γ), which can recruit and activate microglia [88]. A time course study of earthworms (Eisenia fetida) exposed to the 4.56 ± 0.14 and 3.55 ± 0.10 μg cm⁻² for 24 and 48 h (LC50), respectively, of PPF found a significant relationship between increases in percent inhibition of ACHE and increase in time of exposure from 8–48 h [90]. | MIE: Event: 12 Acetylcholinesterase (ACHE), Inhibition AOP16: Acetylcholinesterase inhibition leading to acute mortality | | | | |

(Continued)
| Stressors | Category | Molecular Target | Effect | Empirical Support | Involved KEs | Involved AOPs | Link to tau-driven AOP |
|----------|----------|------------------|--------|-------------------|-------------|--------------|-----------------------|
| Propofol | Sedative drugs | HMG-CoA reductase | Increased cholesterol serum levels result in impaired cholesterol biosynthesis | Treatment of rats with propofol induced increased levels of serum total cholesterol, possibly due to the increase in HMG-CoA reductase activity, a rate limiting step in cholesterol biosynthesis. Propofol at 2 and 4 mg/kg increased the levels of serum total cholesterol by 74% and 55%, triglycerides by 97% and 115%, and LDL-C by 45% and 73%, respectively, while HDL-C decreased by 41% and 54%, respectively [91]. | | | Cholesterol dysmetabolism |
| RDX | iGABARs | | Directly blocks chloride conductance through the ion channel. | Event: 667 iGABAR chloride channel, Binding at picrotoxin site | AOP: Blocking iGABA receptor ion channel leading to seizures | Seizures |
| Retene | Alkyl-polycyclic aromatic compounds (PACs) | CYP1A1, CYP1B1 | Oxidation of macromolecules before conjugation with GSH, decreased cellular ATP production, MMP, oxidative phosphorylation and mitochondrial protein complexes I, II, and IV activity | Exposure of human neuroblastoma SK-N-SH cells to retene at 0.5–40 μM for 24 h, increased in levels of ROS, significantly decreased MMP, up-regulated pro-apoptotic genes and down-regulated antioxidative genes expression, elevated cytochrome c protein levels and lipid peroxidation in a concentration-related manner [24]. | | | Mitochondrial dysfunction, Oxidative stress |
| Rotenone | Isoflavone pesticides, insecticides, piscicides | LC3-II expression, Beclin-1 expression, mTOR | Oxidative stress induced increased in numbers of autophagic vacuoles, autophagy, and apoptosis | 1. Robust increase in steady state expression of LC3 (LC3I and LC3II), upon rotenone treatment compared to untreated cells, and a significant decrease in phosphorylation of Akt and beclin1, in human SHSY-5Y cells [93]. 2. Exposure to rotenone at a higher dose (10 μM) decreased mTORC1 activity, in human SHSY-5Y cells. Rotenone-treated cells showed ~2 fold increase in ROS generation compared to untreated cells [93]. | Event: 177 | AOP: Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment. AOP3: Mitochondrial dysfunction and Neurotoxicity | Oxidative stress, Dysfunctional autophagy |
| Complex I | Inhibition of ETC, increased Ca^{2+} levels and reduced AKT phosphorylation, Mitochondrial complex I inhibitor | | Mouse brain concentrations of rotenone (20–30 nM) triggered approximately a 50% inhibition of Complex I [12]. | | | Glucose dysmetabolism via mitochondrial dysfunction |
| Stressors Category | Molecular Target | Effect | Empirical Support Involved KEs | Involved AOPs Link to tau-driven AOPs |
|-------------------|------------------|--------|-------------------------------|--------------------------------------|
| Silica (SiO2-NPs), Silver (AgNPs) nanoparticles | Nanoparticles PSD-95, NR2B expression | Reduction in these synaptic markers reflect reduction in synaptic activity | 1. In brains of immature rats subjected to a low dose of AgNPs, decreased expression of several NMDA receptor complex-related proteins, such as GluN1 and GluN2B subunits, scaffolding proteins PSD95 and SynGAP, as well as neuronal nitric oxide synthase (nNOS) [94]. 2. AgNPs oral exposure significantly decreased levels of the presynaptic proteins synapsin I and synaptophysin, as well as PSD-95 protein which is an indicator of postsynaptic densities, in hippocampal region of rat brain [95]. |
| GSK3B | Induction of tau hyperphosphorylation | Exposure of human SK-N-SH to SiO2-NPs significantly increased the intracellular levels of ROS and the hyperphosphorylation of tau at Ser262 and Ser396 accompanied by increased GSK3B activity [96] |
| APP | | | |
| Sodium azide (NaN3) | Gas-forming inorganic compound Complex IV | Inhibition of the mitochondrial ETC, oxidative stress | Exposure of in rat PC12 cells to NaN3 inhibited cytochrome oxidase in the mitochondrial ETC and accumulation of mitochondrial ROS. NaN3-induced apoptosis was associated with the expression levels of Pgc-1α family proteins and Cox IV in mitochondria-mediated signaling pathway [97]. Mitochondrial dysfunction, Oxidative stress |
| Spiroxamine | Fungicides CYP51, DHCR7 | Impairment of cholesterol biosynthesis | Exposure of human hiPSCs and mouse neuroblastoma Neuro-2a cells to spiroxamine induced decreased cholesterol biosynthesis either by inhibiting CYP51 or DHCR7 [48]. Cholesterol dysmetabolism |
| Taxol & epothilones | Microtubule interacting drugs (MSAs) Tubulin (TUBB) | MSAs bind to polymerized tubulin. Impairment in axonal transport leads to an inadequate supply of the neuronal periphery. | 1. Disruption of microtubule dynamic instability decreased transport of horseradish peroxidase in dorsal root ganglia neurons resulting in less microtubule crosslinks. Intact axonal transport regained after taxol wash-out (1 day treatment, 2 days wash-out) [98]. 2. Taxol inhibited anterograde fast (and retrograde) axonal transport in rat sciatic nerves [99] and anterograde transport in SK-N-SH human neuroblastoma cells and mouse sciatic nerves [100]. 3. Suppressed microtubule dynamic instability had inhibitory effects on anterograde fast axonal transport in isolated squid axoplasm [101]. Impaired axonal transport |

| (Continued) |
| Stressors                  | Category                  | Molecular Target                  | Effect                                                                 | Empirical Support                                                                                                         | Involved KEs | Involved AOPs                          | Link to tau-driven AOP                      |
|---------------------------|---------------------------|-----------------------------------|------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|--------------|----------------------------------------|---------------------------------------------|
| 2,3,7,8-tetrachlordibenzo-p-dioxine (TCDD) | Dioxin-like compounds    | LC3-II expression                 | Oxidative stress induced increased in numbers of autophagic vacuoles, autophagy, and apoptosis | In human neuronal cell line SHSY5Y, expression of LC3II was significantly increased in cells exposed to 200 nM TCDD, compared with control cells [102]. | TDA15A15     | AOP145: Sustained AHR Activation leading to Rodent Liver Tumours, AOP130: Aryl hydrocarbon receptor activation leading to early life stage mortality, via reduced VEGF, AOP131: Aryl hydrocarbon receptor activation leading to early life stage mortality, via increased COX-2, AOP132: Aryl hydrocarbon receptor activation leading to uroporphyria | Oxidative stress, Dysfunctional autophagy |
|                           |                           | AHR                               | Potent aryl hydrocarbon receptor (AHR) ligands                        | TCDD toxicity in human SHSY5Y neuroblastoma cells depended on dioxin concentration and time of incubation, with a main role of aryl hydrocarbon receptor at low nanomolar TCDD concentrations (3 nM at 24h). Induced apoptosis by the disruption of calcium homeostasis, affecting membrane structural integrity [103]. | MIE: Event 165 Activation, Long term AHR receptor driven direct and indirect gene expression changes, Event: 18 Activation, AhR | AOP145       | AOP145: Oxidative Stress Leading to Hypertension | Oxidative stress                           |
|                           |                           | GSK3β                             | tau hyper phosphorylation                                            | Acute exposure to TCDD (25 μg/kg body weight) induced neuronal toxicity in the cortex of female Sprague-Dawley rats. Exposure of rat PC12 cells to TCDD induced activation of GSK3β and decreased β-catenin in neural cells [104]. | Tau hyperphosphorylation | AOP145       | AOP145: Oxidative Stress Leading to Hypertension | Oxidative stress                           |
|                           |                           | LC3-II & Beclin expression        | Oxidative stress induced increased in numbers of autophagic vacuoles, autophagy, and apoptosis | Low concentrations of TOCP induced autophagy and inhibited neurite outgrowth in a dose-dependent manner in human neuroblastoma SH-SY5Y cells. After treatment with TOCP, both LC3-II and the ratio of LC3-II/LC3-I, and autophagy proteins Atg5 and Beclin 1 were increased [105]. | | AOP149       | AOP149: Oxidative Stress Leading to Hypertension | Oxidative stress                           |
|                           | Organophosphorus based compound | LC3-II & Beclin expression        | Oxidative stress induced increased in numbers of autophagic vacuoles, autophagy, and apoptosis | Low concentrations of TOCP induced autophagy and inhibited neurite outgrowth in a dose-dependent manner in human neuroblastoma SH-SY5Y cells. After treatment with TOCP, both LC3-II and the ratio of LC3-II/LC3-I, and autophagy proteins Atg5 and Beclin 1 were increased [105]. | | AOP149       | AOP149: Oxidative Stress Leading to Hypertension | Oxidative stress                           |
|                           | Ultra-Fine Particles (UFP) | SH containing proteins            | Oxidation of intracellular GSH, resulting in the formation of GSGG which alters the redox state of the cell | Exposure to 50 μg/mL UFP for 6 hours led to a decrease in GSH levels (from 17.1 ± 1.8 μM to 12.0 ± 2.4 μM) and an increase in GSSG (from 0.62 ± 0.26 μM to 1.60 ± 0.2 μM) in human aortic endothelial cells [106]. | | AOP149       | AOP149: Oxidative Stress Leading to Hypertension | Oxidative stress                           |
| Viruses | Infectious pathogens | BACE-1 and nicastrin | Effect | Empirical Support |
|---------|----------------------|----------------------|--------|------------------|
| HSV-1/2/3, HIV | Infectious pathogens | BACE-1 and nicastrin | A large proportion of Aβ plaques contain viral or bacterial DNA. Infections activate the amyloidogenic pathway of APP processing while inhibiting Aβ degradation, leading to intracellular Aβ accumulation [107]. | 1. HSV-1 infection of human and rat neuronal cultures activated amyloidogenic pathway of APP processing while inhibiting Aβ degradation, leading to intracellular Aβ accumulation [107]. |
| | | | | 2. Cell culture experiments revealed that intracellular concentrations of Aβ were significantly increased as early as 24-h post-infection and that infected cells increased their expression of both BACE-1 and nicastrin, a component of γ-secretase [108]. |
| | | | | 3. Antiviral treatments greatly reduced Aβ accumulation in HSV-1 infected cells. Treatment with the antiviral agent Acyclovir reduced the intensity of intracellular Aβ staining to 28% of that in untreated infected cells, while also reducing the levels of BACE-1 and nicastrin [109]. |
| | | | | 4. In cell culture aggregates from human AD brains, treatment with the HIV factor Tat led to increased concentrations of soluble A and a reduced activity of the Aβ-degrading enzyme neprilysin [110]. |

| Voriconazole | Antifungal drug | CYP46A1 | Decreased 24S-OHC levels, reduction of HMG-CoA reductase levels, downregulation of cholesterol synthesis and hypocholesterolemia |
|--------------|-----------------|---------|------------------------------------------------------------------|
| Intraperitoneal injections of voriconazole in mice reduced the levels of 24S-hydroxycholesterol in the brain, inhibiting the CYP46A1 [111]. |

| Zink (Zn) | Metals | Binding to Aβ oligomers, Aβ1-40-Zn2+ and Aβ1-42-Zn2+ | Neurotoxic soluble Aβ oligomers, affecting the calcium ion channel activity in synapsis, through disrupting nerve signal transmission and damage mitochondrial causes to increase free radical lead to cell death. |
|-----------|--------|------------------------------------------------------|------------------------------------------------------------------|
| | | | Zn\textsuperscript{2+} binding decreased the solvation energy (increase hydrophobicity) of Aβ oligomer, which enhanced the aggregation propensity, and that a higher concentration of Zn\textsuperscript{2+} could reduce aggregation kinetics. Aβ peptide can reduce Cu\textsuperscript{2+} to Cu\textsuperscript{+}, and Fe\textsuperscript{3+} to Fe\textsuperscript{2+}, facilitating the generation of reactive oxygen species H\textsubscript{2}O\textsubscript{2} and OH\textsuperscript{-} radical [112]. |
been also linked to glucose dysmetabolism via mitochondrial dysfunction. Other cases, in the AOP48, its MIE, binding of agonist, ionotropic glutamate receptors, such as NMDARs, and in the AOP10, its MIE, binding at picrotoxin site, iGABAR chloride channel, have been also linked to synaptic dysfunction and hyperphosphorylation of tau, respectively. These connections among events of different AOPs may support the usefulness of these networks for filling the gaps in understanding how same stressors-MIE interactions can lead to different adverse outcomes or different stressors-MIE interactions can lead to same adverse outcomes.

Despite the fact that some of the selected AOPs in this proposed AOP network have not approved by OECD yet, they can still provide useful information relevant to the adverse outcome of concern, memory loss. The available data for these AOPs still under development may need reassessment for confirming their potential involvement in the tau-driven AOP after endorsement by OECD.

Moreover, this presented network of AOPs for memory loss is consisting of individual AOPs which have earlier been linked to memory or cognitive related disorders, based on existing knowledge. These AOPs are available in AOP-wiki, providing all relevant information and allowing scientists to comment or update them. Of course, the developed AOPs are endorsed by OECD requiring extensive review of the provided data by experts.

It is worth noticing that human studies were prioritized for the empirical support used for weighing the links between the presented stressors and the potential MIEs, in this attempt to create this proposed AOP network. However, most of the available studies for the exposures of neurotoxicants have been performed on animals. This limitation may lead to potential weaknesses of our AOP network to adverse outcomes such as memory loss in humans. More human data are needed to further support the evidence for implicating these neurotoxicants or plausible MIEs in memory loss in humans.

CONCLUSIONS

The application of AOP network, using existing data, can serve a useful tool for a better understanding of the complexity of biological systems and for predicting the adverse effects. A proposed AOP network for the tau-driven AOP may help to contribute into unravelling of the interactions among existing mechanistic data linked to memory loss as an early phase of sAD pathology.
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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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