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Pesticides, cognitive functions and dementia: A review

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ABSTRACT

Pesticides are widely-used chemicals commonly applied in agriculture for the protection of crops from pests. Depending on the class of pesticides, the specific substances may have a specific set of adverse effects on humans, especially in cases of acute poisoning. In past years, evidence regarding sequelae of chronic, low-level exposure has been accumulating. Cognitive impairment and dementia heavily affect a person's quality of life and scientific data has been hinting towards an association between them and antecedent chronic pesticide exposure. Here, we reviewed animal and human studies exploring the association between pesticide exposure, cognition and dementia. Additionally, we present potential mechanisms through which pesticides may act neurotoxically and lead to neurodegeneration. Study designs rarely presented homogeneity and the estimation of the exposure to pesticides has been most frequently performed without measuring the synergic effects and the possible interactions between the toxicants within mixtures, and also overlooking low exposures to environmental toxicants. It is possible that a Real-Life Risk Simulation approach would represent a robust alternative for future studies, so that the safe exposure limits and the net risk that pesticides confer to impaired cognitive function can be examined. Previous studies that evaluated the effect of low dose chronic exposure to mixtures of pesticides and other chemicals intending to simulate real life exposure scenarios showed that hermetic neurobehavioral effects can appear after mixture exposure at doses considered safe for individual compounds and these effects can be exacerbated by a coexistence with specific conditions such as vitamin deficiency. However, there is an overall indication, derived from both epidemiologic and laboratory evidence, supporting an association between exposure to neurotoxic pesticides and cognitive dysfunction, dementia and Alzheimer's disease.

1. Introduction

Pesticides are a broad category of various, widely-used chemicals, mainly applied in agriculture to protect the crops from pests; the nature of pests ranges from insects and birds, to fungi and microorganisms (Zaganas et al., 2013). Depending on the desired target and mechanism of action, there are several classes of pesticides, the most representative being organophosphates (OPs), organochlorines (OCs), carbamates, pyrethroids and neonicotinoids (Costa et al., 2008). Though these compounds do not exert a specific toxicity, they may result in human

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poisoning.

More specifically, OPs and carbamates both inhibit the enzyme acetylcholinesterase (AChE), leading to the accumulation of acetylcholine (ACh) in the synapses. Although their acute toxicity symptoms are similar, poisoning caused by carbamates is usually quickly reversible, as opposed to OPs. Furthermore, OPs have been linked to more chronic adverse effects compared to carbamates, such as organophosphate-induced delayed polyneuropathy (OPIDP), neuropsychological sequelae and developmental neurotoxicity (Dardiotis et al., 2019b; Roldan-Tapia et al., 2005; Ruszkiewicz et al., 2019). Pyrethroids slow down the activation and inactivation of the sodium channels, leading to hyperexcitability, and only a few human poisonings have been reported with these substances (Ray and Fry, 2006). OCs, nowadays banned in most countries worldwide, have diverse sites of action, such as the sodium channels of the axonal membrane or the chloride channels, antagonizing γ-aminobuturic acid (GABA) inhibition. Pyrethroids και OCs have been reported to have acute and chronic side-effects, the latter mostly affecting the function of the liver and the organs of the reproductive system (Costa et al., 2008). Neonicotinoids, on the other hand, exert their action and their toxicity via the activation of nicotinic receptors; these newer compounds are relatively safe, since they are specialized for insect rather than mammalian receptors (Costa et al., 2008). Additionally, pesticides, besides these adverse effects, have been reported to be associated with carcinogenesis (Falzone et al., 2016; Rapisarda et al., 2017), with numerous studies tying them to various forms of cancers (Brody et al., 2004; Brown et al., 1990; Hardell and Eriksson, 1999).

Highly water-soluble pesticides, such as 2,4-D, a known herbicide, are less persistent in the environment and most likely to biodegrade quickly and not end up bioconcentrated in organisms. Lipid-soluble pesticides such as DDT and DDE, a member of the OC group, are especially prone to bioaccumulation (Katagi, 2010). Although very little is known about pesticide detoxification in the central nervous system (CNS) and detoxifying systems are highly polymorph, leading to great inter-individual variability in susceptibility to the neurotoxic effects of insecticides, chronic pesticide effects during the period of cerebral development are also an issue of particular interest (Cassereau et al., 2017).

Pesticide molecules, small and lipophilic in nature, can enter from blood to brain and then in neurons, glial cells and brain microvessels (Pulgar, 2018). Pesticides target and disrupt blood- brain barrier receptors in the CNS (Moura et al., 2019), enhancing pesticidal chronic toxicity and affecting the physiological process of receptor-mediated transcytosis, as well as blocking pharmaceutical transport and delivery through the brain endothelial cells towards the parenchyma, thus leading to drug ineffectiveness and other adverse effects (Moura et al., 2019).

Accumulating epidemiologic evidence over the past decades suggests that repeated exposure to low levels of pesticides negatively affects the CNS in various ways (Stephens et al., 1995), while agrichemical exposure has been identified as a risk factor for a plethora of adverse CNS effects (London et al., 1997). Repeated or even single acute episodes have been associated with long-term pesticide exposure, but also low-level chronic exposure has been reported to be more strongly implicated in acute episodes, though relevant studies have not been consistent (Baldi et al., 2011; Jamal et al., 2002; Kamel et al., 2003). Additionally, pesticides have been linked to a series of neurological diseases, such as amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD), with the latter presenting the strongest association (Dardiotis et al., 2013; Freire and Koifman, 2012; Kamel et al., 2012). Pesticide exposure has been eventually associated with cognitive impairment and dementia, including that due to Alzheimer's disease (AD) (Dardiotis et al., 2019c).

Taking into account the burden associated with such disease, particularly but not only in the Western countries and their aging populations, and the associated costs in terms of both economic loss and low quality of life, identifying the factors that pertain to these disorders and that could halt their progression if modified, is crucial; regarding the matter in hand, highlighting pesticide safety issues for individuals. Pesticides, due to their wide-spread use, affect the majority of people and if they are really implicated in neurodegenerative processes such as dementia, their restriction could substantially contribute in reducing the incidence of these diseases. Several studies on animals and human subjects have been conducted in order to pinpoint possible links between pesticides and cognition/dementia and AD, yet, to our knowledge, no consensus in the scientific community on the matter exists, and recent reviews on this issue are lacking. Therefore, in this article, we have attempted to identify the mechanisms underpinning the toxicity relevant to cognitive impairment, AD and dementia separately for each substance and for pesticides in general. Moreover, we aimed to present the results of studies on pesticides and dementia/cognitive impairment, to provide further insight in the way they interact.

2. Possible mechanisms of pesticide-associated cognitive impairment

The multifactorial nature of pesticide-related cognitive impairment is consistent with experimental results showing distinct roles for pesticide exposure, years of exposure and age of exposed individuals. The extent to which each of the aforementioned factors contributes to cognitive deficits remains under-explored. Several theories have been suggested and the most dominant one hybridizes the various approaches and offers a combined interpretation under the suggested theoretical models. The main culprit, incriminated by the overwhelming majority of relevant studies, could be the oxidative stress induced by the substances; neuronal cells are more susceptible to oxidative stress due to their high polyunsaturated fat content in the myelin sheaths, low anti-oxidative capabilities, enzymatic systems with transient metals that aid in the production of free radicals, and high oxygen demand and glucose metabolism rate (Chen et al., 2015; Mostafalou et al., 2012). Studies have also investigated the effect pesticides have on the pathological hallmarks of Alzheimer's disease (AD)- the archetype of cognitive impairment in humans- such as the Aβ amyloid plaques and the Tau protein dysfunction (Chen et al., 2015; Palop and Mucke, 2010), with some studies also describing the implication of epigenetic modifications (Chen et al., 2015). As it is already well-known, pathologically, amyloid plaques and neurofibrillary tangles are the main forms of aggregated proteins involved in AD (Nousia et al., 2019, 2018). Amyloid plaques are visible protein aggregates derived from the dimers and oligomers of brain amyloid-β protein, while neurofibrillary tangles are composed of a compact filamentous network of helical filaments of hyperphosphorylated tau protein. Together these neuropathological changes are thought to result in the loss of synapses and neuronal cell death, leading in cognitive dysfunction (https://pathways.nice.org.uk/pathways/dementia).

Paraquat (PQ), one of the most commonly used herbicides, has been the focus of numerous studies. Its main mechanism of action is the induction of oxidative stress and mitochondrial damage (Baltazar et al., 2014; Drechsel and Patel, 2008), as it can enter the organelles and use electrons from the electron transport chain to generate reactive oxygen species (ROS) (Cocheme and Murphy, 2008) and enhance H2O2 production (Castello et al., 2007). Chen et al. (2015) revealed that PQ-exposed mice, regardless of their Amyloid Precursor Protein (APP) gene status, had lower complex I and IV activities, and that genetically modified rats with triple peroxiredoxin 3 (PRDX3, anti-oxidative mitochondrial enzyme, central to H202 removal) activity were protected against oxidative stress (Chen et al., 2015; Chen, 2012). In another study conducted by the same researchers, PQ-exposed mice performed more poorly than controls in cognitive tests, while the markers of mitochondrial damage correlated directly with the impairment in associative learning and memory skills; again, PRDX3 overexpression attenuated differences in performance (Chen et al., 2015; Chen, 2012). PQ

also led to an increase in Aβ amyloid aggregation in APP carrier mice, a phenomenon linked to oxidative stress, since PRDX3-overexpressing APP subjects showed suppressed Aβ amyloidogenesis (Chen, 2012). Additionally, the involvement of the NLPR3 inflammasome, a key component in inflammation commencement in response to ROS in neuronal damage (Kepp et al., 2011), was evinced by the elevation of IL-1b and caspase-1 in PQ-exposed mice regardless of their APP status (Chen et al., 2015). Similarly, PRDX3-overexpressing mice showed decreased levels of these markers, further validating the role of H2O2 in CNS inflammation (Chen et al., 2015).

PQ has also been reported to affect the glutaminergic system in PD. It has been shown to enhance glutamate release from non-NMDA receptors, leading to a raised influx of calcium and the stimulation of nitrogen oxide species, which can in turn induce mitochondrial damage alongside other ROS (Shimizu et al., 2003). Autophagy also seems to play a part, since it degrades oxidated-by-ROS proteins and its inhibition has been shown to accelerate cell death after exposure to PQ (Gonzalez-Polo et al., 2007); it was also noted in neurons of AD patients (Nixon et al., 2000). Finally, PQ exposure has been linked to increased p-Tau via the activation of a phosphorylating kinase, and tubulin alterations, hinting towards cytoskeleton remodeling (Wills et al., 2012).

OPs constitute a class of pesticides encountered worldwide, with widespread use, and therefore, exposure. Though their main mechanism of action involves the inhibition of AChE and the consequent toxic accumulation of Ach in the synapses (Dardiotis et al., 2019b), current evidence suggests that non-cholinergic systems are implicated in the neuropsychological and chronic manifestations of OP exposure, such as the Gulf War Illness (Androutsopoulos et al., 2013; Jokanovic, 2009; Vucinic et al., 2017). Kaur et al. (2007) described deficits in mitochondrial complex I, II and IV activities after chronically exposing rats to dichlorvos; the researchers pinpointed oxidative stress as the culprit, after finding lower glutathione (GSH) and lower superoxide dismutase (SOD) activity levels (Kaur et al., 2007). Abou-Donia (2003), describing the entity of OP-ester induced chronic neurotoxicity (OPICN), which involves many neurobehavioral symptoms such as attention and memory deficits, included oxidative stress in its main mechanisms, via the critical depletion of mitochondrial energy, the activation of proteolytic enzymes, and DNA fragmentation, all giving way to apoptosis (Abou-Donia, 2003). Consequently, OPs considerably disrupt mitochondrial function and lead to oxidative stress in the CNS (Middlemore-Risher et al., 2011).

Flaskos et al. (2011) have extensively studied the effects of OPs, chlorpyrifos (CP) in particular, in typical neuronal development, and their studies have shown that AChE inhibition is not involved in the noted disruptions; other pathways involve the deregulation of transcription factors central to neurite growth and of enzymatic biomarkers of CNS-cell differentiation (Flaskos, 2012; Flaskos et al., 2011). CP also seems to affect typical development via alterations in structural proteins and glial cell proliferation (Garcia et al., 2002, 2005). Concerning Aβ amyloidogenesis, the in vivo studies that have focused on CP have so far yielded contradictory results. In an animal model, the amyloid levels were raised in the hippocampus and the cortex of exposed mice, while this change has been accompanied by memory deficits (Salazar et al., 2011); nevertheless, Peris-Sampedro's (2014) similar two-phase study failed to replicate the same findings (Peris-Sampedro et al., 2014).

Other mechanisms, such as the OP-triggered induction of a xanthine oxidase, may also play a role (Gultekin et al., 2000) in cognitive impairment, and OPs' versatile activity does not seem to stop at enzymes, but rather extends to the genetic level as well. OPs affect DNA methylation in several hundred genes, and this has been demonstrated to occur in vivo in both human and animal models alike (Hodjat et al., 2017; Xing et al., 2014; Zhang et al., 2012a, b). Abou-Donia (2003) in their OPICN review, reported that OPs also seem to induce the AChE gene expression, enhancing apoptosis, and implicated the glutamatergic system as well, with the activation of NMDA glutamate receptors and the subsequent calcium influx in the post-synaptic neurons, that leads to degeneration (Abou-Donia, 2003). As Terry (2012) concluded, the OP delayed toxicity may also involve axonal disruption and neurotrophins (Terry, 2012) (see (Reddy et al., 2012; Slotkin, 2006) for similar findings in AD).

Finally, the aforementioned findings cannot undermine the importance of OPs' main mode of action; AChE inhibition. It has been widely acknowledged that acetylcholine excitoxicity, through repeated stimulation, quickly consumes the available cellular ATP since it requires more energy (Dettbarn et al., 2006). This action may also constitute another route through which OP exposure results in mitochondrial dysfunction, since prolonged depletion of ATP has been shown to reduce mitochondrial membrane potentials, which in turn leads to electron transfer enzymatic complexes' dysfunction and the formation of ROS (Carlson and Ehrich, 1999; Milatovic et al., 2006; Mostafalou et al., 2012).

Additional pesticide classes seem to lead to cognitive impairment through the same mechanisms activated by PQ and OPs. Maneb has been shown to obstruct the glutathione system (Baltazar et al., 2014). OCs, such as DDE and DDT, aggravated Aβ amyloidogenesis in in-vivo models (Li et al., 2015; Richardson et al., 2014) and their lipophilicity has motivated research tying obesity and dementia to OCs (Lee et al., 2016). Amyloidogenesis has also been implicated in triazine- and pyrazole-associated cognitive impairment, as it has been shown to induce the production of Aβ42 over Aβ40, another hallmark of AD, via the potentiation of amyloid secretases (Cam et al., 2018; Portelius et al., 2016). Lastly, animal models involving carbamates and pyrethroids have suggested Tau protein malfunction as a plausible mechanism underlying cognitive impairment (Chen et al., 2015).

Moving on, various pesticides bind to growth factors (IGF1, TGF-α, EGF, EDGF and VEGF), chemokines, hormones, transmitters [e.g. G protein-coupled receptors (GPCRs), involving fast-responding GABA receptors (Tanaka, 2019), muscarinic or nicotinic acetylcholine receptors, glycine and 5-HT3], and integrin, and thus play a key role in the activation of a secondary downstream intracellular messenger system. The activation of this secondary signaling through PLC, IP3/DAG, PKC (Yamasaki et al., 2009), adenylatecyclase, cAMP and phosphatidylinositol signal pathway, deregulate gene expressions related, among others, to cellular immune responses (Chaffey, 2003),vascular permeability (Fan et al., 2018), β-amyloid precursor protein expression, neuronal death, synaptic dysfunction, and memory and learning, the deficits of which are pathological findings in AD (Del Prete et al., 2014).

Synthetic pesticides, such as carbamates, are inducers of nuclear factor-kappa B (NF-κB) –a transcription factor that induces a plethora of genes, some of which involved in the process of inflammation- activity through ROS (Bowie and O'Neill, 2000; Schieven et al., 1993), lipoxygenases, TNFα and IL-1β (Westbrook et al., 2012). Pesticide-induced NF-kB activation (Gutierrez et al., 2005) alongside growth factors such the brain-derived neurotrophic factor (BDNF) (Zaheer et al., 2001), the Wnt/β-catenin signaling (Kobayashi et al., 2015), IL-1β (Chung et al., 2017), TNFα (Reid et al., 2009) and TNFR (Qiu et al., 2004) cytokines, PKAc (Kaltschmidt et al., 2006) or alteration of testosterone (Hofbauer et al., 2002), estrogen (Michael et al., 2005) and aromatase levels (Hofbauer et al., 2002), transcriptionally (Martín Millán, 2015) and post-transcriptionally (Jia et al., 2017) affect gene expressions (Santoro, 2016), but are also implicated in processes of synaptic plasticity (Boersma et al., 2011) and memory (Meffert et al., 2003).

Fungicides that act as AChR agonists, such as fludioxonil, fenhexamid, mepanipyrim, cyprodinil, pyrimethanil, and chlorpyrifos-methyl (OP and pyrethroid combination), directly and indirectly, via ER, AR and multiple other receptor pathways, transcriptionally remodel gene expression, resulting in the deregulation of neural differentiation, observed and in birth defects (Medjakovic et al., 2014). Furthermore, pesticide ligands of NRs and AChR disrupt AHR mRNA/HES1 oscillations and the MASH-1/ Notch signaling pathway (Akahoshi et al., 2006). Organotin, for example, activates RXR-PPAR heterodimers that exert their mutagenic and carcinogenic capabilities (Prival et al., 1977),

and deregulate the behavioral and circadian rhythms (Ema et al., 1991), resulting in circadian clock disruption, mood and CNS disorders (Logan and McClung, 2019), and global prevalence of obesity by both HFD-induced disruption of metabolic rhythms (Eckel-Mahan et al., 2012) and ER/FFAs/PPARγ signaling activation (Liu et al., 2013), which suppresses the Per1 gene expression (Killilea et al., 2017; Kawai et al., 2010; Kawai et al., 2010; Killilea et al., 2017).

Preactivation of the suprachiasmatic nucleus (SCN) by AChR agonist-pesticides, such as CP, carbendazim and thiabendazole, blocks the glutamate-induced phase resetting of the SCN electrical activity rhythm (Mukai and Tischkau, 2007), suggesting that chronic pesticidal activation directly within the SCN impacts the ability of the clock to respond to phase-resetting stimuli e.g. the daily rhythm of thyroid hormone secretion that is governed by SCN-signalling via the rhythmic TSH secretion (Fahrenkrug et al., 2017). Similarly, AChR-binding pesticides change the behavioral and electrical activity of circadian clock genes Per1 and Bmal1 in the liver and the SCN (Amakura et al., 2016), attenuating light-induced induction of Per1 in the SCN (Takeuchi et al., 2008), providing an essential mechanism for pesticidal AChR activation on light-induced changes by daily photic cues transmitted from the retina (Barakat et al., 2005) via the retinohypothalamic tract in behavioral rhythmicity (Popovska-Gorevski et al., 2017).

At this point, we would like to also bring attention to another factor of pesticide toxicity; their absorption. A recent study showed that a high percentage of the pesticides studied (81.4 %) exhibited high intestinal absorption and a 38.5 % displayed brain permeation (Chedik et al., 2017). Since many neurodegenerative disorders seem to be induced by insecticides and herbicides, most notably OPs, OCs, phenoxyacetic acids and triazine compounds (Mostafalou and Abdollahi, 2017), the knowledge of their gut absorption and brain permeation kinetics and dynamics is necessary for predicting chronic and direct toxicity in the neurovascular unit. Infants and young children are particularly sensitive to these contaminants, as their brains and organ systems are not fully developed and the field of neurodevelopmental pesticide toxicity is gaining more ground in the scientific community. For this reason, it is important for future researchers to be aware of the mechanisms of chronic pesticide neurotoxicity, which we have summarized in Fig. 1, and the various other biological mechanisms which exceed the purposes of this manuscript, but have been presented in Fig. 2.

In conclusion, the pathways through which pesticides cause cognitive impairment exhibit great heterogeneity and no single mechanism has been identified. Oxidative stress and mitochondrial dysfunction seem to play the dominant role, with several other modes of actions, such as epigenetic alterations and amyloid production elevation, contributing considerably to cognitive impairment as well.

3. Animal studies

Ever since suspicions on the effects pesticides seem to have on cognition were raised, several research groups have attempted to conduct experiments in order to investigate this association and elucidate the mechanisms that underlie it. Experiments on rats remain the most popular, with a wide variety being conducted mainly via the administration of pesticides on subjects and the subsequent assessment of their performance on tasks designed to test specific aspects of the subjects' cognitive functioning, (e.g. attention span or learning ability), such as mazes and hidden target platforms (Abdullah et al., 2011; Levin et al., 2010; Phillips and Deshpande, 2016; Terry et al., 2003). Here, we have included 15 animal studies, that we believe have provided important results, and connect them with findings from other studies and reviews as well.

More specifically, Levin, Slotkin et al. have presented a series of studies on rats following pesticide exposure, mainly OPs. The studies have exhibited cognitive impairment to be closely linked to deficient Ach function in the synapses (Levin et al., 2001, 2010; Slotkin, 2004, 2006), even at concentration levels that do not lead to diffuse cholinesterase inhibition (Slotkin, 2004, 2006). Visuospatial memory performance has also been shown to be highly dependent on ACh function; also, in their experiments, they have measured several indices tied to ACh and serotonin function, known to be affected by developmental exposure to OPs, such as 5HT1A/2R, activity of choline acetyltransferase (ChAT), and the concentration of α4β2 nicotinic ACh receptors (nAChRs) (Levin et al., 2010; Yatham et al., 1999, 2000). The same researchers have located deficits in ACh presynaptic activity, accompanied by a rise in compensatory mechanism indices, in regions where ACh mediates several important behaviors such as reward, similar to what has been found in AD (Bissette et al., 1996; Levin et al., 2010; Siokas et al., 2020; Slotkin et al., 1994; Stamati et al., 2019; The Canadian Study of Health and Aging, 1994). Testing a non-toxic prenatal exposure to parathion, they described a long-lasting effect on

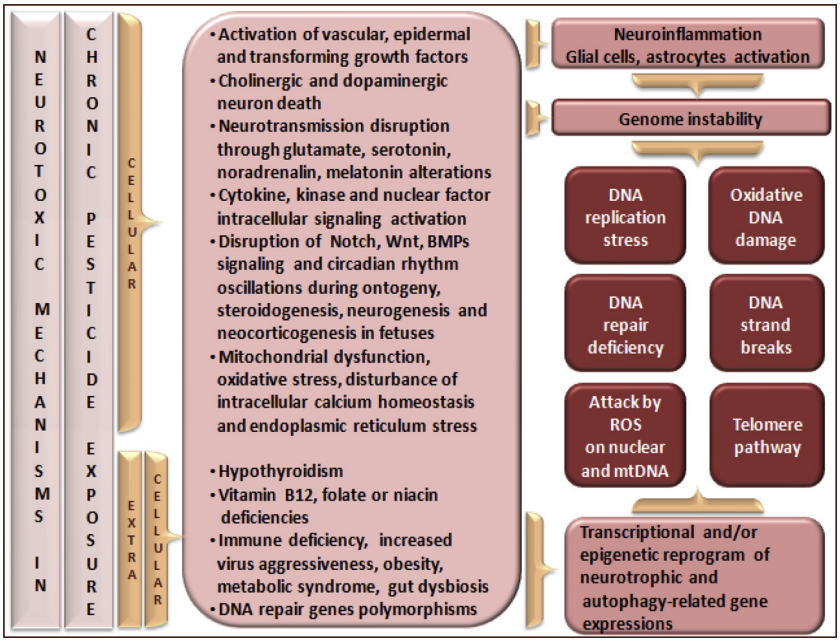

Fig. 1. Cellular mechanisms of dementia and cognition decline due to long - term pesticide exposures.

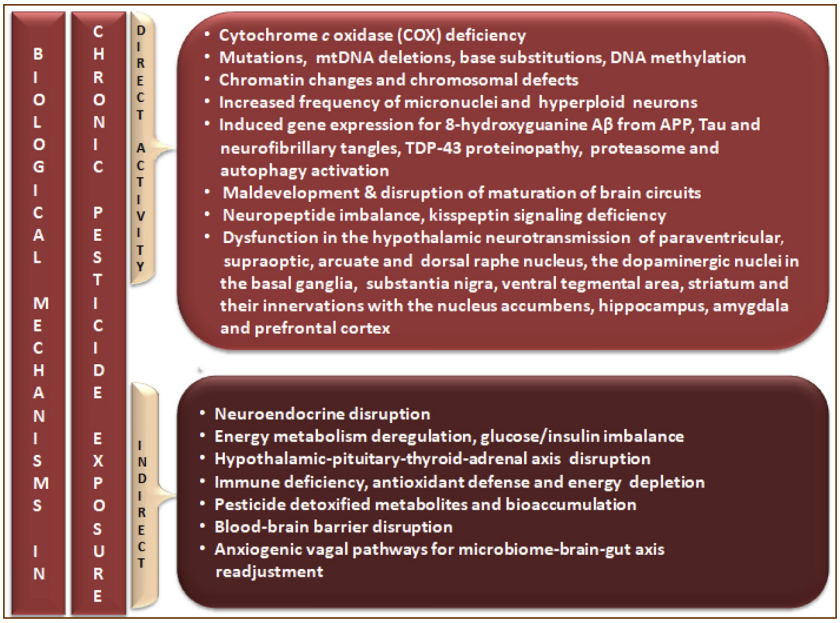

Fig. 2. Direct and indirect long-term pesticide activities during dementia.

several brain regions of relatively young rats, with the pathological findings being akin to those found in subjects of deep senescence. Crucially, parathion effects were not found to be subject to sex differences; exposed male rats did not present better performance scores than female rats in visuospatial memory tasks, as expected based on their sex (Levin et al., 2001, 2010). A possible explanation could be the faster recovery and plasticity that female brains present when exposed to these substances (Levin et al., 2010). In an another relevant study, visuospatial impairment was also noted in malathion-exposed test subjects, at concentrations that did not inhibit AChE, thus implying that additional mechanisms are implicated as well (dos Santos et al., 2016).

Some studies have also highlighted the role of oxidative stress in the reported cognitive deficits (Gubandru et al., 2013; Sayapina et al., 2017). Jain et al. (2013) found that triazophos-exposed rats presented learning and memory deficits, and had much lower levels of BDNF and GSH in their hippocampi (Jain et al., 2013). Deficits in mitochondrial complex activity were further noted in chlorpyrifos (CP)-exposed rats' hippocampus neurons, whose activity was positively correlated to performance in object location tasks, suggesting that damage in the respiratory chain of these neurons was the primary cause of the cognitive impairment (dos Santos et al., 2016). In Akande et al.'s (2014) study, taurine, a known anti-oxidant, was found to counteract the oxidative processes caused by the exposure to CP and lead acetate in exposed rats (Akande et al., 2014); CP has been shown to compromise both learning and memory skills in exposed tested subjects (Ambali and Aliyu, 2012). It has also been shown that BDNF is involved in memory and learning skills located in the hippocampus (Alonso et al., 2005; Linnarsson et al., 1997; Numakawa et al., 2011; Siokas et al., 2019), therefore its depletion could reflect cognitive deficits, alongside oxidative stress. Chen et al. studied PQ exposure and found poorer performance in associative learning and memory tests in exposed mice, in direct correlation to mitochondrial damage, while mice over-expressing an anti-oxidating enzyme were found to be relatively protected (Chen et al., 2015; Chen, 2012).

Finally, in a similar vein, several animal models have attempted to replicate the Gulf War Illness, which is now considered to be caused by exposure to OP nerve gas Sarin, with substances such as diisopropylfluorophosphate and permethrin, and further explore its effects (Abdullah et al., 2011; Hattiangady et al., 2014; Phillips and Deshpande, 2016). These studies showed that the exposed mice presented disturbances in circadian rhythms, psychomotor skills, endocrine and immune system proteins, as well as anxiety-like or depression-like behaviors, all commonly reported by Gulf War veterans (Abdel-Rahman et al., 2004; Abdullah et al., 2011; Hattiangady et al., 2014; Phillips and Deshpande, 2016). Abdullah et al. (2011) reported a delayed cognitive impairment in exposed mice, in learning and memory skills, and attributed it to compensatory mechanisms that lower synaptic ACh levels in response to the chronic elevation of the substance after exposure to chemicals such as pyridostigmine bromide (Abdullah et al., 2011). The researchers also reported astrogliosis simultaneous to the cognitive impairment manifestation, which aligns with the findings of other studies (Abdel-Rahman et al., 2004; dos Santos et al., 2016), thus providing converging evidence on an underlying inflammatory/immune-mediated etiology of the cognitive impairment (Abdullah et al., 2011). Phillips and Deshpande (2016) and Hattiangady et al. (2014) reported depression and deficits in motivation and spatial memory, and attributed them to hippocampal lesions revealed by histological analyses, a finding that was also captured in studies involving Gulf War veterans (Hattiangady et al., 2014; Phillips and Deshpande, 2016). In line with previous studies' hypotheses, the activation of the glutaminergic system, as a response to AChE inhibition, could explain the damage to the hippocampus neurons via an elevation in calcium levels and the subsequent excitoxicity (Deshpande et al., 2010).

All in all, there is a wide variety of experiments with pesticides based on animal models, mainly involving rodents and organophosphates, yet their results are equally versatile; it seems that several mechanisms are involved in the pathogenesis of cognitive impairment following exposure to these substances, which were extensively described in the preceding sections.

4. Human studies

4.1. Pesticides and cognitive and neuropsychological function

Besides the notion that pesticide exposure may enhance the risk of dementia, it has long been suggested in the scientific community that such an exposure, if chronic, may affect specific cognitive functions even without reaching a diagnosis of dementia. Several studies have examined this potential association, with some paying particular attention to differentiating between chronically-exposed subjects and those suffering from episodes of acute pesticide poisoning, an event that could influence the final outcome (Ames et al., 1995; Kamel et al.,

2003; Mackenzie Ross et al., 2010; Steenland et al., 2000; Stephens and Sreenivasan, 2004). A wide array of tests has been administered by scientific groups to evaluate participants' cognitive functioning. Some studies have applied gold-standard, widely acknowledged tests such as the Mini Mental State Examination (MMSE), while others have used neurobehavioral batteries, such as the World Health Organization's Neurobehavioral Core Test Battery, which includes numerous tasks and questions. Since the parameters and variables examined were numerous and heavily differentiated from task to task and from study to study, we deemed it better not to include the full extent of numerical values and statistics that each study presented. Instead, we concisely describe their results and encourage the readers to turn to the original articles for further details and numerical values. In this section, we have included 30 relevant studies, spanning from 1992 to 2019, with the last search performed in August 2019.

Early studies failed to show a link between pesticides and cognitive impairment. Daniell et al. (1992) compared 49 OP applicators to 40 controls with self-reporting questionnaires and measurements of cholinesterase serum levels, and found no clinically important effects in the neuropsychological performance of the subjects before and after the spraying season (Daniell et al., 1992). In a similar vein, Ames et al. (1995) compared 45 chronically-exposed-to-anticholinesterase-agents subjects to 90 controls, and found no evidence of increased neurological sequelae in the test parameters (Ames et al., 1995). London et al. (1997) performed a cross-sectional study using exposure questionnaires, measurements of cholinesterase levels and a wide array of neurobehavioral tests in 163 OP applicators and 84 non-exposed workers. According to the results, the study revealed weak effects of occupational exposure on the participants' cognitive and behavioral scores. A number of factors have been implicated for the inconsistencies found, or the lack of effects, such as misclassification of exposure, cross-cultural differences and confounders, namely alcohol consumption and history of head trauma (London et al., 1997). After assessing 40 OP-exposed farmers and 40 age-, sex- and education-matched controls, Bayrami et al. (2012) found increased incidence of clinical symptoms such as eczema and nausea, alongside elevated biochemical markers of oxidative stress, but no evidence of impaired cognitive functions in any of the tests performed (Bayrami et al., 2012). Finally, in a prospective longitudinal study, Berent et al. (2014) evaluated 53workers exposed to CP, an important member of the OP group, and 60 referent workers, yet found no evidence of cognitive impairment; surprisingly, Berent et al.'s (2014) study reported higher cognitive function scores for the exposed subjects in specific domains one year after the initial evaluation (Berent et al., 2014). The authors mention higher marriage and education rates in CP workers, (Berent et al., 2014), a factor which could have influenced the results, since, to our understanding and knowledge, a higher education level and a married life which could be in accordance with lower stress levels, both contribute to higher cognition scores. The overall findings hint towards cognitive impairment in exposed individuals as a consequence of the interaction among several factors, including exposure itself.

Additional studies found evidence in favor of neuropsychological effects stemming from pesticide exposure, though such effects were not deemed to be major. Steenland et al. (2000) assessed 191 current and former applicators exposed to CP, along with 189 controls, yet found no big group differences either in clinical performance scores, or in comparisons conducted between formerly and currently exposed workers; worse cognitive performance in specific domains was only reported for 8 subjects that had a history of acute CP poisoning. Exposed subjects in general, did exhibit higher reporting frequency of fatigue, memory deficits, and tension (Steenland et al., 2000). In an observational study by Salvi et al. (2003), 37 farmers exposed to OPs for 3 months were monitored, and no differences in MMSE and word span scores between them and the general populous were noticed; almost half of them, however, had a diagnosis of either generalized anxiety disorder or major depression. Twenty-five of these subjects were re-evaluated after a 3-month period free of OP exposure, and the occurrence of concurrent psychiatric diagnoses had considerably dropped (Salvi et al., 2003).

As can be so far understood, psychological effects stemming from pesticide exposure constitute a frequently reported finding of studies focusing on possible links between cognitive functioning and pesticide exposure. Stephens et al. (1995) compared 146 OP-exposed farmers and 143 non-exposed subjects via the use of occupational questionnaires and a wide array of neuropsychological tests. Besides the exposed group's poorer mental health, the study revealed significant impairment in the group's information processing speed and sustained attention abilities; no effects were reported for short-term memory and learning skills (Stephens et al., 1995). Jamal et al. (2002) performed a case-control study of 79 OP-exposed farmers nested within a larger cross-sectional study of 685 subjects. Participants were administered detailed questionnaires and neuropsychological battery tests. Though there were no signs of memory deficits, the exposed group exhibited slower processing speed compared to the control group. This specific study has primarily focused on the peripheral neuropathology of the subjects, reporting that this subgroup of individuals was also characterized by depression and anxiety with evidence of peripheral nerve damage (Jamal et al., 2002). Slow processing speed for exposed individuals has also been reported by Stephens and Sreenivasan (2004), who applied the sensitive Syntactic Reasoning test, among others, on 37 sprayers chronically exposed to CP, other OPs and carbamates, in the biochemically-proven absence of recent exposure and with no history of acute poisoning, versus 57 non-exposed workers (Stephens and Sreenivasan, 2004). Cole et al. (1997) conducted a cross-sectional study comparing 144 farm-involved rural citizens and 72 age- and education-matched non-farm-involved individuals. The study reported general cognitive deficits for the farm cohort, which were mainly evident in spatial, psychomotor and motor functions; the subgroup of OP and carbamate applicators were also found to perform worse than controls in attention tests (Cole et al., 1997). Lastly, addressing the class of fumigants, Calvert et al.'s(1998) study with 123 fumigation workers and 120 controls showed a connection between high-level exposure to sulfuryl fluoride and lower scores on cognitive and visual memory tests; yet, this specific study failed to show an over-generalized drop of performance in the fumigation worker's cognitive function abilities (Calvert et al., 1998).

Some studies have reported more global cognitive impairments following chronic exposure to pesticides. In a cross-sectional study by van Wendel de Joode et al. (2001), 36 chronically DDT-exposed workers, alongside 31 controls, performed consistently worse in the neurobehavioral tests administered, with a mean performance decrease of about 20 %, which considerably deteriorated as exposure years increased; drop in cognitive performance in the same study was also found to associate positively with the workers'exposure levels. Verbal attention, and visuomotor speed and sequencing were the two domains most heavily influenced, while exposed subjects also reported psychiatric symptoms at a higher frequency (van Wendel de Joode et al., 2001). In another cross-sectional study (Kamel et al., 2003) with 288 farm workers(exposed to unspecified pesticides) and 51 controls, workers showed consistent deficits in cognitive, psychomotor and balance functions, with the deficits being especially stronger for the experimental subjects who had performed farm work one year before testing. Crucially, poorer performance persisted even after the subjects that had suffered from acute poisoning were removed from the sample, which suggests that chronic exposure does indeed affect the nervous system (Kamel et al., 2003). Deficits in numerous neurobehavioral tests were also reported in Farahat et al.'s study (2003) with 52 OP-exposed workers and 50 age- and education-matched controls. Participants were administered questionnaires, neurobehavioral battery tests and serum measurements. According to the findings, there was impairment in visuomotor and verbal speed, visual and auditory memory, and verbal abstraction; subjects also exhibited higher neuroticism scores. Impairment levels were found to positively correlate with the duration of

occupational exposure, while no subject showed biochemical evidence of acute OP poisoning at the time of testing (Farahat et al., 2003). Further evidence in favor of chronic cholinesterase-inhibitor exposure leading to neuropsychological impairment has been provided by Roldan-Tapia et al. (2005), who assessed 40 farm workers and 26 controls with methods similar to those of Farahat et al. (2003). Impairments were reported for perceptive and visuospatial processing, as well as integration time only for those individuals who wereOP exposed for more than 10 years. Though Roldan-Tapia et al. (2005) used no syntactic reasoning tasks,there seemed to be no great group effect in reasoning skills; picture completion and similarities test scores, with educational level as a covariate, showed relative risks of 1.35 (0.042–2.66) and 0.94 (0.26–1.62) respectively (Roldan-Tapia et al., 2005).

Following the same line of reasoning, Rothlein et al. (2006) compared the performance of 96 agricultural, OP-exposed workers to 45 controls. The former group exhibited poorer scores in the majority of the neurobehavioral battery tests, namely, in sustained attention, information processing and motor speed, as well as coordination. Though urinary OP metabolites were associated with lower scores in some of the tests, this specific association was rather deemed to most likely reflect an approximation of the exposure level due to the metabolites' short life and the nature of the tests, rather than a sensitive predictor of cognitive performance (Rothlein et al., 2006). Baldi et al.'s (2011) follow-up *PHYTONER* study with 614 vineyard workers (unspecified pesticides) showed that the workers exhibited low performance in the neurobehavioral tasks testing processing speed, attention and strategic thinking, with ORs spanning from 1.35 to 5.60; cognitive performance was associated with pesticide exposure, with direct exposure only elevating the risk by some decimals compared to the indirect exposure condition (Baldi et al., 2011). Effects of direct vs. indirect exposure (mostly to cholinesterase inhibitors) were also investigated by Corral et al.'s (2017) study with 32 directly-exposed, 32 indirectly-exposed workers, and 38 controls. The directly-exposed group performed worse in executive functions, verbal fluency, visual and auditory memory tests than the indirectly-exposed workers, who in turn did worse than the control group. Both exposed groups showed higher decline rates than controls, a finding which suggests that low-level chronic exposure, either direct or indirect, does influence cognitive functioning (Corral et al., 2017). Finally, Jamal et al. (2016) compared 187 OP-exposed sprayers to 187 controls, and found that the former group exhibited considerably worse performance inpsychomotor speed, selective attention, divided attention, verbal, non-verbal, and prospective memory, spatial functioning, and initiative, alongside higher rates of anxiety and depression. The study also addressed the fact that the sprayers were negligent to protection measures, such as face masks, gloves and showers, although these were available (Jamal et al., 2016).

The nature of occupation has also been found to play an important role in exposure and, par consequence, to the selection of experimental subjects in relevant research. A particular group often tested in pesticide-cognition studies comprises of sheep dippers and farmers, whose profession entails chronic exposure to low levels of OPs. Stephens et al. (1995), as mentioned before, found information-processing speed and sustained attention deficits, besides poorer overall mental health, in 146 dippers (vs. controls) (Stephens et al., 1995). Similarly, Jamal et al. (2002) reported slower processing speed for 79 sheep farmers (Jamal et al., 2002). Mackenzie Ross et al.'s (2010) study was the first to include subjects that retired on the grounds of ill-health, also assessing the PON1 genotype, since mutations are thought to confer susceptibility to OP poisoning (Androutsopoulos et al., 2011; Dardiotis et al., 2019b, 2018; Mackenzie Ross et al., 2010), and excluding farmers with a history of acute poisoning or other conditions that might have accounted for their reported poor health. The cohort of 128 sheep farmers in Mackenzie Ross et al.'s (2010) study exhibited greater impairments in memory, response speed, fine motor control, mental flexibility and strategy making, alongside higher rates of anxiety and depression, compared to the 78 unexposed controls, though verbal and visuospatial abilities remained largely intact. Some parameters were also found to correlate positively with the duration of exposure (Mackenzie Ross et al., 2010).

The observation of an exponential increase in dementia and cognitive decline prevalence with age has motivated the inclusion of older people in studies focusing on links between exposure and cognitive impairment. Within the context of the *Maastricht Aging Study*, Bosma et al. (2000) have examined 830 non-demented 50-to-80-year-old individuals and reported that exposed (to unspecified pesticides) individuals had a five-fold risk of mild cognitive dysfunction as compared to the non-exposed group (Bosma et al., 2000). Steenland et al. (2013) assessed 400 elderly residents of government-run clinics. Those who had been occupationally exposed to unspecified pesticides in the past performed worse on dementia screening tests, while and a trend of performance decrease was observed for individuals reporting more years of exposure (Steenland et al., 2013). Kim et al. (2015) studied 644 elderly participants of the *National Health and Nutrition Examination Survey* of the U.S., also looking for possible associations between cognitive decline and OC pesticides. All OC compounds were inversely correlated with cognition scores. OPs or pyrethroids did not give rise to any correlation, which, according to the authors, may be attributed to the high lipophilicity of OC compounds, a trait that allows them to stay within the adipose tissue of an organism and be released in small quantities over time (Kim et al., 2015). In a similar vein, Lee et al. (2016) studied 989 people of 70 years or older enrolled in the *Prospective Investigation of the Vasculature in Uppsala Seniors*. According to the results, the measure of 3 OC pesticides after adjusting for weight change was a good predictor of cognitive impairment (Lee et al., 2016). In a large study conducted by Tang et al. (2016), 3,471 urban and 4,429 rural residents over the age of 50 were examined for risk factors pertaining to cognitive impairment, including exposure to pesticides, with an OR of 4.68 95 % CI: 1.27–17.21) (Tang et al., 2016). Similarly, Paul et al.'s (2018) study with a subset of 430 individuals over the age of 60 from the *Sacramento Area Latino Study on Aging* found that OP-exposed subjects exhibited faster cognitive decline rates in 3MSE and SENAS (Spanish English Neuropsychological Assessment Scales) tests when compared to the non-exposed group (Paul et al., 2018). Rohlman et al. (2007) studied 56 adolescents and 119 adults spraying OPs occupationally; adolescents scored higher than adults, while the profession duration (in years) was found to be positively correlated with deficits in cognitive performance tests(Rohlman et al., 2007). The overall data prompts us to think about the underlying dimensionality of the various factors, including age, years of exposure and pesticides, which may all act synergistically towards cognitive impairment.

Experimental evidence in favor of the multifaceted nature of cognitive impairment in an exposure context also derived from Sullivan' et al. (2018) study with 159 Gulf War veterans, who were exposed to pesticides or pyridostigmine bromide. High levels of exposure in both groups were associated with slower information-processing speed, visual memory and attention deficits, as well as mood deterioration. Crucially, the high-pesticide exposure group was found to perform worse than the pyridostigmine bromide exposure group on several cognitive tasks, with the worst level of performance being reported for the visual memory recall test (Sullivan et al., 2018). Finally, data from a population-based cohort study of older adults (HEllenic Longitudinal Investigation of Aging and Diet-HELIAD) in Greece, suggested that non-demented individuals who reported that they had been living in areas near sprayed fields, presented an overall poorer neuropsychological performance, particularly evident in language, executive and visual-spatial functioning, and attention (Dardiotis et al., 2019c).

Table 1 presents an overview of the selected and included studies that have so far investigated the relationship between pesticide exposure and various cognitive functions in humans.

Table 1
Selected studies examining the relationship between of pesticide exposure and cognitive functions in humans.

Authors/Year/ Country	Pesticides	Cognitive and Psychomotor Functions	Tests	Study Design	Sample Description	Exposure Assessment	Findings
Daniell et al./ 1992/USA	Organophosphate insecticides	Motor speed Motor coordinationProcessing speed Memory	Finger tapping Hand-Eye Coordination Continuous Performance Symbol Digit Pattern Memory	Prospective longitudinal	49 apple orchard pesticide applicators and 40 controls – Initial evaluation and follow-up evaluation 1month after the end of spraying season	Questionnaire – occupational exposure + cholinesterase serum levels	No evidence of clinically significant adverse neuropsychological effects
Ames et al./1995/ USA	Organophosphate insecticides	Motor speed Attention Motor coordination Processing speed Memory	Finger Tapping Sustained Attention Hand-Eye Coordination Simple Reaction Time Symbol Digit Pattern Memory Serial Digit Learning	Cross-sectional	45 male subjects with history of moderate cholinesterase inhibition and 90 controls	Cholinesterase serum levels from previous reports	No evidence of chronic or long term neurologic sequelae
Stephens et al./ 1995/United Kingdom	Organophosphate insecticides	Short term memory Sustained attention Processing speed Long term memory	Digit Span Visual Spatial Memory Simple Reaction Time Symbol-Digit Substitution Synaptic Reasoning Category Search Serial Word Learning	Cross-sectional	146 sheep farmers exposed to organophosphates and 143 controls	Questionnaire – occupational exposure	Worse performance of sheep farmers on tests of sustained attention and processing speed – No group differences on short term memory and learning
Cole et al./1997/ Equador	Organophosphate and carbamate insecticides	Attention Visuospatial function Psychomotor function Motor function	Digit Span Benton Visual Retention Digit Symbol Simple Reaction Time Santa Anna Pursuit Aiming Test Digit Vigilance Trails A & B Block Design	Cross-sectional	144 farmers (23 consumers, 28 exposed, 123 applicators) and 72 controls	Questionnaire – occupational exposure + cholinesterase serum levels	Worse performance in the farmers' group
London et al./ 1997/South Africa	Organophosphate insecticides	Dexterity Clerical speed Visuospatial function Attention Motor Speed Apprehension Encoding Short-term memory Semantic access	Santa Anna Pursuit Aiming Digit Symbol Substitution Benton Visual Retention Digit Span Simple Reaction Time Inspection Time Reaction Time Continuous Number Checking Short-term Memory Scanning Manipulating Numbers I, II and III Animal Postures I and II Speaking Arrows Stimulus Resistance Pointing Arrows Echopraxis	Cross-sectional	163 fruit farmers and 84 controls	Questionnaire – occupational and non-occupational exposure + cholinesterase serum levels	Weak evidence of neurobehavioral effects
Calvert et al./ 1998/USA	Fumigants: methyl bromide and sulfuryl fluoride	Motor Coordination Processing speed	Hand-eye Coordination Simple Reaction Time Continuous	Cross-sectional	123 structural fumigation workers and 120 controls	Questionnaire – occupational exposure	Worse performance on specific cognitive functions (Pattern Memory Test and Santa Anna Dexterity Test)

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Table 1 (*continued*)

Authors/Year/ Country	Pesticides	Cognitive and Psychomotor Functions	Tests	Study Design	Sample Description	Exposure Assessment	Findings
		Memory Psychomotor function	Performance Symbol Digit Pattern Memory Serial Digit Learning Santa Anna Dexterity				
Steenlandet al./ 2000/USA	Organophosphate insecticides: chlorpyriphos	Attention Processing Speed Memory Motor Coordination	Digit Span Continuous Performance Simple Reaction Time Symbol Digit Pattern Memory Hand-eye coordination	Cross-sectional	191 termiticide applicators and 189 controls	List of professional applicators by the State of North Carolina – occupational exposure + TCP levels in the urine	Non significant differences
Van Wendel et al./ 2001/Costa Rica	Dichlorodiphenyltrichloroethane (DDT)	Attention Processing Speed Visuomotor function Motor function	Digit Span Digit Vigilance Digit Symbol Trails Pursuit Aiming Simple Reaction Time Finger Tapping Santa Anna Dexterity Purdue Pegboard	Cross-sectional	36 retired malaria-control workers and 31 controls	Questionnaire – occupational exposure	Worse performance for DDT exposed workers
Jamal et al./ 2002/United Kingdom	Organophosphate insecticides	Psychomotor performance Learning Memory	Motor Screening Reaction Time Matching to sample visual search Pattern Recognition Spatial Recognition Paired Associate Learning Spatial Span Rey Auditory Verbal Learning	Case-control	79 sheep farmers	Questionnaire – occupational exposure	slowing down of processing speed – No evidence of memory impairment
Kamel et al./ 2003/USA	Pesticides	Processing speed Psychomotor function	Digit Span Symbol Digit Latency Tapping Santa Anna	Cross-sectional	288 farm workers and 51 controls	Questionnaire – occupational exposure	Worse performance on specific tests (Digit Span, Tapping, Santa Anna) and weak effect on others tests (Symbol Digit)
Salvi et al./2003/ Brazil	Organophosphate (OP) pesticides	Orientation Immediate and short term memory Naming Sequencing	Mini mental Word Span	Longitudinal	37 workers in agriculture of tobacco exposed to OP for 3 months – 25 of these workers after 3 months off OP exposure	Questionnaire – occupational exposure + Serum acetylcholinesterase	No influence of exposure to OP
Farahat et al./ 2003/Egypt	Organophosphorus (OP) pesticides	Verbal abstraction Visuomotorspeed Problem solving Attention Memory	Similarities Digit Symbol Trailmaking part A & B Block Design Paced Auditory Serial Addition Test (PASAT) Letter Cancellation Digit Span Benton Visual Retention Story Recall parts A & B	Cross-sectional	52 male workers in cotton crops and 50 controls	Questionnaire – occupational exposure + Serum acetylcholinesterase	Worse performance on specific tests (Similarities, Digit Symbol, Trailmaking part A & B, Letter Cancelation, Digit Span, Benton Visual Retention)
Stephens/2004/ United Kingdom	Organophosphates (OP)	Processing Speed Memory Verbal abilities	Simple Reaction Time Digit Span Symbol-Digit	Cross-sectional	37 orchard sprayers exposed to OP and 26 pig farm workers and 31	Questionnaire – occupational exposure	Slower performance on Synaptic Reasoning Test

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Table 1 (*continued*)

Authors/Year/ Country	Pesticides	Cognitive and Psychomotor Functions	Tests	Study Design	Sample Description	Exposure Assessment	Findings
			Substitution Synaptic Reasoning Location Recognition Category Search Serial Word Learning		construction workers not exposed to OP	+ DEP and DETP in the urine	
Roldan–Tapia et al./2005/ Spain	Organophosphates (OPs) and carbamates	Attention Reasoning Memory Perception Visuomotorskills Expressive language Motor performance	Stroop "A" Cancellation Letter Trail Making Digit Vigilance Picture Completion Similarities Digit Span Rey-Auditory Verbal Learning Rey-Osterreich Complex Figure Benton Visual Retention Logical Memory Benton Visual Form Discrimination Poppelreuter Block Design Boston Naming Luria	Cross-sectional	40 farm workers and 26 controls	Questionnaire – occupational exposure + Serum cholinesterase level	Worse performance on specific tests
Rothleinet al./ 2006/USA	Organophosphates	Psychomotor functions Processing speed Attention Memory	Finger Tapping Simple Reaction Time Progressive Ratio Symbol Digit Digit Span Selective Attention Serial Digit Learning Continuous Performance	Longitudinal	96 agricultural workers and 45 controls	DAP metabolites in the urine	Worse performance on the majority of tests
Rohlman et al./ 2007/USA	Pesticides	Response speed Coordination Coding Complex functioning Attention Memory Motivation Learning	Finger Tapping Symbol Digit Simple Reaction Time Digit Span Progressive Ratio Selective Attention Serial Digit Learning Continuous Performance Match-to-Sample Reversal Learning	Cross-sectional	119 adults and adolescents currently working in agriculture and 56 adults and adolescents not currently working in agriculture	Questionnaire – occupational exposure	Worse performance on specific tests (Digit Span, Match-to-Sample, Continuous Performance)
Ross et al./2010/ United Kingdom	Organophosphate pesticides (OPs)	Memory Response speed Mental flexibility Language Fine motor control Strategy making Reaction time Visuospatial skills	Wechsler Adult Intelligence Scale-III Wechsler Memory Scale-III Trail Making A& B Stroop Graded Naming Verbal fluency California	Cross-sectional	127 sheep farmers and 78 controls	Questionnaire – occupational exposure	Worse performance on specific tests (response speed, memory, mental flexibility, fine motor control, strategy making)

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Table 1 (*continued*)

Authors/Year/ Country	Pesticides	Cognitive and Psychomotor Functions	Tests	Study Design	Sample Description	Exposure Assessment	Findings
			Computerised Assessment Package				
Bayrami et al./ 2011/Iran	Organophosphate pesticides (OPs)	Orientation Registration Attention Calculation Recall Language Visual Construction	Mini Mental State Examination	Cross-sectional	40 horticulture farmers and 40 controls	Questionnaire – occupational exposure + Serum cholinesterase level	No significant group difference in cognitive function
Baldi et al./2011/ France	Pesticides	Orientation Registration Attention Calculation Recall Language Visual Construction Memory Processing speed	Mini Mental State Examination Wechsler Paired-Associates Benton Visual Retention Five words Trail Making Part A Isaacs Set Finger Tapping Stroop Wechsler Similarities	Prospective case control	614 workers classified as directly exposed, indirectly exposed or non-exposed – 4 year follow-up	Questionnaire – occupational exposure	Worse performance on tests
Steenland et al./ 2013/Costa Rica	Pesticides	Orientation Registration Attention Calculation Recall Language Visual Construction	Mini Mental State Examination		400 elderly subjects	Questionnaire – occupational exposure	Worse performance on tests
Berent et al./ 2014/USA	Chlorpyrifos (CPF)	General ability Attention/information processing Memory-visual Memory-verbal Problem solving Psychomotor	Wide Range Achievement Test-Third Edition Reaction Time Rapid Visual Information Processing Signal Detection Matching to Sample Visual Search Change Score in milliseconds Delayed Matching to Sample CogniSyst Story Recall Stockings of Cambridge Moves Motor Performance Series	Prospective longitudinal	53 CPF workers and 60 controls – Baseline and 1 year later evaluation	TCPy in the urine, plasma butyrylcholinesterase (BuChE) activity and red blood cell acetylcholinesterase activity (AChE)	No evidence for impaired neurobehavioral domains
Kim et al./2015/ USA	Organochlorine pesticides	Visuospatial and motor speed of processing	Digit-Symbol Substitution Test	Cross-sectional	644 elders	Serum organochlorine pesticides	p,p′-dichlorodiphenyltrichloroethane (DDT), p,p′-dichlorodiphenyldichloroethylene (DDE), trans-nonachlor, oxychlordane, heptachlor epoxide, and β-hexachlorocyclohexane showed statistically significant or marginally significant inverse associations with cognitive score after adjusting for covariates
Jamal et al./ 2016/India	Organophosphates		Subjective Neuro-cognition Inventory	Cross-sectional		Questionnaire – occupational exposure	neurocognitive impairment

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Table 1 (*continued*)

Authors/Year/ Country	Pesticides	Cognitive and Psychomotor Functions	Tests	Study Design	Sample Description	Exposure Assessment	Findings
		Attention Memory Psychomotor retardation			187 pesticide sprayers of mango plantation and 187 controls	+ serum acetylcholinesterase	
Lee at al/2016/ Sweden	Organochlorine (OC) pesticides	Overall cognitive function	Diagnostic criteria for AD/MCI	Prospective	989 men and women aged 70 years – Baseline examination and 10 year follow-up	Plasma concentrations of 3 OC pesticides	Increased risk of cognitive impairment (MCI and dementia)
Tang et al./2016/ China	Pesticides	Orientation Registration Attention Calculation Recall Language Visual Construction	Mini Mental State Examination	Cross-sectional	7900 individuals aged ≥50years (4429 from rural area and 3471 from urban area)	Questionnaire – occupational exposure	Increased risk of cognitive impairment (OR = 4.68 95 %CI 1.27–17.21)
Corral et al./ 2017/Chile	Organophosphate pesticides	Memory Executive functions Attention Language Visuoconstruction	Mini Mental State Examination Digit Span Rey-Osterrieth Complex Figure Stroop d2 Test of Attention Frontal Assessment Battery Semantin Verbal Fluency	Cross-sectional	32 agricultural workers directly exposed to pesticides, 32 individuals environmentally exposed to pesticides and 38 controls	Questionnaire – occupational exposure + Place of residence - environmental exposure	Higher cognitive deficits in both exposed groups
Paul et al./2018/ USA	Organophosphorus	Overall cognitive performance	Modified Mini Mental State Exam	Prospective cohort	430 persons aged ≥ 60 years – Baseline examination and every 12–15 months for up to seven study visits	Place of residence - environmental exposure	Faster cognitive decline for persons with high OP exposure
Sullivan et al./ 2018/Gulf War	Organophosphates Carbamates Organochlorines Pyrethroids DEET	Language Attention/executive domain Psychomotor domain Visuospatial domain Memory	Boston Naming Trail-making Computerized Continuous Performance Wisconsin Card Sorting Finger Tapping Grooved Pegboard Hooper Visual Organization Rey OsterriethComplex Figure Stanford-Binet Copying California Verbal Learning		159 Gulf War military pesticide applicators	Environmental exposure report by the Department of Defense and questionnaire - environmental exposure	Worse performance of persons with high pesticide exposure
Bosma et al./ 2000/ Netherlands	Pesticides	Main cognitive domains	Stroop Colour Word Verbal Learning Letter Digit Coding Word FLuency	Prospective cohort	830 non-demented people aged 50–80 years – Baseline examination and follow-up after 3 years	Questionnaire – occupational exposure	Increased risk of Mild Cognitive Dysfunction
Dardiotis et al./ 2019/Greece	Pesticides	Main cognitive domains and overall performance	composite, memory, executive, visual-spatial, language and attention z-scores	Cross sectional analysis	1,397 non-demented (1,218 cognitively healthy individuals and 179 individuals with MCI)	Self-reported-pesticides exposure	Non-demented individuals who reported that they had been living in areas near sprayed fields, had poorer neuropsychological performance. The poorer performance was evident in language, executive and visual-

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Table 1 (*continued*)

Authors/Year/Country	Pesticides	Cognitive and Psychomotor Functions	Tests	Study Design	Sample Description	Exposure Assessment	Findings
							spatial functioning, and attention. These associations remained after excluding subjects with MCI.

4.2. Pesticides and dementia

Dementia refers to an acquired objective cognitive or behavioral impairment that represents a decline from a previous level of performance that interferes with daily function (McKhann et al., 2011). Dementia is an umbrella term that covers many types of severe cognitive impairment, including AD, vascular dementia (VaD), frontotemporal dementia (FTD) and Lewy body dementia (LBD). The symptoms of the different dementias partly overlap and partly differentiate one another; however, the diagnosis of dementia is not easy, as many concomitant diseases and atypical symptoms may exist, such as psychosis (Dardiotis et al., 2019a; Nussbaum et al., 2017; Rayner et al., 2006; Siokas et al., 2018; Tsatsakis et al., 2019a). Unfortunately, many of the epidemiological studies that have investigated the association between pesticide exposure and cognitive impairment have focused on a general diagnosis of dementia without making a distinction between its different phenotypes, or the different pesticides involved. Here, we have included 18 studies, spanning from 1984 to 2015.

Occupational exposure to unspecified pesticideshas been associated with increased risk of all-cause dementia [Hazard Ratio (HR) = 1.38 and 95 % Confidence Interval (95 %CI) = 1.09–1.76)] in the Cache County Study (Hayden et al., 2010). This prospective cohort study enrolled 3,084 residents of the agricultural community of Cache County, UT, aged 65 years and above without dementia, and assessed their cognitive status at baseline and after 3, 7 and 10 years. The participants completed detailed questionnaires and after adjustments for various factors, an increased risk of dementia was shown for the exposed group. The study also evaluated the risk of a specific type of dementia, namely AD, in late life after occupational pesticide exposure and found a similar association (HR = 1.42 and 95 %CI 1.06–1.91) (Hayden et al., 2010). In another study, Povey et al. (2014) examined the effects of pesticides, otherwise unspecified, on the incidence of neuropsychiatric disorders in British sheep farmers (Povey et al., 2014). The results showed no association between dementia and low dose chronic exposure -defined as ever handling a pesticide concentrate. However, the outcome was different for the participants who sought advice for pesticide poisoning. In the latter, acute exposure showed a considerable association with dementia [Odds Ratio (OR) = 6.94 and 95 %CI = 3.44–14.00] (Povey et al., 2014). The Prospective Netherlands Cohort Study by Koeman et al. (2015) investigated the effects of various occupational factors, pesticides included, on non-vascular dementia (Koeman et al., 2015). Unexpectedly, the authors found an inverse association between pesticides and non-vascular dementia among men. A possible explanation for this oddity was the healthier living habits of farmers, who were however exposed to higher levels of pesticides (Koeman et al., 2015). Finally, a population-based case-control study in Taiwan compared patients with a history of acute OP or carbamate poisoning with age- and sex-matched controls, and found a hazard risk of 1.98 (95 % CI = 1.59–2.47) for all-cause dementia for the exposed subjects (Lin et al., 2015). However, in a follow up analysis of the data stemming from the Canadian Study of Health and Aging, Medehouenou et al. (2014) measured polychlorinated biphenyls (PCBs) and OCs plasma concentrations in 2023 participants and after adjusting for several factors such as ApoE4 allele, age, and sex, found no, or an inverse association between the measured concentrations and dementia; higher concentrations of some PBC and OC metabolites were tied to a reduced prevalence of dementia [e.g.: PCB105: OR = 0.87, 95 % CI = 0.77–0.99; PCB 118:OR = 0.86, 95 % CI = 0.74–0.99, HCB (OC) partially adjusted OR = 0.85, 95 % CI = 0.75–0.97; fully adjusted OR = 0.87, 95 % CI = 0.77–0.99, p,p′-DDT (OC) partially adjusted OR = 0.82, 95 % CI = 0.72–0.93; fully adjusted OR = 0.84, 95 % CI = 0.74–0.96], and the majority showed no higher or lower risk (Medehouenou et al., 2014).

The most common form of dementia is AD. AD patients typically exhibit gradually progressive cognitive and behavioral impairment with functional decline. AD has been extensively reported to affect many,

and common symptoms include deficits in short term memory, language processing, and executive functions (Apostolova, 2016). Several epidemiological studies have examined the relationship between pesticide exposure and risk of AD with conflicting results. There are four case-control studies, three prospective cohort studies and one ecological study that have so far focused on the aforementioned association, from 1985 until 2011. Three of the case-control studies showed no association between pesticide exposure and AD, while the fourth found increased risk of AD. More specifically, French et al. (1985) obtained information from 78 male patients with AD and 76 controls in order to assess various possible risk factors for AD, and observed a much commoner occurrence of head trauma among patients (OR = 4.5, Confidence Limits = 1.44–15.69), but no difference in occupational exposure of any kind, including unspecified pesticides (OR = 0.8, Confidence Limits = 0.29–2.19) (French et al., 1985). These findings have been replicated by Gun et al. (1997) in Australia, as well as by Gauthier et al. (2001) in Canada (Gauthier et al., 2001; Gun et al., 1997), whereby no relation was reported between generic pesticide exposure and risk of AD. On the contrary, the Canadian Study of Health and Aging, (1994) designed to identify risk factors for AD (among other purposes) found an increased risk of AD tied to occupational exposure to unspecified pesticides (OR = 2.17 and 95 %CI = 1.18–3.99) after examining 258 patients with probable AD of recent onset and 535 controls. The risk also seemed to be higher for less educated subjects (The Canadian Study of Health and Aging, 1994).

Considerably different results were yielded by three prospective cohort studies, spanning from 2001 to 2010. Besides the Cache County Study by Hayden et al. (2010) mentioned above, a population-based longitudinal study by Tyas et al. (2001) in Canada analyzed the data of 694 subjects who were cognitively intact on baseline examination and for whom cognitive status assessment was obtained after 5 years (Hayden et al., 2010; Tyas et al., 2001). On this follow-up examination, 36 subjects were diagnosed with AD. The study found an increased risk of AD associated with occupational exposure to fumigants/defoliants [Risk Ratio (RR) = 4.35 and 95 %CI 1.05–17.90]. However, the increased risk was found lower, though still somewhat elevated for pesticides/fertilizers as a whole (RR = 1.45 and 95 %CI = 0.57–3.68) (Tyas et al., 2001). The PAQUID Study (2003) in France, on the other hand, was a large prospective cohort study comprised of 1507 elderly participants aged ≥65 years, who were followed-up for 5 and 10 years (Helmer et al., 2006; Ramaroson et al., 2003). Questionnaires with detailed occupational background questions were used to collect information on participants 'occupational exposure to pesticides, while the place of residence was manipulated as an index of environmental exposure. The authors analyzed 96 cases with AD during the follow-up session and found a strong association between occupational exposure and AD in men (RR = 2.39 and 95 %CI = 1.02–5.63). No association was revealed for women, which was partially explained by the fact that pesticides are mixed and mainly applied by men; no major association was found for environmental exposure either (Baldi et al., 2003). Finally, Parron et al. (2011) conducted an ecological study in Spain in order to examine whether environmental pesticide exposure was associated with various neuropsychiatric conditions (Parron et al., 2011). The study population included 17,429 patients who were recruited from hospital records. Environmental exposure in Parron et al.'s (2011) study was deemed high or low based on selected geographical areas. The analyses showed an increased risk of AD in populations living in districts with high generic pesticide use (OR = 2.10 and 95 %CI = 1.96–2.25) (Parron et al., 2011). In general, when examining etiological relationships between risk factors and AD or more generally any chronic disease, cohort studies are preferable to case-control studies, due to the absence of recall bias (Yan et al., 2016). However, the well conducted case-control studies, with excellent exposure assessment, and particularly cohort-nested case-control studies can still be more reliable, particularly when compared to the limited reliability of the evidence yielded by the ecologic studies.

Possible associations of pesticide exposure with the other, less common forms of dementia have been less explored. Vascular dementia (VaD), the second commonest form of the disease (Lin et al., 2015), is diagnosed on the basis of cognitive impairment with vascular contribution manifested by history, physical examination, cognitive tests and neuroimaging data (Smith, 2016). Various risk factors for VaD were studied in a follow up publication from Lindsay et al., (1997) mentioned before, which recruited 129 VaD patients of recent onset and 535 controls. The diagnosis of VaD was made on the basis of the ICD-10 criteria as well as on clinical grounds without routine neuroimaging. According to the study's findings, occupational exposure to unspecified pesticides and fertilizers was elevated for the VaD patients only (OR = 2.60, 95 %CI = 1.30–5.23) (Lindsay et al., 1997). Results were similar in Hebert et al.'s (2000) study, based on the second phase of the Canadian Study of Health and Aging. The DSM-IV and NINDS-AIREN criteria were used to identify VaD patients and all subjects underwent a CT scan. Analysis of the data of 105 VaD patients and 802 controls revealed that exposure to pesticides or fertilizers was an important risk factor for VaD (OR = 2.05, 95 %CI = 1.03–3.85) (Hebert et al., 2000).

Frontotemporal dementia (FTD) is a progressive neurodegenerative disorder mainly characterized by behavioral changes and/or language deficits, usually affecting adults in their fifties to sixties. There are several clinical subtypes of FTD, including behavioral variant FTD, semantic, non-fluent/agrammatic and logopenic-variant primary progressive aphasia, as well as FTD associated with motor neuron disease (MND) (Finger, 2016). A study on the medical and environmental risk factors for FTD including pesticides in general was carried out in Netherlands by Rosso et al. (2003). This case-control study included 80 FTD patients and 124 controls matched on age, sex and surrogate informant relations. The study found no association between exposure to pesticides and sporadic FTD. The only major risk factor identified was head trauma. It follows that more studies, including prospective cohort studies, are needed to shed more light on potential relationships between exposure to pesticides, either occupational or environmental, and FTD. It would be particularly interesting to examine possible associations between FTD and MND, given the fact that numerous epidemiological studies have found that the risk of MND, of ALS in particular, is associated with pesticide use (Kamel et al., 2012; Vinceti et al., 2012, 2017).

Lewy Body Dementia (LBD) includes both Parkinson Disease Dementia (PDD) and Dementia with Lewy Bodies (DLB). There is a clinicopathologic overlap between these two entities, alongside several differences. PDD occurs after an established diagnosis of Parkinson's disease, while in DLB, parkinsonian signs develop with or after the presence of dementia (Armstrong, 2019). A case-control study by Hubble et al. (1998) in Kansas provided evidence in favor of a genetoxin interaction as a risk factor for PDD (Hubble et al., 1998). The analysis of the data of 43 PDD patients and 51 PD patients without dementia showed that subjects exposed to unspecified pesticides and carrying a susceptibility-for-PDD allele had a predicted probability of PDD reaching 83 % (Hubble et al., 1998). Review of relevant literature indicates a relationship between pesticides and PD (Brown et al., 2006; Freire and Koifman, 2012), that may be influenced by genetic factors, implying an increased risk of developing PD after exposure to pesticides, for genetically susceptible people (Dardiotis et al., 2013). This association suggests that potential links may exist between pesticides and LBD as well, since PD and LBD share similar pathophysiological and clinical characteristics (Zaganas et al., 2013).

All the aforementioned epidemiological studies assessed exposure to pesticides either by using questionnaires and/or the place of residence in order to clarify occupational and/or environmental exposure, respectively. Few case-control studies were based on biomarkers of exposure, i.e. have examined serum pesticide levels in AD patients and controls. The study by Singh et al. (2013) in India, for example, measured serum levels of various OCs in 70 patients with AD and 75 controls, and found significantly increased levels of β-

Table 2
Selected studies examining the relationship between of pesticide exposure and risk of dementia in humans.

Authors/Year/Country	Clinical Entity	Study Design	Sample Description	Exposure Assessment	Adjustments - Matching	Findings
Hayden et al./ 2010/ USA	Dementia and AD	Prospective cohort	3084 non-demented people ≥65 years – Baseline examination and follow up evaluations after 3, 7, 10 years	Questionnaire – occupational exposure	Adjusted for age, sex, education, baseline MMSE score, APOE ε4 status	Increased risk of all-cause dementia (HR:1.38 95 %CI 1.09–1.76) Increased risk of AD (HR:1.42 95 %CI 1.06–1.91)
Povey et al./ 2014/ Great Britain	Dementia	Cross-sectional	1350 British sheep farmers who were ≥13 years in 1970	Questionnaire – occupational exposure [Acute exposure was defined as ever seeking advice for pesticide poisoning – Low dose chronic exposure was defined as ever handling the pesticide concentrate]	Adjusted for age, sex, region, ever smoker, lambing, slaughtered livestock, somatic score	No association between dementia and ever handling the pesticide concentrate (OR = 1.18 95 %CI 0.61–2.29) Increased risk of dementia in those ever seeking advice for pesticide poisoning (OR = 4.27 95 %CI 1.85–9.83)
Koeman et al./ 2015/ Netherlands	Dementia (non-vascular)	Case cohort	682 males and 870 females who had died with non-VaD out of 120.852 subjects who had enrolled in the Netherlands Cohort Study in 1986	Baseline questionnaire in 1986 - occupational exposure	Adjusted for age, smoking, physical activity, BMI	Inverse association of non-vascular dementia among men (HR = 0.61 95 %CI 0.40 – 0.93)
French et al./1984/ USA	AD	Case control	78 male AD patients and 76 controls	Questionnaire – occupational exposure	Matching by age, sex, race	non-statistically significant differences between AD patients and controls (OR = 0.8 95 %CI 0.29–2.19)
Canadian Study of Health and Aging (CSHA)/ 1994/Canada	AD	Case control	258 AD patients and 535 controls	Questionnaire – occupational exposure	Adjusted for age, sex, education, residence	Increased risk of AD (OR = 2.17 95 %CI 1.18–3.99) – the increased risk was greater in participants with low education level
Gun et al./1997/ Australia	AD	Case control	170 AD patients and 170 controls	Questionnaire – occupational exposure	Matching by age, sex	non-statistically significant differences between AD patients and controls (OR = 2.54 95 %CI 0.41–27.06)
Gauthier et al./2001/ Canada	AD	Case control	67 AD patients and 67 controls	Residential history - environmental exposure + Questionnaire – occupational exposure	Matching by age, sex - Adjusted for education level, family cases, APOE ε4 allele	Non-statistically significant differences between AD patients and controls (OR = 0.97 95 %CI 0.38–2.41)
Tyas et al./2001/Canada	AD	Prospective cohort	694 cognitively intact subjects – Baseline examination and follow up evaluation after 5 years	Questionnaire – occupational exposure	Adjusted for age, education, sex	Increased risk of AD after exposure to fumigants/defoliants (RR = 4.35 95 %CI 1.05–17.90) – non- statistically significant association with AD after exposure to pesticides (RR = 1.45 95%CI 0.57–3.68)
Baldiet al./2003/France (PAQUID Study)	AD	Prospective cohort	1507 French elderly ≥65 years – Baseline examination and follow-up evalutaion after 5, 10 years	Questionnaire – occupational exposure + Place of residence - environmental exposure	Adjusted for age, smoking, education	Increased risk of AD in men (RR = 2.39 95 %CI 1.02–5.63)
Parron et al./2011/Spain	AD	Ecological	17.429 patients diagnosed with various neuropsychiatric disorders, including AD	Different selected areas -environmental exposure	Adusted for age, gender	Increased risk of AD (OR = 2.10 95 %CI 1.96–2.25)
Lindsay et al./1997/Canada (Canadian Study of Health and Aging, CSHA)	VaD	Case control	129 VaD patients and 535 controls	Questionnaire – occupational exposure	Adjusted for age, sex, education, residence in community or institution	Increased risk of VaD (OR = 2.60 95 %CI 1.30–5.23)
Hebert et al./2000/Canada (Canadian Study of Health and Aging, CSHA)	VaD	Prospective case control	105 VaD patients and 802 controls	Questionnaire – occupational exposure	Adjusted for age, sex, education, region	Increased risk of VaD (OR = 2.05 95 %CI 1.03–3.85)
Rosso et al./2003/ Netherlands	FTD	Case control	80 FTD patients and 124 controls	Questionnaire – occupational exposure	Adjusted for age, sex, surrogate informant relationship	No association with FTD
Hubble et al./1998/USA	PDD	Case control	43 PD patients with dementia and 51 PD patients without dementia	Questionnaire – occupational exposure		Increased risk of PDD in combination with a genetic trait (CYP 2D6 29B + allele) (OR = 3.17 95 %CI 1.11–9.05)
Singh et al./2012/India	AD	Case control	70 AD patients and 75 controls	Serum levels		Increased levels of β-HCH, dieldrin and pp'-DDE in AD patients

(*continued on next page*)

Table 2 (*continued*)

Authors/Year/Country	Clinical Entity	Study Design	Sample Description	Exposure Assessment	Adjustments - Matching	Findings
Richardson et al./2014/USA	AD	Case control	86 AD patients and 79 controls	Serum levels	Adjusted for age, sex, race/ethnicity, education, APOE genotype, location	Increased levels of DDE in patients with AD
Medehouenou et al./2014/ Canada (Canadian Study of Health and Aging, CSHA)	Dementia and AD	Cross-sectional	2,023 participants, 574 with dementia, 399 with AD in particular	Polychlorinated biphenyls and OCs plasma concentrations	Adjusted for blood collection period, total plasma lipids, age, sex, education, apolipoprotein E e4 allele, tobacco and alcohol use, rural/urban residence, and comorbidities	Elevated plasma PCB and OC pesticide concentrations were not associated with higher prevalenceof all-cause dementia and AD
Lin et al./2015/Taiwan	Dementia	Case control	9,616 patients hospitalized for acute OP and CM poisoning and 38,510 controls	Hospital records	Adjusted for age, sex and year of hospitalization	1.98-fold increased risk of dementia compared with the control cohort (95 % CI = 1.59–2.47)

hexachlorocyclohexane (β-HCH), dieldrin and pp'-dichlorodiphenyldichloroethylene (pp'-DDE) in the patients (Singh et al., 2013). Richardson et al. (2014) also found elevated levels of dichlorodiphenyldichloroethylene (DDE) in the serum of patients with AD, thus supporting the reliability of epidemiological studies that show increased risk of AD after pesticide exposure (Richardson et al., 2014). Table 2 presents an overview of the included studies that have so far investigated the relationship between pesticide exposure and risk of dementia in humans.

5. Discussion

The research on the intricate mechanisms and the relations between pesticides and cognition is far from being complete. Considering that many studies have indeed reported an association between exposure and cognitive impairment, it would probably be naïve to assume that they were all mere coincidences and that chronic exposure to deeply harmful substances such as pesticides leaves the brain unscathed.

However, there are many reasons which may explain why researchers could not reach a consensus on this issue so far. First of all, a sadly big number of studies did not assess exposure to specific substances, pesticide categories, or compounds. In many occasions, exposure to and application of pesticides are assessed via questionnaires and personal interviews, where the subjects cannot name the exact compounds, or are heterogeneous, and no specific pesticide class can be pinpointed. It has also been challenging to reliably quantify exposure levels, as few biomarkers exist, and many of them do not accurately depict chronic exposure; they may have short half-lives, or different pathways may be activated in acute and chronic exposure instances. Animal studies have shown that prolonged exposure to low-doses of OPs that do not produce AChE inhibition or clinical symptoms of OPs intoxication leads to inflammatory responses that can in turn lead to neuronal damage (Chapman et al., 2006; Collombet et al., 2005; Henderson et al., 2002; Lim et al., 2011). It is speculated that OP-induced inflammation has a big impact on the pathogenesis of OP neurotoxicity so new biomarkers of inflammation used in epidemiological and biomonitoring studies could help for a better evaluation of OPs impact on cognitive function and risk of dementia.T he methods of assessment tend to also be inherently fairly unreliable and subject to bias, for example recall bias in the case of questionnaires (Tsatsakis et al., 2016, 2018), and a relatively small number of studies included biochemical measurements, such pesticide levels or their respective biomarkers, for example the bio-scavenger butyrylcholinesterase (BChE) in the case of OPs. Additionally, the populations under study exhibited broad heterogeneity in terms of exposure, extending from workers directly and indirectly exposed, to unexposed subjects; population heterogeneity has also been further broadened by differences in ethnicity. There should be a consensus between the studies analyzed regarding what counts as chronic exposure and if the period under study is relevant or sufficient for developing the neurological toxic effects. Sometimes the delayed neuropathies can manifest outside of the timeline of the study. For example, the study of Daniel et al. (Daniell et al., 1992), that could not show any evidence of clinically significant adverse neuropsychological effects, evaluated the pesticide applicators before the spraying season and at 1 month after the end of the spraying system, while in the studies that showed a significant correlation between worse neurological performance and pesticide exposure, the follow-up period was usually more than 1 year (Baldi et al., 2011, 2003; Berent et al., 2014; Bosma et al., 2000; Helmer et al., 2006; Lee et al., 2016; Tyas et al., 2001). In fact, the studies that reported a positive association, when compared to those that did not, seemed to include larger populations and larger periods of time, ranging from months to even 10 years. Conclusively, besides determining what chronic exposure means, specific cut-off points in the timeframes of the studies should be established, for proper comparisons to be feasible in the future; is one month after the pesticide applying period enough? Should

subjects be studied at least 3 months or even years after acute/occupational exposure? How many years of inhabiting a high-exposure area are deemed enough for a subject to be named “chronically exposed to low doses”? All these questions should be addressed if the scientific community wishes to reach dependable conclusions.

Confounding factors also play a crucial role in assessing risk and associations. Firstly, polymorphisms in the genes involved in pesticide metabolism can also lead to different response after exposure to the same level of pesticides. For instance, regarding OPs, mutations in the genes of BCHE, cytochrome P450 enzymes (CYP2D6, CYP2C19, CYP3A), paraoxonase-1 (PON1) and glutathione S-transferase (GSTM1, GSTT1) have been shown to be involved in increased risk after exposure to OP pesticides (Howard et al., 2010). Consequently, further studies that analyze the correlation between genetic variability in genes associated with enzymes implicated in pesticide metabolism, can better simulate the real life cases. It would also be beneficial for studies to incorporate adjustments for risk factors proven to associate with cognitive deficits, such as head trauma. As can be seen, in most cases, controls and cases were age- and sex- matched, while few further adjustments were made. It is also important to address whether subjects had a history of acute poisoning when handling the subject of pesticides, since some studies did show a difference between those who had and those who did not (Lin et al., 2015; Povey et al., 2014). One study also addressed the matter of adequate self-protection (Jamal et al., 2016); this would have been particularly interesting if more studies had included such a section. It is possible that the extent of the protective measures’ application may heavily vary from country to country and from field of work to field of work, and so discrepancies in the results may result from this factor as well.

Study designs rarely presented homogeneity either, so drawing clear conclusions from the available studies still remains difficult. Moreover, the estimation of the exposure to pesticides has been most frequently performed without measuring the synergic effects and the possible interactions between the toxicants within mixtures, and also overlooking low exposures to environmental toxicants (Docea et al., 2018; Tsatsakis et al., 2019b, 2016; Tsatsakis et al., 2017). Exposure to such substances known to affect cognitive function could also occur alongside pesticide exposure, and influence the results. One such example is lead, with studies suggesting that it aggravates AD (Wu et al., 2020; Zhou et al., 2018), so adjustments for potential exposure confounders are crucial in drawing accurate results. It is possible that a Real-Life Risk Simulation approach may represent a robust alternative in order for the safe exposure limits and the net risk that pesticides confer to impaired cognitive function to be examined (Hernandez and Tsatsakis, 2017; Kostoff et al., 2018; Tsatsakis et al., 2017). Previous studies that evaluated the effect of low dose chronic exposure to mixtures of pesticides and other chemicals with the intention of simulating real life exposure scenarios showed that hermetic neurobehavioral effects can appear after mixture exposure at doses considered safe for individual compounds and these effects can be exacerbated by the coexistence with conditions such as vitamin deficiency (Tsatsakis et al., 2019c, d). Finally, some study limitations, such as inadequate size with consequent lack of statistical precision and selection bias particularly in case-control studies, markedly affected the epidemiologic studies, thus hindering the possibility to adequately assess the effect of these compounds on cognitive function in humans.

Another point that arises when one wants to answer the question of whether pesticides can cause cognitive deficits, is the question of what happens with the general population. Most studies have examined groups of people that have been exposed, such as pesticide applicators. However, a few studies did involve people indirectly exposed via their occupation and did show a degree of cognitive impairment, while the study of Parron et al. (2011), for instance, did not even assess occupational exposure; they only used geographical areas with higher pesticide use and found a higher risk for AD (Parron et al., 2011). It is no news that the general population becomes exposed to pesticides via water, nutrition and other factors (Murphy et al., 1983), and that pesticides reach groups not traditionally linked to exposure in a variety of ways; for example, the levels of OC pesticides were found higher than the European Union highest allowed level in Nigerian personal care products (Adekunle et al., 2018). Population-based studies assessing pesticide exposure in general are few and far between, such as the aforementioned HELIAD study, where individuals with some degree of environmental exposure to pesticides showed poorer cognitive performance (Dardiotis et al., 2019c). It seems that if we are to accept that pesticides do in fact affect cognition in directly exposed individuals, the general population is not “safe” either, since exposure is so widespread and low doses seem to suffice in producing chronic sequelae without ever needing to surpass the levels of acute poisoning.

Nonetheless, there seems to be enough scientific evidence to support the existence of adverse effects of pesticides on individuals’ mental condition. Although pesticide exposure may not be the sole factor involved in dementia and cognitive impairmentin general, it may be enough to trigger the process, often in combination with other factors such as genetic polymorphisms and other toxicants, as it promotes phenomena such as oxidative stress. Therefore, we think it is safe to assume that modifying pesticide use may protect humans against dementia. Such perspective, of course, needs to be further explored and validated by future studies. In the next step, further studies with larger cohorts of participants, comparable study-designs and a Real-Life Risk Simulation approach are needed to better elucidate the relationship between pesticides and cognitive impairment. Special attention should be paid to identifying the specific pesticide categories most detrimental to cognition, as well as the toxicological mechanisms through which they act.

6. Conclusions

Given the complicity of the matter, the relationship between pesticides and cognitive impairment seems particularly tricky to decipher. There is a plethora of studies available, either on animals or involving humans, which have attempted to shed light on this subject, but their results are considerably heterogeneous. It is not to wonder, however, since the study designs and the parameters in hand are completely different from study to study and many aspects remain underexplored. The evidence seems to hint towards the existence of an association between pesticides and cognitive impairment but in order for this association to be confirmed, future research groups should try to follow similar designs, addressing the points analyzed above. Absolute proof of this association could have a tremendous effect in preventing dementia, at least in part, via adequate self-protection measures or specific compound restriction.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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