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The association between chronic Dicofol exposure and the risk of Alzheimer's disease

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THE ASSOCIATION BETWEEN CHRONIC DICOFOL EXPOSURE AND THE RISK OF ALZHEIMER'S DISEASE

by

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THE ASSOCIATION BETWEEN CHRONIC DICOFOL EXPOSURE AND THE RISK OF ALZHEIMER'S DISEASE

EOIN MORIARTY

ABSTRACT

Alzheimer's Disease (AD) is a disorder that causes progressive cognitive impairment. It's a unique neurodegenerative disorder in which beta amyloid plaques and neurofibrillary tangles begin to preoccupy brain tissue. AD has been a historically difficult disease for society to examine partially because it's etiology is still not well understood. One of the risk factors associated with AD development that has been researched is exposure to pesticides, primarily focused on the pesticide Dichlorodiphenyltrichloroethane (DDT).

Studies have shown that DDT disrupts both the neurological and endocrine systems. As a result, this disruption leads to hyperexcitability of axons as well as a loss of neuroprotection thereby causing an increased susceptibility to Alzheimer's Disease. Although DDT has been extensively studied, other pesticides are still used in large amounts throughout the world. One pesticide that's particularly used to do it's low cost and availability is Dicofol. Dicofol is a DDT derivative in which it's structure is similar to the structure of DDT. Although Dicofol is similar to DDT in terms of structure and use, research into the effects of Dicofol exposure has not been extensively performed. The following study has been proposed in order to investigate a relationship between Dicofol exposure and the risk of developing AD.

The study performed is a retrospective cohort study of 1134 people consisting of pesticide applicators collected from the Agricultural Health Study. A licensed neurologist using the Mini Mental Status Exam will assess if cognitive impairment is present in the participants. Those that are believed to be cognitively impaired will then undergo neuroimaging to confirm the presence of beta amyloid plaques, which are indicative of AD. Finally a Chi Square analysis will be used in order to determine if there are associations between exposure status and AD diagnosis.

This study has both clinical and public health significance. As stated previously, dicofol is still used widely in many countries due to it's availability and low cost. Therefore, many agricultural workers may be unknowingly exposing themselves to a pesticide that can increase their susceptibility to neurodegenerative disease. This concern is primarily the reason as to why a study such as the one proposed is needed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

3MS.....Modified Mini Mental Status

AD..... Alzheimer's Disease

AHS…….…….Agricultural Health Study

ALS..... Amyotrophic Lateral Sclerosis

ApoE .....Apolipoprotein

APP ..... Amyloid Precursor Protein

BGT.....Bender Gestault Test

CI.....Confidence Interval

CSF……..Cerebral Spinal Fluid

CT ..... Computed Tomography

DCBP ..... Dichlorobenzophenone

DDE .....Dichlorodiphenylethylene

DDT ..... Dichlorodiphenyltrichloroethane

DHEA…….....Dehydroepiandrosterone

DSST.....Digital Symbol Substitution Test

E2 ..... Estradiol

EEG..... Electroencephalogram

EPA……....……Environmental ProtectionAgency

ER .....Estrogen Receptor

FDG..... Fludeoxyglucose

FSH ..... Follicle Stimulating Hormone

LBD..... Ligand Binding Domain

LH .....Luteinizing Hormone

MD ..... Molecular Docking

MoCA ..... Montreal Cognitive Assessment

MRI .....Magnetic Resonance Imaging

NMDA .....N-Methyl-D-Aspartic Acid

OC ..... Organochlorine Pesticides

OR ..... Odds Ratio

PCBs .....Polychlorinated Biphenyls

PET .....Positron Emission Tomography

PL .....Prolactin

SEM .....Structural Equal Modeling

TSH .....Thyroid Stimulating Hormone

INTRODUCTION

Background

Alzheimer's disease (AD) affects approximately 5.5 million people, most of whom are older than 65. AD is a neurodegenerative disease that affects short-term memory, cognition, speech, and the ability to perform daily activities. It's a devasting disease that causes families to watch their loved ones gradually disappear.

Since it was first discovered, researchers have been investigating the genetic susceptibility of the disease, the underlying pathology, the progression of cognitive decline, the loss of capacity as the disorder develops and AD related risk factors, such as head injuries, lower education, and exposures to environmental toxicants.

Statement of the Problem

One risk factor that appears to be associated with AD is the exposure to pesticides. Dichlorodiphenyltrichloroethane (DDT), for example, has been associated with developing neurodegenerative diseases later in life.[1] Further, research has found DDT disrupts both the nervous and endocrine systems.[2] Studies have reported calcium channel gates in neurons stay constantly open following DDT exposure, leading to hyper-excitability and resulting in synaptic disruption. It's been hypothesized that this synaptic disruption could cause neurodegeneration, leading to AD. It has also been reported that DDT affects estrogen receptors, resulting in an increase in estrogenic response. Although estrogen is known to be neuroprotective, an increase in the estrogenic response could result in a negative feedback loop, causing an overall decrease in estrogen and, ultimately, decreasing estrogen-related neuroprotection.[3] This decrease in

neuroprotection then causes an increased risk of developing AD.[4] It's been hypothesized that this decrease in estrogen-related neuroprotection then causes an increased risk of developing AD.[5] Although DDT was a useful pesticide and lowered the risk of malaria in the United States, it was banned in the 1970s because the health and environmental risks were too high. However, despite this ban, alternative pesticides such as dicofol were still being used before being banned. Dicofol's chemical structure differs from DDT in that it has an additional hydroxyl functional group on C-1. While there's evidence on early DDT exposure and developing neurodegenerative disorders, such as AD, very little is known about whether exposure to chemicals similar to DDT, such as Dicofol, puts an individual at greater risk of developing AD.

Hypothesis

Long term dicofol exposure will be associated with an increased risk of developing Alzheimer's disease.

Objectives and specific aims

Objective: To find a positive association between dicofol exposure and an increased risk of Alzheimer's Disease. Specifically, this study aims to:

- Recruit a cohort of subjects who were previously exposed to dicofol and are now old enough to develop AD and a control group of similar age, location, and education status who were never exposed to dicofol.
- Obtain serum samples to measure exposure/pesticide concentration through gas chromatography

- To assess each cohort for Alzheimer's disease by examining them in a physical, neurological and psychiatric exam as well as a Modified Mini Mental State (3ms) exam
- Examine an association between long term dicofol exposure and an increased risk of developing Alzheimer's disease.

REVIEW OF THE LITERATURE

Overview

Alzheimer's disease (AD) is a neurodegenerative disorder that occurs most commonly in those over the age of 60. According to the National Institute on Aging, more than 5.5 million people in the United States over the age of 65 may have AD-related dementia. There are more than 700,000 AD-related deaths in the United States yearly.[6] The average time from diagnosis to death is 8 years, however those suffering from the disease can live 20 or more years after diagnosis. AD-related dementia is characterized by cognitive decline with early deficits in short-term memory, language, and executive functioning. Although patients have trouble remembering recent events and information, their older memories remain in the initial stages of the disorder until eventually they lose their long-term memory. AD patients have word-finding difficulty and have trouble expressing their emotions. In addition, AD dementia produces significant problems with planning, multi-tasking, organizing, making decisions, or insight into the fact that they have a neurodegenerative disease.

AD is classified into different categories based on severity. Mild dementia involves some neurological impairment, such as short-term memory loss, however the patient can still function independently. With moderate dementia, the patient shows dependence in many areas but is independent in self-care. In severe dementia, the patient requires assistance in all basic activities, including using the restroom, grooming and dressing. Alzheimer's dementia also affects the caregivers who devote their time and emotions to caring for the patient and can cause a great deal of caretaker stress and an increased risk of depression.[7]

Alzheimer's Disease Pathophysiology

Neuritic plaques are formed when amyloid protein undergoes incorrect enzymatic cleavage of the Amyloid Precursor Protein (APP), resulting in a fibrillar form of amyloid protein known as beta-amyloid.[8] In comparison, neurofibrillary tangles are formed when the microtubule associated protein tau becomes hyperphosphorylated, misfolds and begins to aggregate with other mis-folded tau proteins resulting in the neurofibrillary tangles.

Although plaques and tangles are hallmark characteristics of Alzheimer's disease, they do not form immediately. Rather they progress insidiously for years before a diagnosis is made. The progression of Alzheimer's disease begins with accumulation of beta-amyloid plaques years to decades before symptoms occur, resulting in synaptic dysfunction[7]. Tau proteins then begin to misfold, leading to injury within the neurons. The injured neurons then begin to atrophy and symptoms begin to arise, progressively leading to dementia.

Organ System disruptions related to Alzheimer's Disease

In addition to the biomolecular changes that lead to Alzheimer's disease, disruptions in other organ systems may lead to an increased risk of susceptibility. For example, disruptions in the endocrine system, specifically changes in estrogen levels, have been found to be associated with an increased risk of Alzheimer's disease. Estrogen

is a sex hormone produced in the follicles of ovaries and the corpus luteum/placenta.[9] Estrogen is also produced in smaller amounts in the liver and adrenal glands. Estrogen receptors (ER) are highly populated in the brain. For example, ER-alpha is located mainly in the hippocampus and ER-beta is located in the forebrain and cerebral cortex. Studies have shown that estrogen is beneficial in the protection of brain tissue, however, the mechanism has not been fully explained. Estrogen promotes the growth and survival of cholinergic neurons, increases cholinergic activity, has antioxidant properties, and promotes the metabolism of APP. APP is a precursor protein of beta-amyloid, the main component of amyloid plaques in AD. As a result, it is strongly suggestive that a decrease in estrogen results in a decrease in neuroprotection, thereby, increasing the risk of developing AD in the future.

Genetic Determinants

There are two different types of Alzheimer's disease, familial and sporadic. Familial Alzheimer's Disease is rare (approximately <1% of Alzheimer's Disease) and usually occurs within families due to dominant mutations in the genes Presenillin 1, Presenillin 2, and APP. Sporadic Alzheimer's disease is more common. There is no single causative gene for the disease but mutations in the Apolipoprotein (ApoE) gene can increase the risk of developing the disease. The ApoE gene is responsible for a protein that helps form lipoproteins. Lipoproteins are essential in packaging molecules, including estrogen, for transport throughout the body.[11] There are three alleles of the

ApoE gene: ε2, ε3, ε4. Those with the ε2 allele are more likely to develop Alzheimer's disease dementia later in life in comparison to those with the ε4 allele. The ε3 allele is the most common allele on the ApoE gene and is believed to have a neutral role in Alzheimer's disease dementia while the ε4 allele increases ones risk in developing AD with an earlier age of onset.

Gender

In addition to genetics, there is an increased risk of developing AD in women. Women have a 1/6 chance of developing AD in their lifetime compared to men who have a 1/10 chance.[12] More than half of the AD population in the U.S. are women. It is possible that women have an extended period of time in which they can develop the disease as they generally live longer than men and AD is an age-related disease. Thus, women may be more likely to develop Alzheimer's disease. However, the longevity of women is not the only reasoning behind their increased susceptibility to AD. Women with the ApoE-4 gene variant were found to have an even more substantially increased risk of developing Alzheimer's due to the fact that ApoE is involved with estrogen transport[13].

Diagnosis

The diagnosis of Alzheimer's disease has improved immensely since it was first discovered, primarily due to an increased knowledge of the clinical manifestations and the biology underlying the disease. The original criteria for AD diagnosis required extensive revision including that the histological changes associated with AD can be seen in people who are cognitively normal. In addition, the presence of Lewy bodies, vascular dementia, behavior variant fronto-temporal dementia, and primary progressive dementia were all omitted in the criteria due to the fact that these disorders could not be recognized when AD was first discovered[14]. There were also no CT, MRI, or PET imaging or CSF assays when AD was first discovered. Also noted in the original Alzheimer's disease criteria, memory impairment was the primary cognitive deficit and this has since been redacted. It is now understood that symptoms such as posterior cortical atrophy and primary progressive aphasia can occur before memory impairment. The genetics of AD, specifically APP, presenilin 1 and presinilin 2 gene mutations, were also not included in the original criteria. Therefore, it was evident that new criteria were needed for the evaluation and diagnosis of AD.

The National Institute of Aging notes that AD is ultimately diagnosed by examining the brain tissue in an autopsy, or biopsy in some cases, for areas of atrophy primarily near the hippocampus and the frontal temporal lobes. Physicians diagnose Alzheimer's disease by asking family members questions about the patient such as the status of their overall healthy, what prescriptions they have, any past medical problems,

any difficulty pursuing daily activities, or any changes in behavior or personality. Clinicians may also perform memory, problem solving, attention, or counting tests, as well as blood and urine screens, to exclude any other reasons for the current symptoms. Finally, physicians may diagnose their patients through brain scans. CT, MRI, and PET scans can be administered in order to assess for AD or exclude other possible causes for the patient's symptoms.

Comparing Imaging Modalities

When using an MRI to assess for AD, two factors can be detected.[15] First, atrophy can be assessed, as well as changes in the tissue that would cause visualized vascular damage as white matter hyperintensities in T2-weighted images. In terms of atrophy, the typical pattern that is seen in AD begins with the entorhinal cortex, followed by the hippocampus, amygdala, and parahippocampus. Because increased cerebral atrophy occurs early in the disease in severely affected individuals, MRI imaging can be a marker of disease progression and used as a potential outcome measure in trials. The main benefit of using an MRI is its availability. Both US and European guidelines recommend structural imaging such as MRI for the diagnosis of Alzheimer's, as well as other forms of dementias. The benefit of MRI in comparison to CT is that MRI does not require exposure to ionizing radiation, eliminating the risk of carcinogenecity. Because an MRI can measure atrophy, it can reflect cumulative neuronal damage and, therefore, is one of the more beneficial modalities of imaging when assessing and diagnosing AD. It should

be noted, however, that although atrophy is strongly correlated with cognitive decline, patterns of atrophy overlap with other diseases that are separate from AD. Additionally, MRI is limited in that it cannot detect the histopathological changes associated with AD, such as amyloid plaques or neurofibrillary tangles.

PET imaging in AD is generally used to identify synaptic activity. The brain relies exclusively on glucose as its source of energy. Therefore, using the glucose analog fludeoxyglucose (FDG) in a PET scan, one can appropriately evaluate brain metabolism when it is labeled with Fluorine-18. The resting activity of the brain requires the most energy, in which the highest amount of glutamaturgic synaptic signaling is found in the cortex. FDG uptake is strongly correlated with synaptic activity and, therefore, PET scans using FDG are widely accepted as a bio-marker for brain metabolism. In AD patients, hypometabolism characteristically occurs in the limbic and associated regions of the brain. AD patients have visibly low synaptic activity in certain regions under PET including: the posterior midline cortices of the parietal and posterior cingulate gyri, the inferior parietal lobule, the posterolateral portions of the temporal lobe, the hippocampus and the medial temporal cortices. Throughout course of AD, the metabolism of the brain decreases and bilateral asymmetry is commonly seen early on PET scans.

Once AD is established, deposition of amyloid plateaus but, along with cognitive function, FDG uptake continues to decline. High amyloid deposition in the parietal regions was found to be associated with FDG hypometabolism, which raises the possibility of local toxicity. This relationship between high amyloid deposition in the

parietal regions and FDG hypometabolism may be inaccurate, however. Studies have found this association to not be significant primarily because the amyloid burden seen in the subjects were already plateaued. In addition, this relationship was found to be weaker in the frontal regions of the brain in which the highest amount of amyloid is found. Additional research is needed to further investigate the relationship but FDG metabolism appears to change with amyloid build-up. In fact, this may illustrate an intermediate stage between the beginning of pathological changes and the development of degeneration, as well as synapse disruption.

FDG PET scans have become a popular marker of neurologic dysfunction in AD. FDG PET scans are valuable in two instances: when confounding incidences, such as frontotemporal lobar degeneration (FTLD) occur, FDG PET scans can alter an ambiguous AD diagnosis to a FTLD diagnosis; and when hypometabolism occurs prior to the presence of cognitive changes, FDG PET can predict the rate at which cognition decreases in the future. FDG PET scans are clearly beneficial, however, the limitations of these scans are largely due to their price and limited availability. In addition, these scans are further limited because they require IV access and expose patients to radioactivity despite it being low-risk.

Amyloid PET scans are used to reflect beta-amyloid pathology but do not reflect a clinical diagnosis. The substrate for all known beta-amyloid tracers is fibrillar beta-amyloid specifically in the beta-sheet confirmation. When discussing beta-amyloids, it's important to differentiate between fibrillar and nonfibrillar forms and to avoid using

terms based on visual descriptions due to the differing amount of beta-amyloid seen in the visually descriptive plaques. Visually descriptive plaques such as compact, cored, and neuritic plaques have larger amounts of amyloid compared to fleecy and amorphous plaques. There may be an association between the amount of signal in an amyloid PET scan and the concentration of amyloid oligomers. The amyloid PET scan is beneficial because it allows for definitive proof of beta-amyloid accumulation in areas of the brain (Figure 1).

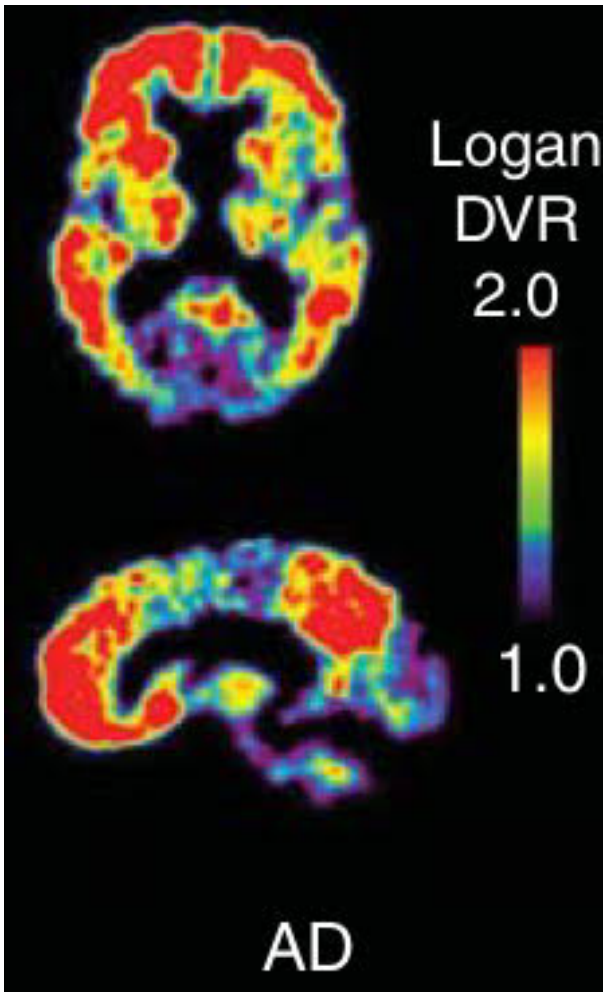


Figure 1: PiB PET image of AD subject showing heavy beta-amyloid deposition[15].

Beta-amyloid PET scans are extremely useful in diagnostics. A study performed by Johnson et. al in 2012 found that, in 17 research groups groups in which an amyloid PET scan was performed on clinically diagnosed AD patients, 96% were found to be positive for a heavy amyloid burden using the diagnostic criteria as the gold standard.[15] Thus, those 4% of amyloid-negative patients may have been misdiagnosed as having AD. Alternatively, the scans may not have been sensitive enough and eventually the patients who were amyloid-negative would become positive over time. However, follow-up of the amyloid-negative subjects found that none became positive over 5 years and, therefore, sensitivity was not the cause for the scans providing amyloid-negative results. Additionally, in the case of diseases similar to AD such as MCI, nine studies provide results in which MCI patients were scanned and found to be amyloid positive. Given that, it's apparent that the most prevalent use of amyloid PET scans would be in elderly patients that have normal cognitive function in which cerebral beta-amyloidosis could be detected in the "invisible stage." By discovering cerebral beta-amyloidosis in asymptomatic patients, it allows more opportunity for providing treatment. Similar to the FDG PET scan, the major limitation of amyloid PET scans are pricing and availability. When comparing the amyloid PET scan to other imaging modalities such as an MRI or FDG PET, it provides a definitive diagnosis in which it can clearly show that beta-amyloid is present, in comparison to MRI and FDG PET scans which are not as specific.

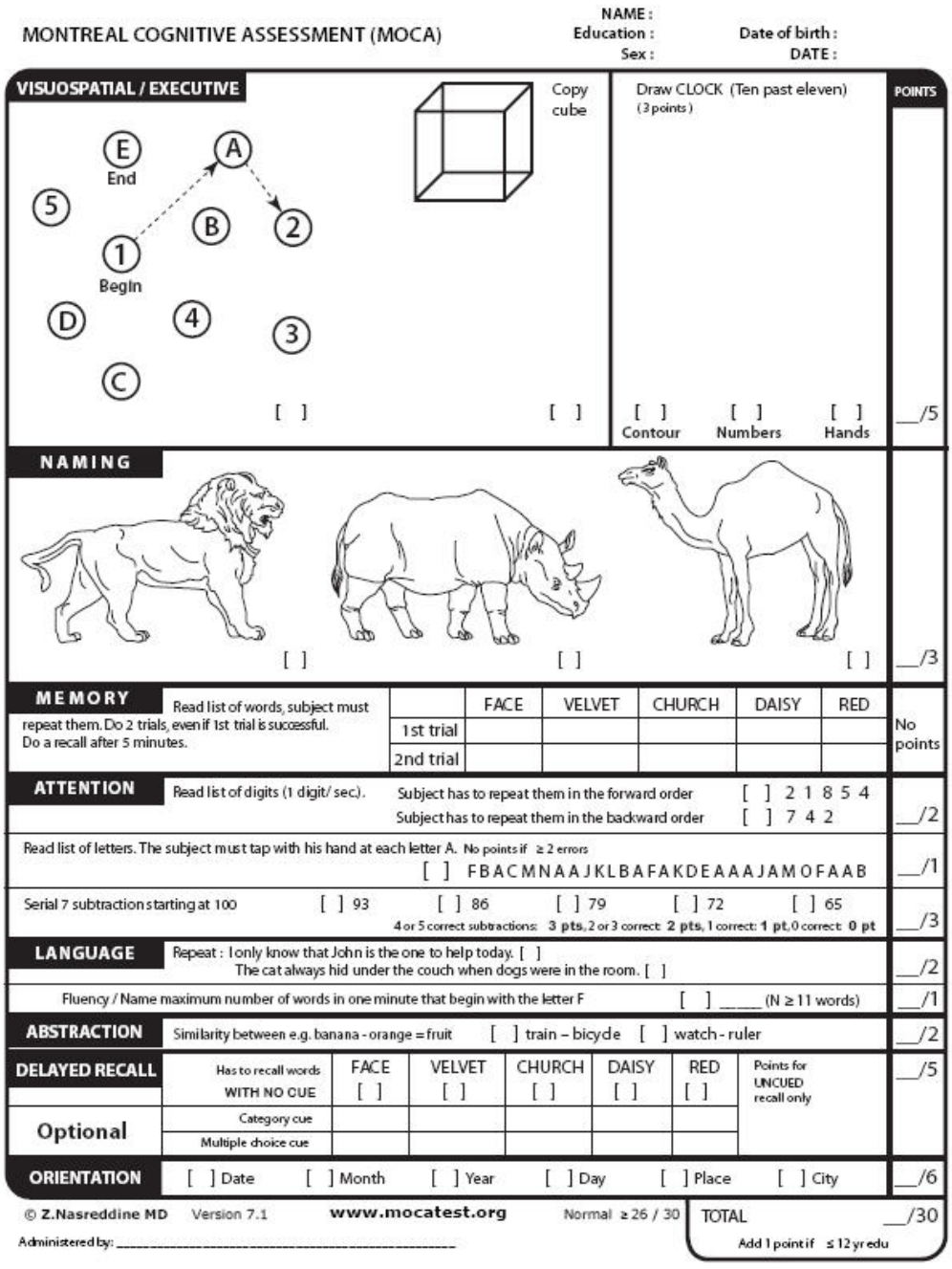
In addition to amyloid PET scans, cerebral spinal fluid (CSF) biomarkers can be used in AD diagnosis. Decreases in beta-amyloid in CSF occurs as early as, if not earlier than, changes seen in the amyloid PET scan and in those that are pre-symptomatic.

Differences in amyloid levels in CSF and using a PET scan appear to be insignificant, as they both appear useful in assessing for beta-amyloid accumulation. CSF measurements are advantageous in comparison to amyloid PET scans because they are not as expensive and can provide similar information on whether or not beta-amyloid is accumulating within the brain. However, because amyloid PET scans are more dynamic and correlate directly with beta-amyloid load, they appear to be the superior method. In addition, a major limitation to assessing amyloid levels in CSF is that the levels are correlated strongly to only brain regions adjacent to CSF spaces compared to amyloid PET scans which cover all brain regions. However, if one needs to only determine the presence of beta-amyloid within the brain, measuring levels using CSF can be more cost efficient.

Assessment

In order for a patient to be diagnosed with Alzheimer's disease, their cognitive function must be assessed using formal testing protocols such as the Montreal Cognitive Assessment (MoCA) or detailed neuropsychological testing. Screening protocols such as the MoCA examine a patient's orientation, attention and working memory, episodic memory, language, spatial awareness, and executive function (Figure 2). A MoCA score <26 suggests cognitive impairment in many patients. In some patients, especially those who are cognitively high functioning, scores above 26 can occur when the patient has diagnosable AD. In these cases, more detailed neuropsychological assessment is necessary to determine whether cognitive impairments exist. Once the patient is

determined to be cognitively impaired, reversible causes of cognitive impairment, including B12 or folate deficiency, thyroid dysfunction, depression, and medication side effects, must be ruled out via blood test.



MONTREAL COGNITIVE ASSESSMENT (MOCA)

NAME :
Education :
Sex :
Date of birth :
DATE :

VISUOSPATIAL / EXECUTIVE — POINTS

Copy cube

Draw CLOCK (Ten past eleven) (3 points)

E End, A, 5, B, 2, 1 Begin, D, 4, 3, C

[ ] [ ] [ ] Contour [ ] Numbers [ ] Hands __/5

NAMING

[ ] [ ] [ ] __/3

MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION Read list of digits (1 digit/ sec.).

Subject has to repeat them in the forward order [ ] 2 1 8 5 4

Subject has to repeat them in the backward order [ ] 7 4 2 __/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors

[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B __/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt** __/3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]

The cat always hid under the couch when dogs were in the room. [ ] __/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] ____ (N ≥ 11 words) __/1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler __/2

DELAYED RECALL

	FACE	VELVET	CHURCH	DAISY	RED	
Has to recall words WITH NO CUE	[ ]	[ ]	[ ]	[ ]	[ ]	Points for UNCUED recall only
Optional Category cue						
Optional Multiple choice cue						

__/5

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City __/6

© Z.Nasreddine MD Version 7.1 www.mocatest.org Normal ≥ 26 / 30

TOTAL __/30

Add 1 point if ≤ 12 yr edu

Administered by: _____

Figure 2: MoCA test courtesy of mocatest.org

In addition to assessing cognitive function, patients with suspected Alzheimer's disease must be assessed for functional independence. Often the Functional Activities Questionnaire is used.[16] The questionnaire contains 10 questions that determine whether the patient can perform various daily tasks, such as paying bills, shopping, organizing their home, and understanding current events. Each question is scored from 0 to 3. A score of 0 indicates that the patient has difficulty performing the action but can do it by themselves. A score of 1 indicates that the patient requires assistance to perform the task. A score of 3 indicates that the patient is completely dependent on someone to assist them. Any total score greater than or equal to 9 qualifies the patient as impaired.

Treatment

There are four current medications for treating those diagnosed with Alzheimer's disease:[17] Donepezil, Galantamine, Rivastigmine, and Memantine. These medications, however, provide only symptomatic relief for a limited duration and are not curative[17]. Cholinesterase inhibitors such as Donepizil, Galantamine, and Rivastigmine prevent the breakdown of acetylcholine, a neurotransmitter which is involved with functional learning and memory. These medications are generally administered with Memantine, an N-Methyl-D-aspartic acid (NMDA) receptor antagonist, which blocks the toxic effects associated with excess glutamate often found with cholinesterase inhibitor use. NMDA

receptor antagonists regulate glutamate, thereby reducing the chance of a chronic increase in calcium, which has been associated with neuronal injury.

In addition to medication, Alzheimer's disease patients are also advised to make lifestyle changes in order to reduce the progression of the disease. These lifestyle changes include increasing cardiovascular exercise, maintaining a Mediterranean diet, and trying various new complex projects to stimulate cognitive function.

Pesticides related to Alzheimer's disease

AD does have a genetic susceptibility seen with familial Alzheimer's, however, these cases make up <1% of the diseases' incidence. The majority of Alzheimer's disease cases occur sporadically or because of exposures or other events occurring during the patient's lifetime. A possible cause includes exposures to certain neurotoxicants throughout their lifetime. An important example of these neurotoxicants are Dichlorodiphenyltrichloroethane and dicofol.

Dichlorodiphenyltrichloroethane (DDT) is a pesticide first used on crops in the United States during the 1940s around World War II, in order to reduce the incidence of malaria and typhus in the United States. Before it was banned, DDT was one of the most popular pesticide due to its low cost and effectiveness. Approximately 1,350,000 pounds of DDT was used before it was banned, according to the Environmental Protection Agency.[18] DDT belongs to a group of pesticides, organochlorines, that have very low

solubility in water and very high fat solubility, thereby increasing resistance to degradation while allowing the insecticide to move freely through the axonal membrane. The active structure of DDT involves phenyl rings connected by a mono-substituted methylene bridge ensuring stability in its active state.

DDT's mechanism of action on insects involves preventing axonal sodium gates from closing when the axonal membrane has been depolarized in the peripheral nervous system. As a result, an excess of sodium ions flows through the axon and causes hyperexcitability throughout the axon, leading to neurotoxicity. Because axons are hyperexcited in the setting of DDT exposure, common symptoms seen with DDT toxicity are body tremors, vomiting, and convulsions. According to the National Pesticide Information Center, DDT is broken down within the body into less toxic metabolites, such as DDD, DDE, and DDA. These metabolites are slowly excreted through urine, feces, or breast milk. In the setting of starvation, DDT can be released from fat stores into the circulatory system, causing hepatotoxicity and neurotoxicity.[19]

According to the National Pesticide Information Center, DDT is moderately toxic to animals and humans; the LC50 for inhalation is between 0.2-2 mg/L. DDT exposure can be measured in blood or fat. The soil half-life of DDT can range between 2-15 years and as long as 150 years in an aquatic environment, thus making it very dangerous and persistent for aquatic wildlife. Because of its long half-life and availability to be built up in aquatic environments, fish began to accumulate amounts of DDT. Those fish were then eaten by predators who accumulated larger amounts of DDT until the primary predator

received the highest dose, therefore resulting in biomagnification. Eventually, due to the environmental and wildlife repercussions caused by DDT use, the EPA banned the substance in the United States in 1972.

Although DDT is banned in the United States, it is still considered a cost-efficient pesticide in lower income countries, such as those in Africa, South America, and Asia, which continue to use it to reduce the risk of malaria. In addition, contamination of soil and water in the U.S. through long-term DDT use have resulted in chronic exposure to the population despite the fact that it is no longer actively used. In terms of occupational hazards, farmers are considered to be those with the highest risk of exposure, thereby making them more susceptible to chronic diseases possibility associated with exposure, such as AD.[20]

DDT not only affects the nervous system; the organochlorine can also affect the endocrine and reproductive systems. Microvesicles, such as those containing DNA, RNA, lipids, and transcriptional factors, are used in communication between cells. DDT disrupts the thyroid by preventing the Thyroid Stimulating Hormone (TSH) receptor from attaching to the raft containing compartments of cells[21]; the TSH receptor is prevented from activating and becoming internalized. In addition, DDT can induce the formation of extracellular viscose that contains the TSH receptor and autoimmune responses against the receptor can develop, leading to Grave's disease. Although this appears unrelated to AD or neurodegenerative disorders, it is important to acknowledge the effects that pesticides can have on organ systems throughout the body.

Another study pertaining to the effects of pesticides on other organ systems includes a study performed by Jaga et. al investigating DDT's effects on estrogen receptors in the reproductive system[22]. The researchers note that because estrogen is more studied in females, they limited their scope to investigating DDT's relationship to breast tissue and the uterus. The researchers used an E-SCREEN assay on MCF-7 cells to measure proliferation of epithelial cells found in breast in uterine tissue. DDT was found to cause an estrogenic response in those exposed; DDT induced the formation of 16-alpha-hydroxyesterone, a derivative of estrogen, which has been found to be more estrogenic and genotoxic. These findings are important because a higher ratio between 16-alpha-hydroxyesterone and 2-hydroxyesterone has been found to be a risk factor for breast cancer. O,p'-DDT, a metabolite, competes with estrogen to bind the estrogen receptor (ER). A second mechanism could hypothetically cause a synergism between DDT and estrogen, allowing the substances to bind to two separate sites on the same ER and, thereby, enhancing the estrogenic response.

Dicofol is an organochlorine derived from DDT. Dicofol was developed in 1986 as an acaricide and used on a variety of produce and crops. The EPA temporarily banned dicofol in 1990 due to concerns that there was DDT contamination when processed.[23] Presently, dicofol is manufactured carefully to ensure that it contains less than 0.1% DDT. The structure of the acaricide is very similar to DDT, however, it has a hydroxyl functional group on C-1 in comparison to the hydrogen at that positione on DDT (Figure 3). Dicofol's mechanism of action is unclear, however, it is speculated to involve

overstimulation of peripheral nerves and previous studies have noted an association of the pesticide with endocrine system disruption.

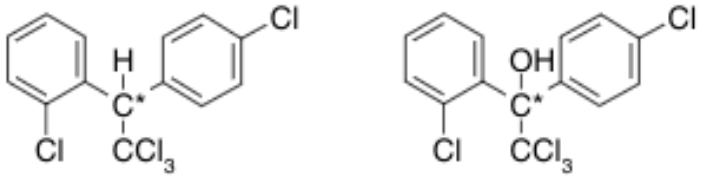


Figure 3: Chemical structures of o,p'-substituted DDT (left) and dicofol (right) (CAS No. 789-02-6 and 10606-49-9, respectively). Chiral carbon atom denoted by ''*". Courtesy of Hoekstra et al, 2006

Similar to DDT, dicofol is stored within fat and is excreted through feces. The LC50 of dicofol in rats was noted to be >5mg/l for 4 hours.[24] Dicofol is considered to be moderately toxic to humans, causing symptoms ranging from nausea and vomiting to fatigue with respiratory exposure to severe cases of convulsions and death from respiratory failure. The WHO reported that the soil half-life of dicofol is approximately 60 days. However, it is very persistent in water, thereby making aquatic wildlife susceptible to toxicity. In addition, dichlorobenzophenone (DCBP), a degradation product of dicofol, has also been attributed to its toxicity. DCBP is similar to the DDT metabolite DDE and works similarly to DDE; they have both exhibited antiandrogenic properties in African green monkey kidney cells (COS-7) and in human mammary carncinoma cells (T47D).[25] Therefore, if dicofol and DCBP both have antiandrogenergic properties, they could potentially reduce the amount of estrogen in the body and, thereby, reduce

neuroprotection in the nervous system. This decrease in neuroprotection could result in an increased risk for developing Alzheimer's disease.

Existing research

The relationship between organochlorine pesticides, such as DDT, and nervous system function has been examined extensively. Multiple studies have found that long-term exposure to DDT increases the risk of developing neurodegenerative disorders, including AD. However, very little is known about pesticides similar to DDT. Dicofol was used worldwide after DDT was banned because it was thought to be a non-toxic replacement. However, very little is known about a possible relationship between dicofol exposure and risk for developing AD. Because dicofol may affect the endocrine system in ways that are similar to DDT, it may also damage the nervous system years after exposure. Therefore, investigation into the association between long-term exposure to dicofol in populations, such as agricultural workers or pesticide sprayers, and their risk of developing AD is warranted. It should also be noted that unfortunately in most of the studies listed, most sprayers sprayed multiple pesticides thereby causing it to be very difficulty to identify each pesticide's individual contribution.

Acute Neurologic Effects of DDT exposure

Although DDT was banned in the U.S. during the early 1970s, countries continued to use the pesticide because they felt that the benefits in malaria control outweighed the environmental and health risks. Misra et al, examined the effects of DDT exposure on the cognitive functions of agricultural workers in India who continued to use

the substance despite it being banned in higher economic countries.[26] Workers who sprayed DDT on crops for at least a year and had sprayed DDT at least a week before testing (N=29) were recruited for the study. Each subject recorded a detailed history of their diet and drug intake to rule out any extrinsic factors that would negatively affect nervous system function. They underwent a physical, neurological, and psychiatric exam. The intellectual capacity of each subject was determined using a combination of the Koh's block design test, the Passalong test, immediate memory tests, picture completion and pattern-drawing test, memory function tests, and Bender Gestalt Test (BGT) visual motor exam. This combination of assessments was known as the Bhatia battery performance test. Nine of the subjects who had an abnormal Bender Gestalt score underwent an electroencephalogram (EEG). To measure DDT exposure, serum samples were obtained from each of the 17 sprayers and 12 controls and assessed using gas liquid chromatography for a total of 29 subjects. Any subject with evidence of malnutrition was excluded from the study. In addition to these 29 workers who sprayed 5% DDT year-round without the use of protective equipment or face masks, the investigators identified 26 healthy individuals who had not been exposed to pesticides for controls and were matched based on age, sex, and socioeconomic status.

The exposed group had an average age of 39.3 years and were exposed to DDT on average for 12.7 years. Despite the chronic exposure, those that sprayed DDT considered themselves healthy with mild cognitive symptoms that didn't interfere with daily activities. Within the exposed group, 27.5% reported psychological symptoms, such as irritability, anxiety, forgetfulness, and depression, and 10.3% reported worsening

symptoms following spraying. Additionally, 24% of exposed workers noted that they had "soft" neurological signs, such as hyperactivity of deep tendon reflexes, tremors, fasiculations, and loss of reflexes.

Those that sprayed DDT had higher BGT scores than the controls in both age categories ($P<0.1$ below 40 years, $P<0.05$ above 40 years, mean serum DDT in sprayers=0.212 ppm and in controls 0.007 ppm, BGT scores in sprayers below 40 years=58.54, BGT scores in controls below 40 years=38.50, BGT scores in sprayers above 40 years=64.56, BGT scores in sprayers below 40 years=40.50). Of the nine workers with abnormal BGT scores, 55% showed significant EEG changes, including decrease in alpha activity, low voltage record, asymmetry. Serum DDT levels in the exposed group showed a definitive correlation with the duration of exposure ($r=0.32$, 8.5x higher levels DDT compared to controls) but there was a higher correlation found between BGT scores and DDT levels ($r=0.46$).

This study is informative as it was one of the first to assess effects of occupational exposure to DDT. The study does have limitations, however. Because of the small sample size (n=29) and the recruitment of only male subjects leads to the question of the effects of DDT exposure on women and, thereby, revealing the need for more research with a larger, diverse study population. A small sample size in this study is an issue because it increases the risk of a Type II error. In addition, the study notes that the subjects were exposed to other pesticides besides DDT such as BHC and Malathion in small quantities. Even though the study notes the exposures were in small quantities, it could potentially skew the results of the study. It should also be noted that the study does not investigate

DDT-related pesticides or DDT analogues, such as dicofol, thereby requiring further research into their association with neurological effects.

Chronic effects of DDT exposure on the nervous system

Van Wendel et al. investigated the correlation between long-term exposure to DDT and nervous system outcomes in older populations.[30] Because the researchers wished to study a population known to have long-term exposure to DDT, they recruited former malaria-control workers with known pesticide exposures in Costa Rica and assessed CNS function utilizing their neurobehavioral outcomes. They hypothesized that, in comparison to a control group, those exposed to DDT for a long period of time would have a significantly worse neurobehavioral performance.

The register of the Ministry of Health in Costa Rica was used to recruit 59 retired men ranging in age from 55-70 who had been exposed to DDT between 1955 and 1996. Only those that had been exposed to DDT for a minimum of 2 years prior to the study were included. The control group was comprised of 64 male retired guards and drivers of similar age and educational attainment as the exposed group. Of those approached for consent, 36 men from the exposed group and 31 men from the control group agreed to participate in the study. Exclusion criteria for the case group included previous epilepsy diagnosis, cerebral hemorrhage diagnosis, prior diagnosis of intoxication of a cholinesterase-inhibiting pesticide or recent exposure to a pesticide. The study was unspecific as to how long since the subjects were exposed to the pesticide. Exclusion criteria for the control group included Parkinson's disease diagnosis, childhood spastic

paralysis diagnosis, and previous DDT or cholinesterase inhibiting pesticide exposure. Each subject underwent clinical, neuropsychological, and psychiatric assessments. Neurobehavioral assessment outcomes (N=17) were used to assess their cognitive, motor, and sensory functions. In addition, the subjects were divided into high, medium, and low exposure subgroups. Odds ratios were calculated using multiple logistic regression and adjusted for potential confounders, such as age, history of loss of consciousness, visual acuity, and smoking habits.

Participants with long-term DDT exposure performed more poorly than the control group (up to 20% decrease in mean performance for cognitive sequencing, trails-A at 95% CI). In the high exposure subgroup, scores were significantly poorer on outcomes that assess visual-motor speed and verbal attention. In addition, the high exposed group reported significantly more neuropsychological and psychiatric symptoms than the control group (OR=3.98 CI at 95% 1.02-15.63). Therefore, the tests in cognitive, motor, and sensory domains show a significant exposure-effect relationship with DDT.

This solidifies previous research on the effects of DDT on neurobehavior, while demonstrating that DDT has chronic, as well as acute effects on the nervous system. It is limited, however, similar to the previous study in that only men were selected for this study. The study also suffers a potential assembly bias as it did not detail specifically how the DDT exposure was measured.

Assessing Occupational Exposure and the risk of developing AD

A recent meta-analysis published in January 2019 by Gunnar Gunnarsson et. al investigated a correlation between pesticide exposure and Alzheimer's disease as well as other neurodegenerative diseases, such as Amyotrophic lateral sclerosis (ALS) and Parkinson's disease.[31] The researchers applied the Meta-analysis of Observational Studies in Epidemiology (MOOSE) and Grading of Recommendations, Assessment, Development and Evaluations (GRADE) guidelines to conduct a meta-analysis.

The authors identified 31 studies that pooled indicated a 50% increased overall risk of developing AD, ALS and PD following pesticide exposure (weighted RR for AD = 1.50; 95% CI 0.98-2.29).

The study was limited due to a potential publication bias and because they did not specify which pesticides each case had been exposed to, what dosage were they exposed to or the exposure length. These factors could influence the results, as some pesticides such as DDT are known to causes neurodegenerative changes, while others are known to be benign. Therefore, the results may be skewed depending on which pesticides the subjects were exposed to. In addition, the length and intensity of pesticide exposure could affect the risk of developing neurodegenerative disease. Therefore, by not distinguishing the length of exposure, the results could again be biased. It also did not specify which pesticides were related to development of specific neurodegenerative outcomes and is not informative for specific contributions of DDT or dicofol to the development of a neurogenerative disorder.

The risk of AD in the setting of ApoE genotyping and pesticide exposure

Richardson et al. (2004) conducted a case control study investigating the association between developing AD and elevated serum dichlorodiphenylethylene (DDE) levels, while also determining whether ApOE genotype modifies susceptibility to developing AD in this association.[32] DDE is a metabolite of DDT that was used as a replacement for DDT in many countries. The researchers also aimed to investigate the relationship between DDT/DDE exposure and APP accumulation.

Serum samples were obtained from Alzheimer's Disease Research Centers at the University of Texas Southwestern Medical Center and Emory University. The samples were from control patients and patients with AD who were diagnosed in the centers between 2002 and 2008. Inclusion criteria for the control group consisted of a Mini - mental Status Exam (MMSE) score between 28-30, no abnormalities in brain structure determined by MRI and/or a normal neurologic exam. AD cases met NIH and the Alzheimer's Disease and Related Disorders Association criteria based on either their clinical exam results and MRI imaging or laboratory assessments that would rule out other medical causes for their dementia. Serum samples and genotyping were taken at enrollment when the MMSE was administered.

In total, 79 controls and 86 AD samples were collected from prior studies and across the two sites, including 94 women and 71 men. In order to determine the correlation between serum DDE levels and brain DDE levels, researchers obtained 11 autopsy samples from patients who died at an average age of 85.7 years from the AD Center at Washington University. Before dying, these patients were diagnosed with AD

using the National Institute of Deurological and Communicative Disorder-Alzheimer's Disease and Related Disorders association criteria with histological confirmation of AD via brain autopsy. Serum samples were collected approximately 193 days before death and the brain samples were collected 12 hours postmortem.

The ApoE genotyping was determined using TaqMan PCR.

Serum DDE levels were assessed using gas chromatography/mass spectrometry and were expressed in the context of free cholesterol levels with a detection limit of approximately 100 pg/mL. To determine the pesticide concentration in the collected brain tissue, 150mg of temporal cortex analytes were analyzed via gas chromatography/mass spectrometry.

To investigate the association of APP levels with in vitro exposure to DDT/DDE, differentiated neuroblastoma cells (SH-SY5Y cells) were used. The cells were exposed to DDT or DDE for 48 hours, washed, and fixed in paraformaldehyde. The cells were then incubated using primary antibodies against APP and MAP2 and then fluorescently labeled with secondary antibodies. Microscopic images using a fluorescent illuminator were taken. The mean density/intensity (SEM) was then calculated 3 times.

Unconditional logistic regression controlling for age, sex, and location controls was used to calculate the ORs and the 95% CIs for the association between serum DDE levels and AD diagnosis in both UTSW and Emory locations. To determine the probability of having AD and to evaluate a decrease in MMSE score per tertile of DDE level in a study population, while controlling for age, sex, race/ethnicity, education, APoE genotype and location, the researchers used generalized estimating equations. To

determine whether the ε4 allele of the ApoE genotype modified the association between DDE levels and MMSE scores, the researches stratified the data by genotype or used generalized estimating equations to create an interaction model.

DDE concentrations were found in 70% of the control group and 80% of AD cases with a mean structural equal modeling (SEM) of 3.8x higher in the AD cases than in the control cases (2.64 ng/mL cholesterol, $P<0.001$). In the AD samples, no other organochlorine pesticide was found to be elevated besides DDE when compared to the controls. When neuronal cells were exposed to DDT or DDE for 48 hours, the APP levels increased by approximately 50% (confirmed using fluorescent microscopy at 20x magnification), indicating an accumulation in amyloid protein and supporting the theory that exposure to the pesticide leads to an increase in APP, a protein well known to be found in excess in AD patients on autopsy. In addition, the researchers found that the presence of the ApOE ε4 allele was not associated with an increased AD diagnosis (OR, 3.70; 95% CI, 2.97-4.60; $P < 0.0001$) therefore they were unable to justify that the ApOE ε4 allele would modify the association between exposure and developing AD. In addition similar ORs were observed (OR, 3.60; 95% CI, 1.23-10.57; $P < 0.001$) when non detects were assigned to a value of zero.

The relatively large sample size for this study and use of two locations geographically are strengths of this study. Because the levels of DDE mimic the levels found in the most recent National Health and Nutrition Examination Survey, the results are likely generalizable to the U.S. population. The study is limited, however, in that only organochlorine pesticides were investigated and in that it excludes other known harmful

pesticide exposures, such as organophosphates. In addition, the researchers note that 17 of the 86 AD patients had levels of DDE that were undetectable, as well as control patients with extremely elevated DDE levels, therefore suggesting that DDE exposure may only be associated with AD progression in a certain number of cases or possibly due to unique genetics in certain individuals or other extrinsic factors.

Their findings present a significant association between exposure to DDE and AD, as well as demonstrating that ApoE alleles did not modify the association in this study, it's still a possibility that could be concluded with additional research. A possible underlying mechanism in the association between pesticide exposure and AD was identified, as APP levels were elevated when neuronal cells were acutely exposed to DDT and DDE. This research is important because, by determining a mechanism by which these pesticides can cause neuronal damage, treatment-involving inhibiting APP-cleaving enzymes could be considered for workers who have occupational exposure to DDE, in order to inhibit the buildup of APP and reducing the risk of neuronal damage.

Confirming the Association between DDT and AD

The hypothesis that pesticide exposure earlier in life may lead to later development of neurodegenerative disease has received considerable attention. A review of the literature on this hypothesis was conducted by Yan et al. (2016) who hypothesized that pesticides such as DDT are related to an increased risk of developing Alzheimer's disease due to their ability to cause mitochondrial and oxidative stress in neurons.[33]

In their systematic review, 1529 total articles were initially included, however, most were removed because they were either duplicates, not pertinent to the research, did not assess pesticide exposure, did not address AD as an outcome, or did not use a case control or cohort study design. The systematic review was narrowed to seven studies, four of which were case-control and three of which were cohort (Table 1). In total, 6,835 participants were included in the seven studies, of which 1,050 were AD patients.

Study	Design	Assessment of Exposure	Diagnosis of AD	Case	Control	Adjustments
Tyas et al	Cohort	Subjects self reported either being exposed vs. never being exposed (Prospective)	3MS for diagnosing cognitive impairment, NINCDS-ADRDA for AD diagnosis	36 patients (mean age: 79.8) with AD after 5 year follow up	658 controls (mean age: 73.7)	Age, education, sex
Baldi et al	Cohort	Subjects self reported either being exposed vs. never being exposed (Prospective)	3MS for diagnosing cognitive impairment, NINCDS-ADRDA for AD diagnosis	96 patients (mean age: 79.2) with AD after 5 year follow up	1507 controls (ages >67, mean age: 78.4)	Smoking status, education

Hayden et al	Cohort	Subjects self reported either being exposed vs. never being exposed (Prospective)	3MS for diagnosing cognitive impairment, NINCDS-ADRDA for AD diagnosis	344 patients (mean age: 74.1) after 7.2 year follow up	3,048 people (mean age: 74.1)	Age, sex, education, mini-mental status score, ApoE4 status
Gun et al	Case-control	Respectively using proxy reports for job history and code into JEM	NINCDS-ADRDA was used for AD diagnosis	170 patients with AD (male mean age: 77.4 , female mean age: 77.1)	170 controls (male mean age: 77.1, female mean age: 76.7)	N/A
CSHA et al	Population-based case-control	Respectively using proxy reports for risk factor information	3MS for diagnosing cognitive impairment, NINCDS-ADRDA for AD diagnosis	258 patients with AD (mean age: 84.1) diagnosed within 3 years	353 controls (mean age: 79)	Age, sex, education, residence in community or institution
Gauthier et al	Population-based case-control	Respectively using proxy reports for job history and code into JEM	3MS for diagnosing cognitive impairment, NINCDS-	68 patients with AD ages >70 years	68 controls ages >70 years	Education, family history, ApoE4 status

			ADRDA for AD diagnosis			
French et al	Case-control	Respectively using proxy reports for risk factor information	N/A	78 male patients with AD	76 hospital controls	N/A

Table 1: Characteristics of the 7 studies used in the study by Yan et. al

In their meta-analysis, the authors used a fixed-effects model to calculate summary ORs and 95% CI. They found the OR for the relationship between pesticide exposure and development of AD to be 1.34 (95% CI: 1.08-1.75) suggesting that there was a positive association.

Pesticide exposure was associated with an increased risk of AD in all subgroup analyses, however, only half of the subgroups reached statistical significance. In the cohort studies, there was an increased odds of developing AD (OR=1.39, 95% CI=1.08, 1.75) in comparison to no significant increased odds of developing AD seen in the case control studies (OR=1.24, 95% CI=0.78, 1.97).

A strength of the study is that there appears to be no publication bias. An Egger's test and Begg's test was performed indicating no evidence of publication bias (Egger, P=0.66, Begg, P=0.81). However, the study also has many weaknesses in regards to bias. For example, because the patients with AD were selected from hospitals in the case-control studies, there is the possibility of an admission rate bias that would need to be investigated further. Another weakness of the study is due to the fact that only select

cohort studies exhibited a positive association between pesticide exposure and AD risk, while case control studies failed to show any association. Therefore, because cohort studies are generally preferred in investigating etiological relationships, such as AD and pesticides, recall bias may be present. The design of the study causes limitations. The study includes incidence of AD in association with pesticide use rather than the prevalence of AD, therefore excluding ecological studies, which may help support their theory. Another limitation to the study was that the case-control studies had a much smaller sample size. The smaller sample size reduces the statistical power in comparison to the cohort studies. This lack of statistical power indicates a need for a new study with a larger sample sizes to confirm their results or a larger sample size with a more common exposure. The smaller sample size may have also contributed to the lack of significance noted previously. They also didn't acknowledge which specific pesticides were being used as well as the length of exposure. In conclusion, a more extensive meta-analysis is warranted in the future due to the fact that a small number of identified studies were used (n=7).

This study suggests a positive association between AD and exposure to pesticides at a low dose for long periods of time. The authors hypothesized that low dose long-term exposure to pesticides such as paraquat, dieldrin, organochlorines, and organophosphates is a risk factor for developing AD, however, they noted that a larger sample size is required to validate a causal relationship.

Organochlorine Pesticides and decrease in cognition in the setting of the elderly

Because AD is largely a geriatric illness, a study performed by Kim et. al (2015) explored whether patients who had higher serum concentrations of organochlorines would have greater declines in cognition as they age than persons with lower exposures to organochlorines.[34] They based this hypothesis on the known persistence of organochlorines in the body due to their lipophilic properties, as well as their ability to easily cross the blood-brain barrier. The researchers noted that despite OC pesticides being banned decades ago, their liphophilic properties allowed them to be stored in adipose tissue. Therefore, those who were exposed may have experienced either leaching of OC from their own fat stores or from eating the fatty meat of animals previously exposed to OC pesticide resulting in increased OC pesticide concentrations.

Subjects were recruited using the National Health and Nutrition Examination Survey (NHANES), which measured serum OC levels in the 1999-2004 data collection and also their cognitive function aged 60-85 in the 1999-2002 data collection. The sample size of was 644. The Digit Symbol Substitution Test (DSST), a component of the Wechsler Adult Intelligence Test, was used to assess cognitive function. The DSST assesses visuospatial and motor speed-of-processing, has an executive function component, and is sensitive in measuring frontal lobe executive function. Patients were asked to draw symbols under a corresponding number in a period of 120 seconds with a max score of 133. Participants who were unable to complete the sample items did not continue testing.

OC concentrations were measured using venous blood samples and gas chromatography, as well as mass spectrometry for quantification. Six OC pesticides were

chosen (DDT, DDE, beta-hexachlorocyclohexane, trans-nonachlor, oxychlordane, and heptachlor epoxide). Leach OC concentration was divided by the total lipid value for standardization.

The DSST score and dichotomous DSST score (< 25% percentile) were used as study outcomes. The continuous measure represented the general populations DSST scores and the dichotomous reflected severe impairment. The first and second tertiles of pesticide exposure measure were combined to increase stability. Gender, race, ethnicity, level of education, income, smoking status, body mass index, presence of heart disease, diagnosed hypertension, and diagnosed diabetes were adjusted for in the study to prevent confounding. In addition, the results accounted for stratification and clustering in the NHANES to prevent any bias.

A significantly lower DSST score was associated with aging and the difference between the lowest and highest age quintile DSST scores were approximately 16 symbols correctly substituted. An absolute low cognition score (DSST score <25% percentile, $p < 0.01$) had a clear pattern of interaction with pesticide exposure, except in the cases involving DDT and DDE exposure. However, both DDT and DDE had an increased prevalence of exposure in the lowest quartile DSST score in all age groups ($p < 0.01$).

The pesticides beta-heachlorocyclohexane, trans-nonachlor, oxychlordane, and hepatachlor epoxide were found to modify the association between age and cognitive function in the elderly population in the U.S. The association between increased age and lower DSST scores strengthened as OC pesticide concentration in serum increased. Other studies suggested that intrinsic and extrinsic factors, such as gender, education, ApoE,

physical activity, or food intake, alter the decline in cognition with aging but these previous studies had not investigated the role of OC pesticides in that association. The interactions of DDT and DDE with age were not significant but were associated with a lower DSST score in all age groups suggesting that DDT's effect on cognitive function does not involve age.

The study was limited because length of OC exposure was not included, thereby preventing the study from separately exploring the association of acute and chronic OC exposure with DSST scores. Secondly, because the study was cross-sectional, attributing causality is difficult and, therefore, a prospective study would be more appropriate. Cognitive function was only evaluated using the DSST, thereby limiting the outcomes and revealing a need for a collection of tests to expand on other areas of cognition.

Dicofol's effect on the Endocrine System

Previous research had hypothesized that DDT and DDT-related pesticides, such as dicofol, affect estrogen levels. Zhuang et al (2012) investigated the differences in conformational changes between the estrogen receptors when bound with DDT analogs, whether these differences result in different physiological responses, and what molecular moiety of DDT and its analogs favor binding to the receptors[35].

The researchers performed twelve molecular docking (MD) simulations using the DDT analogues with human ERα and ERγ ligand binding domains (LBD) and the conformational changes of each LBD was studied. The structures of DDT and its analogs with the ERs were modeled using MD methods taken from X-ray crystal structures

provided by the Cambridge Crystallographic Data Centre. Each algorithm was performed 10 times. The structures of ERα and ERγ LBDs when in complex with DDT and its analogs were optimized using a MolProbity server.

In terms of analyzing the binding of each compound to an ER, they were able to simulate 4,500 conformations of ERα LBD and 5,000 ERγ LBD. After evaluating for structural stability of hydrogen bond formation, the investigators discovered that aromatic-aromatic stacking was occurring and proposed that it helped stabilize the molecules. In addition, the binding affinities for each pesticide with the ERs were increased.

They were able to observe the structural changes of DDT analogs when they bind to ERs resulting in different moieties such as the aromatic ring stacking resulting in an increase in both binding and affinities. It was suggested that because these pesticides are able to increase their binding and affinity to estrogen receptors, they are more likely to illicit endocrine disruption. Therefore, because DDT analogs could bind to ER in aromatic ring stacking, endocrine disruption would occur.

Research of dicofol on the nervous system

Unfortunately, the research on dicofol and its effects on the nervous system post-exposure is very minimal. When using PubMed.org, there were no articles found investigating dicofol and its effects on the nervous system.

Many studies listed above investigated different aspects of pesticide exposure and neurodegenerative disease. Researchers have investigated the acute and chronic

neurologic effects of DDT exposure, the risk of developing AD with occupational exposure, the risk of developing AD with pesticide exposure and a genetic predisposition, as well as the association between organochlorine pesticides and decrease in cognitive function. Although these studies are beneficial, they mainly focus on DDT in terms of pesticide exposure. The research on an association between DDT derivatives such as dicofol and the development of AD is scarce. Therefore, a proposed study in which those who were exposed to dicofol for an extensive period of time in the past should be conducted to determine exposures and investigate relationships between exposure concentrations and development of AD.

Chapter 3: METHODS

Study design

A retrospective cohort study of 1134 people consisting of pesticide applicators and their spouses will investigate associations between Dicofol exposure and risk of developing AD. The retrospective cohort design has two advantages: it allows an efficient means of examining an outcome that develops slowly over years and is effective for identifying rare exposures, such as Dicofol.

Study population and sampling

The study will sample participants aged 65 and older (probable age range 65-85) in the Agricultural Health Study (AHS), assembled in 1993-1997. Pesticide applicators from Iowa and North Carolina (N=57,310) were enrolled when they received or renewed their pesticide-use licenses, of whom 84% of eligible applicators completed an enrollment questionnaire.[38] Enrollees were sent home with an additional questionnaire to enroll their spouses with 32, 345 (75% of those where were eligible) enrolling in the AHS.

Inclusion criteria for this study include those that are currently >65 years old (per questionnaire data obtained at enrollment). Exclusion criteria include: history of head trauma, ischemic/hemorrhagic stroke, or medical record confirmation of diagnosis of any other neurodegenerative diseases (e.g., Parkinson's disease, Lewy Body Dementia, vascular dementia, or epilepsy).

A sample size of 1134 participants (n=567 in the exposed and non-exposed groups) is required to adequately power the study. This was calculated assuming an α of

0.05, a β of 0.2, a 1:1 recruitment of exposed to unexposed, risk of developing Alzheimer's in the unexposed group of 0.13 (calculated risk of developing AD over the age of 65 as supported in a study performed by Brookmeyer et. al, 2011) and an OR of 1.6 (calculated in a study performed by Kamel et. al, 2012 for another neurological disorder). Brookmeyer et al. investigated the national estimates of the prevalence of AD, concluding that at age of 70 years old, the prevalence is 0.13. Kamal et al. (2012) used a cross sectional analysis with the AHS participants to investigate if pesticide exposure was associated with development of with amyotrophic lateral sclerosis (ALS), finding that the OR for ALS diagnosis was 1.6. While ALS is much less common than AD, this should provide a conservative basis for determining sample size for AD.

For recruitment, 1500 Dicofol-exposed and unexposed participants from the AHS cohort will be approached to participate in the study in order to reach an appropriate sample size.

Exposure assessment

Half of the study sample will consist of pesticide applicators who reported being exposed to Dicofol on their enrollment take home questionnaire and the other half will consist of controls who did not report being exposed to Dicofol. This information will be obtained from the AHS "Take Home Applicator" data set (Agricultural Health Study, 1993).

Exposure or non-exposure to Dicofol will be based on participants' answers to questionnaires about this exposure completed in 1993-1996 when the cohort was

assembled. It will be characterized as a binary exposure variable (yes/no), as no exposure dosage or frequency data are available.

Participant assessments

The pesticide applicators and their spouses provided information on demographics such as chronic medical conditions, lifestyle (smoking history, alcohol and drug use etc.), specific pesticide use, agricultural hazards and practices through the self-administered questionnaires in the AHS (Agricultural Health Study, 1996; Alavanja et al., 1996). When approached about the current study, those agreeing to participate will complete updated questionnaires on these variables. Access to their electronic medical record data (EMR) will also be requested to allow exclusion of participants who meet exclusion criteria based on medical conditions.

Diagnosis of AD

AD diagnosis will be determined in two ways. If EMR data for a participant indicate she/he has been diagnosed with AD, the participant will automatically be deemed as having the disease.

Other participants will undergo a diagnostic assessment to determine whether diagnosis of presumptive AD is likely. The initial assessment will be conducted in the neurology study clinic and will include a neurological examination (consisting of assessing cranial nerves, gait, etc.) and use of the Mini-mental Status Examination (MMSE) to determine

likelihood of cognitive impairment consistent with AD. The clinic neurology specialist will assess the patient while considering their past medical history, a past surgical history, allergies, medications, and a social history. Participants identified through neurological examination and likely AD based on MMSE score will then undergo an Amyloid PET scan with study Radiology personnel in order to determine the presence of a heavy amyloid burden in areas such as the frontotemporal region of the brain, which has been shown to be associated with the diagnosis of AD. Criteria for deciding if there is enough amyloid burden will be decided by a licensed Radiologist. Participants who meet both MMSE cut-off criteria and amyloid PET criteria will be identified as AD cases.

MMSE

The MMSE is a 30-item screening examination of cognitive function that includes 5 domains: Orientation to Time, Orientation to Place, Immediate Recall, Attention, Delayed Verbal Recall, Naming, Repetition, 3-Step Commands, Reading, Writing, and Copying. These individual scores are added together for total score. Because MMSE scores vary by years of formal education, education-adjustment cutoffs for AD on the MMSE will be: 22 or below among participants who completed 7th grade or less; 24 or less for those with an education of 8th grade or high school (without graduating); 25 or less among participants with a high school diploma; and 26 or less for those with a college education or higher. Scoring below the education-adjustment cutoffs indicates the subject is possibly moderately impaired.

Recruitment

Patients chosen from the AHS will be approached primarily by research and administrative personnel associated with the study using a developed script. Those consenting to be enrolled will be sent a packet consisting of a questionnaire involving different aspects of their lifestyles, as well as general information on the study such as the goals. A written consent form will be included in the packet with a return address back to the study's personnel at one of the two designated sites.

The study will maintain two project neurology clinics located in Iowa (Lutheran Hospital in Des Moines) and North Carolina (Wakemed Raleigh Hospital in Raleigh). Because patients will likely have been treated by various hospitals in Iowa and North Carolina, the electronic medical records (EMR) for each participant will be obtained after acquiring the written consent originally sent to each participant. If their EMRs confirm that they have no history of diagnoses meeting exclusionary criteria, they will be asked to come to the neurology clinic in their respective state for assessment (unless the EMR confirms existing AD diagnosis).

Data analysis

A chi-square analysis of the data will be used to determine a possible association between exposure status and AD diagnosis. Using the chi-square test, the association between categorical variables (whether the participant had been exposed to Dicofol in the past and diagnosis of AD or negative) will be explored. The proportion of participants with a positive AD diagnosis independent of Dicofol exposure and the proportion of participants

with a positive AD diagnosis associated with Dicofol exposure will be identified. In addition, because smoking, high cholesterol, and alcohol use may act as a confounder for the risk of developing AD, they will be adjusted for through stratification. It should also be noted that age is also a potential confounder, in which if one group has a larger proportion of older participants in comparison to the other group; the data may be altered.

Timeline and resources

Because the study is a retrospective cohort study, the timeline for this proposal is divided into three phases and will cover a 1-year period.

Month 1-3

Phase one of the study will include IRB application and approval. Resources such as study personnel hired for the project and approved by IRB will be assembled to begin recruitment. EMRs will be reviewed for each consenting participant.

Month 4-8

Once the required sample size is met, the second phase will require those selected to come be assessed at their closest study neurology clinic site, which will be used as an additional resource. The study neurological site teams will include a board-certified neurologist, Neurology PA, or Neurology NP to conduct neurological examination procedures and MMSEs. All travel expenses will be covered and each participant will receive a $100 gift card for their participation as incentive.

Patients identified as likely AD through the neurological examination and MMSE will be undergo Beta Amyloid PET Scanning at a site staffed by radiologists and technicians associated with the study.

Month 9-12

Phase three of the study will consist of data analysis and preparation of publications.

Institutional Review Board

Participants are required to meet with a neurologist to assess cognition using the MMSE, as well as an Amyloid PET Scan to assess for Beta-Amyloid accumulation in the brain. Because those included in the AHS will need to be contacted and assessed in-person, the AHS data used will need to be re-identified. In addition, data will be collected through personal interaction with the participants by study personnel. Therefore, an IRB application would need to be submitted. The IRB proposal would likely be an expedited review. The expedited review would specifically be in the Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving x-rays or microwaves. category. The IRB approval must be obtained before the study begins.

LIST OF JOURNAL ABBREVIATIONS

Alzheimers Dement	Alzheimer's & Dementia
Cold Spring Harb Perspect Med	Cold Spring Harbor Perspectives in Medicine
Curr Alzheimer Res	Current Alzheimer Research
Curr Neurol Neurosci Rep	Current Neurology and Neuroscience Reports
Environ Health	Environmental Health
Environ Health Perspect	Environmental Health Perspectives
Ind Health	Industrial Health
Int J Environ Res Public Health	International Journal of Environmental Research and Public Health
Interdiscip Toxicol	Interdisciplinary Toxicology
J Endocrinol Invest	Journal of Endocrinological Investigation
JAMA Neurol	JAMA Neurology
Med Hypotheses	Medical Hypotheses
Nat Rev Endocrinol	Nature Reviews. Endocrinology
Pharm Res	Pharmaceutical Research
Reprod Biol Endocrinol	Reproductive Biology and Endocrinology
Reprod Toxicol	Reproductive Toxicology
Sci Rep	Scientific Reports

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