Exh D

Greenamyre

I. QUALIFICATIONS

My name is John Timothy Greenamyre, MD, PhD. I am a board-certified neurologist, movement disorder specialist and internationally recognized neuroscientist. I am Professor and Vice-Chair of Neurology at the University of Pittsburgh, where I am also the Director of the Pittsburgh Institute for Neurodegenerative Diseases. I have been listed one of the Best Doctors in America since the 1990s. I was selected by my peers worldwide to serve as the Chair of the prestigious Gordon Research Conference on Parkinson's Disease in 2019. I am Chair of the Scientific Advisory Board (SAB) of the Parkinson's Foundation and serve on the SABs of the Michael J Fox Foundation and the American Parkinson Disease Association. I am Editor-in-Chief of the highly respected scientific journal, *Neurobiology of Disease*, which handles 1200-1400 manuscript submissions per year. I have authored about 200 publications, more than half of which deal with Parkinson's disease, and which have garnered more than 30,000 citations by other authors. I have delivered more than 200 invited lectures, both nationally and internationally.

I have been asked by plaintiffs' counsel to provide my opinion as to whether occupational exposure to the pesticide paraquat caused or substantially contributed cause to the development of Parkinson's disease in Jerry Mills, Ronald Niebruegge, Charles Schmidt and Carroll Rowan, who I understand are Plaintiffs in this action.

II. METHODOLOGY AND OPINIONS

To evaluate this question, I engaged in what is known as a differential etiology -- a process generally used by physicians to determine and then treat the most likely cause of a person's disease. In short, all plausible causes of the disease are considered, and then, based on the particulars of the patient's circumstances, they are ruled out, one-by-one, until the most likely cause is left. Here, the potential causes or risk factors for Parkinson's disease include genetics, advancing age, head trauma and exposure to chemicals, namely pesticides.

Because the issue in this case is whether the pesticide paraquat, specifically, played a causative role in Plaintiffs' developing Parkinson's disease, I analyzed at length the reasons why it would be appropriate to consider paraquat as a plausible cause of Plaintiffs' Parkinson's disease. It is my opinion, to a reasonable degree of scientific and medical certainty, that exposure to paraquat can contribute to the development of Parkinson's disease generally. It is my further opinion that Plaintiffs' exposure to paraquat during their decades spraying the pesticide substantially contributed to his development of the disease. To assess the above considerations, I reviewed the relevant literature regarding the association between paraquat exposure and Parkinson's disease, including the animal studies and epidemiological evidence. I reviewed the Plaintiffs' medical records and his deposition. I examined Plaintiffs and interviewed them in order to make a qualitative assessment of their paraquat exposure to determine if it put them at increased risk of Parkinson's disease. I also considered the latency period between their exposure and diagnosis (and probable onset of disease) to assess the extent to which the exposures of concern played a probable role in the development of their Parkinson's disease.

III. INTRODUCTION TO CLINICAL PARKINSON'S DISEASE

A. General Aspects

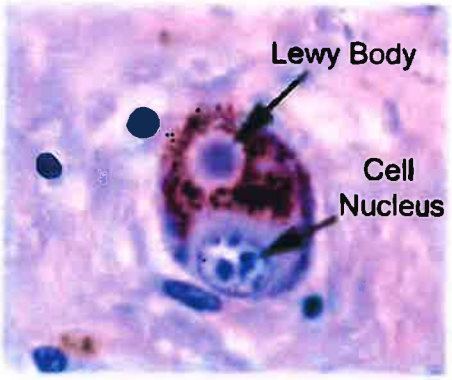
Parkinson's disease (PD) is a chronic, inexorably progressive, ultimately fatal, neurodegenerative disease that affects about 1 million people in North America. It is the fastest growing neurological disorder in the world[1].

Clinically, PD is characterized by **tremor at rest** (shaking), **rigidity** (increased muscle tension on examination), **bradykinesia** (slowed movements) and **postural instability** (balance problems). Other motor problems include: loss of facial expression, shuffling gait, reduced arm swing when walking, stooped posture, soft voice, small handwriting. There are also non-motor symptoms including loss of sense of smell, constipation, REM sleep behavior disorder, depression, anxiety – and in later stages, dementia and psychosis (with hallucinations)[2].

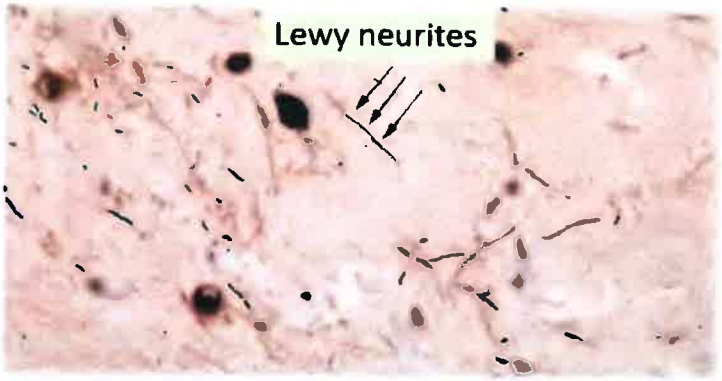
PD affects many parts of the nervous system, but the brain is particularly vulnerable. One of the pathological hallmarks of PD is the degeneration of a small group of nerve cells, called dopaminergic neurons, deep in a brain region called the substantia nigra. These cells produce a chemical neurotransmitter called dopamine that the brain uses to control motor function. As these neurons degenerate, the symptoms of PD emerge.

Another pathological hallmark of PD is the presence of Lewy bodies (Figure 1, left panel)[3] and Lewy neurites (Figure 1, right panel)[4] in the remaining neurons of the brain. This Lewy pathology consists primarily of a build-up of insoluble clumps of a protein called alpha-synuclein[5]. The abnormal accumulation of alpha-synuclein leads to cellular dysfunction and degeneration. Mapping the distribution of alpha-synuclein protein pathology allows scientists to determine which brain regions are affected by the disease and how the disease spreads from one region to another.[6]

Figure 1



A Lewy body inside a cell



In the affected areas of the brain, aggregated alpha synuclein can be seen in the branches of cells. Alpha synuclein has been stained brown on a section of brain from a person with Parkinson's disease.

B. How PD symptoms begin and progress

Although current diagnostic criteria for PD depend on the motor symptoms described above (tremor, rigidity, bradykinesia and postural instability, etc.), there is now abundant evidence and consensus that there is a prodromal phase in which non-motor symptoms manifest before the eventual emergence of the classical motor signs of PD. For example, loss of the sense of small (anosmia) typically precedes the diagnosis of PD by years to decades in many cases[7]. This is consistent with pathological data showing accumulation of alpha-synuclein in the olfactory bulb of people in the earliest stages of PD before motor symptoms appear[8]. Similarly, a large subset of PD patients experience constipation for years or decades prior to being diagnosed[7] long before motor symptoms appear, there is pathological evidence of alpha-synuclein accumulation in the enteric nervous system, or the part of the nervous system that controls the motility (or movement) of the gastro-intestinal system[9] Lastly, a large fraction of PD patients has a history of physically acting out their dreams while sleeping (REM sleep behavior disorder or RBD) many years prior to being diagnosed with PD[10]. This is because they have abnormal alpha-synuclein deposits in regions of the brain that control sleep[11].

Years after the prodromal non-motor symptoms begin, the typical signs and symptoms of PD emerge. Usually, the initial symptoms are unilateral, meaning they manifest on only one side of the body at first. For many people, the initial symptom is an intermittent resting tremor in one of the hands, or the dragging of one of the legs, or the loss of normal arm swing while walking on one side of the body. This may be accompanied by a loss of facial expression and a general slowing of movements and of activities of daily living.

C. Diagnosis

There is no specific diagnostic test for PD. Instead, the diagnosis is based on the presence of typical motor symptoms and reinforced by a history of typical prodromal symptoms (anosmia, constipation, RBD). It is common for PD to be misdiagnosed (or undiagnosed), especially in its early stages, when symptoms are mild and nonspecific. Diagnostic certainty increases with duration of the illness and is further supported by a clear-cut response to dopaminergic medication, such as levodopa. The absence of such a clear-cut response to this kind of treatment is a sign practitioners use to rule out PD.

D. Other symptoms

PD is a progressive neurodegenerative disease meaning the symptoms worsen over time. A variety of symptoms, both "motor" and "non-motor" may develop over time:

Motor

- Decreased facial expression
- Decreased arm swing while walking
- Small handwriting
- Impaired fine dexterity
- Difficulty initiating or maintaining gait (gait freezing)
- Soft, hoarse speech

Neuropsychiatric symptoms

- Depression, apathy, anxiety
- Anhedonia – the inability to feel pleasure
- Attention deficit
- Hallucinations, illusion, delusions
- Dementia
- Obsessional behavior (often drug induced)
- Confusion
- Delirium (could be drug induced)
- Panic attacks

Sleep disorders

- Restless legs and periodic limb movements
- RBD
- Excessive daytime drowsiness
- Vivid dreaming
- Sleep-disordered breathing

Sensory symptoms

- Pain
- Paresthesia, or an abnormal sensation such as tingling, burning or numbness of the skin
- Olfactory disturbance (such as loss of sense of smell)

Autonomic symptoms

- Bladder disturbances – urgency, nocturia, frequency
- Sweating
- Orthostatic hypotension – which increases risk of falls
- Sexual dysfunction – hypersexuality (likely drug induced), erectile dysfunction
- Dry eyes

Gastrointestinal symptoms

- Drooling of saliva
- Ageusia (loss of sense of taste)
- Dysphagia/choking
- Reflux, vomiting
- Nausea
- Constipation
- Unsatisfactory voiding of bowel
- Fecal incontinence

Other symptoms

- Fatigue
- Blurred vision
- Seborrhea (dandruff)
- Weight loss

E. Staging of PD

Various clinical "staging" schemes have been developed to account for the fact that symptoms worsen over time. One of the most commonly used scales is the modified Hoehn & Yahr Scale[12], which only considers motor function:

Stage	Characteristics
0	No signs of disease
1	Unilateral involvement only; minimal or no functional impairment
1.5	Unilateral disease, plus axial (midline) involvement
2	Bilateral disease, without impairment of balance
2.5	Mild bilateral disease with recovery on pull test
3	Mild to moderate bilateral disease; some postural instability; physically independent
4	Severe disability; still able to walk or stand unassisted
5	Wheelchair bound or bedridden unless aided

F. Symptomatic treatment

The most effective single medication available for the treatment of PD's symptoms is levodopa, a medication that helps replace the missing dopamine neurotransmitter[13]. Most of the other currently available medications for PD augment levels of dopamine or mimic dopamine synthetically. These drugs, deep brain stimulation (DBS) and focused ultrasound all treat a subset of the motor symptoms of PD. Furthermore, all available treatments of PD *only treat or mask the symptoms* and do not affect the underlying disease process – PD progresses relentlessly despite treatment.

IV. POTENTIAL CAUSES OF PARKINSON'S DISEASE

A. Genetic Risk Factors

The vast majority of PD cases are not inherited. Together, all of the known genes implicated in some way in PD account for **only about 10% of cases**; the remaining 90% have no known genetic link (Table 1)[14]. Additionally, even though an individual may carry a genetic mutation associated with PD, the two most common PD gene mutations (in genes called LRRK2 and GBA) have "incomplete penetrance", meaning that simply carrying the mutation does not mean one will necessarily develop PD. For example, less than 10% of people carrying a GBA mutation will ultimately develop PD. In these cases, an environmental trigger may lead to onset of PD[15].

Table 1

	Mutation	Note	Year of discovery	Proposed disease mechanism	Inheritance	Frequency	Nominated by GWAS	Multiple independent families reported*	Functional evidence†	Negative reports published‡	Confidence as actual PD gene§
SNCA	Missense or multiplication	Often with dementia	1997, 2003	Gain of function or overexpression	Dominant	Very rare	Yes	++	++	+	Very high
PRKN	Missense or loss of function	Often early onset	1998	Loss of function	Recessive	Rare	No	++	++	+	Very high
UCHL1	Missense		1998	Loss of function?	Dominant	Unclear	No	–	+		Low
PARK7	Missense	Often early onset	2003	Loss of function	Recessive	Very rare	No	++	++	+	Very high
LRRK2	Missense		2004	Gain of function	Dominant	Common	Yes	++	++	+	Very high
PINK1	Missense or loss of function	Often early onset	2004	Loss of function	Recessive	Rare	No	++	++	+	Very high
POLG	Missense or loss of function	Atypical PD	2004	Loss of function?	Dominant	Rare	No	++	+	+	High
HTRA2	Missense		2005	Unclear	Dominant	Unclear	No		–	– –	Low
ATP13A2	Missense or loss of function	Atypical PD	2006	Loss of function	Recessive	Very rare	No	++	++	+	Very high
FBXO7	Missense	Often early onset	2008	Loss of function	Recessive	Very rare	No	++	++	+	Very high
GIGYF2	Missense		2008	Unclear	Dominant	Unclear	No	+	+	– –	Low
GBA	Missense or loss of function		2009	Likely loss of function	Dominant (incomplete penetrance)	Common	Yes	++	++	+	Very high
PLA2G6	Missense or loss of function	Often early onset	2009	Loss of function	Recessive	Rare	No	++	++	+	Very high
EIF4G1	Missense		2011	Unclear	Dominant	Unclear	No	–	+	– –	Low
VPS35	Missense		2011	Loss of function	Dominant	Very rare	No	++	+	+	Very high
DNAJC6	Missense or loss of function	Often early onset	2012	Loss of function	Recessive	Very rare	No	++	+	+	High
SYNJ1	Missense or loss of function	Often atypical PD	2013	Loss of function	Recessive	Very rare	No	++	+	+	High
DNAJC13	Missense	Same family as *TMEM230*	2014	Unclear	Dominant	Unclear	No	+	+	–	Low
TMEM230	Missense	Same family as *DNAJC13*	2016	Loss of function?	Dominant	Unclear	No	–	+		Low
VPS13C	Missense or loss of function		2016	Loss of function	Recessive	Rare	Yes	++	+	+	High
LRP10	Missense or loss of function		2018	Loss of function?	Dominant	Unclear	No	–	+		Low

GWAS genome-wide association study. PD Parkinson's disease *In this column, ++ denotes ≥4 families reported; + denotes ≥2 and <4 families reported; – denotes 1 family reported; – – denotes no reported families. †In this column, ++ denotes ≥4 disease-related reports; + denotes ≥1 and <4 disease-related reports; – denotes no disease-related reports. ‡Reports that could not replicate the finding that this gene is a PD gene. In this column, + denotes no negative reports; – denotes ≥1 and <4 negative reports; – – denotes ≥4 negative reports. §Sum of the scores in the three preceding columns, with each + adding 1 and each – subtracting 1; very high denotes a score of ≥5; high denotes a score of 4; medium denotes a score of 2 or 3; low denotes a score of ≤1

Table: Mutations that have been reported to cause Parkinson's disease

B. Environmental Risk Factors

Aside from the small percentage of PD cases that can be attributed to bad genetic luck, the great majority of PD cases are "sporadic" and have no obvious cause. That said, there are certain environmental factors that are generally considered to contribute to one's risk of developing PD. In the case of PD, the chemicals generally thought to contribute to the risk are pesticides[16, 17, 18].

1. Biological Actions of Paraquat

a. Observations from Cell and Animal Studies

i. Paraquat is a Redox Cycler that Causes the Oxidative Damage that is Generally Understood to Lead to the Development of PD

The pesticide most commonly associated with PD in the scientific literature is the herbicide paraquat[19, 20, 21]. The literature shows that systemic long-term paraquat exposure in animals elicits PD-like symptoms and loss of dopamine producing neurons in the substantia nigra. This occurs because of paraquat's high potential to redox cycle[22, 23]. "Redox" refers to oxidation and reduction, which is a type of chemical reaction between molecules that involves a loss or gain of electron(s). Oxidation and reduction always occur together. Reduction is a gain of an electron or electrons. Oxidation, the opposite, is a loss of an electron or electrons. In the first step of the redox cycle, paraquat dication (PQ++) is reduced (gains an electron) to form a free radical monocation (PQ+). In the presence of oxygen, the paraquat radical will rapidly re-oxidize (lose an electron) to re-form the dication with the simultaneous production of superoxide anion. This second redox reaction generates a reactive oxygen species, or "ROS," called superoxide radical that is known to interfere with the biological function of cells by causing oxidative "stress" or damage. This second redox reaction also returns the paraquat molecule to its original 2+ state allowing the cycle to repeat and create more superoxide radicals. Thus, paraquat is known as a redox cycler that can undergo many rounds of electron transfer in the presence of a reductant and molecular oxygen. In essence, a single molecule of paraquat can produce many, many molecules of superoxide radical and is therefore a potent cause of oxidative stress or damage.

Oxidative damage – such as that caused by paraquat – has long been thought to be important in the development of PD[24, 25, 26]. Early studies of postmortem human brains showed loss of the antioxidant, glutathione, and oxidative damage to proteins, membrane lipids and DNA/RNA in people who died with PD[27, 28, 29]. Among other deleterious effects, oxidative damage causes protein damage and misfolding. One of the proteins affected in this scenario is alpha synuclein[30], which aggregates to form Lewy bodies, a pathologic hallmark of PD[31, 32]. Normally, damaged or misfolded proteins are detoxified via degradation by the ubiquitin proteasome system. However, paraquat-induced oxidative damage also affects the ubiquitin proteasome system and impairs its activity[33, 34]. As a result, there is a toxic accumulation of damaged or misfolded proteins including alpha-synuclein[33, 34]. Another system, the autophagy-lysosomal pathway, also degrades damaged and misfolded proteins. However, like the ubiquitin-proteasome system, it too is susceptible to paraquat-induced oxidative damage[34, 35] – and this also contributes to accumulation and aggregation of alpha-synuclein. Thus, one result of cellular oxidative damage is disruption of the cellular "garbage disposal" systems (ubiquitin proteasome system and autophagy lysosome pathway) which, in turn, leads to toxic accumulation and aggregation of proteins, such as alpha-synuclein.

ii. Paraquat Activates Microglial Cells Causing Inflammation that is Generally Understood to Initiate or Exacerbate the Development of PD

There is strong genetic and pathological evidence that inflammation plays an important role in the neurodegeneration of PD[36]. This inflammation may play either an initiating or a secondary role – and it may specifically involve only inflammatory systems in the central nervous system (CNS), or the peripheral immune system[37]. The primary immune cells in the CNS are microglia. The activation of microglia (inflammation), is a process in which microglia may release inflammatory cytokines (chemical messenger molecules) that can alert other immune cells and damage neurons.

In healthy brain tissue, microglia are mostly in a "ramified" or resting state, in which they have a small cell body and extend many thin membrane branches (like antennae) that insert amongst the neurons in the surrounding brain tissue. These antenna-like branches conduct constant surveillance and look for signals of pathogen invaders (viruses, bacteria) and of dead or dying cells. Upon stimulation, microglia become activated and withdraw their branches, increase their volume and behave like macrophages (a kind of white blood cell). In the activated state, microglial cells can phagocytose (eat) pathogens and also can eat and eliminate damaged neurons or other brain cells. In this manner, microglia basically clean up the brain tissue and when the immediate threat is removed, they may return to their resting state. In some cases, however, microglia remain pathologically activated, and rather than protecting neurons, they may provoke neurodegeneration[38]. Reactive oxygen species, such as the superoxide radical produced by paraquat's redox cycling, is one of the triggers that activates microglia.[22, 39]

In turn, paraquat can hijack reducing equivalents from a microglial enzyme (called NADPH oxidase) and generate more damaging superoxide radical via redox cycling[22]. Paraquat steals an electron from the enzyme, becomes paraquat radical, then delivers the extra electron to molecular oxygen, generating more superoxide radical. In this way, paraquat ROS production can activate microglia which make more NADPH oxidase and thereby amplify the ability of paraquat to redox cycle. This leads to persistent neurotoxic microglial activation and neurodegeneration.

iii. The Oxidative Damage Caused by Paraquat Leads to an Accumulation of Alpha-Synuclein, One of the Hallmarks of PD

As discussed above, one of the pathological hallmarks of PD is the presence of alpha-synuclein-containing Lewy bodies and Lewy neurites in remaining neurons. Alpha-synuclein is a small, natively unfolded protein (Figure 2)[40] contained within neurons and other cells[30]. The first genetic cause of PD to be discovered was in fact a mutation in the gene that encodes alpha-synuclein[41].

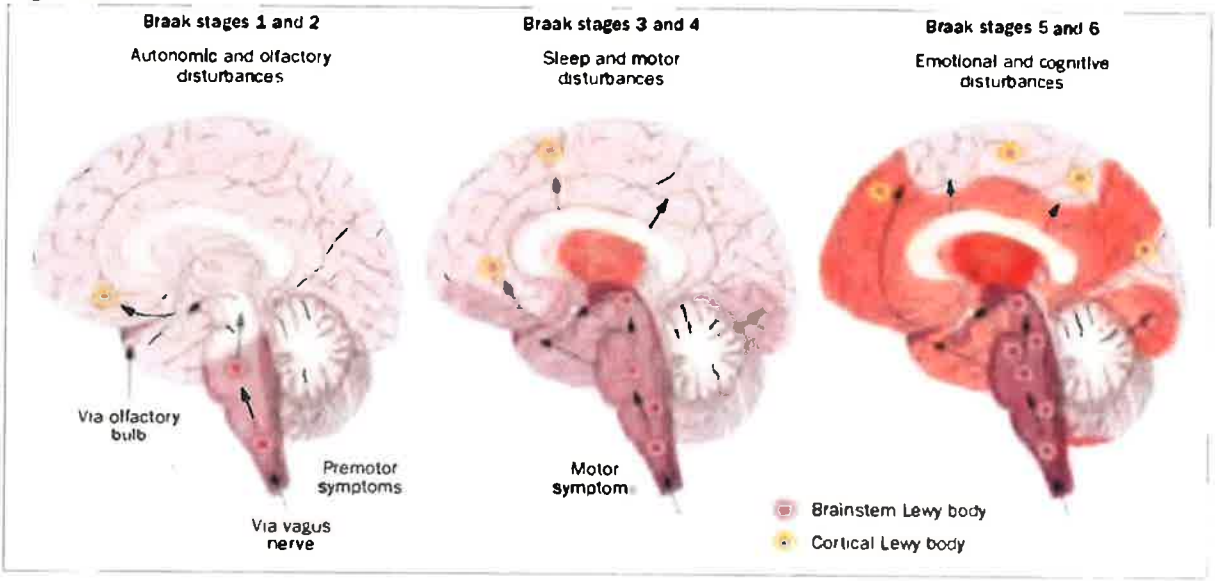
Figure 2

Structure of alpha-synuclein

Cellular levels of alpha-synuclein are normally regulated by the ubiquitin proteasome system and the autophagy lysosome pathway. As noted above, these "garbage disposal" systems may be damaged by paraquat-induced oxidative stress. As a result, there is abnormal accumulation of alpha-synuclein protein. As alpha-synuclein levels increase, it is much more likely to begin to aggregate. Small aggregates (oligomers) of alpha-synuclein protein cause cellular toxicity including damage to mitochondria[42].

In addition, when alpha-synuclein protein misfolds it can induce similar misfolding in other alpha-synuclein protein molecules. This process of "self-templating" leads to further toxicity and spread of pathology throughout the nervous system. In this process, small oligomers made in one neuron can be released from that cell and taken up by an adjacent neuron and transfer the templating process to that neuron[43]. In this way, an initiating event far from the substantia nigra dopamine neurons may eventually lead from one cell to the next until it ultimately reaches the dopamine neurons and causes them to degenerate. In this context, there is compelling experimental and human clinical/pathological data (delineated by Braak et al.[6]) indicating PD may begin in the gastrointestinal system or the nasal epithelium (olfactory bulb) in the nose with alpha-synuclein templating and cell-to-cell spread that eventually reaches the substantia nigra (Figure 3)[44]. In fact, a direct (mono-synaptic) connection between the olfactory bulb and the substantia nigra has been discovered recently[45].

Figure 3



What might initiate alpha-synuclein accumulation and misfolding in the nasal epithelium or GI tract? As previously discussed, oxidative stress, such as that produced by paraquat, causes alpha-synuclein to misfold and accumulate[46]. In this way, alpha-synuclein pathology initiated in the nose or the gut may lead to degeneration of the substantia nigra dopamine neurons and result in symptoms of PD. This pathogenic cascade of events is widely accepted as likely by a large consensus of PD scientists[47]. An important aspect of this scheme is that the initiating trigger for these events is not even required to get into the brain. Indeed, the oxidative stress induced by paraquat could occur in the nose or the gut and lead, via cell-to-cell transmission of alpha-synuclein, to the neurodegeneration characteristic of PD. Thus, although there is good evidence that paraquat can enter the brain, *it needn't do so to cause PD.*

V. HOW PARAQUAT GETS FROM THE ENVIRONMENT INTO THE BODY AND BRAIN

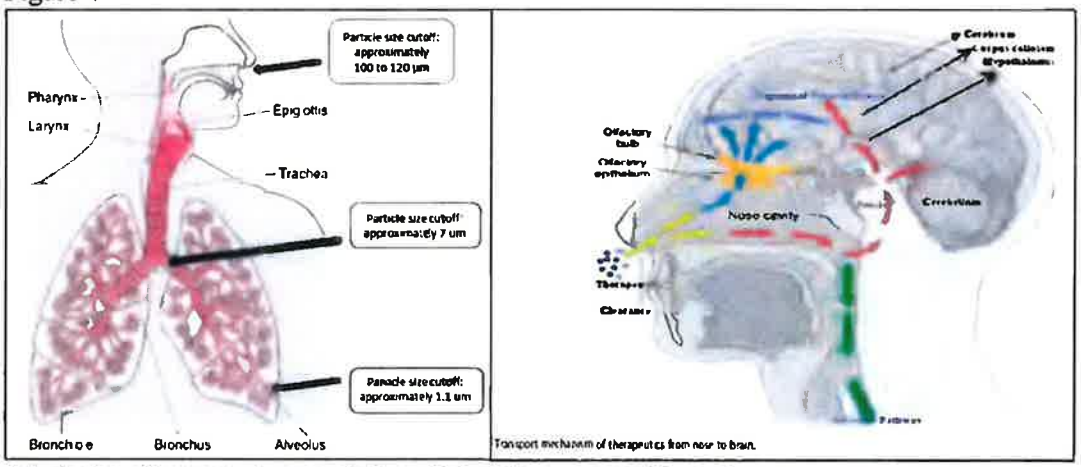
In agricultural workers who mix and/or apply paraquat (including field workers, applicators, pilots, mixers, loaders and bystanders), there are three likely routes of entry into the human body: inhalation, ingestion and absorption through the skin. Each of these routes of exposure can lead to systemic (in the bloodstream) paraquat toxicity. Once paraquat is in the bloodstream it will reach the brain.

Baharuddin et al.[48] found significant differences in absorption of paraquat depending on the method of application by the farm worker. *Manual application* of paraquat on vegetation was associated with significant side-effects including neurologic symptoms as well as substantial elevation of liver enzymes consistent with systemic absorption of sufficient amounts to cause hepatotoxicity. In a study partially funded by Syngenta, Lee et al.[49] reported detectable levels of paraquat in the urine of a subgroup of paraquat sprayers in Costa Rica, presumably absorbed through inhalation. Wesseling et al.[50] reported three fatal cases of paraquat toxicity due to skin exposure in workers who sprayed the herbicide. Tungsanga et al.[51] reported a case of skin absorption of paraquat through the scrotum, which was associated with serious toxicity. Chui et al.[52] studied the pulmonary absorption of [^{14}C]paraquat via gastric, dermal, aerosol, or tracheal administration in rats and documented much higher pulmonary absorption than through gastric or dermal application.

A. Inhalation

People can be exposed to paraquat via the inhalation of airborne particulates (paraquat aerosols). Smaller aerosol particles/droplets are more readily respirable (i.e., available to the bronchia and alveoli of the lungs, where gas exchange occurs) with a particle diameter cutoff of 3 μm for adults and 5 μm for children[53]. This is further supported by a rat study[54] which concludes that toxicity is a complex function of particle size and in the rat "it appears that the most effective size is in the region of 3 μm, as larger particles do not reach the alveolar regions and finer particles are probably not retained there." Larger particles (>10 μm) are excluded from the bronchia and alveoli of the lungs (Figure 4, left panel)[55], but still accessible to the nasal passages (Figure 4, right panel)[56], which have an upper limit of acceptance of 100-120 μm with inhalation efficiency inversely proportional to particle size[57, 58]. *Notably, although larger particles may not reach the alveoli, once in the nasal passages, they may be swallowed along with nasal mucous. Additionally, nasal paraquat may be absorbed into the nasal epithelium and olfactory bulb. This is particularly true with paraquat because of its water solubility. Thus, the size of the water droplets doesn't impede access.*

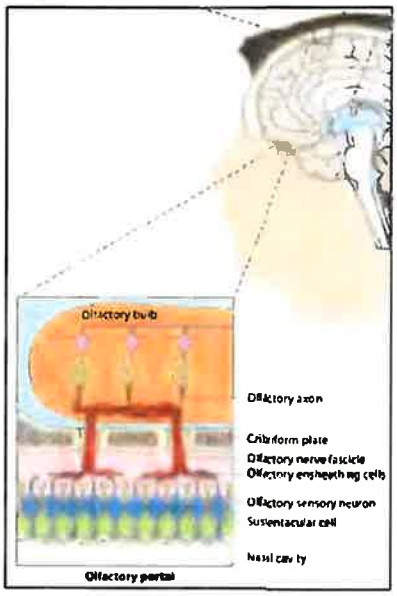
Figure 4



Inhalation of paraquat aerosols is sufficient to cause parkinsonism. A recent study demonstrated that mice exposed to paraquat aerosols using an inhalation chamber show accumulation of paraquat in the brain far

removed from the olfactory bulb[59]. The exposed animals exhibited motor abnormalities (decreased grip strength) and exposed males showed abnormalities of the dopamine synthetic enzyme, tyrosine hydroxylase.

Figure 5



The Olfactory system as a portal to the brain. The olfactory system comprises the odor-detecting sensory system that exists in the peripheral nervous system within the nasal cavity and, within the CNS, the olfactory bulb and higher processing centers of the brain. Within the nasal cavity, the olfactory epithelium lines part of the nasal cavity. The axons of olfactory sensory neurons penetrate the basement membrane beneath the epithelium, and the axons of the olfactory nerve course through the lamina propria toward the brain, penetrating the skull through the cribriform plate and entering the brain at the olfactory bulb (Figure 5)[60]. *Olfactory sensory neurons are directly exposed to the external environment via the nasal cavity and can transfer pathogens or chemicals to the olfactory bulb and into other regions of the brain.* Recent research has discovered a direct (monosynaptic) connection from the substantia nigra to the olfactory bulb[45]. This reinforces the concept of a direct pathway by which inhaled substances and pathogens gain access to the midbrain where neurodegenerative processes lead to PD symptoms[61, 62].

Implications of paraquat inhalation exposure on contracting PD. Inhalation exposure is likely a major route leading to PD via effects on the olfactory system and/or the gut (when paraquat-contaminated mucous is swallowed). An agricultural sprayer, mixer, loader or field operator (e.g., flagger) is very likely exposed during normal operations to the spray mist of paraquat. [63, 64, 65, 66, 67]

Of course, any question regarding the systemic absorption of paraquat and its access to the brain becomes largely moot with the recent evidence that the sentinel sites for the initial pathology of PD are the olfactory bulb and the enteric nervous system. Given that paraquat can cause aggregation of alpha-synuclein after systemic injection in experimental animals (reviewed by Dinis-Oliveira et al.[68]; see also Mitra et al.[69]), inhalation of paraquat would present the nasal mucosa and olfactory bulb with substantial exposure as well as the gut through swallowing contaminated saliva and secretions and lead to alpha-synuclein aggregation, the first step in the pathophysiology of PD. Indeed, Nuber et al.[70] created mice with PD-associated mutant alpha-synuclein expressed in olfactory bulb neurons and exposed them to low dose paraquat. Low dose paraquat caused degeneration of the dopaminergic neurons and the accumulation of insoluble alpha-synuclein.

B. Ingestion

The major function of the digestive tract is to digest and absorb the foods we eat, but toxins may also be absorbed from the gut. After being taken up in the blood, a toxin like paraquat can be quickly distributed throughout the body where it can cause damage, in sites far from the gut, such as the brain.

Ingestion exposure can occur by inadvertent non-dietary ingestion of soil, dust, or chemical residues on surfaces or objects that are contacted via hand-to-mouth or object-to-mouth activity. Additionally, *individuals who are exposed to airborne paraquat aerosol particles, may ingest paraquat by swallowing mucous from the contaminated nasal cavity*[71]. While ingested paraquat can certainly have systemic effects

and enter the brain, there is increasing evidence suggesting that the ingestion of paraquat may initiate the pathology of PD in the digestive tract. For instance, it has been shown that oral ingestion of paraquat in mice causes alpha-synuclein pathology in the myenteric neurons of the gut[72].

Further, a recent study demonstrated the existence of a direct connection between the vagus nerve and the substantia nigra, thereby refining the path by which alpha-synuclein travels from gut to brain[73]. The same group did a study showing that a very low dose of paraquat (1 mg/kg/day) administered orally with lectin (such as that from beans, wheat or tomatoes) causes an alpha-synucleinopathy, which spreads from the gut to the substantia nigra and causes degeneration of the dopamine neurons[74]. Cutting the vagus nerve prevents the brain pathology and neurodegeneration. *Thus, although paraquat can enter the brain and the substantia nigra dopamine cells, it need not do so to initiate the pathology that results in Parkinson's disease. Indeed, paraquat's effects on the gut are sufficient to cause Parkinson's disease.*

C. Dermal

Dermal exposure is a known route of occupational exposure. Dermal exposure results in absorption immediately after paraquat comes into contact with the skin or eyes. Absorption will continue as long as paraquat remains in contact with the skin[75]. Also, if the skin area that is exposed is broken, abraded, irritated or cut, it will allow more paraquat penetration[69]. Once paraquat is absorbed through the skin, it becomes systemic (enters the bloodstream). Perspiration has been shown to enhance dermal absorption of pesticides[76].

VI. PARAQUAT-INDUCED PD: EPIDEMIOLOGICAL STUDIES & ANIMAL MODELS

A. Observations from Epidemiological Studies

Epidemiological studies support a causal connection between use of pesticides and PD. The environmental chemical consistently associated with PD is paraquat.

- The existence of a potential association between paraquat and Parkinson's disease was known by no later than 1985, when Barbeau et al reported a high correlation between Parkinson's disease incidence and pesticide use in Quebec Canada, suspecting paraquat as the cause[77].
- A 1990 case control study in which personal histories of 57 cases and 122 age-matched controls were compared found a statistically significant increased risk of developing Parkinson's disease with paraquat exposure[78].
- A 1992 Canadian population-based case-control study of persons who were exposed to herbicides and could specifically recall their exposure history reported that one individual who used paraquat between the ages of 26 and 31 years was the only herbicide-exposed case in the study whose onset of symptoms occurred before the age of 40[79].
- A 1997 case control study in Taiwan, where paraquat is commonly sprayed on rice fields, found a dose-dependent relationship between paraquat and Parkinson's disease: the risk for Parkinson's disease increased in parallel with cumulative lifetime exposure to paraquat. The odds ratio for Parkinson's disease incidence was found to be as much as 6.4 times higher among subjects who had been exposed to paraquat for more than 20 years compared with age-and-sex matched controls[80].
- A 2007 study, which examined a cohort of 80,000 licensed private applicators and spouses, found that farm workers exposed to paraquat had twice the expected risk of developing Parkinson's disease[81].
- A 2008 case-control study in East Texas found that exposure to paraquat increased a person's risk of getting Parkinson's disease by 3.5 times[82].
- In a 2009 case-control study, self-reported occupational use of paraquat was found to increase the risk of Parkinsonism by 280%[83].
- A 2011 study that looked at 110 PD cases and 358 controls, found PD associated with the use of paraquat at an odds ratio of 2.5 with a 95% confidence interval of 1.4–4.7. In other words, people who used paraquat were likely to develop Parkinson's disease approximately 2.5 times more often than non-users[20].
- A 2013 meta-analysis published in Neurology found that 'exposure to paraquat ... was associated with about a 2-fold increase in risk' of Parkinson's disease[18].

Thus, to summarize, numerous epidemiological studies have reported that exposure to paraquat significantly increases the risk of developing PD.

B. Observations from Animal Studies

Human PD is a complex and heterogeneous disorder. As such, no single animal model of PD captures all the clinical and pathological features of the human disease. Nevertheless, there are a variety of relevant biological endpoints when determining whether a given system accurately reflects aspects of a human disease. For example, PD is defined pathologically, by a **loss of substantia nigra dopamine neurons** together with **alpha-synuclein pathology**, including Lewy bodies and Lewy neurites. If the loss of dopamine neurons is sufficient, there will be a loss of dopamine and concomitant behavioral changes, but the brain has compensatory mechanisms such that a threshold of degeneration (roughly 50%) must be reached before dopamine levels decline and motor abnormalities emerge. In addition, inflammation (microglial activation) is an early and persistent feature of PD, as is evidence of oxidative stress and damage of biomolecules.

1. Loss of substantia nigra dopamine neurons.

In testing the toxicity of a chemical that is related to PD, such as paraquat, ***the clearest and most unequivocal endpoint after exposure is neurodegeneration and loss of dopamine neurons in the substantia nigra***. Such a selective loss of nigral dopamine neurons in mice and rats has been reported repeatedly in scientific publications after various routes of exposure to paraquat, including intraperitoneal (ip), subcutaneous (sc) and oral.

Mice:

- An early report of paraquat-induced nigral degeneration was from Di Monte's group. Using C57BL/6 mice, they reported a *dose- and age-dependent **25-33% loss of nigral neurons** that occurred with as little as 1 mg/kg paraquat given weekly for 3 weeks*[84]. In another publication, they found that paraquat exposure increased levels of alpha-synuclein in dopamine neurons[85].

- Andersen's lab found a **29% loss of nigral neurons**[86] when mice were treated with paraquat (7 mg/kg every 2 days for 10 doses).

- Smeyne found a larger **50% loss of nigral neurons**[87] in C57BL/6J mice exposed to paraquat (10 mg/kg twice weekly for 3 weeks).

- Cory-Slechta's group reported that paraquat produced a **30-37% decrease in dopamine neurons**[88] across age groups in C57BL 6J mice, with the 18-month-old animals showing the greatest mean loss.

- Fernagut found a **27% loss of dopamine neurons**[89] in C57BL/6J mice after paraquat dosing (10 mg/kg twice weekly for 3 weeks).

- Purisai et al reported about **25% loss of dopamine neurons**[90] in C57BL/6J mice after paraquat dosing (10 mg/kg twice weekly for 2 weeks).

- Jones showed that C57BL/6J mice (2.5 - 4 months old) had a **31% loss of nigral dopamine cells**[91] after exposure to paraquat (5 mg/kg, once weekly for 3 weeks).

- Tieu and colleagues found that in 12-week-old C57BL/6J mice, paraquat exposure (10 mg/kg twice weekly for 5 weeks) produced a **22% loss of nigral neurons**[92].

- Hayley and colleagues reported about **30% loss of dopaminergic neurons**[93] in C57BL/6J mice after paraquat exposure (10 mg/kg twice weekly for 3 weeks).

- Louise Marks (Syngenta report number XM7258 - 2007): In C57BL/6 mice, administration of 10 mg/kg of paraquat once a week for three weeks resulted in **a statistically significant reduction, 23.7 ± 6.9% SD, (p< 0.001, n=9) in dopaminergic neuronal cell number in the substantia nigra. This was associated with an increase in alpha-synuclein protein in substantia nigra.**

- Louise Marks (Syngenta report number XM7371 - 2007): In C57BL/6 mice, administration of 10 mg/kg paraquat dichloride, once a week for 3 weeks, resulted in a **statistically significant reduction (21.2 ± 4.3% SEM, n=9, p<0.01) in dopaminergic cell number in the substantia nigra pars compacta** (SNpc).

- Despite statements by Syngenta (on Paraquat.com): "The [Syngenta] research work has been, and will continue to be, published in peer-reviewed scientific journals and the results communicated to relevant regulatory agencies", ***the company chose not to publish or otherwise make public these results that confirm paraquat-induced nigral degeneration.***

In addition to these publications, a Syngenta-sponsored review by Smeyne et al[94] *attempted a comprehensive literature survey of paraquat's effects on dopamine neurons in mice. Supplemental data (S3 Table 1) found 38 studies that reported that paraquat kills dopamine neurons and only 9 studies that it does not. Of the negative studies, most were funded by Syngenta – and Louise Marks' positive studies (above) were not mentioned. Thus, there are numerous independent studies from independent labs that confirm paraquat kills substantia nigra dopamine neurons in mice.*

Rats:

- In a recent study by Anselmi et al74, Sprague-Dawley rats received paraquat (+ lectin) **orally** at a dose of 1 mg/kg/day for 7 days. Four weeks later, they were found to have almost **50% nigral cell loss.**

- In 2020, Cristóvão et al.[95] reported a new model of paraquat exposure that simulates aspects of dermal exposure. Using **subcutaneous** osmotic pumps, male Wistar rats received paraquat (2.5 mg/kg/day for 4 weeks) delivered as a low-level continuous infusion. **The number of dopaminergic neurons in the substantia nigra, quantified at week 8, was reduced by 41%.**

Monkeys:

- A Syngenta summary (SYNG-PQ-02601798) of a study by Professor Di Monte using squirrel monkeys treated with paraquat (2.5 mg/kg *s.c.* weekly for 6 weeks) showed that while there was no absolute loss of nigral dopamine neurons (at 4 weeks after the end of treatment), ***about 10,000 out of 61,000 (16%) dopamine neurons stopped making tyrosine hydroxylase (TH). Because TH is required to make dopamine, this means that paraquat rendered about 10,000 (16%) of these essential nerve cells nonfunctional.***

2. Alpha-synuclein pathology

The presence of Lewy bodies and Lewy neurites containing alpha-synuclein is a pathological hallmark of PD. As noted above, paraquat-induced oxidative stress leads to accumulation, misfolding, oligomerization and aggregation of alpha-synuclein, and it also enhances cell-to-cell transfer of pathological alpha-synuclein:

Mice, rats & monkeys

- Manning-Bog et al. reported that treatment of C57BL/6 mice with paraquat (10 mg/kg weekly for 3 weeks) led to **accumulation and aggregation of alpha-synuclein in nigral dopamine neurons**[85].

- Wills et al (2012) showed that treatment of C57BL/6 mice with paraquat (10 mg/kg twice weekly for 6 weeks) **more than doubled alpha-synuclein levels**[34].

- Anselmi et al. reported on Sprague-Dawley rats that received paraquat (+ lectin) **orally** at a dose of 1 mg/kg/day for 7 days. They found that **paraquat initiated synuclein pathology in myenteric neurons of the gut, which then ascended cell-to-cell through the vagus nerve, ultimately reaching substantia nigra, where it caused neurodegeneration**[74].

- Musgrove et al found in mice that exposure to the ROS-generating agent, **paraquat, resulted in enhanced production of oxidatively modified forms of alpha-synuclein, increased alpha-synuclein aggregation into oligomeric species, and marked degeneration of neurons.** ***Enhanced oxidative stress also affected neuron-to-neuron protein transfer, causing an increased spreading of alpha-synuclein from the nucleus of the vagus toward higher brain regions***[46].

- A Syngenta summary (SYNG-PQ-02601798) of a study by Professor Di Monte using squirrel monkeys treated with paraquat (2.5 mg/kg *s.c.* weekly for 6 weeks) showed ***"An up regulation of alpha-synuclein was also noted in brain samples taken 2, 4 & 8 weeks post PQ dose."***

In summary, animal studies using mice, rats and monkeys show that paraquat (administered i.p., s.c., or orally) (1) kills nigral dopamine neurons and (2) initiates alpha-synuclein pathology. These are fundamental features of Parkinson's disease.

VII. WHERE PARAQUAT INITIATES PD PATHOLOGY

Having established (1) the mechanism of cellular toxicity of paraquat (redox cycling and oxidative stress), (2) routes of exposure (inhalation, ingestion and dermal), (3) that paraquat exposure increases the risk of developing PD in humans, and (4) that animals exposed to paraquat develop PD pathology, the next question is how and where the PD pathology is initiated at various sites in the human body.

A. In the Brain

Paraquat can be absorbed into the bloodstream via inhalation, ingestion, or skin exposure. Once in the bloodstream, paraquat can exert its toxic effects far from the initial site of exposure. It has been demonstrated that paraquat can gain access to the brain across the blood-brain-barrier (BBB) by active transport involving a neutral amino acid transporter[96, 97]. Once past the BBB, the paraquat dication (PQ++) can be reduced by microglial NADPH oxidase to paraquat monocation (PQ+) and can then be carried into dopamine neurons by a dopamine transporter[92]. *In this way, after exposure, paraquat in the bloodstream may cross the BBB, enter the brain and be specifically targeted to the dopamine neurons of the substantia nigra, the degeneration of which leads to many of the motor symptoms of PD.*

Once inside the dopamine neurons of the substantia nigra, paraquat undergoes repeated cycles of oxidation and reduction by stealing electrons from mitochondrial complex I[98] and donating them to molecular oxygen (O_2). In this way, each molecule of paraquat produces many superoxide anions, which can, in turn, produce hydrogen peroxide and hydroxyl radical. All of these reactive oxygen species can damage biomolecules like DNA, RNA, protein, membrane lipids and metabolites. Additionally, since paraquat exerts its redox cycling in the vicinity of mitochondria, it leads to damage of the mitochondria themselves, which then become leaky and can produce more reactive oxygen species[99], independent of paraquat. Moreover, redox cycling of paraquat leads to membrane damage, making them leaky and allowing critical solutes to cross membranes inappropriately. Oxidative damage to proteins can make them misfold or aggregate or become otherwise nonfunctional. It has been demonstrated that paraquat exposure can increase the accumulation and aggregation state of alpha-synuclein protein. *Paraquat-induced oxidative stress also facilitates cell-to-cell transfer and templating of pathological alpha-synuclein.*

Damaged or misfolded proteins, such as alpha-synuclein, are normally degraded and detoxified by the ubiquitin proteasome system and or the autophagy lysosomal pathway. Redox cycling of paraquat, however, leads to damage and dysfunction of these "proteostatic" mechanisms. As a result, alpha-synuclein accumulates, misfolds, aggregates and is subject to aberrant templating. These abnormal forms of alpha-synuclein spread from one neuron to the next, leading to progressive degeneration of the substantia nigra, as well as extension of pathology to other brain regions, which leads to worsening and additional symptoms.

In the brain, paraquat also affects microglia, the resident immune cells of the brain. One of the triggers that activates microglia is reactive oxygen species, such as superoxide or hydrogen peroxide, including those produced by the redox cycling of paraquat. In turn, paraquat can hijack the microglial enzyme, NADPH oxidase, and generate more damaging superoxide via redox cycling. In this way, paraquat-induced ROS production can activate microglia, which then amplify the ability of paraquat to redox cycle. This can lead to persistent neurotoxic microglial activation[38] and continued neurodegeneration.

In summary, after systemic absorption of paraquat, the following scheme applies:

(1) Paraquat (PQ++) crosses the BBB via the neutral amino acid carrier.
(2) PQ++ is reduced to PQ+ by microglial NADPH oxidase.
(3) PQ+ is selectively transported into substantia nigra dopamine neurons by the dopamine transporter.
(4) *Redox cycling of paraquat causes mitochondrial damage, general oxidative stress, and accumulation of abnormal alpha-synuclein, which leads to neurodegeneration and initiation of alpha-synuclein, with spread of pathology.*

B. In the Olfactory System

Paraquat has been shown repeatedly to induce an increase in total alpha-synuclein, as well as phosphorylation at serine-129 and oligomerization of the protein[45, 72, 74]. These abnormal pathogenic forms of alpha-synuclein exert cellular toxicity and the concomitant misfolding and templating of alpha-synuclein leading to neuron-to-neuron transmission of pathology[61, 100]. It has also been shown repeatedly that initiation of alpha-synuclein pathology in the olfactory bulb leads to widespread alpha-synuclein pathology, including in the substantia nigra[62, 100]. Moreover, initiation of alpha-synucleinopathy in the olfactory bulb can lead to degeneration in the substantia nigra[58,100]. *Thus, although paraquat can cross the blood brain barrier and enter the brain and specifically target substantia nigra dopamine neurons, it need not do so to cause substantia nigra degeneration.*

In summary, after nasal exposure to paraquat, the following scheme applies:

(1) Inhalation of paraquat aerosol particles results in deposition of paraquat in the nasal cavity.
(2) Nasal paraquat enters the olfactory system, including the olfactory bulb.
(3) Within olfactory neurons, paraquat induces alpha-synuclein pathology, including abnormally misfolding and templating of the protein.
(4) *Misfolded alpha-synuclein spreads transneuronally, eventually entering the substantia nigra dopamine neurons.*
(5) *Misfolded alpha-synuclein causes mitochondrial and other cellular toxicities resulting in neurodegeneration and emergence of motor symptoms.*

C. In the Gastrointestinal Tract

The propulsion of ingested food (or toxins) through the gastrointestinal (GI) tract is controlled by a part of the nervous system called the enteric nervous system (ENS). The neurons of the enteric system are located in the walls of the stomach and intestines. It has been recognized for decades that in PD, neurons of the ENS accumulate Lewy bodies, similar to those in the brain[9]. After the more recent discovery that Lewy bodies are composed largely of alpha-synuclein, it has been determined that there is alpha-synuclein pathology in the GI tract, possibly preceding the diagnosis of PD. Such pathology is thought to cause the constipation that is reported by many patients prior to their diagnosis.

The vagus nerve connects the brainstem to the ENS and helps control its function. With the recognition that the earliest alpha-synuclein pathology in PD is found in the nucleus of the vagus nerve (dorsal motor nucleus of the vagus), scientists have explored whether the early alpha-synuclein pathology seen in the GI tract might travel to the brain via the vagus nerve. Retrospective clinical studies showed that people who

had undergone surgery to cut their vagus nerve (vagotomy), an older treatment for peptic ulcer disease, were less likely to develop PD[101, 102]. There are also multiple experimental studies showing that alpha-synuclein pathology initiated within the ENS (in the GI tract) spreads via the vagus nerve to the brain; severing the vagus nerve prevents the transfer of synuclein pathology to the brain74[103, 104]. *Importantly, a direct connection between the nucleus of the vagus and the substantia nigra has been discovered. Thus, there is a clearly defined pathway from the gut to the brain region responsible for many of the motor symptoms of the disease.*

As discussed previously, after inhalation of paraquat, much of the toxin ends up in the nasal cavity, including the nasal mucosa. As mucous is constantly swallowed, paraquat is ingested and finds its way to the GI tract where it can enter neurons of the ENS. A recent experimental study in rats demonstrated that a very low dose of paraquat (1 mg/kg/day) administered orally with lectin (such as that found in beans, wheat or tomatoes) causes an alpha-synucleinopathy that spreads from the gut to the substantia nigra and which causes degeneration of the dopamine neurons; cutting the vagus nerve prevents the brain pathology and neurodegeneration[74]. *Thus, although paraquat can enter the brain and the substantia nigra dopamine cells, it need not do so to initiate the pathology that results in Parkinson's disease. Indeed, paraquat's effects on the gut are sufficient to cause Parkinson's disease.*

In summary, after ingestion exposure to paraquat, the following scheme applies:

(1) Inhalation of paraquat aerosol particles results in deposition of paraquat in the nasal mucosa.
(2) As mucous is swallowed, it ends up in the GI system, where paraquat can enter neurons of the ENS.
(3) Paraquat leads to alpha-synuclein pathology, including abnormally misfolding and templating of the protein.
(4) Misfolded alpha-synuclein spreads transneuronally, up the vagus nerve to the nucleus of the vagus, eventually entering the substantia nigra dopamine neurons.
(5) Misfolded alpha-synuclein causes mitochondrial and other cellular toxicities resulting in neurodegeneration and emergence of motor symptoms.

VIII. GENERAL CAUSATION-Mr. Mills

In summary, and based on my review of the relevant literature, it is my opinion to a reasonable degree of scientific and medical certainty: that paraquat can enter the human body via various methods of exposure; that once in the body, paraquat can initiate pathology in the brain, gut or olfactory system, due to its well-established propensity to redox cycle; that paraquat induced pathology ultimately converges on the substantia nigra; that paraquat selectively targets and damages dopamine neurons in the substantia nigra; and that the death of a sufficient amount of dopamine neurons ultimately leads to the symptoms of Parkinson's disease. ***I therefore conclude, in general, that exposure to paraquat can contribute to the development of Parkinson's disease and therefore have ruled it in as a potential cause of Mr. Mills' Parkinson's disease.***

Other potential causes of PD to be considered include:

Genetics– One of Mr. Mills' physicians ordered testing of genes associated with Parkinson's disease as part of his evaluation of Mr. Mills. A CLIA (Clinical Laboratory Improvement Amendments) certified lab, Invitae, performed the analysis. His physician received the results and they were forwarded to me. Mr. Mills tested negative for all 16 genes analyzed (ATP13A2, ATP7B, DCTN1, DNAJC6, FBXO7, GCH1, LRRK2, PARK7, PINK1, PRKN, PRKRA, SLC6A3 SNCA, SPR, TH, & VPS35). Furthermore, 23andMe testing for the most common genetic risk factors for PD (mutations in LRRK2 and GBA) was also negative. I have relied on these separate tests and results as I would in my professional practice. Thus, there is no reason to consider genetics as a cause of Mr. Mills' PD.

Head trauma/ traumatic brain injury – Mr. Mills has no history of a significant head injury or concussion. As such, this can be ruled out as a contributing factor to his PD.

Welding – Individuals who are employed as welders have increased risk of developing an atypical form of "parkinsonism", distinct from typical PD, that is believed to be caused by exposure to manganese (used in welding rods). This risk factor is irrelevant to Mr. Mills' PD because (1) he did only occasional small repair welding jobs that took only 5 – 10 minutes, and (2) he has typical PD, not atypical parkinsonism.

Exposure to other pesticides or toxicants – There has been no suggestion that Mr. Mills ever used or was exposed to other pesticides or toxicants that significantly increase the risk of PD.

History of drinking well water – Epidemiological studies have variably linked drinking well water in rural areas with risk of developing PD; however, drinking well water is simply believed to be a surrogate for use of agrichemicals[79]. Thus, Mr. Mills' history of drinking well water does not appear to be an independent risk factor for PD – instead, if there is any impact of drinking well water, it is likely to simply be a reflection of his use of paraquat on the orchard.

IX. SPECIFIC CAUSATION

A. Does Mr. Mills have PD?

Mr. Mills underwent a video (WebEx) interview on March 19, 2020

Mr. Mills is an 89-year-old man who says his first symptom of Parkinson's disease began about 10 years ago as a tremor involving his right hand. He initially noticed this when he was trying to shave in the morning. Within one to two years of noticing this tremor, he sought medical attention and received a

diagnosis of Parkinson's disease. He was eventually referred to a neurologist who started him on Sinemet, 1 tablet TID, without much benefit at first. He was subsequently seen by a movement disorders specialist (Dr. Norris) at Washington University in St. Louis, where the diagnosis was confirmed. His Sinemet dose was increased eventually to 3 tablets TID with good resolution of his tremor and other symptoms. Over the intervening years, his symptoms have progressed and include generally slow activities of daily living, gait difficulties with poor balance (he has had no falls). He has difficulty getting in and out of a car or a low, soft chair - and he also difficulty rolling over in bed. Because of complaints of nighttime leg cramping, he was recently told to increase his Sinemet to 2 tablets at midnight in addition to his three daily doses of Sinemet. This resulted in resolution of his leg cramping. Currently he does not have wearing off symptoms between doses of Sinemet, but he admits to occasional dyskinetic movements.

In addition to these motor symptoms, he has a number of *typical nonmotor symptoms* of PD, including constipation, anosmia, RBD (REM sleep behavior disorder), anxiety and depression.

Risk factors: there has been no immediate family member diagnosed with Parkinson's disease. He has no history of significant head injury or concussion or loss of consciousness from a blow to the head. Welding is not a risk factor for typical Parkinson's disease.

Neurologic exam (limited in this video interview): It was clear that Mr. Mills has moderate loss of facial expression (hypomimia). He has mildly hypophonic speech. He has a mild, intermittent, bilateral tremor of his hands at rest. No dyskinesia was seen. Finger taps were slow on the right – and on the left, were decrementing, interrupted and difficult to perform.

Based on his (1) history, (2) motor and (3) non-motor symptoms and (4) response to medication – and (5) a limited neurologic exam, it is clear that *Mr. Mills has typical idiopathic Parkinson's disease.*

B. Mr. Mills' paraquat exposure

Having concluded that exposure to paraquat can contribute to the development of Parkinson's disease and having ruled it in as a *potential cause* of Mr. Mills' Parkinson's disease, it is necessary to consider the specifics of his paraquat exposure. Mr. Mills used paraquat for weed control in his orchard starting in about 1978 and continuing until about 2017. During this period, he sprayed paraquat about every other year (or ~20 seasons). Each application required two 8-hour days, so his total application time over this timespan amounted to about 320 hours. When mixing or spraying paraquat, he wore rubber gloves (much, but not all, of the time), along with conventional long-sleeved shirt, pants and regular (not waterproof) boots. The spray nozzles on his rig would regularly clog, and to fix this, he took off his gloves to clear the clog. He states that this allowed his hands to get wet with paraquat solution. He further states it would be impossible to clean the nozzles while wearing protective gloves. *Thus, it is clear that Mr. Mills had a substantial and prolonged exposure to paraquat prior to the onset and diagnosis of his Parkinson's disease.*

C. Specific causation

Plaintiffs' counsel have provided to me the definition of "proximate cause" and "burden of proof" in Illinois civil lawsuits. I have been asked to assume that these definitions are applicable to my opinions. Having determined that (1) paraquat can cause PD; (2) that Mr. Mills has PD; (3) that other causes of PD can be ruled out; and (4) that Mr. Mills had a substantial and prolonged exposure to paraquat, it is my expert opinion, to a reasonable degree of scientific and medical certainty, that *it is more likely than not that paraquat exposure caused Mr. Mills' PD.*

X. GENERAL CAUSATION-Mr. Niebruegge

In summary, and based on my review of the relevant literature, it is my opinion to a reasonable degree of scientific and medical certainty: that paraquat can enter the human body via various methods of exposure; that once in the body, paraquat can initiate pathology in the brain, gut or olfactory system, due to its well-established propensity to redox cycle; that paraquat induced pathology ultimately converges on the substantia nigra; that paraquat selectively targets and damages dopamine neurons in the substantia nigra; and that the death of a sufficient amount of dopamine neurons ultimately leads to the symptoms of Parkinson's disease. ***I therefore conclude, in general, that exposure to paraquat can contribute to the development of Parkinson's disease and therefore have ruled it in as a potential cause of Mr. Niebruegge's Parkinson's disease.***

Other potential causes of PD to be considered include:

Genetics– One of Mr. Niebruegge's physicians ordered testing of genes associated with Parkinson's disease as part of his evaluation of Mr. Niebruegge. A CLIA (Clinical Laboratory Improvement Amendments) certified lab, Invitae, performed the analysis. His physician received the results and they were forwarded to me. Mr. Niebruegge tested negative for all 16 genes analyzed all 16 genes analyzed (ATP13A2, ATP7B, DCTN1, DNAJC6, FBXO7, GCH1, LRRK2, PARK7, PINK1, PRKN, PRKRA, SLC6A3 SNCA, SPR, TH, & VPS35). Furthermore, 23andMe testing for the most common genetic risk factors for PD (mutations in LRRK2 and GBA) was also negative. I have relied on these separate tests and results as I would in my professional practice. Thus, there is no reason to consider genetics as a cause of Mr. Niebruegge's PD.

Head trauma/ traumatic brain injury – Mr. Niebruegge has no history of a significant head injury or concussion. As such, this can be ruled out as a contributing factor to his PD.

Welding – Individuals who are employed as welders, have increased risk of developing an atypical form of "parkinsonism", distinct from typical PD, that is believed to be caused by exposure to manganese (used in welding rods). This risk factor is irrelevant to Mr. Niebruegge's PD because (1) he did only occasional small repair welding jobs that took only several minutes, and (2) he has typical PD, not atypical parkinsonism.

Exposure to other pesticides or toxicants – There has been no suggestion that Mr. Niebruegge ever used or was exposed to other pesticides or toxicants that significantly increase the risk of PD.

History of drinking well water – Epidemiological studies have variably linked drinking well water in rural areas with risk of developing PD; however, drinking well water is simply believed to be a surrogate for use of agrichemicals[79]. Thus, Mr. Niebruegge's history of drinking well water does not appear to be an independent risk factor for PD – instead, if there is any impact of drinking well water, it is likely to simply be a reflection of his use of paraquat on the farm.

XI. SPECIFIC CAUSATION

A. Does Mr. Niebruegge have PD?

Mr. Niebruegge underwent a video (WebEx) interview March 19, 2020

Mr. Niebruegge is a 67-year-old man who says his first symptoms of Parkinson's disease began in July 2007 when he was told that when he walked, he tended to keep his left arm flexed – and he didn't swing it normally. In retrospect, family members told him they saw symptoms at least a couple of years before that: he was slow, unsteady and took small steps. Within a few months, he received a diagnosis of Parkinson's disease. He initially saw a local neurologist, Dr. Goldring, who started him on Sinemet, which helped with some of his symptoms. He was subsequently seen by a movement disorders specialist (Dr. Criswell) at Washington University in St. Louis, where the diagnosis was confirmed – and he has been followed there since then. Over the intervening years, his symptoms have progressed and include generally slow activities of daily living, and gait difficulties with poor balance. He has difficulty getting in and out of a car or a low, soft chair - and he also difficulty rolling over in bed. He complains of poor fine dexterity and he has developed freezing of gait. Currently he does not have wearing off symptoms between doses of Sinemet, but he did notice this in the past.

In addition to these motor symptoms, he has a number of *typical nonmotor symptoms* of PD, including constipation, anosmia, RBD (REM sleep behavior disorder), and occasional anxiety.

Risk factors: there has been no immediate family member diagnosed with Parkinson's disease. He has no history of significant head injury or concussion or loss of consciousness from a blow to the head. Welding is not a risk factor for typical PD. No known exposures to toxins or pesticides that significantly increase the risk of PD.

Neurologic exam (limited in this video interview): It was clear that Mr. Niebruegge has moderate loss of facial expression (hypomimia). He has mildly hypophonic speech. He has a mild, intermittent tremor of his left hand at rest. No dyskinesia was seen. Finger taps were slow on the right – and on the left, were decrementing, interrupted and difficult to perform. After several attempts, he was able to stand from a seated position without pushing himself up with his arms.

Based on his (1) history, (2) motor and (3) non-motor symptoms and (4) response to medication – and (5) a limited neurologic exam, it is clear that *Mr. Niebruegge has typical idiopathic Parkinson's disease.*

B. Mr. Niebruegge' paraquat exposure

Having concluded that exposure to paraquat can contribute to the development of Parkinson's disease and having ruled it in as a *potential cause* of Mr. Niebruegge's Parkinson's disease, it is necessary to consider the specifics of his paraquat exposure. Mr. Niebruegge used paraquat for weed control on his no-till farm starting in about 1970 and continuing until the early 1990s. During this period, estimates he sprayed paraquat 15-30 days (8 hours/day) each year. In his deposition, he estimates 2,300-2,400 hours of exposure over more than 20 years. While applying paraquat, when the spray boom clogged, he went behind the rig (where he had just sprayed, and where the weeds were wet with paraquat) and he took off his gloves to fix the nozzles. *"You couldn't do it with your gloves on."* As a result, he would regularly get paraquat solution on his hands. Furthermore, he said in his interview that his shirt and pants were commonly wet with paraquat solution from walking in the sprayed weeds. Additionally, he said that while spraying paraquat on the farm, it was impossible not to drive through the recently sprayed mist as he turned and reversed direction. As a result, he remembers inhaling paraquat solution. He tried to use PPE, such as gloves, but he noted: *"And in practical application, you can't use the stuff and not be exposed to it." Thus, it is clear that Mr. Niebruegge had a substantial and prolonged cutaneous and inhalation exposure to paraquat prior to the onset and diagnosis of his Parkinson's disease.*

C. Specific causation

Plaintiffs' counsel have provided to me the definition of "proximate cause" and "burden of proof" in Illinois civil lawsuits. I have been asked to assume that these definitions are applicable to my opinions. Having determined that (1) paraquat can cause PD; (2) that Mr. Niebruegge has PD; (3) that other causes of PD can be ruled out; and (4) that Mr. Niebruegge had a substantial and prolonged exposure to paraquat, it is my expert opinion, to a reasonable degree of medical and scientific certainty, that *it is more likely than not that paraquat exposure caused Mr. Niebruegge's PD.*

XII. GENERAL CAUSATION—Mr. Schmidt

In summary, and based on my review of the relevant literature, it is my opinion to a reasonable degree of scientific and medical certainty: that paraquat can enter the human body via various methods of exposure; that once in the body, paraquat can initiate pathology in the brain, gut or olfactory system, due to its well-established propensity to redox cycle; that paraquat induced pathology ultimately converges on the substantia nigra; that paraquat selectively targets and damages dopamine neurons in the substantia nigra; and that the death of a sufficient amount of dopamine neurons ultimately leads to the symptoms of Parkinson's disease. ***I therefore conclude, in general, that exposure to paraquat can contribute to the development of Parkinson's disease and therefore have ruled it in as a potential cause of Mr. Schmidt's Parkinson's disease.***

Other potential causes of PD to be considered include:

Genetics– One of Mr. Schmidt's physicians ordered testing of genes associated with Parkinson's disease as part of his evaluation of Mr. Schmidt. A CLIA (Clinical Laboratory Improvement Amendments) certified lab, Invitae, performed the analysis. His physician received the results and they were forwarded to me. Mr. Schmidt tested negative for all 16 genes analyzed all 16 genes analyzed (ATP13A2, ATP7B, DCTN1, DNAJC6, FBXO7, GCH1, LRRK2, PARK7, PINK1, PRKN, PRKRA, SLC6A3 SNCA, SPR, TH, & VPS35). Furthermore, 23andMe testing for the most common genetic risk factors for PD (mutations in LRRK2 and GBA) was also negative. I have relied on these separate tests and results as I would in my professional practice. Thus, there is no reason to consider genetics as a cause of Mr. Schmidt's PD.

Welding – Individuals who are employed as welders, have increased risk of developing an atypical form of "parkinsonism", distinct from typical PD, that is believed to be caused by exposure to manganese (used in welding rods). This risk factor is irrelevant to Mr. Schmidt's PD because (1) though Mr. Schmidt had some occupational welding exposure in the 1960s and did occasional small repair welding jobs after that which took less than an hour, (2) he has typical PD, not atypical parkinsonism. Welding is not a risk factor for typical PD.

Exposure to other pesticides or toxicants – There has been no suggestion that Mr. Schmidt ever used or was exposed to other pesticides or toxicants that significantly increase the risk of PD.

History of drinking well water – Epidemiological studies have variably linked drinking well water in rural areas with risk of developing PD; however, drinking well water is simply believed to be a surrogate for use of agrichemicals[79]. Thus, Mr. Schmidt's history of drinking well water does not appear to be an independent risk factor for PD – instead, if there is any impact of drinking well water, it is likely to simply be a reflection of his use of paraquat on the farm.

XIII. SPECIFIC CAUSATION—Mr. Schmidt

A. Does Mr. Schmidt have PD?

Mr. Schmidt underwent a video (WebEx) interview March 19, 2020

Mr. Schmidt is an 86-year-old man who says his first symptoms of Parkinson's disease began in 2004 when he was told that he had a "pill-rolling" tremor of his right hand. His PCP later diagnosed him with PD that year, and he was started on Sinemet in 2008, which helped his tremor. In the intervening years, his symptoms have progressed and include generally slow activities of daily living, and gait difficulties with poor balance and falls (requiring a cane). He has difficulty getting in and out of a car or a low, soft chair. He sleeps poorly and has frequent nocturia. Currently he has wearing off symptoms 4 hours after each dose of Sinemet, and he also has dyskinetic head movements sometimes.

In addition to these motor symptoms, he has a number of *typical nonmotor symptoms* of PD, including constipation, anosmia, RBD (REM sleep behavior disorder), and occasional anxiety.

Risk factors: there has been no immediate family member diagnosed with Parkinson's disease. Other than two head injuries sustained as a young child for which he apparently received no medical care or treatment, he has not sustained a significant head injury or concussion or loss of consciousness from a blow to the head. Given the extreme time delay between these events and his Parkinson's onset, I find no correlation.[105] Welding is not a risk factor for typical PD. His history reflects no known exposures to toxins or pesticides that significantly increase the risk of PD.

Neurologic exam (limited in this video interview): Mr. Schmidt has moderate loss of facial expression (hypomimia). He is moderately bradykinetic. He has moderately hypophonic speech. He has a mild, intermittent tremor of his right hand at rest. No dyskinesia was seen. Finger taps were slow and decrementing bilaterally.

Based on his (1) history, (2) motor and (3) non-motor symptoms and (4) response to medication – and (5) a limited neurologic exam, it is clear that *Mr. Schmidt has typical idiopathic Parkinson's disease.*

B. Mr. Schmidt's paraquat exposure

Having concluded that exposure to paraquat can contribute to the development of Parkinson's disease and having ruled it in as a *potential cause* of Mr. Schmidt's Parkinson's disease, it is necessary to consider the specifics of his paraquat exposure. Mr. Schmidt used paraquat for weed control on his farm starting in about 1968 and continuing for about 7-10 years thereafter. During this period, estimates he sprayed paraquat 4-5 days (8 hours/day) each year. Thus, his estimated exposure to paraquat was at least 280 hours. While applying paraquat, when the spray boom clogged, he took off his gloves to fix the nozzles. *"You can't you can't handle the tools and equipment with gloves on. It's just impossible."* As a result, he would regularly get paraquat solution on his hands. Furthermore, he said in his interview that his shirt and pants were commonly wet with paraquat solution from walking in the sprayed weeds. *"You walk through the paraquat mist and get it on your clothes. It was unavoidable. There was a mist that was obvious. It was on the vegetation. It was on the equipment that you were working with. When you work with paraquat, you see it all the time."* Additionally, he said that while spraying paraquat on the farm, it was impossible not to drive through the recently sprayed mist as he turned and reversed direction. *"There's a mist or a drift in the air for a period of time, 5 minutes from the time you apply it until it all settles down...by that time you've made the turn you come back through it." Thus, it is clear that Mr. Schmidt had a substantial and prolonged cutaneous and inhalation exposure to paraquat prior to the onset and diagnosis of his Parkinson's disease.*

C. Specific causation

Plaintiffs' counsel have provided to me the definition of "proximate cause" and "burden of proof" in Illinois civil lawsuits. I have been asked to assume that these definitions are applicable to my opinions. Having determined that (1) paraquat can cause PD; (2) that Mr. Schmidt has PD; (3) that other causes of PD can be ruled out; and (4) that Mr. Schmidt had a substantial and prolonged exposure to paraquat, it is my expert opinion, to a reasonable degree of scientific and medical certainty, that *it is more likely than not that paraquat exposure caused Mr. Schmidt's PD.*

XIV. GENERAL CAUSATION---Mr. Rowan

In summary, and based on my review of the relevant literature, it is my opinion to a reasonable degree of scientific and medical certainty: that paraquat can enter the human body via various methods of exposure; that once in the body, paraquat can initiate pathology in the brain, gut or olfactory system, due to its well-established propensity to redox cycle; that paraquat induced pathology ultimately converges on the substantia nigra; that paraquat selectively targets and damages dopamine neurons in the substantia nigra; and that the death of a sufficient amount of dopamine neurons ultimately leads to the symptoms of Parkinson's disease. ***I therefore conclude, in general, that exposure to paraquat can contribute to the development of Parkinson's disease and therefore have ruled it in as a potential cause of Mr. Rowan's Parkinson's disease.***

Other potential causes of PD to be considered include:

Genetics– One of Mr. Rowan's physicians ordered testing of genes associated with Parkinson's disease as part of his evaluation of Mr. Rowan. A CLIA (Clinical Laboratory Improvement Amendments) certified lab, Invitae, performed the analysis. His physician received the results and they were forwarded to me. Mr. Rowan tested negative for all 16 genes analyzed all 16 genes analyzed (ATP13A2, ATP7B, DCTN1, DNAJC6, FBXO7, GCH1, LRRK2, PARK7, PINK1, PRKN, PRKRA, SLC6A3 SNCA, SPR, TH, & VPS35). Furthermore, 23andMe testing for the most common genetic risk factors for PD (mutations in LRRK2 and GBA) was also negative. I have relied on these separate tests and results as I would in my professional practice. Thus, there is no reason to consider genetics as a cause of Mr. Rowan's PD.

Head trauma/ traumatic brain injury – Mr. Rowan slipped on the ice in 1991 sustaining facial fractures but intracranially there was no evidence of injury to the brain. As such, this can be ruled out as a contributing factor to his PD.

Welding – Mr. Rowan did not weld.

Exposure to other pesticides or toxicants – There has been no suggestion that Mr. Rowan ever used or was exposed to other pesticides or toxicants that significantly increase the risk of PD.

History of drinking well water – Mr. Rowan's home was on city water, not well water.

IX. SPECIFIC CAUSATION

A. Does Mr. Rowan have PD?

Mr. Rowan underwent a video (WebEx) interview March 19, 2020

Mr. Rowan is a 91-year-old man who says his first symptoms of Parkinson's disease began in about 2000 with generalized slowness, gait difficulties and a right hand tremor. He believes he was formally diagnosed with PD 5-6 years later. He was subsequently seen by a movement disorders specialist (Dr. Norris) at Washington University in St. Louis, where the diagnosis was confirmed – and he has been followed there since then. As his symptoms worsened, he underwent bilateral DBS (deep brain stimulation) which helped his tremor, but which resulted in the adverse effect of severely hypophonic speech. At this point, his speech is generally unintelligible, his gait is unsteady (he requires a walker), and he cannot dress himself or perform his ADLs (activities of daily living). He occasionally has mild dyskinesias.

In addition to these motor symptoms, he has a number of *typical nonmotor symptoms* of PD, including constipation, anosmia and severe RBD (REM sleep behavior disorder).

Risk factors: there has been no immediate family member diagnosed with Parkinson's disease. Other than a fall in 1991, he has no history of significant head injury or concussion or loss of consciousness from a blow to the head. Welding: he never welded. No known exposures to toxins or pesticides that significantly increase the risk of PD. His house was on city water.

Neurologic exam (limited in this video interview): It was clear that Mr. Rowan has severe loss of facial expression (hypomimia) with parting of his lips. He has severely hypophonic speech and is unintelligible. He was extremely bradykinetic. No tremor was seen. No dyskinesia was seen. Finger taps were decrementing, interrupted and extremely difficult to perform.

Based on his (1) history, (2) motor and (3) non-motor symptoms and (4) response to medication – and (5) a limited neurologic exam, it is clear that *Mr. Rowan has typical idiopathic Parkinson's disease.*

B. Mr. Rowan's paraquat exposure

Having concluded that exposure to paraquat can contribute to the development of Parkinson's disease and having ruled it in as a *potential cause* of Mr. Rowan's Parkinson's disease, it is necessary to consider the specifics of his paraquat exposure. (This information was obtained from Steven Rowan, the plaintiff's son, who grew up on the farm and remembers Mr. Rowan's paraquat use.) Mr. Rowan used paraquat for weed control around fruit trees, grape vines and berry plants starting in about 1972 and continuing for about 10 years. At this time, Carroll Rowan was able to simply buy Ortho Paraquat or Gramoxone without a license or training. He used a handheld sprayer to kill the weeds around his fruit trees and plants each spring and often times would spray again later in the season. Each application took about three 8-hour days. His son remembers that the handheld sprayer was leaky, and that Carroll often had paraquat on his hands, and splashes on his pants or shirt. When he finished spraying, he would clean up and change his clothes in the basement. *Thus, it is clear that Mr. Rowan had a substantial and prolonged (at least 240 hours) exposure to paraquat prior to the onset and diagnosis of his Parkinson's disease.*

C. Specific causation

Plaintiffs' counsel have provided to me the definition of "proximate cause" and "burden of proof" in Illinois civil lawsuits. I have been asked to assume that these definitions are applicable to my opinions. Having determined that (1) paraquat can cause PD; (2) that Mr. Rowan has PD; (3) that other causes of PD can be

ruled out; and (4) that Mr. Rowan had a substantial and prolonged exposure to paraquat, it is my expert opinion, to a reasonable degree of scientific and medical certainty, that *it is more likely than not that paraquat exposure caused Mr. Rowan's PD.*

SUMMARY COMMENT

I was also provided a large number of medical records and bills generally related to the medical care and treatment and home health care of Carroll Rowan related to his Parkinson's disease. In my opinion, the medical care and home health care is reasonably related to and made necessary by his Parkinson's disease. The medical bills for the services provided seem fair and reasonable to me.

Signed:  July 10, 2020

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