



Pesticides and Parkinson's Disease. A well-established causal relationship. Measures to protect all citizens.

Submission by Parkinson Québec

To the Committee on Agriculture, Fisheries, Energy and Natural Resources,

whose purpose is to examine the impacts of pesticides on public health and the environment, as well as current and future innovative alternative practices in the agriculture and food sectors, with due regard for the competitiveness of Quebec's agri-food sector.

Author

Romain Rigal, MSc, Service Development Coordinator, Parkinson Québec

Editor

Nicole Charpentier, BSc OT, Executive Director, Parkinson Québec



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DAVID SUZUKI**
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EXECUTIVE SUMMARY

Parkinson Québec is giving a voice to people who are living with Parkinson's disease, their loved ones, caretakers and all Quebec citizens who are at risk of developing this disease due to pesticide exposure.

The suggestions outlined below are backed by the most recent evidence from toxicological and epidemiological studies examining and demonstrating the association between pesticide exposure and the development of Parkinson's disease.

Given that:

- Today, more than 25,000 Quebecers suffer from Parkinson's disease (PD). This number is expected to double by 2040 in every country with industrialized agriculture.
- PD is an incurable neurodegenerative disease. More than 90% of PD cases are the result of interactions between environmental factors and individual genetic susceptibility.
- The onset of the disease is related to non-exclusive situations such as chronic, low-level exposure to nerve agents, limited exposure accompanied by delayed symptoms due to a decline in nerve cell protection and an increased susceptibility to toxins that worsens with age.
- PD is characterized by the progressive degeneration of dopaminergic neurons and the accumulation of neuronal inclusions, or Lewy bodies, mainly composed of a malformed protein known as alpha-synuclein.
- Both motor (e.g. tremor, rigidity) and non-motor symptoms (e.g. cognitive disorders, depression) are extremely debilitating for someone who is already slowly losing their independence.
- The direct costs associated with the disease are estimated at more than 22,400 US dollars per patient per year. These costs exclude human and social costs as well as indirect costs.



560, rue Ontario Est
Montréal (Québec) H2L 0B6
514-861-4422
parkinsonquebec.ca



50, rue Saint-Catherine Ouest, bureau 540
Montréal (Québec) H2X 3V4
514-871-4932
info@davidsuzuki.org

- From 2000 to 2019, no less than 8 meta-analyses concluded that exposure to pesticides almost doubles the risk of developing PD.
- Occupational exposure to pesticides doubles or even triples the risk of developing PD. In Quebec, pesticide applicators and grain producers are particularly at risk. While personal protective equipment is effective in safeguarding against acute reactions, it does not protect against the risk of developing PD.
- Pesticide exposure within the home is sometimes greater than occupational exposure but is not controlled under any regulations. People who experience thirty days of lifetime exposure to herbicides within their home are 1.7 times more likely to develop PD. Residential exposure within 500 metres of where a combination of rotenone, maneb and ziram have been applied, almost doubles the risk of developing PD.
- The frequency, intensity and cumulative number of lifetime days of exposure are intimately related to an increased risk of developing PD. People who experience 10 days of exposure per year are 2.5 times more likely to develop PD.
- Being exposed to pesticides during neurodevelopment contributes to the risk of developing Parkinson's disease by damaging the dopaminergic system and increasing its vulnerability to any subsequent exposures. People who have been exposed to pesticides during their childhood are up to 6 times more likely to develop PD.
- In animal models, exposure during pregnancy leads to pesticide sensitization, which in turn, makes adults more vulnerable to any subsequent exposures.
- Simultaneous exposure to several different pesticides, as is the case in our everyday environment, leads to a potentiation of the toxic effects of each product.
- For more than 30 years, paraquat (herbicide), rotenone (insecticide), MPP+ (herbicide), MPTP (precursor to the nerve agent MPP+) and manganese have all been routinely and interchangeably used to trigger the death of dopaminergic neurons and chemically mimic PD in animal models.
- In animal models, pesticides have indistinctly reproduced damage to dopaminergic neurons, brain structures and potentially, the clinical symptoms of PD.
- Pesticides have a negative effect on the genes that protect and ensure that dopaminergic neurons are functioning properly. They also activate genes that predispose humans to PD.
- The risk of developing PD following pesticide exposure has already been outlined in the Québec Pesticide Strategy 2015-2018.

We encourage the parliamentary committee to intervene at a provincial level by providing the following suggestions:

- Prohibit the use of rotenone, paraquat and maneb products.
- Recognize Parkinson's disease as an occupational disease for all individuals who meet the following criteria:
 - Suffer from Parkinson's disease, which has been diagnosed and confirmed through an examination by a qualified medical specialist in neurology.
 - Have experienced occupational exposure to pesticides for 5 years or more.
 - The term "pesticides" refers to products that are intended for agricultural use and maintaining green spaces, as well as veterinary biocides and pesticides—whether authorized or not at the time of application.

- Occupational exposure refers to handling or using these products by coming into contact with them or inhaling them; coming into contact with crops, exposed surfaces, treated animals or when performing maintenance on machinery intended for pesticide application.
- Period of time between the last risk of exposure and the diagnosis of the disease is less than 5 years (time before receiving care).
- Create a compensation fund for people who meet the above criteria, but who have been exposed to pesticides outside of their professional environment or in an environment that is not covered under the CNESST health plan.
- Provide all farmers or farmworkers and their families with free or low-cost compulsory protection. This program could be financed by levies on pesticide sales.
- Prohibit the use of all pesticides that are capable of inducing neurodevelopmental toxicity, regardless of whether or not they have carcinogenic properties.



- Position Quebec as a leader in good agriculture practices by adjusting allowable exposure levels to the lowest thresholds recognized worldwide.
- Revise and update the Québec Pesticide Risk Indicator in real-time based on independent scientific data.
- Reduce pesticide residues in food by prohibiting the preharvest use of all pesticides, including glyphosate herbicides, and by adopting maximum residue limits that are consistent with best scientific knowledge.
- Authorize the sale of new products in Quebec based on independent studies that evaluate the potential synergies between pesticides.
- Evaluate the health hazards of pesticides based on actual agricultural practices, including simultaneous exposure to multiple products and not simply non-compliant exposure situations.
- Promptly organize an awareness and prevention campaign that targets the Quebec population in order to protect the health and development of future generations from the risks associated with pesticides.
- Support Parkinson Québec in developing and deploying an awareness campaign that focuses on the health impacts that pesticides have on healthcare professionals, such as doctors, neurologists and health care personnel.
- Put economic deterrence measures in place, such as an environmental tax, related to the purchase or application of pesticides, particularly in the agricultural sector. This green tax could be reinvested into the development of organic farming strategies.
- Support farmers in transitioning to organic farming practices by offering significant financial incentives.
- Prohibit the use of pesticides both inside and outside of health facilities, schools, retirement homes, buildings and residences.
- Restrict the use of pesticides within a 2 km radius of residences, buildings and public roads.
- Require that a public announcement be made before pesticides are to be applied in spreading areas, in order to minimize secondary exposures. These announcements should be followed by information campaigns that include strategies to protect children and residences, such as closing windows and shutting off air conditioning systems, as well as banning children from playing outside during or after application.
- Impose significant fines on companies or individuals who violate these regulations.
- We also encourage the parliamentary committee to intervene at the municipal level by providing the following suggestions:
- Prohibit all forms of cosmetic pesticide use in municipalities that do not already have such regulations.
- Prohibit distributors from selling cosmetic pesticides in municipalities that have already adopted such regulations.
- Deploy an awareness campaign that targets citizens and businesses in order to discourage them from using household pesticides indoors.
- Deploy an education campaign that targets citizens and businesses in order to teach them about the various eco-friendly alternatives available that can replace household pesticides.
- Update the pesticide standards and guidelines for drinking water and modernize the Drinking Water Regulation to include all pesticides.

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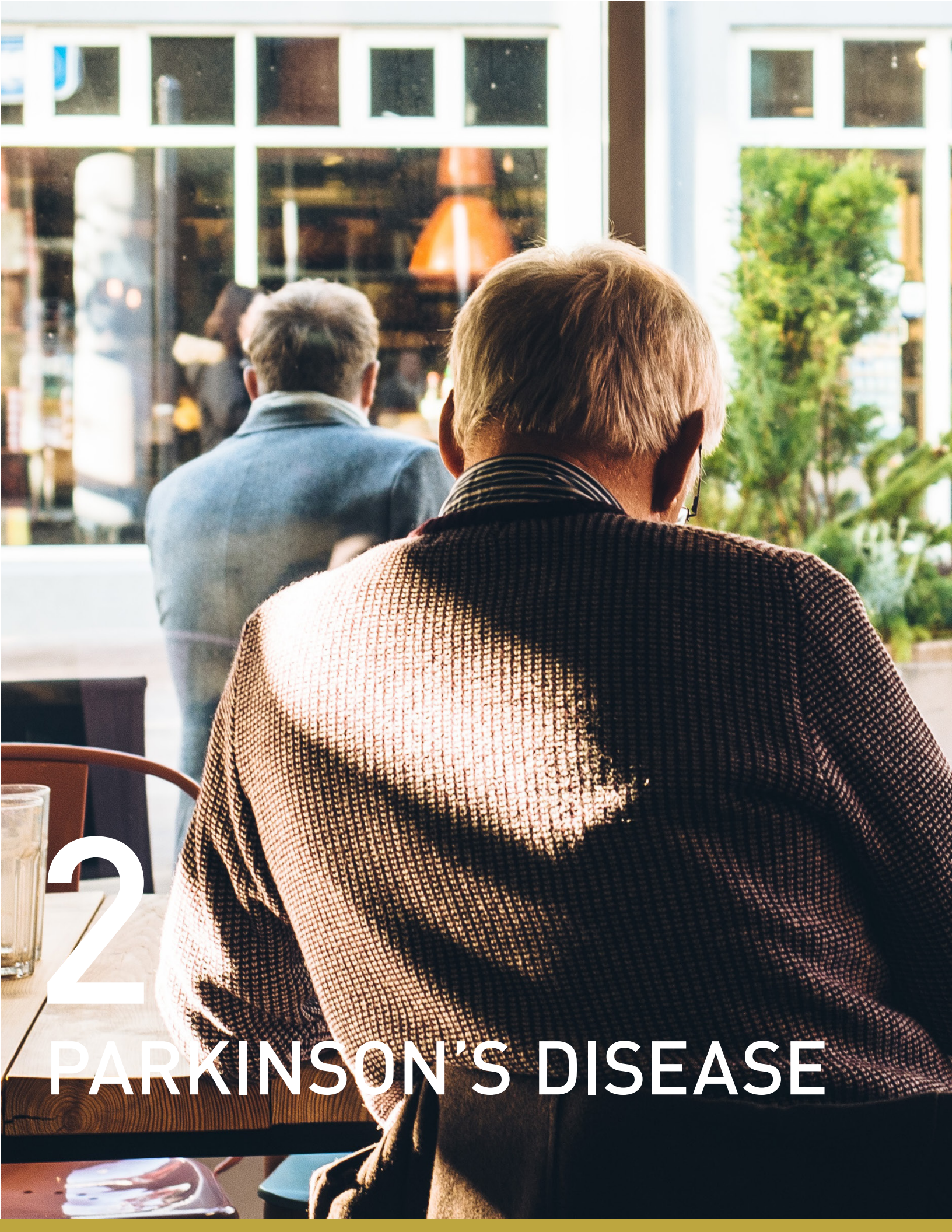
For nearly two decades, Parkinson Québec and its 12 regional offices and organizations have been working toward educating and increasing awareness among people suffering from Parkinson's disease, as well as those around them.

PARKINSON QUÉBEC STRIVES TO:

- inform and empower those affected by the disease so that they can enjoy an active and fulfilling life;
- support those affected by the disease on their journey to finding the freedom to move, regardless of how far along the disease has progressed;
- invest in education and research that contributes to developing pharmacological and other forms of treatment, innovative interventions and more efficient technology in order to counteract the negative impacts that the disease has on everyday life;
- ensure that the rights and interests of those affected by Parkinson's disease are protected.

We support the relevant steps taken by the Committee on Agriculture, Fisheries, Energy and Natural Resources to examine the impacts of pesticides on public health and the environment, as well as current and future innovative alternative practices in the agriculture and food sectors, with due regard for the competitiveness of Quebec's agri-food sector.

This ground-breaking initiative is crucial in helping Quebec society more effectively tackle the challenges of an ageing population and the growing impact that environmental factors have on the development of chronic diseases.



2

PARKINSON'S DISEASE

2.1 HOW MANY PEOPLE IN QUEBEC ARE LIVING WITH PARKINSON'S DISEASE?

More than 25,000 Quebecers suffer from Parkinson's disease (PD).¹ Parkinson's disease is the second most common neurodegenerative disease after Alzheimer's. Most people are diagnosed with the disease at the age of 60, and it affects more men than women (a 3:2 male/female ratio).² We estimate that nearly 2,400 Quebecers are diagnosed with Parkinson's disease every single year.³

From 1990 to 2015, the number of people living with PD worldwide doubled to more than 6 million. The latest estimates predict that this number will double again by 2040. The increase in life expectancy and a growing exposure to chemical compounds, such as heavy metals and pesticides, are now two of the most significant factors contributing to the development of this pandemic.⁴ Parkinson's disease, a neurological disease that was quite rare for most of human history, is now well on its way to becoming a large-scale scourge on society.

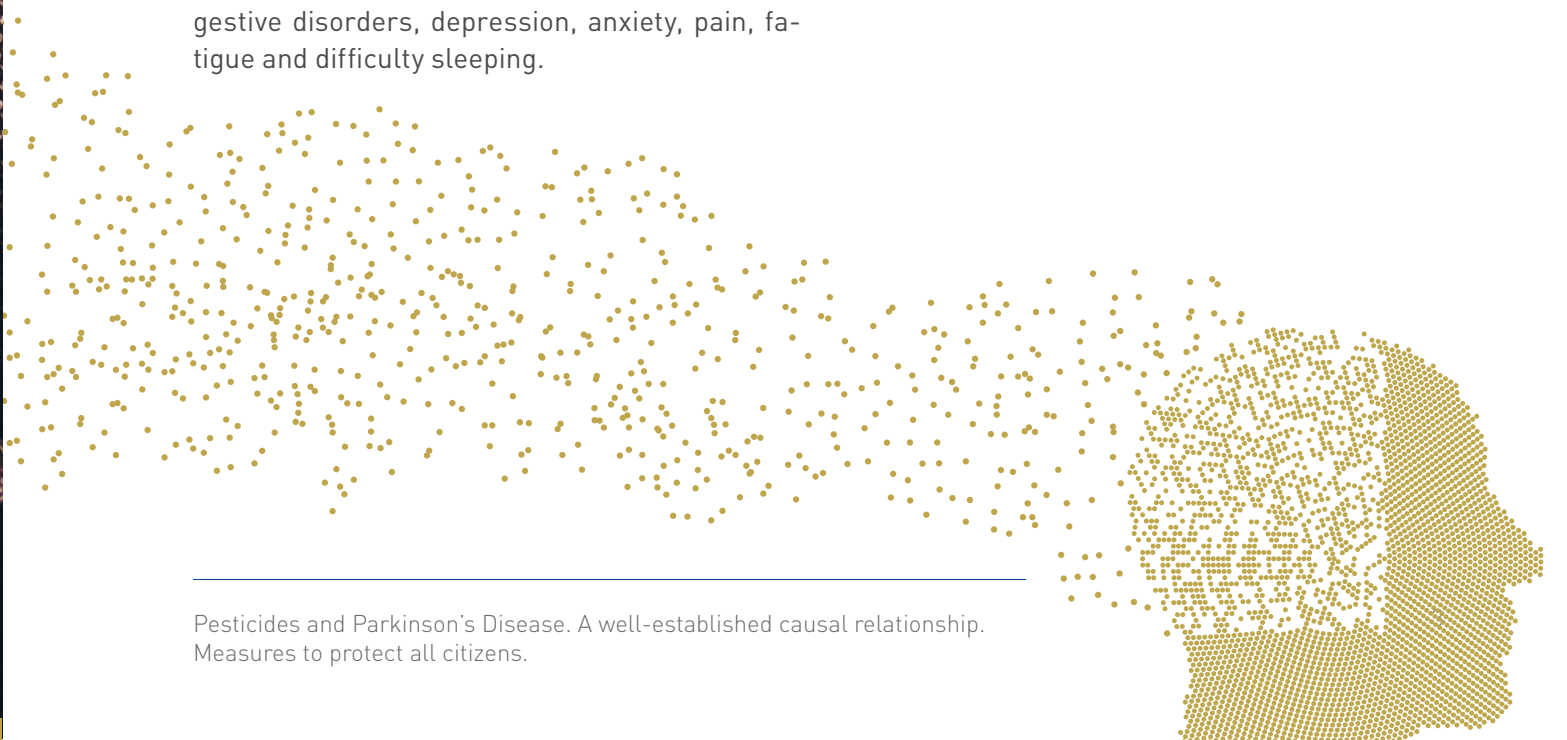
2.2 WHAT ARE THE CLINICAL SYMPTOMS OF THE DISEASE?

Since its classification in the late 19th century and even through to the 20th century, Parkinson's disease was largely understood, studied and treated as a movement disorder. The most common clinical symptoms of the disease include rest tremor, rigidity, difficulty moving and postural instability.⁵

It is only recently that we have gained a deeper understanding of this disease and now recognize non-motor symptoms such as cognitive and digestive disorders, depression, anxiety, pain, fatigue and difficulty sleeping.

PD is a neurodegenerative disease, meaning that the symptoms of the disease continue to worsen over time. The severity and diversity of the symptoms can vary from person to person, making the disease extremely debilitating. The affected person gradually loses their independence and must heavily rely on both the help of those around them, as well as any current treatment options available to them. There are no therapeutic treatment options currently available that can cure or even slow the progression of PD.

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2.3 WHAT CAUSES THE DISEASE?

The debate surrounding whether environmental or genetic factors are to blame for the etiology of Parkinson’s disease have been around as long as the disease itself. After all, PD was only recognized for the first time near the end of the 19th century. Since then, evidence to support both arguments has come to light.

Recently, the genetics theory has attracted a lot of attention as it has successfully been able to identify whether members of the same family possess the clinical and pathological symptoms of Parkinson’s disease as a result of mutations in one or several specific genes.^{6,7} However, hereditary forms of parkinsonism are extremely rare (5 to 10%).⁸ In other cases, some people have minor sporadic genetic mutations, which may also indicate a predisposition to the disease.^{5,6}

Most cases of Parkinson’s disease are idiopathic, meaning that the cause is unknown. The very first

theories on environmental causality that were suggested more than 100 years ago drew significant evidence from cases of PD that were caused by external agents (e.g. viral postencephalitic parkinsonism or neurotoxin-induced secondary parkinsonism).

Today, the disease is considered to be multifactorial. This stems from the interaction between environmental toxins and individual genetic susceptibility.⁵ Recent work on animal models suggests that the onset of the disease is linked to non-exclusive situations such as chronic, low-level exposure to nerve agents, limited exposure accompanied by delayed symptoms due to a decline in nerve cell protection and an increased susceptibility to toxins that worsens with age.⁹ These works are particularly relevant when interpreting the epidemiological and toxicological data reviewed in this submission.

2.4 WHAT PROCESSES LEAD TO THE ONSET OF PD SYMPTOMS?

Even though the causes of PD remain uncertain, the processes that lead to its development and the onset of the first symptoms are now well-documented. The Braak model (Figure 1) is particularly useful in understanding how external agents are capable of triggering PD and how the symptoms of the disease worsen depending on the degree of neuronal damage.¹⁰

The Braak model suggests that an environmental toxin is responsible for triggering PD by entering the body through the nose or the digestive tract and then targeting the neurons in the olfactory bulb or vagus nerve. This toxin interferes with neurons in three ways, by:

- a. Provoking malformations and triggering the aggregation of a protein that is crucial to the release of molecules that relay information. In cases of PD, this protein is called alpha-synuclein. Its accumulation within cellular inclusions, or Lewy bodies, is one of the telltale signs of PD.¹¹ In addition to their toxicity to the affected neurons, these aggregates also have the ability to infect the surrounding neurons;
- b. Disrupting the normal functioning of mitochondria and depriving the neurons of their source of energy. Moreover, this loss of energy also produces oxidative derivatives capable of initiating cell death;

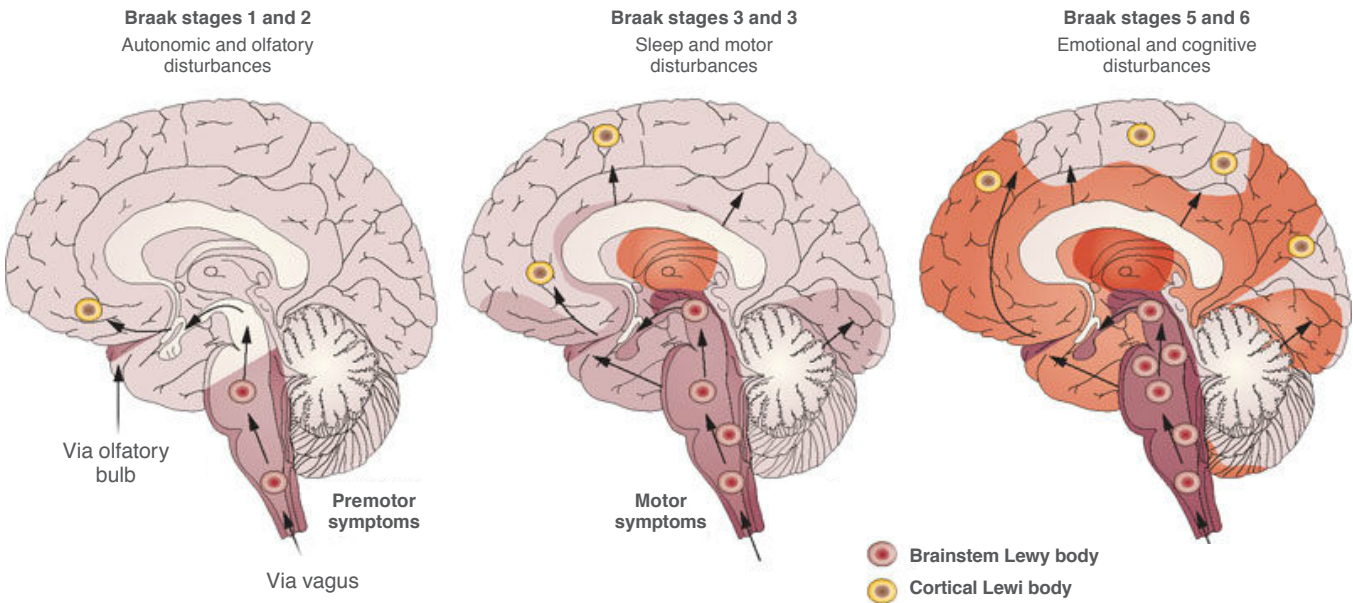


FIGURE 1. Progression of PD according to the Braak model.

c. Causing inflammation as a result of the residue released during neuronal death.

These three combined processes cause the neurons in the central nervous system to progressively die.

These protein clusters gradually make their way along the olfactory and vagus nerves, which respectively connect the nose and the digestive tract to the brain, causing the neuronal damage that coincides with the onset of various symptoms.¹² That is why the onset of the disease is characterized by non-motor symptoms, such as a loss of smell or constipation.

These aggregates gradually move toward the part of the brain responsible for controlling

movement, also known as the substantia nigra. In this part of the brain, a molecule known as dopamine acts as the chemical messenger between neurons. The progressive degeneration of dopaminergic neurons leads to a loss of dopamine, which then triggers the appearance of various motor disorders. This is another one of the hallmarks of PD.¹³

The cognitive and emotional symptoms of the disease appear when the contaminated neurons have gone beyond the substantia nigra and have spread to the cortical areas of the brain. Moreover, non-motor symptoms are caused by damage to other neuronal systems, such as the GABAergic, cholinergic and serotonergic systems.¹¹

3

WHAT ARE THE IMPACTS OF LIVING WITH PARKINSON'S DISEASE?

3.1 WHAT IS IT LIKE LIVING WITH PARKINSON'S DISEASE?

66-year-old Jean lives with his wife Germaine in Mégantic. He was diagnosed with Parkinson's disease at the age of 57. He has worked on his family's farm for his entire life. Nowadays, he can no longer continue farming as he has been severely impaired by the disease.

Jean has a fixed, mask-like expression. Even if he was good-natured before the disease, he can now no longer display any emotion. The disease has crippled all of the muscles in his body, including those in his face. However, his mouth and vocal cords have been spared. His voice is monotone and somewhat muted.

Watching him take small "awkward" steps and constantly lose his balance, his wife confides that he used to be "a strong man." Jean and Germaine started to suspect that the disease was looming when Jean was no longer able to lift his arms high enough to reach the plates on the top shelves, and his left hand started to tremble ever so slightly near his thumb. Two years later the diagnosis hit: Parkinson's disease. Their neurologist explained to them that some symptoms can appear decades before a diagnosis has been confirmed. In fact, for years, Jean was experiencing vivid dreams that caused him to toss and turn "like the devil," resulting in him no longer being able to share the same bed with his wife as she was "being hit." Moreover, his mood was unstable, and he felt extreme and ungrounded anxiety over the daily worries of the farm.

Jean must now take 17 pills every day to treat his Parkinson's—yet that doesn't even control all of his symptoms. For more than a quarter of his waking hours, Jean feels as though he is carrying a lead weight that prevents him from moving. This feeling occurs when his medications are no longer effective. If he were to take any more medication, his arms and legs would move uncontrollably in all directions. He admits that everything in his life has slowed down. Even just walking, one of the most natural movements, takes time, but "his mind is also slow. Sometimes, he mulls over ideas in his head for minutes before making even the most straightforward decision." This slowness is unfortunately in no way relaxing. The disease also disrupts his sleep cycle and robs him of a good night's sleep.

On top of his Parkinson's medications, Jean also takes antidepressants and anxiolytics. "It's not that he's afraid of the future," says his wife reassuringly, "It's that the disease has also affected his mind. The blue pills that he takes also help to reduce his fear of unexpectedly 'freezing.' Sometimes he 'freezes' for several minutes right in the middle of the road or the grocery store."

Fortunately, Jean has his wife of nearly 50 years, Germaine, by his side, as each day he becomes less and less independent and he knows it.

The names of the people interviewed have been changed.

3.2 IMPACT ON DAY-TO-DAY ACTIVITIES AND QUALITY OF LIFE

Parkinson's disease is a neurodegenerative disease that negatively affects cognitive and motor functions. Patients not only have to deal with slowly losing their strength and abilities, but must also overcome significant emotional tur-

moil. As the disease progresses, those affected become increasingly dependent on their caregivers to help them accomplish simple everyday tasks (e.g. walking, eating, using the bathroom), which may increase feelings of being a social and

personal burden. Their social circles progressively become smaller as their level of impairment becomes more pronounced. The stress of not knowing how quickly their physical condition will deteriorate is only made worse by the fears associated with their financial situation, employability, progressive social isolation and so on.¹⁴ Seeing as PD is an incurable chronic disease, the patient requires family and social support for the rest of their life. This is even more devastating for the 10% of patients who are diagnosed before the age of 50.

The only treatment options available for PD merely aim to relieve the symptoms of the dis-

ease and must be taken for life. Many of the treatments can cause numerous side effects that not only worsen the patients existing symptoms but jeopardize their quality of life.

The quality of life for people living with PD, compared to that of the general population, deteriorates significantly with age and as the disease progresses. According to validated questionnaires like the PDQ-39 and EQ-5D, patients oftentimes report a major decrease in physical and social functioning. These differences have been reported across all age and gender groups, but are more pronounced in younger patients.¹⁵

3.3 IMPACT ON SOCIETY

In 1998, the direct cost of PD in Quebec totalled 110 million dollars.¹⁶ These direct costs include all of the resources associated with PD prevention, detection and treatment (e.g. hospital costs, medications, doctors' fees and expenses paid to other healthcare professionals). However, the direct costs borne by patients and other payers are not included in that figure. These costs should not be ignored. More than one out of every three patients has to make financial sacrifices in order to pay for things like their medications, maintaining their independence and physical activities that help them maintain a healthy lifestyle.¹⁷

The indirect costs associated with PD stem from a loss of productivity due to disability, as well as

premature deaths. Other indirect costs such as the value of time lost from work, leisure activities and time spent with family and friends are also not included.

In 2013, the economic burden of PD in the United States exceeded 14 billion US dollars (approximately 22,800 US dollars per patient per year). Medical expenses for people living with PD were 12,800 US dollars/year higher than those of the general population of the same age. The indirect costs associated with PD have been conservatively estimated at 6.3 billion US dollars (nearly 10,000 US dollars per patient per year).¹⁸ A recent study indicated that this burden has now risen to 52 billion US dollars.¹⁹



4 DO PESTICIDES CONTRIBUTE TO THE DEVELOPMENT OF PARKINSON'S DISEASE?

In 2019, the results of several epidemiological studies, which had been corroborated by discoveries from various toxicological experiments, established a causal relationship between pesticide exposure, pathogenic α -synuclein induction, damage caused to dopaminergic neurons in the substantia nigra and, potentially, the development of the clinical symptoms of PD.¹⁶

The link between pesticide exposure and PD has already been outlined in the Québec Pesticide Strategy 2015-2018.²⁰

This evidence will be reviewed and discussed in the following sections.

4.1 THE STUDY OF THIS CONNECTION THROUGH THE YEARS

In the 1980s, scientists first became suspicious of the link between PD and exposure to chemical agents. At that time, seven young drug addicts who used the derivative 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) from a synthetic opioid, spontaneously developed parkinsonian syndromes.²¹ Over the following 30 years, this molecule was used to artificially replicate the symptoms of PD in animals in order to study its origin and potentially find therapeutic treatments. Although this molecule does not appear to pose any danger to public health, its chemical structure and mechanism of action are very similar to those of many pesticides commonly used in Quebec, including rotenone, paraquat and maneb.²²

In the early 1980s, Quebec's Dr. Barbeau, a pioneer in Parkinson's research, tabled the theory that the development of PD could be caused by frequent exposure to environmental components with a structure similar to that of MPTP.²³ Several years later, he noticed that PD patients were more prevalent in rural parts of Quebec, particularly in Trois-Rivières, where pesticides were being heavily used.²⁴ These findings were replicated in every country with industrialized agriculture, raising suspicions about the effects that the nerve agents in pesticides could have on the development of PD.

4.2 WHAT IS THE RISK OF DEVELOPING PD AFTER BEING EXPOSED TO PESTICIDES?

From 2000 to 2019, no fewer than 8 meta-analyses concluded that pesticide exposure virtually doubles the risk of developing Parkinson's disease.²⁵⁻³²

In the following section, we will present each of these meta-analyses, as well as their respective contributions. We will also include the various techniques used by researchers to reduce any biases likely to skew their results.

Following these initial observations, numerous epidemiological studies have investigated the relationship between PD and pesticide exposure. Between 1983 and 2019, hundreds of studies were carried out worldwide, each applying different experimental designs in order to confirm this association. These studies assessed the impact that pesticides, as a whole or by class, have within different exposure environments (industry professionals, farmers, local residents) and at different exposure intensities.

The results of these successive studies have been combined several times in various meta-analyses. These types of meta-analyses aim to assess the legitimacy of a conclusion and quantify the increased risk of developing PD in people who have been exposed to pesticides when compared with those from an unexposed population.¹

In 2000, Priyadarshi et al. published the first meta-analysis examining the link between PD and pesticide exposure. This analysis, which includes 19 case-control studies published between 1989 and 1999, reveals that pesticide exposure virtually doubles the risk of developing PD (OR = 1.94; CI95%: 1.49-2.53). According to these findings,

people who have been exposed to pesticides are nearly twice as likely to develop the disease than those who haven't. A similar increased risk was found in every country around the world where these studies were conducted. In this analysis, the authors demonstrated that the risk of developing PD increased with the length of exposure, though they could not demonstrate a significant dose-response relationship or single out one particular pesticide over another.²⁵

In 2012, Van Maele *et al.* published a meta-analysis examining the risks associated with occupational exposure to pesticides (particularly among farmers, as well as labourers working on sugarcane and banana plantations). In order to counter the effects of the memory bias (participants do not remember or misunderstand their exposure), which was inherent in the case-control studies used by Priyadarshi *et al.*, the authors only considered 12 prospective studies published between 1985 and 2011, in which participants were monitored over a period of time. Combining studies that used the best methodological designs, in which PD diagnoses were confirmed by a neurologist, reveals that the risk of developing PD is about two and a half times greater in people who experience occupational exposure to pesticides (OR = 2.56; CI95%: 1.46-4.48; n = 4). Exposure on plantations, where pesticides are heavily used and working conditions are inadequately monitored, significantly increases the risk of developing PD.²⁶

1. In meta-analyses, this increase in risk is expressed as an odds ratio (OR). In this report, ORs are interpreted as relative risks (RR), which is a comparative measure of the risk of developing PD among people who have been exposed to pesticides, compared to the risk of an "equivalent" unexposed group of the population. A relative risk of 1.0 indicates that there is no increase in risk. In the first meta-analysis, the OR of 1.94 indicates that the risk of developing Parkinson's disease is 1.94 times greater in people who have been exposed to pesticides when compared with an unexposed population with the same characteristics. Moreover, the OR must be statistically significant in order to be considered. To be statistically significant, this 95% confidence interval should not include a value of 1, which indicates that there is no association between PD and pesticide exposure. The size of this interval is relative to the size of the studies being considered and the variability of their results.

In the same year (2012), van der Mark *et al.* combined the results of 46 studies (39 case-control studies, 4 cohort studies, 3 cross-sectional studies) published between 1989 and November 2010 in order to assess the toxicity of different classes of pesticides and evaluate how different experimental designs could impact the results of these studies. According to this analysis, the risk of developing PD is slightly more than one and half times greater in people who have been exposed to pesticides (OR = 1.62; CI95%: 1.40–1.88). This association is particularly significant for people who have been exposed to herbicides (OR=1.40; IC95%: 1.08–.81) and insecticides (OR=1.50; CI95%: 1.07–2.11). However, the authors were unable to establish a link between fungicide exposure and PD (OR = 0.99; CI95%: 0.71–1.40).³³ As a consequence of this meta-analysis, the French government has recognized PD as an occupational disease among farmers who have been exposed to pesticides for more than 10 years.³⁴ However, according to this analysis, the risk of occupational exposure was not simply limited to farmers. Acknowledging this fact is a step in the right direction; however, a new piece of legislation must be adopted in order to be more inclusive of all individuals whose professions puts them at risk of coming into contact with pesticides.

In 2013, Pezzoli and Cereda delved further into the specific impact that pesticides have on the development of PD. Just like van der Mark, they analyzed 51 case-control studies in order to demonstrate that pesticide exposure, regardless of class, nearly doubles the risk of developing PD (OR=1.76; CI95%: 1.56–2.04). The results for specific classes of pesticides were also replicated, concluding that the risk of developing PD is 1.3 times greater in people who have been exposed to herbicides (OR = 1.33; CI 95%: 1.08–1.65) and 1.5 times greater for those who have been exposed to insecticides (OR = 1.53; CI 95%: 1.12–2.08). Once again, the association between fungicide exposure and PD was insignificant.²⁸ The results of these functional classes of pesticides were consistent with the findings in the previous

meta-analysis, however, they also revealed certain agents that possess disparate toxicological profiles, which could obscure the significant effects of a small number of agents with the same mode of action belonging to the same class.³⁵ Out of all of the pesticides specifically evaluated by the authors, only paraquat had any significant association with PD.

In 2013, more than 10 years after the meta-analysis by Priyadarshi *et al.*, Allen *et al.* were able to confirm the positive association between pesticide exposure and PD. Their analysis of 28 case-control and cohort studies that were published between 1989 and 2012, demonstrates that the risk of developing PD is almost one and a half times greater in people who have been exposed to pesticides (OR = 1.42, CI95%: 1.32–1.52). By conducting a subgroup analysis using a sample of 17 case-control studies, the authors were able to demonstrate that the risk of developing PD is one and a half times greater in people who have experienced occupational exposure to pesticides (OR = 1.49; CI95%: 1.34–1.66). This elevated occupational risk suggests that the increased risk of developing PD is directly associated with the intensity, duration and/or frequency of the pesticide exposure itself. Moreover, a sub-analysis of 6 case-control studies demonstrated that the risk of developing PD increased by 34% (OR=1.34; CI 95%: 1.34–1.66) among at-home pesticide users. Finally, the authors were able to evaluate the specific effects that herbicides (OR = 1.27, CI95%: 1.11, 1.45), insecticides (OR = 1.30; 1.14, 1.47), fungicides (OR = 0.77; CI95%: 0.51–1.16) and rodenticides (OR = 0.90, CI95%: 0.52–1.59) had in the development of the disease, while also taking into account the type of exposure (occupational or not). This study confirms the results yielded by van der Mark and Van Maele on the toxicity of pesticides within an occupational environment. It also provides further insight into the specific harmful effects of herbicides and insecticides and outlines the risks associated with their domestic use. The authors also concluded that unless future case-control studies provided better

exposure characterization, it would be impossible to collect any additional evidence on the association between pesticide exposure and PD.²⁹

In response to the statement made by Allen *et al.* in 2017, Gunnarsson *et al.* adopted a stricter methodology for selecting published studies. He chose to only base his research on studies that met the highest scientific criteria in terms of methodological design, diagnostic and exposure assessment, missing data management and statistical analysis. The analysis of this limited selection of 8 cohort and 15 case-control studies, demonstrates that the risk of developing PD is 1.67 times greater in people who have been exposed to pesticides (OR=1.67; CI95% 1.42–1.97). In addition to carefully selecting each study, this meta-analysis was limited to just two exposure levels (medium and high), thus prioritizing the

number of years of exposure rather than the maximum duration of exposure. This means that the risk must be interpreted for recurrent, moderate-intensity occupational exposure to pesticides. Moreover, the authors conclude that the relative influence of older studies (with higher ORs) on the results of new meta-analyses tends to fade over time and stabilize around a risk increase of about 1.7, or nearly double.³¹ This study was partially funded by two Swedish insurance groups (AFA Insurance and Forte). In response to the publication of these results, both insurance groups occasionally recognized Parkinson's disease as an occupational disease.

The meta-analyses carried out by Ahmed (2017) and Yan (2018) will be reviewed in the following sections.



4.3 IS THIS RISK ASSOCIATED WITH OCCUPATIONAL EXPOSURE?

Occupational exposure to pesticides doubles or even triples the risk of developing PD. In Quebec, pesticide applicators and grain producers are particularly at risk. While personal protective equipment is effective in safeguarding against acute reactions, it does not protect against the risk of developing PD.

PD has long been associated with occupational exposure.^{36,37,37–44,45} Among the many studies that report a positive association to PD, at least three stand out for their relevance, methodological rigour of exposure assessment and analyses on specific pesticides.

A case-control study by Elbaz *et al.*, conducted in France in 2009, demonstrates that the occupational use of pesticides almost doubles the risk of developing PD (OR = 1.8; CI95%: 1.1–3.1) and insists that this risk is dose-dependent. This risk is particularly high among people who use organochlorine or amide insecticides, as well as dithiocarbamate fungicides. People who have been exposed to the above-mentioned pesticides are 2.4, 3.1 and 2.1 times more likely to develop PD, respectively, than people who have never been exposed to these types of chemical compounds.⁴⁶

To overcome the challenges of evaluating particular pesticides, the case-control study used in the Agricultural Health Study (AHS), investigated pesticide toxicity by grouping them together according to their traditional functional classification (herbicide, insecticide and fungicide) instead of by their mode of action (see section 5). People who are exposed to pesticides that are capable of inhibiting mitochondrial complex I are 1.7 times more likely to develop PD. For rotenone, in particular, the risk of developing PD becomes 2.5 times greater (OR = 2.5; CI95%: 1.3–4.7). Similarly, people who use pesticides that are capable of causing oxidative stress, double their risk of developing PD. When it comes to paraquat, the risk of developing PD becomes 2.5 times greater (OR = 2.5; CI95%, 1.4–4.7).⁴⁷

Finally, with the help of a GPS system and California’s database on pesticide use, Wang’s studies on pesticide exposure demonstrate that occupational exposure to each of the 36 organophosphates studied, increased the risk of developing PD. Depending on the product, this increase in risk ranged from 2 to 5. Unsurprisingly, the vast majority of the participants in this study were all exposed to multiple products simultaneously, and therefore, had increased risks.⁴⁸ By using the same methodology, the authors demonstrated that occupational exposure to paraquat, rotenone and ziram tripled the risk of developing PD (OR: 3.09; CI95%: 1.69–5.64).⁴⁸

It should be noted that agriculture-related occupational exposure differs enormously depending on the geographical area being studied, the period of time, and more particularly, the type of agriculture being examined (e.g. grain farming, orchards, livestock). The following is a risk gradient for farmworkers: Professional pesticide applicators → grain producers → livestock producers → dairy producers.⁴⁹

Nevertheless, some studies have found no significant association between PD and professions in the agricultural sector.^{38,40,43,50–52} These studies do however lack rigour in their exposure assessment but were still able to demonstrate a positive association between direct pesticide exposure and PD.⁵³

Given the risks associated with chronic pesticide exposure in farmworkers, training that warns against the dangers of certain products and the use of personal protective equipment is crucial.

Unfortunately, these measures appear to only be moderately effective. Repeated studies carried out worldwide, demonstrate that the vast majority of farmers are aware of the dangers of pesticides, but that half of them rarely use personal protective equipment (PPE), if ever. In fact, PPE use is less common among farmers who operate their farms independently than among farm

employees. When PPE is used, it is more often when pesticides are being prepared, than when they are being applied.^{54,55,56}

PPE use reduces the frequency of incident reports.⁵⁶ However, it does not reduce the risk of being exposed to pesticides, which has been linked to the development of PD.⁵⁷

4.4 IS THIS RISK ASSOCIATED WITH RESIDENTIAL EXPOSURE AND AMBIENT EXPOSURE?

Pesticide exposure within the home is sometimes greater than occupational exposure but is not controlled under any regulations. People who experience thirty days of lifetime exposure to herbicides within their home are 1.7 times more likely to develop PD. Residential exposure within 500 metres of where a combination of rotenone, maneb and ziram have been applied, almost doubles the risk of developing PD.

In general, the intensity of occupational pesticide exposure is higher than that in residential settings or larger exposure environments.⁵⁸ However, pesticide residues in an indoor environment appear to be a greater potential source of long-term exposure when compared to being applied outdoors. In fact, once indoors, these molecules are unlikely to dissipate and will linger until they are able to naturally degrade.⁵⁹ A pilot study conducted in France by Bouvier *et al.* concluded that the general population was exposed to a wider variety of pesticides and sometimes at higher concentrations than in occupational environments.⁶⁰

In 2000, Stephenson raised significant concerns surrounding the residential use of pesticides by the general population within their homes. According to his research, people who use pesticides within their home are 1.7 times more likely to develop PD. People who use insecticides on their gardens are one and half times more likely to develop PD (OR = 1.5). The risk of developing PD is dose-dependent for people who use herbicides on their gardens. The risk is 1.4 times greater if

used for less than 30 days, and 1.7 times greater for users who report a lifetime exposure of at least 160 days.⁶¹

In 2009, with the help of a GPS system and California’s database on pesticide use, Costello *et al.* assessed the impact of ambient exposure to paraquat and maneb within a 500-metre radius around residential homes. Within this 500-metre radius, the risk of developing PD is almost twice as high when compared to the risk to those living outside of this area (OR = 1.75; CI95%: 1.13–2.73).⁶²

In 2011, Wang *et al.* reused the Costello methodology and included exposure to the fungicide ziram and compared the exposure risk in both residential and occupational areas. People living within 500 metres of an area where these three pesticides have been applied (paraquat, maneb and ziram) almost double their risk of developing PD (OR: 1.86; CI95%: 1.09– 3.18) when compared with those living outside of this area.⁶³

4.5 IS THIS RISK DEPENDENT ON THE DOSE AND DURATION OF EXPOSURE?

The frequency, intensity and cumulative number of lifetime days of exposure are intimately related to an increased risk of developing PD. People who experience 10 days of exposure per year are two and a half times more likely to develop PD.

In 2000, Ritz and Yu estimated the parkinsonism-related mortality rate in California between 1984 and 1994. This rate was 17% to 49% higher in counties that used pesticides strictly for occupational purposes, than in counties that did not use these products at all. After adjusting for the demographic characteristics of the population, the risk of dying from PD-related causes was 50% higher in these counties, regardless of exposure intensity and length of residence in the county. They also concluded that a dose-response relationship existed between the amount of insecticides being used per unit area and the development of PD.⁶⁴

In a large case-control study conducted in 5 European countries in 2006, Dick *et al.* demonstrated that a dose-response relationship existed between the average annual pesticide exposure intensity and the development of PD. People who are exposed to pesticides for an average of 10 hours per year at the allowable exposure level are 1.19 times more likely to develop PD than people who have never been exposed to these types of chemical compounds (OR = 1.19, 95% CI 0.90–1.57). People who are exposed to pesticides at the allowable exposure level for an average of 36 hours per year are one and a half times more likely to develop PD (OR = 1.56, CI95%: 1.19–2.04).⁴³

4.6 IS THIS RISK ASSOCIATED WITH EXPOSURE DURING CHILDHOOD?

Being exposed to pesticides during neurodevelopment contributes to the risk of developing Parkinson’s disease by damaging the dopaminergic system and increasing its vulnerability to any subsequent exposures. People who have been exposed to pesticides during their childhood are up to 5 times more likely to develop PD.

In a case-control study conducted in 2008, Hancock *et al.* demonstrated that the risk of an entire family developing PD was dependent on how often pesticides were used each year, but also on the number of accumulated lifetime days of exposure. For example, people who are exposed to pesticides for more than 10 days per year are two and half times more like to develop PD (OR =2,55; CI95%: 1,38–4,73). A lifetime exposure of more than 180 days triples the risk of developing PD (OR = 3,25; CI95%: 1,84–5,73) in people who do not have a family history of PD.⁵⁷

In 2018, Yan *et al.* performed a meta-analysis in order to specifically quantify the dose-response relationship between long-term/low-intensity pesticide exposure and the risk of developing PD. This analysis was based on 10 studies which clearly defined pesticide exposure levels. The results revealed that there was a 5% risk increase after 5 years of exposure (OR = 1.05; CI95%: 1.02–1.09) and an 11% increase (OR = 1.11; CI95%: 1.05–1.18) after 10 years of exposure. In the hopes of determining a point below which the risk of developing PD was no longer significant, the authors demonstrated that just one year’s worth of pesticide exposure corresponds to a risk increase of 1% (OR=1.01, 95% CI: 1.00–1.02).³²



The risks associated with pesticide exposure, particularly to paraquat and maneb, are not limited to exposure during adulthood. Two recent toxicological studies have shown that the combined effects of being exposed to these two products shortly after birth, followed by subsequent exposure during adulthood, increases the risk of degeneration to the substantia nigra and thus the development of PD. Conversely, post-natal exposure only leads to minor changes in adulthood. However, re-exposure during adulthood reveals the hidden toxicity of pesticides.⁶⁵

Similarly, one study investigated the impact of chronic, low-dose exposure to dieldrin in young adult mice. The results revealed changes in redox equilibrium in the substantia nigra, an increase in alpha-synuclein concentration and a decrease in dopamine transmitter activity, all of which are specific PD-related markers.⁶⁶

Sub-analyses of the Costello and Wang studies, reviewed in the previous section, provide interesting epidemiological insights into the results of these animal models. In their studies, these researchers found that people who have been exposed to paraquat and maneb during their childhood and/or adolescence are up to 6 times more likely to develop PD than those who had never been exposed to these types of chemical compounds. By comparison, people who are exposed to pesticides exclusively as adults, only increase their risk of developing PD by one-third.^{62,63}

This toxicological data, which has been backed by various epidemiological studies, is extremely worrying, seeing as in Quebec, 99% of urine samples from children between the ages of 3 and 7 contain metabolites from organophosphorus pesticides.⁶⁷

4.7 IS THIS RISK ASSOCIATED WITH EXPOSURE DURING PREGNANCY?

In animal models, exposure during pregnancy leads to pesticide sensitization, which in turn, makes adults more vulnerable to any subsequent exposures.

In several mouse model studies, exposure to maneb during pregnancy and subsequent exposure to paraquat in adulthood leads to a marked decrease in locomotor activity, changes in dopamine levels and a selective loss of neurons in the substantia nigra. Exclusive exposure to pesticides during pregnancy, not followed by re-exposure during adulthood, does not lead to a decrease in dopaminergic activity.^{68, 69}

Similarly, exposure to organochlorine pesticides throughout various developmental stages also has serious long-term consequences. Exposing mice to dieldrin, heptachlor or endosulfan during pregnancy or lactation, not only affects the neuron function in the substantia nigra of their pesticide-exposed offspring but significantly increases their susceptibility to the neurotoxic effects of MPTP if exposed to it later in life.^{70, 71}

4.8 DOES THIS RISK INCREASE WITH SIMULTANEOUS EXPOSURE TO DIFFERENT PESTICIDES?

Simultaneous exposure to several different pesticides, as is the case in our everyday environment, leads to a potentiation of the toxic effects of each product.

Human exposure to pesticides rarely involves one single agent. Animal models of Parkinson’s disease that have been caused or worsened by the combined effect of several nerve agents are particularly enlightening when it comes to understanding these synergistic effects.

of these two products⁷²⁻⁷⁵ -assisting each other in simultaneously triggering the degradation of the neurons in the substantia nigra, potentiating the toxicity of the pathological alpha-synuclein and inducing parkinsonian symptoms.^{53, 54} These results suggest that maneb helps paraquat cross the blood-brain barrier more easily.

Experiments carried out in mice that were exposed to both iron and paraquat, revealed evidence of toxic substance interactions, even after there had been a long rest period between exposures. Additional experimental evidence to support the additive or synergistic effects of combined exposure to pesticides has been examined in in vivo studies using paraquat in combination with other pesticides.^{42, 43}

Once again, the subgroup analyses from the Costello and Wang studies were able to revise the synergistic effects of paraquat, maneb and ziram. People who experience occupational exposure to these three pesticides are three times more likely to develop PD.^{62, 63}

One of the most convincing results from animal studies comes from the combined exposure to both paraquat and maneb. These studies have successfully demonstrated the synergistic effects

The results of toxicological studies conducted in animal models using maneb or paraquat, support the hypothesis that a multi-target repeated exposure pathogenesis exists and reiterates the results of various epidemiological studies. Together, these studies demonstrate that: 1)

there is an age-related disposition to experiencing degeneration in the substantia nigra as a response to pesticide exposure, 2) the symptoms of the disease worsen when exposed to two differ-

ent pesticides when the first exposure (pre- or post-natal) to these toxins predisposes animals to an increased sensitivity to re-exposure.

4.9 LIMITATIONS OF EXISTING EPIDEMIOLOGICAL STUDIES

Epidemiological studies that examine the association between PD and pesticide exposure are often plagued by limitations related to measuring exposure. Nevertheless, the vast majority of these studies conclude that there is, in fact, a positive association, further confirmed by all the meta-analyses.

When analyzing epidemiological data, it is common to run into several caveats, particularly when examining studies that investigate the association between PD and pesticide exposure. Methodological challenges often occur in epidemiology and stem from case categorization (who is sick and who isn’t?) and the extent of exposure (who has been exposed and at what intensity?).

definition, job-exposure matrix, detailed dose questionnaire, exposure history), especially in retrospective experimentation plans, often underestimate the actual amount of exposure.⁵³ Similarly, exposure during childhood or even during pregnancy are hard to categorize in retrospect.

Therefore, correctly identifying cases of Parkinson’s disease is difficult because no accurate diagnostic test exists. Differences in clinician experiences, the patients access to care and changes in diagnostic criteria are all possible biases. Additionally, PD has a long prodromal stage (pre-diagnosis). Many cases of undiagnosed Parkinson’s disease do in fact exist but are not included in these studies.

The results from epidemiological studies conducted over the last 30 years tend to be disparate, and sometimes even discordant. Initially, these differences could be explained by the difficulties in identifying patient and control cases (population not reached), the diversity of geographical areas studied and the experimental designs (type of studies) used. We now know that they are mainly due to a diversity in exposure assessment methodologies and their respective reliability.³³ Studies using the most reliable methods consistently demonstrate a positive association between pesticide exposure and PD. This association is overwhelmingly confirmed by all of the meta-analyses that have been published to date.

Additionally, evaluating the nature, dose and timing of the exposure poses additional challenges. Most diagnosis methods (self-declared vs. spraying equipment tracking; yes or no binary

4.10 IS PESTICIDE EXPOSURE LINKED TO THE DEVELOPMENT OF PD OR IS IT ONE OF THE CAUSES?

The association between pesticides and PD is not accidental. Extensive evidence from both epidemiological and toxicological studies demonstrates that there is a causal relationship between pesticide exposure and the development of PD.

All of the epidemiological data reviewed to date, indisputably confirms that there is an association between pesticide exposure and PD. However, this association alone does not make it possible to conclude that there is an actual causal relationship between pesticides and PD.

The Bradford Hill criteria are often used to confirm the causality between different events.⁷⁶

1. Strength of association: The vast majority of the epidemiological studies that we have reviewed, conclude that the risk of developing PD doubles with pesticide exposure.

2. Consistency of results: These epidemiological results have been consistent over the course of the last 30 years, regardless of the experimental designs being used or where in the world the studies have been conducted.

3. Temporality: Pesticide exposure always precedes the development of PD. The next section is dedicated to examining the mechanisms of action of pesticides, the series of events following pesticide exposure and how it relates to the development of the clinical symptoms of PD by first contributing to the degradation of the dopaminergic neurons in the substantia nigra and then by producing pathological alpha-synuclein.

4. Biological gradient: The results of epidemiological studies confirm that a dose-response relationship does in fact exist. The risk of devel-

oping PD increases with the intensity, frequency and cumulative duration of pesticide exposure.

5. Biological plausibility: The mechanisms of pesticide-induced PD will be illustrated in the following section as it relates to MPTP, a nerve agent with a structure and mechanism of action similar to that of certain pesticides.

6. Organic coherence: The development and evolution of PD in both animals and humans, whether naturally occurring or neurotoxin-induced, follows the same pattern described in the Braak model.

7. Analogy: For more than 30 years, paraquat (herbicide), rotenone (insecticide), MPP+ (herbicide), MPTP (precursor to the nerve agent MPP+) and manganese have all been routinely and interchangeably used to trigger the death of dopaminergic neurons and chemically mimic PD in animal models.⁷⁷

Additional research is needed in order to better understand the specific toxicity of pesticides, their interactions, dose-response relationships and particular windows of vulnerability in human life. However, there is now more than enough scientific evidence to conclude that a causal relationship between pesticides and the development of PD does exist, thus encouraging the government to take concrete actions to protect its citizens.

5

HOW DO PESTICIDES ACT AT THE BIOLOGICAL LEVEL TO TRIGGER PD?

As we previously discovered, PD has extremely specific neurochemical, pathological and clinical signatures (Sections 3.2-3.4). If pesticides do play a role in the development of PD, they must be able to reproduce, both in vitro and in vivo, these precise characteristics.

In order to better understand the effects that pesticides have on the cellular processes that lead to the death of dopaminergic neurons, we will study the mechanism of action of MPTP, the first chemical agent to be identified and deemed responsible for the onset of PD. We will then see how pesticides such as rotenone, paraquat, maneb and organophosphorus have similar and equally lethal actions.

5.1 THE MPTP MODEL, A TEXTBOOK CASE OF EXTERNAL NERVE AGENTS

Exposure to MPTP causes selective damage in the substantia nigra. It leads to a severe drop in dopamine levels, a massive loss of dopaminergic neurons and inflammation of the neuronal tissue, thus resulting in the traditional symptoms of PD, which are indistinguishable from those experienced naturally.

The link between pesticide exposure and PD has been given particular attention because of the structural similarity between MPTP and some pesticides.⁷⁸ In the early 1980s, discovering that the chemical agent MPTP could induce parkinsonian syndromes in young adults was a turning point in Parkinson's research and in understanding the role that nerve agents play in the development of Parkinson's disease.²¹

At the cellular level, MPTP must cross the blood-brain barrier that protects the brain from inter-

nal and external toxins. It must then be converted into MPP+, which is what truly facilitates neuronal damage. MPP+ is then recaptured by dopaminergic neurons via a highly specific transporter (DAT), making it a prime target. When MPP+ levels become too concentrated within the neuron, it overtakes the mitochondria, the powerhouse of the cell. The inhibition of complex I of the mitochondrial respiratory chain causes a depletion of the cell's capacity to generate energy, thus resulting in its death. The toxicity of MPTP can also be attributed to the production of free

radicals. Free radicals are released when MPTP is being converted into MPP+ and its inhibition of the respiratory chain.⁷⁹

Examining the brains of individuals who have been exposed to MPTP shows a substantial loss of dopaminergic neurons in the substantia nigra, similar to that seen in cases of idiopathic parkinsonism. Similarly, these brains contain a massive accumulation of extraneuronal neuromelanin, an overgrowth of glial cells that support neuronal tissue, and activity in the microglial cells respon-

sible for central nervous system immunity. When all of these observations are taken together, they are characteristic of continuous cell death and inflammation for several years after the initial exposure.⁸⁰

The clinical symptoms that follow are identical to the motor symptoms experienced with PD (resting tremor, stiffness bradykinesia), and are indistinguishable from those of idiopathic Parkinson's disease.⁸¹

5.2 ROTENONE

Rotenone has been used for almost 20 years in order to develop animal models of PD. Even in low doses, rotenone can cause the neuronal lesions and classic pathological symptoms of PD in both humans and animals.

First recorded in the United States in 1947, rotenone has been widely used as a non-selective insecticide in agriculture, gardening and flea control. In 2006, its registration with the US Environmental Protection Agency was withdrawn for use on livestock and pets. This product is still being used in Canada.

Building on the research that had already been done on MPTP, Betarbet *et al.* developed another animal model by exposing rodents to rotenone, an insecticide that is capable of crossing the blood-brain barrier and specifically blocking complex I of the mitochondrial respiratory chain. As in the case with MPTP, this inhibition triggers the production of free oxidizing compounds and activates the process of mitochondria-dependent programmed cell death.⁷⁸

Even at low doses, this model is capable of triggering the selective degeneration of dopaminergic neurons, alpha-synuclein cell inclusions and the overexpression of the PINK1 gene, all of which are specifically related to PD. These pathological symptoms are accompanied by certain charac-

teristic motor disorders, such as hypokinesia, rigidity, unsteady movements, stooped posture and resting tremor.⁸²

Very few epidemiological studies have investigated the specific impact of rotenone on the development of PD. The study by Tanner *et al.* conducted on a cohort of 84,000 pesticide applicators indicates that those who experience exposure to rotenone²⁹ are 2.5 times more likely to develop PD (OR=2.5, CI95%: 1.3–4.7). This study is particularly methodologically valid because of the familiarity of the population with the products they used, and therefore the validity of their self-reported exposure, as well as the diagnosis of cases of PD by neurologists specializing in movement disorders.

In the previous year, in a case-control study conducted in a Texas clinic, Dhillon *et al.* observed a 10-fold increased risk of developing PD (OR=10.9, CI95%: 2.5–48) associated with the use of gardening insecticides such as rotenone.⁸³

5.3 PARAQUAT AND MANEB

When paraquat is specifically combined with maneb, it triggers the characteristic initial stages of dopaminergic neuron degeneration by provoking the accumulation of alpha-synuclein in the brain and causing inflammation in the microglia cells.

Paraquat, a non-selective herbicide, was introduced to the US market in 1961. It is one of the most commonly used pesticides around the world, including Canada, despite being banned in Europe in 2007. Maneb is a fungicide.

Paraquat is able to gain access to the brain and become activated on the surface of microglia cells after exposure. This activation creates superoxide (O₂⁻), which can then penetrate the surrounding neurons. Dopaminergic neurons,

which are very sensitive to oxidative stress, become prime targets for this nerve agent. Despite its MPTP-like structure, paraquat does a poor job of inhibiting mitochondrial complex I. Its toxicity is linked to its strong redox potential. This oxidative stress contributes to lipid peroxidation, and potentially, to the death of dopaminergic neurons in the substantia nigra. Numerous animal studies have reported that paraquat exposure leads to the degeneration of dopaminergic neurons and triggers the onset of motor disorders, such as decreased locomotor activity and postural reflex.⁸⁴⁻⁸⁶ What's more, paraquat encourages the overexpression and aggregation of alpha-synuclein in the substantia nigra of mice.⁴⁰

Several case-control studies have reported higher risks of developing Parkinson's disease in people who are exposed to paraquat.^{47,52,87-90} Tanner *et al.* were able to demonstrate that this risk was exceptionally high (OR 2.5; CI 95% 1.4-4.7).⁴⁷

Additionally, the risk is substantially greater for people with genetic vulnerability. In the AHS study, people with T1-weighted glutathione-S-transferase deficiency (due to a homozygous deletion of the GSTT1 gene) who use paraquat, become 11 times more likely to develop PD (95% CI 3.0-44.6). Unsettlingly enough, this particular genetic variation is found in 20% of the population.⁹¹

5.4 OTHER PESTICIDES

Pesticides use many mechanisms of actions to induce neuronal death. These include activating pathogenic alpha-synuclein, inhibiting the cellular respiratory chain, suppressing acetylcholinesterase, creating oxidative stress and inducing programmed cell death, inhibiting the ubiquitin-proteasome system or directly activating the susceptibility genes related to the disease (Sec-

tion 6). These mechanisms are largely synergistic and potentially contribute to the development of neurological diseases such as PD.

A non-exhaustive list of pesticides with one or more of these properties, as well as epidemiological data that supports an association with PD, is provided below:

- Acephate
 - 2,4-Dichlorophenoxyacetic acid (2,4-D)
 - Aldrin
 - Azinphos-methyl
 - Benomyl
 - Chlordane
 - Chlorfenvinphos
 - Chlorpyrifos
 - DDT
 - Dieldrin
 - Deltamethrin
 - Demeton
 - Diazinon
 - Dibromide
- Diethyldithiocarbamate
 - Dimethoate
 - Diquat
 - Disulfoton
 - Endosulfan
 - Ethephon
 - Ethion
 - Fenamiphos
 - Heptachlor
 - Lindane
 - Malathion
 - Mancozeb
 - Merphos
 - Methamidophos
 - Methidathion
- Methoxychlor
 - Mevinphos
 - Naled
 - Oxydemeton-methyl
 - Parathion
 - Permethrin
 - Phorate
 - Phosalone
 - Phosmet
 - Profenofos
 - Tribufos
 - Trichlorfon
 - Zineb
 - Ziram



6

HOW DO PESTICIDES TRIGGER PD ON A GENETIC LEVEL?

Pesticides have a negative effect on the genes that protect and ensure that dopaminergic neurons are functioning properly. They also activate genes that predispose humans to PD.

Genetic mutations associated with changes in pesticide degradation processes and the cells' ability to respond to damage, is increasingly recognized as the mediating factor between pesticide exposure and PD.

In a meta-analysis examining pesticide exposure, genetic variation and the risk of developing PD, Almhed et al. reviewed 66 studies published between 1995 and 2016 in order to confirm that the risk of developing PD was one and a half times greater in people who had been exposed to pesticides (OR = 1.46, CI95%: 1.21, 1.77).³⁰

Moreover, it demonstrated that the mechanism of action of pesticides on dopaminergic neurons went well beyond the cellular neurotoxicity described above. Pesticides do in fact have an effect on several genes involved in the onset of PD. They have the ability to:

- Decrease the production of detoxifying enzymes, doubling the risk for mutations in the TGS gene (OR = 1.9; CI95%: 1.40–2.75),
- Increase and facilitate the amount of pesticides being carried to the brain, doubling the risk for mutations in the MDR1 gene (OR = 2.06; CI95%: 1.58–2.68),
- Reduce the amount of dopamine being recycled, increasing the risk for mutations in the SLC6A3 gene by 18% (OR = 1.18; CI95%: 1.02, 1.37).

- Promote alpha-synuclein aggregation, increasing the risk for mutations in the SNCA gene by 18% (OR = 1.18; CI95%: 1.02, 1.37).

- Reduce waste disposal linked to the oxidizing action of pesticides, increasing the risk of mutations in the PON1 by 32% (OR = 1.32, CI95%: 1.09–1.60).

- Activate genetic predisposition to PD (PINK1 gene).

When looked at as a whole, the results of gene-environment epidemiological studies conclude that individual genetic susceptibility in interaction with pesticide exposure determines the risk of developing PD. Genetic susceptibility seems to play a more important role for people who are diagnosed at a young age, whereas environmental factors play a more important role for patients who are diagnosed at an older age.⁷⁹

These studies also suggest that susceptibility genes related to PD may skew the results of epidemiological studies. This most likely explains the sometimes discordant results reported so far—which must be taken into account when analyzing any previous studies.²²

7

CONCLUSION

Parkinson's disease is a complex syndrome resulting from the interaction between various environmental and genetic risk factors. For more than 30 years, evidence that supports the causal relationship between pesticide exposure and PD has been growing.

Pesticides have neurotoxic mechanisms of action which are similar to the agents used to create animal models of Parkinson's disease. They indistinctly cause damage to dopaminergic neurons, brain structures and potentially, the clinical symptoms of PD.

Pesticide exposure in both residential and occupational environments increases the risk of developing PD. This risk is dose-dependent. Additionally, exposure during neurodevelopmental phases (such as pregnancy, infancy and adolescence) leads to neuronal damage that increases susceptibility to any subsequent exposures.

Simultaneous exposure to several pesticides, as is the case in our daily living environments, leads to the potentiation of the toxic effects of each product.

The history of the dangers of pesticides is illustrated by the authorization cycle on the market and their restriction or prohibition.^{92,93} gamma-HCH (lindane) Over the years, as society has replaced organochlorines with organophosphorus compounds, pyrethroids and neonicotinoids, it leaves future generations with a legacy of health problems.

There is now enough scientific evidence to support the fact that pesticides do in fact pose a danger to humans, and particularly when it comes to the development of PD. The government now has the solid evidence that it needs to take action and implement concrete steps capable of protecting all citizens.

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