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Revisiting the Paraquat-Induced Sporadic Parkinson's Disease-Like Model

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Abstract

Parkinson's disease (PD) is a major neurodegenerative disorder that affects 1–2% of the total global population. Despite its high prevalence and publication of several studies focused on understanding its pathology, an effective treatment that stops and/or reverses the damage to dopaminergic neurons is unavailable. Similar to other neurodegenerative disorders, PD etiology may be linked to several factors, including genetic susceptibility and environmental elements. Regarding environmental factors, several neurotoxic pollutants, including 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), have been identified. Moreover, some pesticides/herbicides, such as rotenone, paraquat (PQ), maneb (MB), and mancozeb (MZ), cause neurotoxicity and induce a PD-like pathology. Based on these findings, several in vitro and in vivo PD-like models have been developed to understand the pathophysiology of PD and evaluate different therapeutic strategies to fight dopaminergic neurodegeneration. 6-OHDA and MPTP are common models used in PD research, and pesticide-based approaches have become secondary models of study. However, some herbicides, such as PQ, are commonly used by farming laborers in developing countries. Thus, the present review summarizes the relevant scientific background regarding the use and effects of chronic exposure to PQ in the context of PD. Similarly, we discuss the relevance of PD-like models developed using this agrochemical compound.

Keywords Paraquat · Pesticides · Agrochemicals · Parkinson's disease · Neurodegeneration

Introduction

Currently, exposure to agrochemicals remains a reality in Third World countries. With the increasing growth of agriculture, animal breeding, and aquaculture, as well as the indiscriminate use of these compounds, exposure to agrochemicals is a certainty. Although the major concern regarding agrochemical exposure in humans has primarily been to prevent suicide attempts through oral consumption, knowledge regarding the effects of chronic exposure, specifically on the central nervous system (CNS), is limited. The potential relationship between chronic exposure to organophosphates and alterations in the central cholinergic and/or dopaminergic system and the specific possibility that agricultural workers have an increased risk of developing Parkinson's disease (PD) were hypothesized for the first time in 1978 [1]. The US National Research Council concluded that 3% of developmental disabilities result from environmental exposure [2]. Up to 1000 chemicals induce neurotoxicity under experimental conditions, including metals, solvents, and pesticides, but many other chemicals with unrecognized effects exist. Indeed, exposure to air pollutants and heavy metals and the use of pesticides in rural settings are correlated with Parkinsonism [3–6], further supporting the hypothesis that environmental toxins trigger the neurodegeneration of nigrostriatal dopaminergic neurons [7–9]. Moreover, pesticides are often used in combination with other chemicals, and neighboring farmlands usually apply the same or additional agrochemical combinations, suggesting that exposure to multiple potentially toxic chemical agents may exert additive or synergistic effects [10]. According to the Agricultural Health Study (USA), PD risk increases proportionally with an increase in the number of

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days of exposure to pesticides [11]. Similarly, in France, PD has been recognized as a professional disease due to the large number of farmers exposed to pesticides [12]. Although the mechanism by which agrochemicals induce neurodegeneration remains a research focus, mitochondrial dysfunction, protein aggregation, altered dopamine levels, and increased oxidative stress represent the more relevant alterations induced by agrochemical exposure [13]. Although chronic exposure to harmful environmental pollutants can act as a determinant that triggers the neurodegenerative process in the dopaminergic system [9, 14], linking pesticide exposure to PD, the risk associated with new and/or specific agrochemicals remains uncertain.

Parkinson's Disease

Sporadic Parkinson's Disease

PD is the second most prevalent neurodegenerative disorder worldwide, affecting people over the age of 50 years. Although this disease can develop at early ages due to mutations in several genes, including α-synuclein (SNCA, A30P, E46K and A53T) [15], Parkinson's disease-associated protein (DJ-1), and parkin (PRKN) [16] (Table 1), the sporadic presentation of PD, which has an undetermined etiology, is the most common form manifested in up to 90% of all PD patients. Clinically, PD constitutes a chronic, progressively debilitating, irreversible pathology characterized by selective damage of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Although PD symptomatology is well established, symptoms are observed only when approximately 80% of dopamine levels in the putamen are depleted and when approximately 60% of dopaminergic neurons within the SNpc are lost [17] (Fig. 1). On the other hand, the neuropathological findings in the SN*pc* include the selective loss of dopaminergic neurons and presence of intra-cytoplasmic protein aggregates, known as Lewy bodies (LB) [18, 19] and dystrophic neurites (LN) [20] in the remaining surviving neurons.

PD is diagnosed at later stages by verifying the presence of several symptoms, including age, genetic assessments, nonmotor signs (psychotic symptoms, such as isolated diplopia, freezing, and spatial misjudgment) [21], and the manifestation of progressive movement impairments, such as bradykinesia, muscular rigidity, tremor at rest, and postural imbalance [22, 23]. Unfortunately, treatments that reverse or stop damage to the nigrostriatal system are still unavailable.

Etiology and Pathophysiology of PD

Similar to other chronic neurodegenerative disorders, several hypotheses have been developed to account for the pathophysiology of PD. Although aging appears to be the critical event leading to mitochondrial dysfunction and increased oxidative stress, aging does not constitute the only risk factor for PD presentation [24]. In this context, chronic exposure to environmental neurotoxic contaminants, such as paraquat (PQ), also triggers oxidative stress-related mechanisms that might play a pivotal role in the etiology and early progression of PD. Relevantly, despite the preferred hypothesis, oxidative stress constitutes the main mechanism underlying the different pathological hallmarks exhibited by dopaminergic neurons during PD [25].

In this regard, oxidative stress is a common feature of most neurodegenerative disorders. Production of reactive oxygen species (ROS) by mitochondria and enhanced metabolism of molecular oxygen by the electron transport chain (ETC) can render different cell types, particularly neurons, highly vulnerable to oxidative stress [26]. Moreover, the ability of ROS to affect both mitochondrial function and nucleic acids, thereby disrupting gene expression and other cellular processes, will unequivocally result in critical cellular alterations and death. Specifically in PD, oxidative damage is correlated with the formation of LB due to SNCA aggregation and impairments in the ubiquitin-proteasome system (UPS) [27].

Similarly, neurotoxins and pesticides share a common mechanism to induce damage to dopaminergic neurons that is correlated with an increased oxidative status caused by high levels of ROS, anions, and free radicals. Indeed, several drugs, such as rotenone, 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine; 6-OHDA), and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), induce severe

Table 1 Summary of the main etiological factors for PD

Genetic presentation of PD		Environmental presentation of PD	
Autosomal dominant	Autosomal recessive	Neurotoxins	Pesticides
SNCA	PARK2	6-OHDA	Rotenone
LRRK2	PINK1	MPTP	Paraquat
VPS35	PARK7	Methamphetamine	Maneb
	DNAJC6	MDMA	

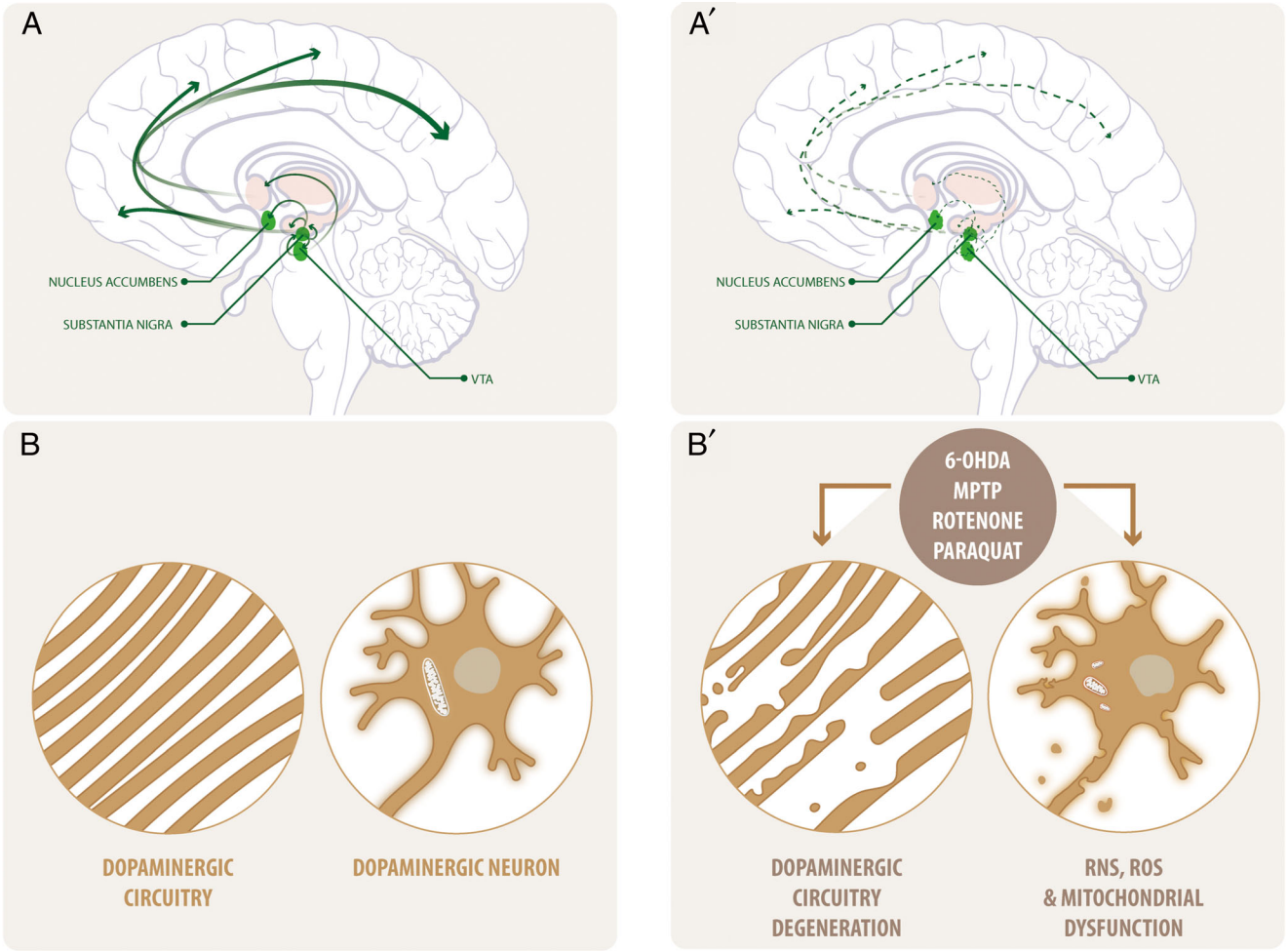

Fig. 1 Central nervous system dopaminergic circuitry. A–B Under physiological conditions, the dopaminergic afferents originating from the SN project to the striatum, and afferents from the VTA to the nucleus accumbens and the frontal cortex. A′–B′ Under pathological conditions, i.e., after exposure to environmental toxins such as PQ or neurotoxins such as 6-OHDA, the dopaminergic circuitry is altered, with a severe loss of neurons within the SN, a subsequent decrease in dopamine secretion, and alterations in neuronal projections. Oxidative damage induced by ROS, RNS, and quinones, among others, and alterations in the ETC within the mitochondria constitute critical markers of the damage induced by these environmental toxins

mitochondrial dysfunction in drug-based animal models of PD, including increased ROS generation and striking sensitivity to stressors [28].

Dopamine Biosynthesis and Dopaminergic Neuron Vulnerability

Relevantly, dopamine metabolism seems to contribute to the increased sensitivity of dopaminergic neurons to oxidative stress. Under physiological conditions, tyrosine is converted to L-DOPA by means of tyrosine hydroxylase (TH), which in turn is transformed to dopamine by the enzyme aromatic amino acid decarboxylase (AADC). These reactions require the presence of Fe^{2+} and tetrahydrobiopterin as cofactors. Once formed, the vesicular monoamine transporter (VMAT) takes up cytosolic dopamine within synaptic vesicles, thereby preventing its oxidation. Additionally, dopamine serves as the precursor molecule for catecholamine synthesis, such as norepinephrine and epinephrine.

Although dopamine degradation is a multistep process, it begins with the activity of the mitochondria-associated monoamine oxidase (MAO). A relevant end product of this reaction is hydrogen peroxide (H_2O_2). The estimated production rate of H_2O_2 during this reaction is over 100-fold greater than that produced during cellular respiration. Notably, dopamine, epinephrine, and norepinephrine can undergo auto-oxidation phenomena, especially when they accumulate in the cytoplasm and in the presence of reactive nitrogen species, leading to the formation of highly reactive semiquinones and quinones [29].

Importantly, under pathological conditions or in the presence of any additional oxidative stressor, such as during PD, compromised dopaminergic neurons could be exposed to an increased load of pro-oxidant molecules. Indeed, in the

presence of Fe^{2+}, H_2O_2 leads to hydroxyl radical formation through Fenton's reaction, and the removal of semiquinones and quinones depends on the activity of antioxidant systems, such as glutathione peroxidase or specific quinone enzymes (NAD(P)H:quinone oxidoreductase-1 (NQO1)). The production of these metabolic products increases and further sustains ROS production, resulting in cell damage and death [30, 31].

At this point, the results indicate that several factors, including dopamine metabolism, neuromelanin production, decreased GSH levels, oxidative/nitrosative metabolites, iron dysregulation, MAO activity, and neuroinflammation, can affect the overproduction of pro-oxidant molecules [32]. Moreover, the increase in pro-oxidant end products potentially occurs via several neurotoxin-dependent reactions. In this regard, mitochondrial dysfunction plays a critical role in the pathological progression of the disease, constituting both an effect of the sustained oxidative stress and a source of additional pro-oxidative mediators.

Although the mechanisms underlying oxidative stress have been widely investigated, researchers have not conclusively identified mechanisms that stop/reverse the subsequent neuronal damage in PD or established whether oxidative stress constitutes a primary cause or is only secondary to the undetermined insult. However, oxidative stress-related response mechanisms, such as activation of the nuclear factor erythroid 2-related factor 2-antioxidant response element (Nfr2/ARE) pathway, can constitute a promising strategy to reduce cell death in PD due to the neuronal response and activation of astrocytes and microglia, as well as the subsequent production of heme-oxygenase-1 (HO-1), γ-glutamylcysteine synthase (γ-GCS), and NQO1.

Sporadic PD Models

Neurotoxins and Pesticides

According to epidemiological studies, the prevalence of sporadic PD is higher among farming communities [33, 34]. Moreover, exposure to pesticides or herbicides elicits a three- to four-fold increase in the risk of developing PD; this increased risk strongly supports the contribution of environmental factors to the etiology of sporadic PD [35, 36]. Approved pharmaceuticals, pesticides, air pollutants, industrial chemicals, heavy metals, and hormones, among other factors, alter gene expression through a broad array of regulatory mechanisms and affect the dopaminergic system; thus, these factors may be associated with several pathological conditions, including PD [37]. Consistent with this finding, several PD-like models are induced by different neurotoxins, including pesticides, resembling the pathological molecular and behavioral hallmarks of the disease (Table 2).

Neurotoxins

6-OHDA

In the late 1950s, Senoh and colleagues isolated 6-OHDA [38, 39] and found that this chemical produces severe denervation of noradrenergic cardiac fibers and selective damage to dopaminergic neurons [40, 41]. 6-OHDA was the first chemical substance discovered that exerted a *specific* neurotoxic effect on neurons containing dopamine, serotonin, and norepinephrine [42]. With a structure similar to that of dopamine and norepinephrine, 6-OHDA uses the same catecholaminergic transport system and causes specific degeneration of dopaminergic and noradrenergic neurons [43]. Once inside neurons, 6-OHDA is rapidly oxidized to hydrogen peroxide and paraquinone, species that are highly toxic to mitochondria by specifically affecting complex I, resulting in increased ROS generation [9]. However, 6-OHDA is unable to cross the blood-brain barrier (BBB); thus, it must be directly applied to the SN or striatum to induce damage. The magnitude of the lesion depends on the amount of 6-OHDA injected, the site of injection, and the species used [19, 43]. This compound is usually injected unilaterally, with the intact hemisphere used as an internal control. This unilateral 6-OHDA injection represents the "hemiparkinson model" [44] and is characterized by asymmetric motor-circling behavior. Importantly, the 6-OHDA model does not mimic all pathological or clinical features of PD. First, 6-OHDA induces an acute effect, which is quite different from the slow and progressive nature of human PD; second, it is not capable of inducing LB formation [9]. Nevertheless, 6-OHDA remains the more developed and better studied model of PD.

MPTP

MPTP is a bypass product of the chemical synthesis of a meperidine analog with potent heroin-like properties. In 1982, a small number of drug users in the USA mistakenly injected heroin contaminated with this neurotoxin and developed a clinical manifestation known as "frozen addicts." Subsequently, in 1984, humans and nonhuman primates developed a severe and irreversible PD-like symptomatology a few days after injecting MPTP [45]. In contrast to the 6-OHDA-based model, the MPTP-based model reproduces nearly all the different features of PD, including rigidity, tremor, slowness of movement, and freezing, depending on the administration regime. Chronic exposure to MPTP causes apoptosis of dopaminergic neurons [46], whereas acute exposure causes necrosis of the SN neuronal pool [47]. Loss of dopaminergic neurons depends on the treatment characteristics and ranges from 20% with a single dose to 50% with acute treatment for 4 days [48]. Unlike 6-OHDA, MPTP is highly lipophilic and crosses the BBB; thus, MPTP has been

Table 2 Advantages and limitations of neurotoxins and pesticides used in in vitro and in vivo PD models

	Advantages	Limitations
6-OHDA	Reproduces a specific loss of SN and ventral tegmental area (VTA) neurons. Oxidative stress is mediated by oxidation products (hydrogen peroxide, quinones and paraquinones, among others).	Administration by stereotaxic injection. Not capable of producing LBs.
MPTP	Systemic administration is possible. Induces a specific loss of SN and VTA neurons. Inhibits ETC complex I.	Neurological and clinical features depend on the administration regime (acute or chronic). Not capable of producing LBs.
Rotenone	Systemic administration is possible. Reproduces a specific loss of SN and VTA neurons. Inhibits ETC complex I. Induces LB formation.	Low reproducibility. High mortality.
Paraquat	Systemic administration is possible. Reproduces a specific loss of SN and VTA neurons. Oxidative stress is mediated by ROS production. Induces LB formation. May interact with other agrochemicals, such as maneb.	Specificity of the dopaminergic neuronal damage has generated some controversies.

administered at the systemic level or directly into the CNS. Once in the CNS, MPTP is metabolized by MAO-B to 1-methyl-4-phenylpyridinium (MPP^+) by glial cells. MPP^+ has a high affinity for the melanin, neuromelanin, and dopamine transporter, resulting in effortless and selective entry into dopaminergic neurons of the SN. In neurons, MPP^+ is sequestered into synaptic vesicles by the VMAT, preventing its interaction with the mitochondria [49, 50]; however, its primary effect is to inhibit complex I of the ETC. The presence of small inclusions containing SNCA has also been observed after only 3 days of treatment [51]. More specifically, an increase in the number of SNCA-immunoreactive neurons in the SNpc and an increase in SNCA mRNA levels were observed [52]. However, the MPTP model has some limitations. Progressive degeneration of nigrostriatal dopaminergic neurons was not observed following acute MPTP treatment, whereas in the long-term treatment, which mimics the main features of PD, motor deficits recovered once the treatment was terminated, clearly deviating from PD pathophysiology [43]. Despite these limitations, the MPTP-based PD model constitutes an easy and more reliable animal model that reflects many features of human PD.

Pesticides

Pesticides are defined as any agent used to kill undesired organisms, such as insects (insecticides), snails and slugs (molluscicide), rodents (rodenticide), plants (herbicide), or fungi (fungicide) [53]. Notably, different methods of pesticide exposure are verified according to its use, including inhalation, ingestion, or dermal absorption [13]. The possibility that pesticides and other environmental toxins may be involved in the pathogenesis of different disorders, including PD, has been suggested [12, 54]. This issue has received special attention because pesticides and their combinations are often used in neighboring farmlands, increasing potential exposure to multiple toxic agents, which can act additively or synergistically [6, 10, 55]. Although pesticides can be categorized according to different characteristics, such as their acute toxicity and/or by their chemical groups, classification according to their mode of action allows establishment of the potential molecular mechanisms leading to PD. In this regard, the majority of pesticides increase ROS levels and the activity of the nitric oxide synthase; they also induce several mitochondrial alterations, defects in the ETC, and changes in mitochondrial movement. These alterations favor protein aggregation and alter dopamine levels [13, 56, 57].

Rotenone

Rotenone is a naturally occurring complex ketone derived from the root of *Lonchocarpus* sp. Rotenone was originally employed by the Indians of Peru as a fish poison registered in 1929 [58]. In 1985, rotenone was shown to induce toxicity in dopaminergic neurons after stereotaxic administration in rats [59]. One of the greatest advantages of rotenone is that it is biodegraded within a few days [60]. Similar to MPTP, rotenone contains a lipid component, which enables it to easily cross the BBB and be rapidly distributed in the CNS; rotenone does not require a dopamine transporter and is not sequestered into synaptic terminals for entry into dopaminergic neurons [61]. In contrast to 6-OHDA and MPP^+, rotenone produces selective damage in the striatum but not in the SN [62] when administered systemically. Thus, the specificity of rotenone for the dopaminergic system remains under debate, raising questions of whether rotenone exclusively acts on mesencephalic dopaminergic neurons or also affects other striatal projection systems [63]. Importantly, rotenone accumulates in some organelles in neurons, such as the mitochondria [64] where it impairs oxidative phosphorylation by inhibiting

complex I of the ETC, similar to MPP^+ and 6-OHDA [65]. Furthermore, microgliosis, increased iron deposits, and formation of LB with ubiquitin and SNCA in nigral cells have been observed in rotenone-based models [9]. The major limitations of the rotenone model include reduced reproducibility, location and extent of the lesion, and a high associated mortality rate [66, 67].

Paraquat and PD

PQ (1,1′-dimethyl-4-4′-bipyridinium dichloride) was developed in the early 1960s by Syngenta as a nonselective quaternary ammonium herbicide. Grant et al. [68] published the first report of cerebral changes induced by PQ poisoning in 1980 using histological techniques to examine hemorrhaging, glial reactions, and meningeal inflammation. However, in 1985, PQ injection was reported to significantly decrease the dopamine concentration and induce behavioral changes similar to those induced by MPP^+ injections [69]. PQ is registered and sold in 90 countries, including the USA, Canada, Australia, Japan, New Zealand, and China. Importantly, China is the world's largest PQ producer, with more than 100,000 t of PQ produced annually, even though its use is forbidden [70] (Table 3).

PQ is highly toxic, and all modes of exposure to PQ constitute a risk factor for PD symptomatology. However, some differences in the absorption rate have been observed. For example, PQ is poorly absorbed in the gut. In contrast, a subcutaneous injection of PQ is rapidly absorbed, with the peak concentration in the blood detected 20 min after administration [71]. Following exposure, compared with other tissues, the lungs exhibit selective accumulation of PQ, resulting in subsequent development of pulmonary edema and other pulmonary damage, which ultimately leads to fibrosis. Liver damage and renal failure may also follow PQ absorption [70, 72]. Regardless of the exposure route, death occurs when the plasma concentration exceeds 1.6 pg/ml 12 h after ingestion [73]. Systemic intraperitoneal (i.p.) administration of low doses of PQ in adult mice and rats generates specific loss of dopaminergic neurons in the SN along with an associated impairment of locomotor activity [55, 74, 75]. In addition, upregulation and aggregation of SNCA occurs [76, 77].

An important feature of pesticides, such as PD-like model inducers, is their ability to cross the BBB and affect the dopaminergic system. Based on our experience and current knowledge regarding the etiology and pathophysiology of the disease, pesticide-based PD models might constitute a more reliable system to examine the different aspects of this highly complex disorder.

An important distinction from other PD sporadic models is that PQ can be used alone or in combination with other agrochemicals, reproducing the exposure conditions observed in an agriculture environment in a more reliable manner. Maneb and mancozeb are some examples of the agrochemicals used in combination with PQ to exacerbate both systemic toxicity and damage to the nervous system. Nociceptin/orphanin (NOP) receptor and prodynorphin-kappa opioid (KOP) receptor systems in the SN and caudate putamen in rats are commonly affected by these combinations, providing further evidence that chronic exposure and interactions between agrochemicals might be implicated in the pathophysiological mechanisms of sporadic PD [55]. Indeed, according to Caputi and colleagues [78], the combination of PQ and maneb exerts neurotoxic effects by altering proteasome function via a decrease in the levels of β1 and Rpt3 proteasome subunit mRNAs. Moreover, this combination alters the opioid system by downregulating the μ and δ opioid receptors in SH-SY5Y neuronal cells, indicating the need for further studies to elucidate the mechanisms involved in models of the environmental etiology of PD.

PQ and the Blood-Brain Barrier

As described above, PQ can cross the BBB. Although this crossing is a slow, inefficient, limited event, detectable levels of this herbicide have been measured in the CNS after systemic injection [79]. Naylor and colleagues [71] confirmed that PQ crosses the BBB by treating adult Wistar-derived Alderley Park male rats with a subcutaneous dose of ^{14}C-labeled PQ. After administration, the PQ concentration in the brain peaked within the first hour and then rapidly decreased, leaving only residual amounts of the pesticide. The study suggested a potential link between the health status of brain microvascular endothelial cells, which form the BBB, and the limited entry of PQ. Importantly, the interaction between PQ and endothelial cells did not result in impairment of BBB function by PQ itself or any PQ radical [80]. Once in the CNS, PQ generally associates with five structures, the pineal gland, cerebral ventricles, anterior portion of the olfactory bulb, hypothalamus, and area postrema [81]. Importantly, this distribution is closely related to the cerebral capillary network [71]. Moreover, PQ persists in the ventral midbrain, with an apparent half-life of approximately 4 weeks [82].

Reportedly, PQ is transported into dopaminergic neurons via the dopamine transporter (DAT) due to the structural similarity between PQ and MPP^+, which also reaches striatal cells in a Na(+)-dependent manner [80, 83]. However, Richardson and colleagues [84] refuted the role of DAT in PQ transport, suggesting that PQ is not a DAT substrate because it is unable to inhibit DAT-mediated dopamine uptake and cause dopaminergic damage. Identification and validation of the molecular mechanisms exploited by PQ to reach different brain areas as well as the dopaminergic system remain open questions; therefore, further studies are required to understand the development of PD-like alterations [85].

Table 3 Regulatory status of PQ products worldwide

Regulatory status	Country	Fundamental effects	References
Forbidden	Sweden (1983)	High acute toxicity.	MAFF 2003
	Hungary (1981)	Systemic irreversible effects.	MSEA 2005
	Finland (1986)	Persistence in soil.	UNEP 1999, 2005
	Austria (1993)	High rate of suicide.	
	Denmark (1995)		
	Slovenia (1997)		
	Germany (1991)		
	Kuwait (1985)		
	Cambodia (2003)		
	Ivory Coast (2004)		
	Syria (2005)		
	United Arab Emirates (2005)		
	Malaysia (2002)		
Non-authorization	Norway (1981)	Voluntary prohibition due to high toxicity.	Berne Declaration 2010
	Switzerland (2002)		SFC 2002
Restrictions	Columbia (1989)	Aerial application is not allowed.	Berne declarations 2010
	Philippines (1989)	Only applied to certain crops.	UNEP 1999
	Indonesia (1990)	Applications by authorized personal only.	US EPA 1997
	South Korea (1987)		
	Uruguay (1992)	Must contain several agents to avoid acute toxicity.	
	USA (1997)		
	Belize (2003)	Commercialization only by a "professional prescription."	
	Chile (2003)		
	Costa Rica (2005)		
	Sri Lanka (2007)		
	Dominican Republic (1991)		

Abbreviations refer to the National Health/Sanitary/Environmental Organizations of each particular country

PQ in Dopaminergic Neurons

According to in vivo studies, PQ exposure depletes GSH and increases the levels of oxidized glutathione (GSSG) in the mouse SNpc [86]. These findings further confirmed that PQ exerts its harmful effects through a mechanism related to oxidative stress (Fig. 2). Furthermore, PQ increases NADPH oxidase expression via the PQ redox cycle and/or altered mitochondrial mechanisms [87]. Interestingly, when cells are pretreated with apocynin, a putative NADPH oxidase inhibitor, PQ-induced ROS generation and dopaminergic cell death are significantly reduced [88]. Consistent with these findings, superoxide dismutase (SOD) and coenzyme Q10 have been evaluated with the intention to reduce oxidative stress and diminish neuronal damage [89, 90]. Moreover, PQ toxicity correlates with DNA fragmentation [91].

PQ also activates other molecular signaling pathways within cells, such as protein kinase delta (PKC δ), extracellular signal-regulated kinase 1/2 (ERK1/2), Jun N-terminal kinase (JNK), and the caspase-3 signaling cascades [92]. Because JNK activation and apoptosis are considered the principal mechanisms by which PQ induces neurodegeneration [93], the blockade of these events by heat shock protein 70 (Hsp70) overexpression revealed a role for Hsp70 in protecting *Drosophila* from PQ exposure [94]. Moreover, considering the antiapoptotic function of Hsp70 and the observed association with PQ, potential mechanisms may involve Hsp70-dependent protective effects on dopaminergic neurons in the PQ-based PD model, including attenuation of oxidative status, such as reduced production of pro-oxidative mediators, stalled JNK activation, and modulation of the caspase-3 cascade [94].

Furthermore, PQ toxicity can modulate the Wnt signaling pathway. Wnt signaling plays an important role in both the development of dopaminergic neurons and their maintenance in the adult brain. Some relevant connections have been made between genetic PD cases and Wnt/β-catenin signaling, such as parkin mutations and their relationship with β-catenin. In mice lacking the parkin gene, increased levels of β-catenin ultimately lead to DA neuronal death [95]. Although it is well

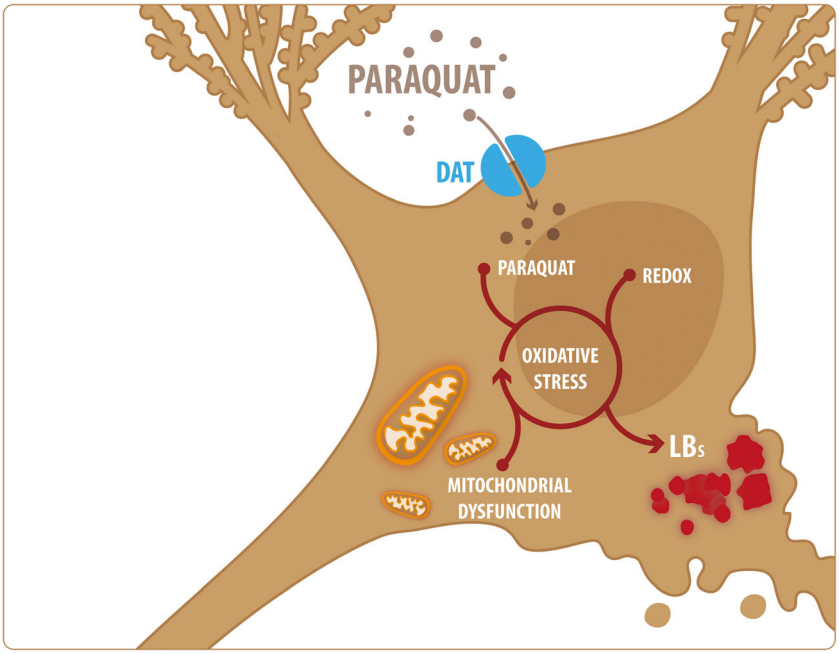

Fig. 2 Molecular events related to PQ-induced damage. PQ crosses the BBB, entering dopaminergic neurons via the dopamine transporter (DAT). In neurons, PQ triggers a vicious cycle related to oxidative stress that involves an imbalance in the redox status, resulting in mitochondrial dysfunction, protein aggregation (the formation of LBs) and further production of pro-oxidant mediators

established that β-catenin has proliferative effects through the Wnt signaling pathway, its unbalance toward increased levels exerts the opposite effect, which might differentiate the roles of Wnt signaling during brain development versus the diseased adult brain [95]. Importantly, this issue indicates that restoration of the Wnt signaling balance might constitute a possible therapeutic target that can reverse the neuronal damage observed in PD. Otherwise, in PC12 cells, PQ inactivates the Wnt pathway, thereby blocking the expression of Wnt target genes and resulting in cell cycle arrest and apoptosis [96]. Moreover, PQ prevents β-catenin from binding to myelin gene promoters, resulting in inhibition of Wnt/β-catenin-dependent myelin gene expression and severe demyelination of nerve fibers [97]. Importantly, these deleterious effects may be counteracted by lithium chloride (LiCl) administration, which blocks PQ-induced oxidative stress and reduces Schwann cell death. Indeed, the combined administration of LiCl + PQ results in normal sensorimotor behaviors and rescues nerve structures in animal models [97]. Moreover, lithium treatment prevents PQ-induced cell death and apoptosis through increasing antiapoptotic protein BCL2 levels, decreasing pro-apoptotic protein BAX expression, and exerting a neurotrophic effect by increasing BDNF expression. More importantly, lithium can decrease production of ROS and activate the redox-sensitive transcription factor NRF2 and its target genes [98].

Although lithium exerts an antioxidant action after chronic treatment in MPTP models [98], the results in the 6-OHDA model indicate that lithium cannot alleviate 6-OHDA-induced degeneration of SNpc dopaminergic neurons [99]. In this regard, additional research is necessary to establish the functional implications of lithium within the pathological scenario of PD.

Parallel to the neuronal toxicity induced by PQ and other pesticides, the neuroinflammatory response is also a component of the exposure outcome and is suspected to induce further degeneration in regions near the primary damaged neurons [8]. As shown in a recent study by Sandström and colleagues [100], PQ not only induces dopaminergic neuron damage but also generates adverse effects on glutamatergic, GABAergic, and glial cells. Indeed, strong astrogliosis and microglial activation along with the downregulation of the M2-neuroprotective microglial phenotype were observed upon PQ exposure, indicating that this chemical compound also affects several CNS cell types in addition to highly sensitive dopaminergic neurons. This finding further supports the hypothesis that the PQ-based PD-like model reliably reflects this complex pathology and represents a valuable tool for PD research.

Importantly, as a common feature of different PD-related neurotoxins, PQ-dependent dopaminergic neuron damage has been attributed to its harmful effects on mitochondria through the formation of PQ radicals that cause oxidative damage [101, 102]. PQ indirectly disrupts mitochondrial function by causing increased mitochondrial ROS formation, most likely due to the interaction between PQ and ETC complex I [9]. In addition, ROS interact with the unsaturated lipids of biological membranes, causing lipid peroxidation in several cellular organelles, ultimately resulting in cell death [103].

Concluding Remarks

Sporadic PD currently constitutes a critical pathology. Reliable models that recapitulate several characteristics of

PD pathophysiology, ranging from the initial alterations to the most characteristic molecular hallmarks and behavioral manifestations of the disease, are required to effectively study this complex disease. Although genetic models of PD are well established, they represent only a small fraction of the cases worldwide. Accordingly, the use of synthetic compounds, which provide the opportunity to reproduce the neuropathological features and symptomatology of PD, constitutes a valuable tool to approach the pathophysiology of this disease. MPTP and 6-OHDA represent two neurotoxins commonly used to induce in vitro and in vivo PD models [4, 5]. However, the third most commonly used PD-like model involves the administration of agrochemicals to reproduce the pathology [4, 5]. Indeed, the correlation between an increased incidence of PD and farming operations was previously demonstrated and strongly suggests that agrochemicals can reproduce the sporadic appearance of PD. In this regard, it seems logical to expect that exposure to PQ or other agrochemicals represents a major risk factor for neurodegeneration, particularly PD. Moreover, simultaneous exposure to multiple agrochemicals has been observed, mainly because of the concomitant use of these compounds in neighboring farmlands, and may increase the risk of developing PD or other neurodegenerative disorders.

Relevantly, as demonstrated by MPTP and 6-OHDA, PQ induces a broad range of effects, indicating the triggering of highly complex cellular and molecular mechanisms that can reproduce the molecular and behavioral hallmarks of PD. Moreover, the study of these mechanisms, the close relation between PQ and increased oxidative stress, and the intrinsic properties of dopamine biosynthesis allow us to understand the increased sensitivity of dopaminergic neurons to PQ exposure and why this compound can more closely recapitulate the gradual, chronic pathological scenario observed in PD.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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