General Introduction
Parkinson’s disease (PD)

Parkinson’s disease (PD) is a progressive neurodegenerative basal ganglion disorder majorly affecting the older people. PD affects roughly five million people world-wide (Dorsey et al., 2007). It is the second most common neurodegenerative disorder (De Lau and Breteler, 2006) and occurs because of a deficiency of available dopamine. The primary motor phenotype is typically characterized by a combination of slowed movement (bradykinesia), resting tremor, postural instability, and rigidity. Many of these motor symptoms arise from the loss of nigral dopamine neurons and resultant striatal dopamine depletion that occurs in the disease (Cannon and Greenamyre, 2010). The formation of intracellular aggregates in surviving dopamine neurons, of mainly composed of the protein α-synuclein, is a pathological hallmark (Spillantini et al., 1997).

Symptoms of PD

The primary symptoms of PD include - hypokinesia, resting tremor, muscle rigidity and muscle weakness accompanied by a deficit of voluntary activity as well as abnormal involuntary motor activity (Bowman and Rand, 1980). While the first component, hypokinesia or akinesia, is typically manifested by difficulty or absence of initiating movement, the second component occurring during voluntary motion is manifested as dystonia or tremors (Bowman and Rand, 1980). Resting tremors occur primarily in the hands and ankles while hand tremors involve the involuntary movement of the thumbs and index fingers in a 'pill rolling' type fashion (Marsden, 1990). Further symptoms include general tetany, deep muscle tremors, arching of the foot, facial rigidity, and slurring of the speech (Marsden, 1990). Dementia, a persistent intellectual impairment, often accompanies advanced PD. Non-motor features are reported to precede the classical motor features, such as olfactory dysfunction, dysautonomia, mood sleep alterations, gastrointestinal dysfunction, cardiac autonomic dysfunction and ophthalmological measures (Tolosa et al., 2009). Jellinger (2001) reported that
the neuronal loss in PD is not merely DAergic with cholinergic, serotonergic and noradrenergic neurons also being adversely affected. The relative contribution made by the different neuronal subgroups towards motor and non-motor clinical features of PD remains a highly researched area.

**Neurochemical and neuropathological features of PD**

The pathological hallmarks of PD are: striato nigral degeneration, nigrostriatal dopaminergic neurons (Fig. 1) accompanied by the presence of intraneuronal proteinaceous cytoplasmic inclusions - “Lewy Bodies” (LBs) (Fig. 2) (Dauer and Przedborski, 2003). PD interferes with the integrated action of the basal ganglia which is composed of four main structures: the striatum, pallidum, subthalamic nucleus and the substantia nigra pars compacta. Striatum is recognized as the input and internal segment of the globus pallidus and the pars reticulata of the substantia nigra as the output structure of the basal ganglia. Dopamine modulates the flow of cortical information through the basal ganglia by reducing the inhibitory basal ganglia output to the thalamus, leading to increase in the activity of the thalamocortical projection neurons. In PD, a compromised dopaminergic pathway leaves an un-antagonized, excitatory cortical input to influence the striatum (Wichmann et al., 1993). Loss of dopaminergic input to the striatum causes the indirect pathway to be stimulated, resulting in greater than normal excitation of the globus pallidus internal segment and substantia nigra pars reticulate which in turn increases the GABAergic output from the internal segment of the globus pallidus and the pars reticulata of the substantia nigra to the thalamus (DeLong, 1990) which results in a reduction of cortical activation, accounting for most of the symptoms of PD.
Fig. 1 The Nigrostriatal pathway
(Source: https://www.atrainceu.com/course-module/1941240-085_parkinsons-module-02)

Fig. 2 Neuropathology of Parkinson’s disease (A) normal nigrostriatal pathway (in red). B) diseased nigrostriatal pathway (in red). (C) Immunohistochemical labeling of intra-neuronal inclusions, termed Lewy bodies, in a SNpc dopaminergic neuron (Source: Dauer and Serge 2003)
Dopamine and PD

PD, a progressive neurodegenerative disorder characterized by movement and postural dysfunction, arise due to the selective loss of catecholaminergic neurons of substantia nigra pars compacta in the midbrain. The degeneration of the melanin-pigmented nigral neurons, accompanied by depletion of dopamine in the striatum, is the neuropathological basis of the movement disorders seen. Clinical symptoms of the disease are displayed upon the loss of approximately 80% of nigral dopaminergic cells and such neural degeneration results in a severe reduction of striatal (e.g., caudate nucleus, caudate putamen) dopamine (Marsden, 1990). Dopamine is the principally affected neurotransmitter within the ambit of parkinsonian pathology (Fig. 3).

Consonant with such dopaminergic neuronal loss, levels of dopamine metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) are also reported to be depressed (Hornykiewicz and Kish, 1987). Studies utilizing immunostaining techniques have discovered significant reductions of the dopamine-specific reuptake transporter (Niznik et al., 1991) and the

Fig. 3 Dopamine release by neurons
(Source: https://www.atrainceu.com/course-module/1941240-085_parkinsons-module-02)
reduction or abnormal distribution of tyrosine hydroxylase (TH) (Kastner et al., 1993), the rate-limiting enzyme mediating dopamine synthesis, in postmortem parkinsonian brain samples. Other pathogenic markers, within the scope of idiopathic parkinsonism are - compromised mitochondrial respiratory complexes NADH CoQ reductase (Schapira et al., 1992) and α-ketoglutarate dehydrogenase (Mizuno et al., 1994) in postmortem brain tissue. Neuropathological studies of PD-related neurodegeneration shed light on the pathogenesis of the disease.

Loss of dopaminergic neurons associated with PD is topologically distinct from the pattern seen in normal aging. While in PD, the cell loss is centralized in ventrolateral and caudal portions of the SNpc, the dorsomedial aspects of SNpc are affected during normal aging (Fearnley and Lees, 1991). Thus, even though age is an important risk factor for PD, the process that produce age-related dopaminergic neuronal death are reportedly different from those of PD with the extent of terminal loss in the striatum being more prominent than the magnitude of SNpc dopaminergic neuron loss (Bernheimer et al., 1973), This suggests that the striatal dopaminergic nerve terminals are the primary targets of the degenerative process and neuronal death in PD. Further, the mechanism of synaptic DA clearance in the striatum appears to be more dependent on DAT than in the prefrontal cortex, where other monoaminergic transporters and the synaptic enzyme (catechol-O- methyl transferase) play a larger role in terminating the actions of DA (Giros et al., 1996). The prefrontal cortex is the primary site of projection of VTA dopaminergic neurons and hence, this difference may be of importance in interpreting the relative resistance of VTA neurons in PD-related neurodegeneration (Fig. 2).

**Genes involved in PD**

While similarities between the inherited and sporadic forms of diseases share common clinical manifestations, these mechanisms can help in the identification of biochemical and molecular pathways involved in the diseases. It has been reported (Dauer and Przedborski, 2003) that genetic mutations in PD
represent only 10% of PD cases. Earliest evidence for the genetic link to PD comes from mutations in the α-synuclein gene. A dominantly inherited form of PD is found to be caused by mutations in the α-synuclein gene (A53T, A30P) (Kruger et al., 1998) and this mutation and is often employed to create transgenic mice to recapitulate the pathophysiology of PD. α-synuclein transgenic mice have afforded considerable evidence to show that A53T mutations can produce severe motor phenotype which can eventually lead to paralysis and even death (Giasson et al., 2002). Mutations in the α-synuclein gene in mice are also found to produce inclusions that resemble ‘Lewy Bodies’ (Masliah et al., 2000). Mutation in LRRK2 gene also causes a dominant form of PD (Zimprich et al., 2004) and it has been reported that LRRK2 mouse model is not specially strong model as it shows only minimal levels of neurodegeneration (Li et al., 2009). Mutations in ‘parkin’ (which accounts for about 50% of the familial cases of PD and 20% of the young-onset PD cases), DJ1 (a redox-sensitive molecular chaperone and regulator of antioxidant gene expression), and PINK1 (phosphatase and tensin homolog—PTEN-induced novel kinase 1, which is localized to the mitochondrial intermembrane space) are reported to cause autosomal recessive forms of PD. Interestingly, knock-out rodent models of these genes do not demonstrate any nigrostriatal degeneration.

**Gene-environment interactions and occurrence of PD**

Many researchers postulate that the majority of cases of PD are influenced by both genetic predisposition and environmental exposures, although the particulars of these interactions are not well understood. The number and diversity of gene–environment interactions that could bear pathogenic relevance to PD are enormous. However, there are limited data pointing to specific interactions (Table 1).
Table 1. Gene–environment interactions linked to human PD. Gene–environment interactions in human Parkinson’s disease.

<table>
<thead>
<tr>
<th>Gene/protein</th>
<th>Alteration</th>
<th>Toxicant</th>
<th>Potential mechanism(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoamine oxidase-B</td>
<td>Polymorphisms</td>
<td>None yet identified, oxidative stress, altered dopamine neurotransmission; toxicant metabolism</td>
<td>Checkoway et al., 1998</td>
<td></td>
</tr>
<tr>
<td>Paraoxonase I</td>
<td>Polymorphisms resulting in altered expression profiles</td>
<td>Organophosphates</td>
<td>Alterations in toxicant metabolism; neurotransmission imbalance (acetylcholine)</td>
<td>Checkoway et al., 1998</td>
</tr>
<tr>
<td>Manganese-containing superoxide dismutase and NAD(P)H:quinone reductase</td>
<td>Polymorphisms</td>
<td>Pesticides</td>
<td>Reduced ability to metabolize pesticides; altered metabolic pathways resulting in increase formation of more toxic metabolites</td>
<td>Fong et al., 2007</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Polymorphisms, mutations</td>
<td>Pesticides, solvents</td>
<td>Reduced ability to metabolize toxicants; altered metabolic pathways resulting in increase formation of more toxic metabolites</td>
<td>Deng et al., 2004; Elbaz et al., 2004</td>
</tr>
<tr>
<td>REP1 in SNCA promoter</td>
<td>Polymorphisms altering expression levels</td>
<td>Paraquat</td>
<td>Paraquat exposure increases risk in those with shorter promoters, removing a protective factor</td>
<td>Gatto et al., 2009</td>
</tr>
<tr>
<td>Dopamine transporter</td>
<td>Polymorphisms altering expression levels</td>
<td>Pesticides</td>
<td>Increased DAT expression, increased toxicant accumulation in dopaminergic neurons; altered DAT expression</td>
<td>Kelada et al., 2006; Ritz et al., 2009</td>
</tr>
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</table>
Mitochondrial complex I function and PD

Abnormalities of mitochondrial complex I have been implicated in neurological disorders as well as late-onset neurodegenerative diseases, such as Parkinson’s disease (PD) (Greenamyre et al., 2001). Schapira et al., (1989) stated that humans with PD suffer from systemic mitochondrial complex I deficiency. The exact role of complex I in these illnesses remains controversial and the mechanisms by which complex I defects cause neurodegeneration are not entirely clear. As the proximal component of the electron transfer chain, complex I contributes to ATP synthesis and maintenance of membrane potential. Therefore, severe defects in complex I activity can be anticipated to produce lower rates of ATP synthesis and also cause graded mitochondrial depolarization (Greenamyre et al., 2001). Thus, the interaction at the level of mitochondrial function is expected to be an important pathogenic mechanism of the interplay between genetic and environmental factors.

Role of oxidative stress and neuroinflammation in PD

There are four key common threads that run across the spectrum of neurodegenerative disease, although not every disease has all features. First, there is increasing awareness of the interplay between a neuroinflammatory component and chronic oxidative stress, and there is growing acceptance that ROS and RNS act together to mediate damage in degenerative disease (Calabrese et al., 2000, Contestabile et al., 2003, Chung et al., 2005, Sayre et al., 1989). The second common feature is the accumulation of unfolded or misfolded proteins in brain cells, leading some workers to refer to AD, PD, HD, and ALS as “protein conformational diseases.” The third common feature, most prominent in AD, PD, and MS is dyshomeostasis of both redox-active (e.g., copper and iron) and redox-inactive (e.g., zinc) metal ions (Salazar et al., 2006, Donnelly et al., 2007). The fourth common feature is abnormal functioning of mitochondria (Beal and Lin, 2006), which play a critical role in metabolism and regulate the entire life cycle of the cell (e.g., in mediating apoptosis). These
features are not independent for example, that small-molecule products of oxidative stress can mediate protein misassembly (Bieschke et al., 2006). Evidence for oxidative stress in AD and PD is consonant with the finding that the areas of the brain affected by these diseases contain abnormally high levels of redox-active metals, particularly iron. An excess of redox-active metals is presumed to be at least partially responsible for the oxidative damage seen to proteins, polyunsaturated lipids, and DNA/RNA in PD (Nunomura et al., 2002) (Fig.4).

**Fig. 4 Mechanism of oxidative stress in PD**  
(Source: Cicchetti et al., 2009)

Despite a well-described clinical and pathological phenotype, which is essentially identical for both the sporadic and the rare familial forms of PD, the molecular mechanisms involved in the pathogenesis is not understood. Mitochondrial dysfunction, oxidative damage, environmental factors, and genetic predisposition are all suggested to be involved. Because oxidative
stress is intimately linked to other components of the degenerative process, it
is not clear whether oxidative stress leads to or is a consequence of, these
events (Kikuchi et al., 2002, Jenner, 2003). There is fairly large evidence that a
defect in mitochondrial complex I, resulting in a 30-40% decrease in complex I
activity in the substantia nigra, may be the central cause of sporadic PD
(Dawson and Dawson, 2003). Evidence that a complex I deficiency and
oxidative stress might underlie PD pathology is that selective inhibitors of
complex I, such as rotenone and MPP\(^+\) (1-methyl-4-phenylpyridinium),
recapitulate much of the pathology of PD (Alam and Schmidt, 2002). Further
evidence for oxidative stress in PD is the finding of oxidative damage to DNA
(Sanchez-Ramos et al., 1994, Zhang et al, 1999 and Kikuchi et al, 2002) and
protein (Alam et al., 1997, Floor and Wetzel, 1998) observed in the nigro-
striatal region of PD brain, as well as immunocytochemical evidence for
protein nitration (Good et al., 1998), glycation (Castellani et al., 1996), and
HNE modification (Yoritaka et al., 1996, and Castellani et al., 2002).

Besides the loss of pigmented nigral neurons, PD is characterized
histopathologically by the presence of Lewy bodies, detergent-insoluble
(Galloway et al, 1992) eosinophilic intraneuronal filamentous inclusions found
predominantly in the substantia nigra and locus coeruleus. Structurally similar
Lewy bodies are also found in cortical neurons in PD and in diffuse Lewy body
disease. The principal protein constituent of Lewy bodies is fibrillar
\(\alpha\)-synuclein. The physiological functioning of normal \(\alpha\) synuclein appears to
involve synapse maintenance and plasticity, and over expression of normal \(\alpha\)
synuclein only modestly affects cell viability. On the other hand, most studies
show that over expression of mutant \(\alpha\) synuclein protein is neurotoxic, most
Deposition of Lewy bodies in sporadic PD may then possibly reflect
posttranslational modifications of \(\alpha\)-synuclein by products of oxidative stress
that affect the peptide behavior in the same way as do the mutations.
Oxidative stressors such as Cu(II) (Paik et al., 1999), Fe/H\(_2\)O\(_2\) (Hashimoto et
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al., 1999), cytochrome c / H₂O₂ (Hashimoto et al., 1999), or nitrating reagents (Hodara et al., 2004) induce aggregation/fibrillization of the protein, and human Lewy bodies and other α-synuclein inclusions are positive to antinitrotyrosine antibodies (Giasson et al., 2002). Evidence for a direct association of α-synuclein aggregation with neurotoxicity comes from a transgenic Drosophila model of PD, where (i) a deletion α-synuclein mutant unable to aggregate was nontoxic and (ii) an aggregation-prone truncation variant resulted in inclusions and enhanced neurotoxicity (Periquet et al., 2003).

There is several evidence to show that increased levels of oxidative stress contribute to PD pathogenesis (Henchcliffe and Beal, 2008), although whether oxidative stress also contributes to the development of dementia is far from understood. Multiple oxidative stress markers have therefore now been examined in PD, in autopsy tissue and body fluids, although it has not yet been possible to examine this process directly in brain tissue of living patients. One study has found increased levels of oxidized glutathione in plasma of individuals with PD (Younes-Mhenni et al., 2007). Multiple independent studies have also detected decreased plasma urate, a potent antioxidant, in PD (Cipriani et al., 2010, Annanmaki et al., 2007, Bogdanov et al., 2008). Elevation of both cerebrospinal fluid and blood concentrations of malondialdehyde in PD patients has been demonstrated (Ilic et al., 1999), and in plasma by Younes-Mhenni et al. (2007). An increase in oxidative damage to DNA has also been reported in leukocytes, plasma, and CSF (Isebe et al., 2009; Younes-Mhenni et al., 2007), of PD patients. 8-Hydroxy-2 deoxyguanosine (8-OHdG) is a product of nucleoside oxidation in DNA and has been found to be elevated in plasma (Bogdanov et al., 2008; Gmitterova et al., 2009) and urine (Seet et al., 2010) of individuals with PD. Coenzyme Q10 (CoQ10), another marker of oxidative stress, was examined in blood from PD patients compared with age- and sex-matched controls in two studies (Gotz et al., 2008; Sohmiya et al., 2004), demonstrating decreased redox ratio in PD (although this did not correlate with disease severity). Many other studies have investigated the influence of
oxidative stress as a potential biomarker. These include studies of superoxide radicals (Illic et al., 1999), glutamate uptake in platelets from PD patients (Ferrarese et al., 2008), and protective enzymes systems such as glutathione reductase or copper and zinc superoxide dismutase (Illic et al., 1999). Overall, these studies shed considerable light on our understanding of PD pathogenesis. However, as yet most measures of individual biochemical markers mentioned above yield variable data in small sample sizes, with similar changes often noted in other neurodegenerative disorders, and therefore they generally have low specificity for PD diagnosis. Thus, none is sufficiently robust to be useful as a diagnostic marker of the disease process in clinical practice. For example, α-synuclein levels in PD overlap with those of controls (Tokuda et al., 2006) and Alzheimer’s disease (Hong et al., 2010; Isobe et al., 2009). Moreover, sensitivity and specificity depend on the cutoff value used, with higher sensitivity accompanied by lower specificity and vice versa. This contrasts with markers in other neurodegenerative conditions, such as the ratio of phosphorylated tau to Ab42 CSF level, which has sensitivity and specificity of about 85% for Alzheimer’s disease (Shaw et al., 2009). Additionally, how these markers may perform in defining endophenotypes, tracking disease progression, and detecting response to targeted therapeutic interventions is unknown.

**Etiology of PD**

**Age**

PD is primarily associated with aged populations – either middle aged or older individuals. However, the pattern of loss of striatal dopamine loss (Kish et al., 1992) and nigral cell (Fearnley and Lees, 1991), in old age are significantly different from that in PD.

**Genetics**

Mutations in the genes encoding parkin and ubiquitin carboxy-terminal hydrolase-L1 (UCH-L1) are found to lead to diseases that are reasonably distinct, from idiopathic PD. Mutations which affect the gene for α-synuclein (a synaptic
protein which is a major component of the filaments associated with Lewy bodies has been identified (Simon-Sanchez et al., 2009). Over-expression of human α-synuclein in mice, has been reported to result in impaired motor responses (Tong et al., 2010).

Metabolic defects

Various metabolic defects have been reported in PD (Steventon et al., 1989; Tanner, 1991) implying that PD patients have less effective detoxification systems. This suggests that PD is the result of increased susceptibility to endogenous or exogenous toxins (Koller et al., 1990). For example, defects in enzyme detoxification systems such as sulfur conjugation, oxidation, and thiol methyl transferase activity could lead to potentiation of relatively low-level neurotoxic exposures in PD patients (Steventon et al., 1989). Proteosomal dysfunctions in metabolic disorders such as diabetes and the possible occurrence of PD have also been investigated.

Environmental factors

Although PD existed long before the introduction of pesticides, the premise is that pesticide exposure contributes to the increased incidence of the disease (Hatcher et al., 2008). Since the discovery of MPTP, the possibility of other similar compounds similar to MPTP, for example, paraquat to induce PD have evoked interest (Brown et al., 2006).

Modeling PD in animals

The available approaches to modeling PD in animals do not depend on disease-related genes. These ‘pathologic’ models are developed using toxins or non-PD-related genetic mutations (Kostic et al., 1997) to mimic the selective degeneration of dopaminergic neurons or exploit the loss of dopaminergic neurons that normally occurs in rodents during early postnatal development (Jackson-Lewis et al., 2000).
**Toxin-based models**

Among the neurotoxins used to induce dopaminergic neurodegeneration, 6-hydroxydopamine (6-OHDA), MPTP, paraquat and rotenone have received the most attention (Dauer and Przedborski, 2003). Interestingly, all of these toxins provoke the formation of ROS. The various toxin models and their characteristics are summarized in Table 2.

**MPTP:** Laboratory understanding of PD is derived from studies on the specific action of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on dopaminergic neurons. MPTP structurally resembles a number of known environmental agents, including well-known herbicides, such as paraquat (Jackson-Lewis and Przedborski, 2007), and rotenone (McNaught et al., 1996), that have been shown to induce dopamine cell degeneration (Betarbet et al., 2000), although mechanistically, the actions of each are likely different. MPTP first is metabolized by the enzyme MAO-B to 1-methyl-4-phenyl-2,3-dihydropyridium (MPDP+) that then deprotonates to generate the corresponding pyridium species, MPP+. Endothelial cells in the microvasculature that make up the BBB contain monoamine oxidases, and several studies have correlated levels of monoamine oxidases with MPTP-induced neuronal loss (Kalaria et al., 1987). Since MPP+ cannot be transported through the BBB, this level of toxification/detoxification can provide a first line of defense against exogenous agents (Smeyne and Jackson-Lewis, 2005). MPTP that is not converted to MPP+ in the periphery rapidly enters the brain where it is processed into glial cells by a number of mechanisms, including monoamine (Brook, 1989) and glutamate (Hazell et al., 1997) transporters or pH-dependent antiporters (Kopin, 1992).

**Rotenone:** Rotenone has become one of the toxic models used to study PD in animals due to its ability to inhibit mitochondrial complex I (NADH dehydrogenase) (Hatcher et al., 2008). Chronic systemic exposure to rotenone has been reported to reproduce several features of PD, including nigrostriatal dopaminergic degeneration and the formation of cytoplasmic inclusions in these
neurons. Rats exposed to rotenone display postural instability, unsteady gait bradykinesia. Rotenone model has been used as an alternative to other classical PD models such as MPTP and 6-OHDA while testing the neuroprotective effects of drugs (Monti et al., 2009). Dawson et al., (2002) reported that limiting factors of rotenone models are the high mortality rate and less-accurate results obtained in cases of oral administration of rotenone.

6-Hydroxydopamine (6OHDA): 6-OHDA is another classic toxin-based animal model of PD. Ungerstedet (1968) first used this neurotoxin to lesion the nigrostriatal dopaminergic pathway in the rat. Mice, dogs, cats and monkeys are all reported to be sensitive to 6-OHDA. Although 6-OHDA is structurally similar to dopamine, the presence of an additional hydroxyl group makes it toxic to dopaminergic neurons. This compound does not cross the blood-brain barrier, which necessitates its direct injection into the SNpc, medial forebrain bundle (MFB), or the striatum.

Animal models of PD

To date several disease models have been developed including both mammalian and invertebrate systems that cover different technical aspects. Rodent or primate models that are closer to the human anatomy allow studying the phenotypic manifestation of environmental influences, age-related processes and gene mutations similar to the human system. However, whole animal mammalian models have some limitations concerning high throughput screening approaches that are used for identification of both genetic and chemical modifiers of certain phenotypes.
<table>
<thead>
<tr>
<th>Toxin</th>
<th>Behavioral alterations</th>
<th>Nigro-striatal damage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral 6-OHDA injection into rodent brain</td>
<td>Quantifiable turning behavior after systemic administration of a dopaminergic agonist. Bradykinesia and impaired paw use on the contra lateral side.</td>
<td>Massive loss of dopaminergic neurons (90%). Dose-dependent loss of striatal dopamine innervations.</td>
<td>Ungerstedet, 1968</td>
</tr>
<tr>
<td>Acute MPTP mouse model</td>
<td>Coordination and motor impairments patent in challenging situations.</td>
<td>Massive loss of dopaminergic neurons (about 70%). Reduced dopamine levels in the striatum.</td>
<td>Jackson-Lewis and Przedborski, 2007</td>
</tr>
<tr>
<td>Chronic MPTP mouse model</td>
<td>Less obvious motor and coordination impairments that are in some cases only detectable in aged mice.</td>
<td>Partial loss of dopaminergic neurons (30–50%). Reduced dopamine innervations in the striatum.</td>
<td>Jackson-Lewis and Przedborski, 2007</td>
</tr>
<tr>
<td>Paraquat in mice</td>
<td>No clear motor impairments detectable.</td>
<td>Little or no cell loss of dopaminergic neurons (25%). Little or no measurable changes in striatal dopaminergic denervation</td>
<td>Przedborski, 2007</td>
</tr>
<tr>
<td>Rotenone</td>
<td>Coordination and motor impairments</td>
<td>Measurable changes in striatal dopaminergic denervation</td>
<td>Greenamyre et al., 2003</td>
</tr>
</tbody>
</table>
There are currently three techniques for producing in vivo rodent models of Parkinson’s disease (Betabert et al., 2002): unilateral lesioning with 6-hydroxydopamine (6-OHDA) (rats), systemic injection of 1-methyl 4-phenyltetrahydropyridine (MPTP) (mice), and systemic injection of rotenone (rats). High-throughput screens using, e.g. rodents, are time and cost-consuming, cover only few individuals and depending on the screening regime application of drugs in large scale format is very complicated or impossible. In addition, the basic requirement for high-throughput or genome-wide screens, namely keeping constant experimental conditions including extrinsic and intrinsic factors, is very hard to fulfill. As mentioned before, the main hallmark of PD is the selective loss of dopaminergic cells that form a neuronal network comprised of tens of thousands of neurons deep inside the brain and send projections to the cortex. This makes monitoring subcellular processes in vivo almost impossible. In order to overcome these difficulties, several mammalian cellular models have been established. However, these cultured cells completely leave out systemic effects that could be observed in whole animal models caused by both genetic and pharmacological manipulation. Using alternative vertebrate and invertebrate models like zebrafish (Danio rerio), fruit fly (Drosophila melanogaster) or the nematode (Caenorhabditis elegans) can overcome limitations of the mammalian system concerning high throughput screening approaches.

**Zebrafish (Danio rerio)**

The zebrafish (Danio rerio) is an attractive vertebrate model for human diseases including Parkinson’s disease (PD) (Bretaud et al., 2004). Zebrafish have a relatively small size (3-4 cm adult size), short generation time (3 months), external development, transparent appearance until early adulthood. Genetic and anatomical correlation with the human central nervous system (CNS) facilitates the amenability of this animal model to the study of human CNS diseases (Spence, 2008). Dopaminergic neurons have been well characterized in the zebrafish and administration of toxins, including 6-hydroxydopamine (6-OHDA)
and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), have been shown to induce DA neuron cell loss in zebrafish. These studies indicate that the zebrafish can be utilized as an effective animal model to elucidate the genetic pathways that underlie PD, determine the downstream effects of environmental toxins and identify new pharmacological strategies that may mitigate PD-associated neurodegeneration.

**Fruit fly (Drosophila melanogaster)**

The utility of the fruit fly as an animal model imparts many advantages for studying the molecular and cellular mechanisms of Parkinson’s disease (PD) (Bilen et al., 2005). It has a short life span (30 days), small size (2.5 mm), short generation time (10 days), a large number of progeny, high degree of conserved biological pathways due to the comparable fly and human genomes and well-defined genetic and pharmacological techniques (Dawson et al., 2002). The availability of Drosophila genome, availability of several mutant strains, development of RNA-interference technologies and targeted gene disruption techniques and transcriptional and proteome profiling studies have all facilitated the amenability of the fly animal model to the study of PD. Several research findings indicate that the Drosophila system has enormous potential to provide mechanistic insights into PD through the identification of genes involved in familial PD, environmental agents involved in sporadic PD, pathways and compounds that may prevent PD pathogenesis (Whitworth, 2011).

**C. elegans**

*C. elegans* is a free-living roundworm belonging to the Phylum *nematode*. It was first used as a biological model system four decades ago and evolved from an exotic organism in biological research into a powerful model organism eight years ago, when its genome was sequenced and RNA interference was discovered (Fire et al., 1998). *C. elegans* is easy and inexpensive to maintain and culture in laboratory conditions on media or agar plates with a bacterial diet of *Escherichia coli* (Brenner, 1974). Hermaphroditic worms have a short life
cycle (~3 days) and a large progeny number (300) (Hope, 1999). Since *C. elegans* has a small body size, *in vivo* assays can be performed in microplates. Its transparent body allows the clear observation of all cells in mature and developing animals. The nematode has an intensively studied genome, complete cell lineage map, established genetic methodologies for mutagenesis, transgenesis, RNA interference (RNAi) and knockout (KO) mutant libraries. This makes it easier to manipulate and study *C. elegans* at the molecular level. As an *in vivo* model, it facilitates detection of end points (e.g., feeding, reproduction, life span, and locomotion) at the organism level and the interaction of a chemical with multiple targets in an organism. Thus, *C. elegans* complements both in vitro and in vivo mammalian models for toxicological studies (Leung et al., 2008).

*C. elegans* can be used to study neurotoxicity and neurodegeneration. The hermaphrodite nervous system comprises of 302 neurons representing 118 characterized neuronal subtypes (Hobert, 2005) with 6393 chemical synapses, 890 electrical junctions, and 1410 neuromuscular junctions (Chen et al., 2006). The male has an additional 79 neurons that are mainly involved in the control of mating. The somatic nervous system is organized into ganglia in the head and tail (Fig. 5).

![Fig. 5 Nervous System of C. elegans (Source: Worm atlas)](image-url)
The nematode *C. elegans* has eight dopaminergic neurons: six are located in the anterior (four CEP and two ADE neurons) and two in the posterior of the animal (two PDE neurons) ([Fig. 6](#)) all of them are arranged in bilateral symmetry. They have specialized ciliated endings which are embedded in the cuticle and therefore are thought to serve as mechanoreceptive neurons. Dopamine has been shown to play a role in locomotion and egg-laying behavior in *C. elegans* (Schafer and Kenyon, 1995, Sawin et al., 2000 and Hills et al., 2004).

Disruption of the worms’ dopaminergic system either by laser elimination of ADE, PDE and CEP neurons, by *cat-2* mutations leading to defects in DA synthesis or by application of the dopamine receptor antagonist such as raclopride eliminates ‘area restricted search behavior’. Conversely, exogenous application of dopamine increases the turning frequency even when worms are off food. The dopaminergic system in *C. elegans* shows a high degree of conservation regarding the respective components and functions in the mammalian CNS. This is also reflected by the extent of sequence similarities of the involved proteins.

The main neurotransmitter systems in the worm are (cholinergic, GABAergic, glutamatergic, DAergic, and serotoninergic) in nature ([Table 3](#)). *C. elegans* is exploited as a reliable model system of neurotoxicity because of phylogenetic conservation between the genetic networks (in terms of neurotransmitter metabolism to vesicle cycling and synaptic transmission) from nematodes to vertebrates. Thus, findings from *C. elegans* can be extrapolated and further confirmed in vertebrate systems.
Fig. 6 Dopamine (DA) neurons of C. elegans hermaphrodites. (a) The cell bodies and processes. (b) Magnification of the anterior region of C. elegans shows only the 6 anterior-most DA neurons. (c) A 7-day-old worm co-expressing both GFP and α-syn in DA neurons display neurodegeneration. (d) Schematic representation of C. elegans anterior DA neurons. (Source: Caldwell et al., 2012)

Table 3: Major functions associated with neurotransmitters in C. elegans
(Source: Worm Book)

<table>
<thead>
<tr>
<th>Major Functions of C. elegans Neurotransmitters</th>
<th>Inhibition</th>
<th>Promotion</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>Muscle contraction</td>
<td>Muscle relaxation</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Locomotion</td>
<td>Deciliation</td>
</tr>
<tr>
<td>Tyramine</td>
<td>Egg laying</td>
<td>Pharyngeal pumping</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Fixed-dependent forward movement</td>
<td>Slower movements in the presence of food</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Locomotion</td>
<td>Deciliation</td>
</tr>
<tr>
<td>GABA</td>
<td>Muscle contraction</td>
<td>Muscle relaxation</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Muscle contractions</td>
<td>Muscle contractions</td>
</tr>
</tbody>
</table>
A new MPP⁺ based PD model using *C. elegans* has been developed that responds with loss of mobility and dopaminergic neurons. Rotenone, which inhibits mitochondrial complex I, has also been reported to elicit similar responses as MPP⁺ (Sherer et al., 2003). In addition, exposure of worms to high doses of 6-OHDA (50mM) for short periods of time has been reported to cause degeneration of dopaminergic neurons in *C. elegans* (Nass et al., 2002). These studies clearly establish responsiveness of *C. elegans* to chemical models of PD in a way that correlates with loss of dopaminergic neurons. Conserved machinery of dopamine signaling, responsiveness to chemical models of PD, availability of transgenic strain to visualize loss of dopaminergic neurons and amenability for being employed in high throughput systems make *C. elegans* an excellent model for studies efficacy of anti-PD drugs and to obtain deeper insights into cyto- and molecular pathology of PD.

Increased production of reactive oxygen species (ROS) is supposed to play an important role in the development of PD (Dauer and Przedborski, 2003). Both pathological and animal model based analyses suggest that dopaminergic neurons are highly sensitive to increased oxidative stress. One source may be metabolic by-product from the dopamine synthesis pathway that leads to early degeneration of these cells during the progression of the disease. Artificial induction of ROS is a useful tool to mimic selective dopaminergic loss in animal models. Moreover, the human substantia nigra contains high levels of neuromelanin, a substance that removes excess cytosolic catecholamines that are not taken up into synaptic vesicles and so has a neuroprotective effect. However, neuromelanin can also interact with organic compounds like pesticides that may contribute to the preferred degeneration of pigmented neurons. Exposure to certain pesticides like paraquat or the insecticide rotenone was shown to increase the susceptibility to develop PD both in humans and animal models.
Various PD-related genetic modifications were shown to disrupt mitochondrial function in *C. elegans* suggesting the possibility that mitochondrial dysfunction is a common feature in familial PD pathology. The toxicity of paraquat is probably mediated by the formation of superoxide radicals. Since paraquat does not easily pass the blood-brain barrier (BBB), its distribution in the CNS is unpredictable and the resulting effects are hard to interpret. In contrast to the mammalian system, *C. elegans* has no structure similar to the BBB – this allows an even systemic application of the drug. The treatment of worms with paraquat leads to increased larval arrest and lethality that can be easily quantified. Rotenone is a natural, highly lipophilic, cytotoxic compound that inhibits the electron transport from the Fe-S center in complex I to ubiquinone. Rotenone is commonly used as an insecticide. Despite the fact that intravenous injection of rotenone produces selective degeneration of nigrostriatal dopaminergic neurons accompanied by α-synuclein-positive cell inclusions in rats, it is not clear if exposure to rotenone can induce PD in humans, although it probably increases the susceptibility to develop the disease. Like paraquat, rotenone led to increased lethality or larval arrest when administered to *C. elegans*.

**Pesticides and PD**

Pesticides are defined as ‘any substance or mixture of substances intended for preventing, destroying, repelling or mitigating pests. Pesticides consist of multiple classes and subclasses and exhibit a vast array of chemically diverse structures (Franco et al., 2010). They are commonly referred to by the organisms designed to control (e.g., herbicides, insecticides, or fungicides) or by their chemical class (organophosphate, organochlorines, triazine) (Alavanja et al., 2004, Voccia et al., 1999). However, pesticides are not always selective for their intended target species and hence, adverse health effects can occur in non-target species including humans. Evidence suggests that pesticide exposure increases the risk of cancer and neurodegenerative diseases. Recent evidence also demonstrates the ability of pesticides to act as endocrine
disruptors, contributing to various adverse effects associated with reproductive and developmental toxicity (Jones et al., 2008).

Although PD existed long before the introduction of pesticides, the thought is that pesticide exposure has contributed to the increased incidence of the disease (Hatcher et al., 2008). Since the discovery of MPTP, parkinsonian inducing effects this arouse the possibility of other similar compounds relevant to MPTP, for example, paraquat to induce PD (Brown et al., 2006).

**Fungicides.** Maneb, or manganese ethylene bis-dithiocarbamate, a fungicide is one with possible parkinsonian symptoms which is suggested to be secondary to exposure to the manganese metal core (Hatcher et al., 2008). Maneb is one of the toxins used to induce PD in animal models but usually in combination with other agents specially paraquat (Thiruchelvam et al., 2000). Maneb has been documented to increase the severity of PD in models which make it one of the good candidates in PD studies (Thiruchelvam et al., 2000).

**Herbicides** *(Paraquat).* The possibility that paraquat *(1,1-dimethyl-4,4-bipyridinium)* may damage the nigrostriatal dopaminergic system and therefore contribute to the neuropathology of Parkinson’s disease (PD) was first proposed following the observation that its chemical structure closely resembles that of MPP+*(1-methyl-4-phenylpyridinium ion)* (Donato et al., 2008). Animal studies confirmed the ability of paraquat to induce selective dopaminergic nigrostriatal degeneration (Kuter et al., 2007).

**Insecticides.** Many of the compounds in this class are, by design, neurotoxic. Similarities between the insect and human nervous systems has lead to cross-toxicity of these compounds (Hatcher et al., 2008).

**Organophosphates.** Although evidence supporting a role for OPs in PD pathogenesis is scant, there are case reports of people with severe Parkinsonism after OP exposure (Bhatt et al., 1999). However, the reversibility of the symptoms and lack of responsiveness to L-dopa are not consistent with PD. A recent family-based case-control study did implicate organophosphates and other insecticides in PD (Hancock et al., 2008). However, it might be that
the effects of OP exposure probably involve disturbance of the balance between the dopamine and acetylcholine systems and not specific pathological changes in the dopamine system which makes them unsuitable in toxic modeling of PD (Karen et al., 2001).

**Organochlorines.** The first clue of a relation between organochlorines and PD was the presence of them in postmortem specimens of PD patients (Hatcher et al., 2008). Kanthasamy et al. (2005) have postulated possible mechanisms for the influence of organochlorines on PD patients.

The mechanisms by which pesticides induce neurotoxicity is depicted in **Fig. 7.** Various studies demonstrating the role of pesticides induce nigrostriatal damage/ PD like outcome in experimental animal models is enlisted in **Table 4.**

![Fig. 7 Mechanism of neurotoxicity of pesticides](Source: Baltazar et al., 2014)
Table 4. Pesticides demonstrated to induce nigrostriatal damage/ PD like outcome in experimental animal models

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Dose/duration</th>
<th>Animal model</th>
<th>Parameter affected</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dieldrin</td>
<td>50ppm, 10 wk</td>
<td>Rats</td>
<td>Small reductions in whole brain dopamine</td>
<td>Wagner and Greene, 1978</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>low doses</td>
<td>Mice</td>
<td>Up regulated dopamine transport in striatal synaptosomes</td>
<td>Kirby and Bloomquist, 1996</td>
</tr>
<tr>
<td>Heptachlor, Permethrin, Deltamethrin Chlorpyrifos</td>
<td>i.p, 3X over 2 wk</td>
<td>C57BL/6 mice</td>
<td>Increased levels of dopamine transporter (DAT) protein in the striatum – HEP, PER decrease in dopamine uptake – CPF released dopamine from striatal terminals - DEL</td>
<td>Bloomquist et al., 2002</td>
</tr>
<tr>
<td>Paraquat , Maneb</td>
<td>0.3 mg/kg PQ, 1 mg/kg MB - post-natal (PN) days 5 to 19</td>
<td>C57BL/6 mice</td>
<td>Minimal nigral dopaminergic cell loss</td>
<td>Thiruchelvam et al., 2002</td>
</tr>
<tr>
<td>Chlorpyrifos Permethrin</td>
<td>s.c, 75 mg/kg i.p, 200mg/kg b.w 3X over 2 wk</td>
<td>C57BL/6 mice</td>
<td>No effect on TH or DAT expression levels</td>
<td>Kou et al., 2006</td>
</tr>
<tr>
<td>Permethrin</td>
<td>200mg/kg b.w, i.p</td>
<td>Male C57BL/6 mice</td>
<td>increased striatal glial fibrillary acidic protein (GFAP) immunopositive neurophil</td>
<td>Dodd and Klein, 2009</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>50mg/kg b.w, s.c</td>
<td>Male C57BL/6 mice</td>
<td>no significant main effect on GFAP immunostaining</td>
<td>Dodd and Klein, 2009</td>
</tr>
</tbody>
</table>
Importance of organophosphorus insecticides (OPI)

OPI are primarily sold as pesticides, but have secondary markets as industrial plasticizers, fuel additives and chemical warfare agents. OPI are additionally been used in public health applications to control insect vectors that might cause pathogenic changes in a host species. Administration of these compounds to animal models generates a substantial amount of information on, their interaction with cytoskeletal elements, axonal transport and their implications in neurodegeneration. Presence of pesticide residues in fruits and vegetables has become a global threat. Frank et al.,(1987) reported residues of OC and OP insecticides along with fungicides and herbicides in fruits. Kumari et al., (2006) showed pesticide residues in Indian fruits and vegetable samples. Fruit samples processed to determine residues of some commonly used OPI viz. monocrotophos, methyl parathion, chlorpyrifos and quinalphos revealed that among the tested OPI, quinalphos was a dominant contaminant with guava containing maximum residues. The probable reason of more frequent residues of quinalphos was attributed to its use on the tested fruits for controlling fruit fly attack close to harvest stage. However, all OPI detected were well within their respective maximum residue limit (MRL) values. Presence of OPI residues in vegetables and fruits is indicative of change in usage pattern of insecticides in India where a shift has taken place from OC to the easily degradable groups of these insecticides in the last decade.

OPI-AChE inhibition and detoxification

OPI exert their main toxicological effects through non-reversible phosphorylation of esterases in the central nervous system and there by inhibiting the acetylcholinesterase enzyme (AChE), which catalyzes the hydrolysis of the neurotransmitter acetylcholine (Pope, 1999). AChE inhibition by OP insecticides leads to accumulation of acetylcholine, interfering with the function of the nervous system and leading to over-stimulation of nicotinic and muscarinic acetylcholine receptors. The OPI are mainly
detoxified through oxidation and hydrolysis. The two main enzyme groups involved in the hydrolysis of these compounds are carboxylesterases (CaEs) and phosphotriesterases (PTEs). The OPI exert most important toxic effects through phosphorylation of serine residues in the active centers of CaEs (Aldridge and Reiner, 1972). The phosphorylation of AChE and Neuropathy Target Esterase are linked with toxic effects. However, in mammals (especially in liver and serum) there is a pool of CaEs of unknown physiological role, of which the inhibition does not cause apparent toxic effects. These esterases, defined as B-esterases, may be considered as enzymes involved in the detoxification of OPs and since, each molecule of CaEs is able to ‘scavenge’ a minimum of one molecule of insecticide before this reaches targets in the nervous system. This detoxification system is very efficient since, the resistance to OPs of some strains of cockroaches and flies (Prabhakaran and Kamble, 1996) is associated with an over expression of B-esterases that remove the insecticides from the media through phosphorylation of their active center.

**OPI and neurotoxicity**

Organophosphorus compounds exert neurotoxicity by three different mechanisms. The primary mode of action is the irreversible inhibition of acetylcholinesterase, which leads to the accumulation of acetylcholine and subsequently results in overstimulation of the nicotinic and muscarinic acetylcholine receptors, producing cholinergic effects (Abou-Donia, 2003). Some of the OPI exert a delayed onset of ataxia known as ‘organophosphorus ester-induced delayed neurotoxicity’ (OPIDN) which is accompanied by a Wallerian-type degeneration of the axon and myelin in the most distal portion of the longest tracts in both the central and peripheral nervous systems. Although largely characterized by chronic neurobehavioral alterations, OPICN involves other molecular, neurochemical, neurophysiological, neuropathological, neuropsychological, and neurological changes (Abou-Donia, 2003). Besides, several studies have reported long-term, persistent, chronic neurotoxicity
symptoms in individuals as a result of acute exposure to high doses that cause acute cholinergic toxicity, or from long-term, low-level, subclinical doses of these chemicals (Lotti, 2002).

**Pesticide used in the study - Monocrotophos**

Monocrotophos (MCP), (dimethyl (E)-1-methyl-2-(methyl-carbamoyl) vinyl phosphate), an organophosphorus insecticide (OPI), is a broad-spectrum systemic insecticide. It is banned in several countries but has found higher production and consumption in India as compared to other OPI (Dewan and Rajendran, 2009). The major food crops on which it is applied are rice, pulses, groundnuts, vegetables and fruits. Amongst vegetables, brinjal (aubergine) and tomato and among fruit crops, mango and grapes have higher applications of MCP. Spices like chillies and tea have also been reported to receive a higher number of applications with MCP. It is also used on castor, citrus, olives, rice, maize, sorghum, sugar cane, sugar beet, peanuts, potatoes, soybeans, cabbage, onion and pepper (Dewan and Rajendran 2009). Levels of MCP above maximum residue limits (MRL) (0.2 µg/g) have also been detected in vegetables such as brinjal, okra, cauliflower and pea in India. According to a recent study conducted in India, the Theoretical Maximum Daily Intakes (TMDI) of MCP was found to be 0.17 mg/d, which was 472% above the Acceptable Daily Intakes (ADI) (0.036 mg/d for an adult (Arora and Singh, 2004). Hence, the continuous consumption of vegetables, even with only moderate contamination levels, can lead to varying degrees of toxic effects in the human population after long-term exposures. In addition, limited data are available regarding the impact of MCP on non-target organisms. Despite commendable efforts undertaken to regulate pesticide use in the country, India does not yet have a clear-cut system to ensure that pesticides are managed in a sound manner that poses only limited risk to health. Several studies have indicated that certain foods contain high levels of pesticide residues (Arora and Singh, 2004).
Aim and Scope
AIM AND SCOPE OF THE PRESENT INVESTIGATION

Parkinson’s disease (PD) is a debilitating motor disorder characterized by selective loss of dopaminergic neurons in substantia nigra pars compacta. The clinical manifestation of PD is characterized by tremor at rest, rigidity, slowness of voluntary movement, postural instability and freezing. Cellular hallmarks of PD include the accumulation of proteinaceous intracellular inclusions named Lewy bodies in surviving DAergic neurons. Although the exact etiology of PD is unknown, both genetic and environmental factors are thought to contribute towards the pathogenesis of PD. Environmental factors or gene–environment interactions are speculated to play a crucial role in the development of PD.

Several epidemiological studies have identified pesticide exposure as a significant risk factor for PD. Evidences suggest that pesticide exposure increases the risk of cancer and neurodegenerative diseases. Studies have demonstrated that drinking well water and living in a rural setting, both of which may increase exposure to agricultural pesticides, increase the risk of developing PD. In addition, exposure to pesticides used in the houses has been linked to PD. However, majority of the studies have not identified specific pesticides or the mechanism by which pesticides damage the dopaminergic system and increase the risk of PD. Many synthetic pyrethroids like cypermethrin and deltamethrin have been shown to cause dopaminergic degeneration and PD like outcomes in rat models. The first clue of a relation between organochlorines (viz., DDT, lindane) and PD was based on the presence of the residues of these insecticides in postmortem specimens of PD patients.

It is well known that organophosphorus insecticides (OPI) are neurotoxic and can cause seizures and convulsions in humans and laboratory animals. Earlier studies have suggested the possible involvement of dopaminergic neurons in OPI-induced neurotoxicity. The importance of alterations in the DA system as a possible causative mechanism underlying the behavioral and functional changes associated with delayed neurotoxicity associated with OPI exposure has been
studied to a limited extent. Dopaminergic neurotransmission has also been reported to be affected in rodents by several OPI thus contributing to the overall spectrum of neurotoxicity of these insecticides. However, comprehensive studies which demonstrate the potential of OPI to elicit and augment dopaminergic neuronal dysfunctions in animal models of PD are lacking.

Several animal models viz., rodent (rat/mice), zebra fish, *Drosophila*, and *C. elegans* have been established to study PD and much of the laboratory understanding of PD is derived from studies on the specific action of neurotoxic chemicals like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) on the dopaminergic neurons. MPTP bears structural resembles to a number of known environmental agents, like herbicides such as paraquat and garden insecticides/fish toxins, such as rotenone have been shown to induce dopamine cell degeneration. The mechanism of actions of each are however different. Several laboratories have employed *C. elegans* as a model organism to inquire the molecular and genetic mechanisms contributing to PD. Further, the availability of transgenic strains of *C. elegans* in various realms of dopaminergic neuronal structure and functions make it a convenient model to study various aspects of PD. Although *C. elegans* has gained popularity in neurotoxicology testing, limited studies have examined ‘pesticide-induced neurodegeneration’ related to PD employing this model.

Accordingly, the focus of this proposal is to employ *Caenorhabditis elegans* and rodents as animal model systems to study the impact of Monocrotophos (MCP) on dopaminergic system and perhaps towards development of PD-like symptoms. Thus, the proposal basically addresses three aspects: a) To study the propensity of monocrotophos (MCP), an organophosphorus insecticide, to elicit dopaminergic neuronal dysfunctions in *C. elegans* and rodents (mice/rat) b) To investigate the interactive role of MCP in chemically-induced models of Parkinson’s disease (PD) in *C. elegans* and rodents (mice/rat) c) To delineate the mechanisms underlying the impact of MCP on the PD outcomes in *C. elegans* and rodents (mice/rat).