Introduction
Parkinson's disease (PD) is the second most prevalent neuro-degenerative disorder after Alzheimer's disease, and its incidence increases with age [1]. The etiology of PD remains elusive and is likely diverse. Recent studies have focused on environmental factors and gene-environment interactions as potential causes.

Even basic epidemiological information remains uncertain. Prevalence and incidence values are difficult to obtain since PD is not a notifiable disease, but it is believed that 1 in every 300 people in Canada and the United States has Parkinson's disease [2]. While genetic susceptibility is believed to play a large role in early-onset PD, most cases of PD seen in North America develop after age 50, suggesting that the majority of PD is caused by a factor other than genetics [3].

Clues to potential environmental risk factors for Parkinson's disease were first discovered in the early 1980s. An increased risk of severe Parkinson's was observed in heroin addicts and determined to be triggered by a chemical found in synthetic heroin (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or MPTP). Later, it was observed that this compound's structure was similar to certain herbicides and pesticides. Since then, various epidemiological studies and laboratory experiments suggest an association between pesticides and Parkinson's disease. We summarize here recent studies in the search for environmental toxins that may contribute to the etiology of Parkinson's disease.

Pathology
Parkinson's disease is marked pathologically by the selective degeneration of dopaminergic neurons, nerve cells that use and produce dopamine as a neurotransmitter substance. These neurons are found in a large cell mass in the base of the brain known as the substantia nigra. Dopamine is released in response to an impulse in the nerve axon and diffuses into the synapse between the nerve ending and the dopaminergic neuron where it interacts with a receptor. As the death of the neurons progresses and dopamine levels drop, signs of the disease (tremors, rigidity, and loss of voluntary movement) become more severe.

Another pathological feature characteristic of Parkinson's disease is the formation of Lewy bodies. Found within the neuron structure, Lewy bodies are deposits of abnormally aggregated neurofilament proteins that contain ubiquitin, ubiquitin C-terminal hydrolase, and alpha-synuclein [4]. Animal studies investigating possible mechanisms for PD use these neurological features to draw similarities between observed effects in rodents and Parkinson's disease.

Epidemiological Studies
The association between pesticide exposure and Parkinson's disease has been investigated in numerous epidemiological studies. A meta-analysis of 19 studies published from 1989 to 1999 found a positive association between pesticide exposure and Parkinson's disease [5]. The majority of the studies reported an elevated risk of PD, and the combined odds ratio was calculated to be 1.94 with 95% confidence intervals of 1.49-2.53. A review of more recent studies shows odds ratios consistent with results from the meta-analysis [6]. Although risk increased with longer exposure durations, no other indication of a dose-response relation was observed.

A weakness found in most epidemiological studies is that exposure is assessed for broad categories like "pesticide" or "pesticide and herbicide" [6]. Even when subgroups were used, they often provided no useful information. In a population-based case-control study investigating the risk of PD with pesticide exposure, farming, well water, and rural living, the subgroups herbicides and insecticides could not be evaluated independently because 90% of the herbicide-exposed subjects were also exposed to insecticides [7]. Another limitation is that subjects seldomly recall the specific class of pesticides used (e.g., carbamates, organophosphates). For those that are able to remember, it is nearly impossible to validate actual pesticide use. While epidemiological studies are suggestive of an association between pesticides and PD, these limitations further emphasize the need for complementary laboratory research.

Mechanisms of Pesticide Toxicity
Although pesticide use has been linked to Parkinson's disease in various epidemiological studies, the exact mechanism of pesticide toxicity still eludes researchers. While several explanations have been proposed, recent advances have been made in three specific areas.

Gene-Environment Interactions
One explanation is that there may be a genetic susceptibility to Parkinson's disease resulting from polymorphisms of specific enzymes involved in the metab-
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olism of pesticides. These enzymes include cytochrome P 450 (CYP), CYP2D6, and glutathione transferase.

In a case-control study in Australia [8], pesticide exposure was a significant risk factor after adjusting for age, sex, and family history of PD (odds ratio of 2.3 [95% CI 1.2-4.4]). Subjects were classified as exposed if they used pesticides more than once-weekly for more than six months before onset of disease. Blood samples were also taken to identify any polymorphisms of glutathione transferases (GSTs). These enzymes are subject to a variety of polymorphisms, with each affecting a different process in the detoxification and metabolism of pesticides. When the authors restricted their analysis to only the participants who reported pesticide exposure, significantly more patients with Parkinson's disease had GSTP1 gene variants. The authors conclude that the polymorphisms influence the ability of the GSTs to detoxify pesticides with neurotoxic effects.

In a study by Hubble et al. [9], researchers investigated the association between PD with dementia and the interaction of environmental and genetic variables. Although none of the genetic markers or environmental factors were independently predictive of PD with dementia, the interaction of cytochrome P 450 450 and pesticide exposure produced a statistically significant odds ratio of 3.17 (95% CI: 1.11-9.05).

The results of these studies are limited by the small population size and recall bias inherent in case-control studies. Also, pesticides consist of a wide range of chemical structures and mechanisms of toxicity and may not all contribute to the development of PD. Despite these weaknesses, the studies suggest genetics may affect susceptibility to Parkinson's disease among the subgroup of people with pesticide exposure.

Mitochondrial Inhibition
Another possible mechanism is the inhibition of mitochondrial respiration at complex I of the electron transport chain, one of the five enzyme complexes of the inner mitochondrial membrane involved in oxidative phosphorylation [10]. When it was discovered that MPTP's metabolite, 1-methyl-4-pyridinium (MPP+), caused mitochondrial dysfunction, a similar mechanism of action was soon reported for Parkinson's disease.

In a study by Betarbet et al. [11], the common pesticide rotenone was shown to reproduce neurological features of Parkinson's disease. Rotenone is extremely toxic to fish, but poses little risk to humans when used properly [12]. The authors were able to show that rotenone produced systemic partial inhibition of complex I in laboratory rats, which resulted in the progressive degeneration of dopaminergic neurons and deficits in motor skills. These results provide a biologically plausible mechanism for the development of Parkinson's disease after exposure to pesticides. The study also emphasizes the need for further research of complex I inhibitors other than pesticides that may exist in the environment.

Questions exist concerning the relevance of this work to humans. The rats in the study by Betarbet et al. were administered rotenone intravenously over a short period of time. A more realistic application would be to extend these studies to primates and examine low-dose long term exposures [13]. Furthermore, the lack of a detectable change in the complex I system of many individuals with Parkinson's disease suggests that this may not be the predominant mechanism.

In response, Betarbet et al. acknowledged that while not all cases of PD are caused by complex I defects, the fact that they are undetectable does not necessarily imply they do not exist [14]. Minimal inhibition of complex I might be undetectable with conventional assays, but still produce neurological damage. Despite the inherent uncertainties, the US EPA is currently reviewing rotenone and the implications of this work [15].

Multiple Exposures
The effect of multiple exposures to chemicals in the environment has been an important area of interest for various health outcomes. Because a variety of chemicals can be applied to a single crop or farm, it is important to understand the effects of pesticide mixtures.

Paraquat is an herbicide that has long been considered a potential risk factor for Parkinson's disease due to its structural similarity to MPP+, the active metabolite of MPTP. When injected directly into the brain, paraquat reduces the level of dopamine and alters behavior. However, systemic administration of this herbicide to rodents shows little evidence of neurotoxicity. While much of the focus has been on paraquat, other classes of pesticides are also known to impair dopaminergic activity. Exposure to maneb, a dithiocarbamate fungicide, has been linked to neurological impairments in agricultural workers.

A study by Thuchelvam et al. [16] revealed that Parkinson's disease may be linked to the combination of these two pesticides. Paraquat and maneb administered individually to mice caused no neurological damage, but when administered as a mixture, produced traits characteristic of PD. Paraquat (5 mg/kg or 10 mg/kg) and maneb (15 mg/kg or 30 mg/kg) were injected into mice once a week for 4 weeks. These levels are considered low and are well below the reported values of LD50, the dose at which 50% of the animals tested are killed. Results of administering the paraquat/maneb mixture showed altered dopamine levels in the substantia nigra, the same area of the brain targeted in Parkinson's disease. Moreover, motor skills in the mice were significantly reduced compared to controls only when combinations of paraquat and maneb were administered together. The authors did not provide a biological mechanism to explain the interactive effects of paraquat and maneb. However, their results suggest the potential for pesticide mixtures to act as etiologic agents for Parkinson's disease and emphasize the need for further investigation of multiple chemical effects.
Conclusion
Exposure to pesticides has been frequently identified as a potential risk factor for Parkinson's disease in epidemiological studies. In addition, recent laboratory experiments have provided evidence for neurological effects and biologically plausible mechanisms that link pesticides to PD.

In response to the many advances made in the field of environmental risk factors, the National Institute of Environmental Health Sciences (NIEHS) announced in August 2002 a $20 million initiative on Parkinson’s disease [17]. The funding will allow three major research centers to collaborate and investigate the environmental and genetic origins of PD. The Parkinson's Institute will investigate the mechanisms of dopamine cell death and the risks associated with metals and pesticides. The Emory Collaborative Center for PD Environmental Research at Emory University in Atlanta Georgia will examine the interaction between pesticides and the proteins involved in maintaining the dopamine within nerve cells. The Center for Gene-Environment Studies in Parkinson’s Disease at the University of California, Los Angeles (UCLA) will study how alterations in the genes that regulate dopamine affect the risk of Parkinson's disease associated with pesticides. The NIEHS is already currently supporting studies at the University of Rochester, the University of Georgia, and the University of Washington [1]. Recent progress in this field suggests that with further research, the etiology of Parkinson’s disease may one day be understood.

References Cited:
4. Lewy Bodies in Parkinson's, http://www.mayo.edu/fpd/6_park/lewy.htm