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Pesticides and Parkinson's disease – a critical review

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Introduction

Parkinson's disease (PD), first described by James Parkinson as the 'shaking palsy', is an idiopathic disease of the nervous system characterised by chronic progressive tremor, bradykinesia, rigidity and postural instability, as a result of loss of dopaminergic neurons in the substantia nigra. In 1983, it was observed that the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) resulted in the development of acute parkinsonism, similar in nearly all clinical, pathological, and biochemical features to the idiopathic disease, in a small group of drug addicts in northern California. This led to an interest in the possible role of environmental toxins in the development of PD and parkinsonism generally and, in particular, to an interest in pesticides, given the close structural similarity of the MPTP metabolite, 1-methyl-4-phenylpyridine (MPP+), to the herbicide paraquat. Since then, numerous epidemiological and toxicological studies have been published, looking at pesticides as a risk factor for PD and parkinsonism and possible mechanisms by which pesticides may act. However, many of these studies present conflicting results and some are of uncertain relevance to humans. In addition, a number of other risk factors have been associated with the development of PD, such as rural living and consumption of well-water. As a result of the uncertainties of the potential role of pesticides in the development of PD and parkinsonism, the Department for the Environment, Food and Rural Affairs (Defra) commissioned the Institute for Environment and Health (IEH) to undertake a critical review of the epidemiological and toxicological literature, summarising the current state of knowledge, and to provide a critical evaluation of the possible role of pesticides in the development of PD and parkinsonism.

A comprehensive literature search was conducted in December 2002 and updated in April 2003 to identify published literature pertaining to the potential role of pesticides in the development of PD and parkinsonism. From this literature search 336 papers were obtained for further review. Additional papers were identified from the reference lists of these papers, current issues of relevant journals and ad hoc searches of the Internet.

Epidemiology

The review of the epidemiological literature identified ten descriptive studies, five cohort studies, 38 separate case–control studies and one meta-analysis that investigated the relationship between PD and pesticide exposure. An association between PD and pesticide exposure was tested for in eight of the ten pesticide-related descriptive studies (two case-series studies, three mortality studies, four prevalence studies and one incidence study). Of these eight, all the mortality studies (one of which was reported only as an abstract) and one prevalence study found a significant association between PD and a marker of pesticide use. One case-series study, two prevalence studies, one looking at hexachlorobenzene exposure, and an incidence study (reported only as an abstract) found no association between PD and pesticide use/exposure. Of the five cohort studies identified (including one reported only as an abstract), three showed an increased risk of PD amongst farming occupations of which two inferred an increased risk of PD from pesticide exposure. One study also identified several other occupational groups with an increased risk of PD. An increased risk of PD following pesticide exposure was observed in the majority of the 38 case–control studies identified, with a majority of those showing a significantly increased risk. The excess risks reported in these studies varied from 1.01 to 7.00, although confidence intervals were wide in many studies, partly due to small numbers.

From the epidemiological studies reviewed, there appears to be a fairly consistent association between prior pesticide exposure and an increased risk of developing PD; this relationship appears to be

reasonably consistent in different populations and countries, although some studies present conflicting results. The heterogeneity between studies may be a result of exposure misclassification. The exposure category, 'pesticides,' represents many hundreds of chemicals and, as a result, one cohort exposed to 'pesticides' may be exposed to a different group of chemicals compared with another cohort said to be exposed to 'pesticides'. It may be that exposure to only a few pesticide compounds results in an increased risk of developing PD; however, differences in exposure to these compounds would be masked by the use of broad 'pesticide' exposure categories in the studies, possibly resulting in the observed heterogeneity. The level of increased risk identified in different studies is variable, although a meta-analysis focusing on pesticide exposure as a risk factor for PD reported a combined odds ratio of 1.94 (95% confidence interval (CI) 1.49–2.53; Priyadarshi *et al*., 2000). However, only 19 of the 34 comparable case–control studies (excluding autopsy studies and conference abstracts) identified were included in the analysis; hence, the limited nature of the dataset might have influenced the overall risk estimate.

In all the studies reviewed, exposure history was collected retrospectively. In all the case–control studies and some of the cohort studies this was done using a questionnaire, thus introducing the potential for recall bias to occur, which could impact on the internal validity of a study. Some studies made efforts to reduce recall bias, either by the involvement of family members and/or carers (Tanner *et al*., 1989; Golbe *et al*., 1990; Koller *et al*., 1990; Chaturvedi *et al*., 1995; Liou *et al*., 1997), although this may have introduced information bias, or by using re-test methods to check the reliability of answers given (Hertzman *et al*., 1990; Koller *et al*., 1990; Butterfield *et al*., 1993; Hubble *et al*., 1993). Those studies that used a re-test method found that the original answers were reliable.

Whilst most of the case–control studies identified looked at 'pesticides' as an exposure category, several studies employed more detailed exposure categories, and significant associations with exposure to herbicides and insecticides as classes of pesticides and PD risk were identified. Based on five studies, findings for an association with fungicide exposure were inconclusive. A few studies that looked at specific pesticide compounds were identified. Seidler *et al*. (1996) identified exposures to organochlorines, alkaline phosphates and carbamates as significant risk factors for PD. Paraquat was shown to be significantly associated with PD in two studies (Hertzman *et al*., 1990; Liou *et al*., 1997) but not in a third (Hertzman *et al*., 1994). One study found a weak non-significant positive association with PD and exposure to DDT (Kuopio *et al*., 1999a).

The relationship between exposure duration and PD risk was investigated in six case–control studies and one cross-sectional study. Four case–control studies and the cross-sectional study found a significant association between increasing pesticide exposure duration and PD risk (Seidler *et al*., 1996; Liou *et al*., 1997; Gorell *et al*., 1998; Chan *et al*., 1998; Engel *et al*., 2001a), although the relationship in one case–control study lost significance after adjusting for smoking, family history, rural living and diet (Chan *et al*., 1998). These studies suggest that PD risk is significantly increased when the duration of exposure to pesticides exceeds a particular threshold (e.g. >10 or >20 years). One case–control study and the cross-sectional study examined the relationship between duration of exposure to herbicides and insecticides. The case–control study found a significant positive trend with increasing duration of herbicide exposure, which was the only significant predictor of PD risk in the study (Seidler *et al*., 1996). The cross-sectional study, after adjustment, did not find a significant relationship, but risk estimates were still elevated in the highest exposure categories (Engel *et al*., 2001a). Only one case–control study examined the relationship between duration of exposure to a specific pesticide compound, paraquat, and PD; there was a significant association with greater than 20 years exposure to paraquat (Liou *et al*., 1997). Overall, these studies suggest that herbicides, possibly paraquat especially, may be risk factors for the development of PD, particularly following extended periods of exposure.

A number of potentially confounding exposures, such as well-water consumption, farming and rural living, have also been found to be associated with an increased risk of PD in a number of studies. In a few of these studies multivariate analyses were performed to examine the relationship between the

various risk factors. In a study by Koller *et al.* (1990), multivariate analysis indicated that drinking well-water was dependent on rural living, suggesting the risk factors were interrelated. In one study, well-water use was found to be positively and independently associated with PD (Zorzon *et al*., 2002), and meta-analysis indicated the overall risk estimate to be 1.26 (95% CI 0.96–1.64; Priyadarshi *et al.* 2001). Several studies have also found farming to be an independent risk factor, in addition to pesticide exposure (Gorell *et al*., 1998; Zorzon *et al*., 2002), and the meta-analysis of Priyadarshi *et al.* (2001) yielded a combined risk estimate of 1.42 (95% CI 1.05–1.91). Despite these studies, there still remains uncertainty as to the exact nature of the relationship between well-water consumption, farming, rural living and pesticide exposure and their relationship to PD risk. As a result, further work is required to clarify the contribution of each risk factor to the overall risk of developing PD and to determine whether any of these factors are confounding.

Overall, it seems unlikely that the relatively consistent association between PD and reported exposure to pesticides could be wholly explained by a combination of chance and selective reporting. Based on the available data, extended exposure to classes of pesticides, such as herbicides and insecticides, especially possibly paraquat, appear to be risk factors for the development of PD. However, further studies are required to understand better the relationship with other potentially confounding exposures, such as well-water consumption and farming, and to elucidate more clearly which particular pesticide exposures may result in an increased risk of developing PD.

Toxicology

Toxicological studies have examined the potential role of a range of pesticides in the development of symptoms of PD in animal models, and potential mechanisms by which the pesticides may act have been investigated. Currently, the available studies are not sufficiently informative to assess fully the potential mechanisms by which pesticides might influence PD development. However, there is some toxicological evidence to support a link between the neurotoxic effects of some pesticides and the mechanisms that are believed to play a particular role in the development of PD.

A number of detailed mechanistic studies have looked at the insecticide rotenone and have shown that, when administered parenterally, rotenone acts on the dopaminergic systems in the nigrostriatal region of the brain that are affected in PD. Its neurotoxic effects are mediated by inhibition of mitochondrial Complex I, formation of Lewy body-like structures and apoptosis, mechanisms that appear to correspond to changes seen during the development of PD; it also results in motor and postural changes.

The herbicide paraquat, in particular, has been studied extensively and, although the evidence is somewhat conflicting, it does appear to be able to cross the blood–brain barrier (BBB) under some circumstances. There is also evidence to suggest that paraquat is taken up into the striatal tissue (Shimizu *et al*., 2003) where it may lead to a depletion of striatal dopamine, a loss of neurons in the substantia nigra and behavioural changes similar to those seen in PD (Liou *et al*., 1996). There are several proposed mechanisms for the neurotoxic effects of paraquat, including the generation of oxygen free radicals and effects on the excitatory amino acid, glutamate, leading to neuronal damage.

There is evidence that dithiocarbamates may interact with other xenobiotic agents to increase neurotoxicity. There is also some evidence that the mechanisms of neurotoxicity associated with exposure to pyrethroids are those that would be applicable to the development of PD. Studies on lindane, DDT and organophosphates suggest that, while these pesticides have neurotoxic actions, they do not act on systems in the brain of relevance to PD. For other pesticides, there are insufficient data to evaluate any possible association with PD.

Of potential importance are a few studies that report dopaminergic neurotoxicity after combined low-level exposure to combinations of factors, such as paraquat and maneb or the combined effects of

pesticides and metals on α-synuclein. Such studies suggest that exposure to multiple low-level environmental neurotoxicants may be a factor in the long-term development of PD.

The examples of PD-like damage induced by MPTP (in man) and rotenone (in animals) provide evidence that, at high doses, some xenobiotics can certainly induce parkinsonism symptoms. However, the toxicology studies identified focused primarily on mechanisms of action after short-term treatments. As a consequence, the routes, doses, and duration of administration employed have not been environmentally or occupationally appropriate. Thus, there still remains considerable uncertainty as to whether the effects seen in animal models, under these treatment regimes, are predictive of changes at lower doses, over longer time periods and via environmentally relevant routes of exposure. However, the difficulties in experimentally modelling the development of a long-term disease, such as PD, must be acknowledged.

Overall, whilst there is some indication of a potential role for some pesticides in the aetiology of PD, there are insufficient data from studies with relevant routes of administration and doses to conclude that exposure to these pesticides at levels likely to be found in the environment would lead to the neurotoxicity observed.

Conclusions

Taking into account epidemiological evidence and toxicological evidence on specific compounds and mechanisms, there does appear to be evidence of a potential role of pesticides in the development of PD. However, the current body of evidence is insufficient to establish causation for any particular pesticide.

In particular, only one epidemiological study has shown a dose–response relationship between a specific pesticide (the herbicide paraquat) and an increased risk of PD. This finding needs to be confirmed in further epidemiological studies on different populations for it to be considered an established association. Furthermore, additional toxicological research should be undertaken to understand better the potential mechanisms by which paraquat might act, employing environmentally relevant routes of exposure and doses in order for the results to be considered predictive of the situation in humans.

There is some toxicological evidence that rotenone and other insecticides, for example permethrin, could also potentially act by mechanisms relevant to the development of PD. However, there is limited supporting epidemiological evidence other than associations with pesticide exposure and PD. The limited epidemiological and toxicological evidence for DDT suggest that this insecticide, now banned in the UK, is unlikely to play a role in the development of PD.

There is currently weak evidence for a role of fungicides in the development of PD. Epidemiological studies found no significant associations between fungicide exposure and PD. However, there is some toxicological evidence that dithiocarbamate fungicides are neurotoxic via effects on the dopaminergic system, although the action is not necessarily specific for the substantia nigra. Overall, further research, particularly toxicological studies at levels of exposure comparable to those experienced in the epidemiological studies, is needed to ascertain whether exposure to fungicides may be a risk factor in PD.

For other pesticides, limited toxicological and epidemiological evidence prevent any firm conclusions being drawn. However, an active programme of research is currently underway, particularly in the USA, which can be expected to provide further insight.

Future research

There are a number of areas that would benefit from further research. Only one meta-analysis of epidemiological studies on PD and pesticides, specifically, has been undertaken and this only included a proportion of the studies currently published. This would benefit from being updated to take account of all available data, giving full consideration to appropriate meta-analysis methodology for combining the studies and taking into account their heterogeneity. There is also a lack of temporal data on the changes in PD incidence and prevalence in the UK. If sufficient historical data exist, it would be helpful to determine whether PD incidence and prevalence have changed substantially over about the past 50 years in the UK. This might help indicate whether there has been any significant change in people's exposure to risk factors pertinent to PD during this period. Finally, no published epidemiological studies on the risk of PD in relation to pesticide exposure in the UK have been identified. Given that UK pesticide exposures may be different to those in other countries (such as the USA) owing to agricultural, regulatory and climatic differences, research among professional users of pesticides in the UK might be needed to provide insight into risks specific to the UK. Such a study would need to be of adequate power, be able to look at exposure to individual pesticide compounds, and make adequate adjustment for significant confounders (e.g. age, smoking, etc.).

Further research on the toxic mechanisms of pesticides in relation to PD would also be beneficial. Research on the interactions of pesticides and various other agents, such as heavy metals, and their relevance to mechanisms potentially involved in PD should be encouraged and developed. Furthermore, future research should also consider the potential for other factors, such as endotoxins, to compromise the integrity of the BBB and therefore increase an individual's susceptibility to exposure from environmental agents such as pesticides. Such studies, where possible, should include routes of administration of relevance to humans (i.e. oral, dermal and inhalation) and should use doses of a similar order of magnitude to those experienced during the normal occupational use of pesticide products.

1 Introduction

Parkinson's disease (PD), first described by James Parkinson as the 'shaking palsy', is an idiopathic disease of the nervous system characterised by chronic progressive tremor, bradykinesia, rigidity and postural instability as a result of loss of dopaminergic neurons in the substantia nigra. In 1983, it was observed that the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) resulted in the development of acute parkinsonism, similar in nearly all clinical, pathological, and biochemical features to the idiopathic disease, in a small group of drug addicts in northern California. This led to an interest in the role of environmental toxins in the development of PD and parkinsonism, more generally, and, in particular, to an interest in pesticides, given the structural similarity of the MPTP metabolite, 1-methyl-4-phenylpyridine (MPP+), to the herbicide paraquat. Since then, numerous epidemiological and toxicological studies have been published, looking at pesticides as a risk factor for PD and parkinsonism and possible mechanisms by which pesticides may act. However, many of these studies present conflicting results and some are of uncertain relevance to humans. In addition, a number of other risk factors have also been associated with the development of PD, such as rural living and consumption of well-water. As a result of the uncertainties of the potential role of pesticides in the development of PD and parkinsonism, the Department for Environment, Food and Rural Affairs (Defra) commissioned the Institute for Environment and Health (IEH) to undertake a critical review of the scientific literature, summarising the current state of knowledge, and to provide a critical evaluation of the possible role of pesticides in the development of PD and parkinsonism.

The aims of this project have been to conduct a systematic review of the scientific literature relevant to an evaluation of any relationship between pesticides and PD and parkinsonism and to critically evaluate the possible role of pesticides in the development of the disease. The project has had four specific objectives:

- to undertake a detailed literature search of all major medical, toxicological and environmental on-line databases to identify relevant reference material;
- to summarise and critically evaluate the epidemiological data on pesticide exposure as a risk factor in the development of PD and parkinsonism;
- to review the available toxicological data on the role of pesticides in the development of PD and parkinsonism and, thereby, to evaluate the mechanistic plausibility of any epidemiological findings; and
- to highlight gaps in the current understanding of the role of pesticides in the development of PD and parkinsonism, more generally, from an epidemiological and toxicological perspective and to recommend further areas for research.

2 Background

2.1 Introduction

This section provides a background to Parkinson's disease (PD) and to the development and use of pesticides in the UK and has been largely based on recent reviews, meta-analyses and some relevant additional papers. This section is not an exhaustive review, but rather aims to provide context for the more detailed epidemiological and toxicological sections that follow.

2.2 Parkinson's disease

2.2.1 Introduction

James Parkinson first described Parkinson's disease in 1817 in *An Essay on the Shaking Palsy* (Parkinson, 1817). It is characterised clinically by parkinsonism (resting tremor, bradykinesia, rigidity and postural instability) and pathologically by the loss of neurons in the substantia nigra region of the brain in association with the presence of ubiquinated protein deposits in the cytoplasm of surviving neurons (Lewy bodies). Parkinson's disease is a progressive condition and while it is not often the primary cause of death, patients with PD experience a 2- to 5-fold increased risk of mortality, the risk of which is strongly associated with the presence of severe extrapyramidal signs, especially bradykinesia (Louis *et al*., 1997).

2.2.2 Clinical features

Parkinson's disease presents as the clinical syndrome of parkinsonism, characterised by four cardinal signs:

- resting tremor;
- rigidity;
- bradykinesia; and
- impaired postural reflexes.

Tremor is the most obvious clinical symptom of PD and often presents in one extremity, worsening with precipitating factors such as stress, fatigue and cold weather (Guttman *et al*., 2003). The tremor of PD is present at rest (as opposed to that of essential tremor, which is present on action) and is, and remains, asymmetrical, starting in one extremity and spreading as the disease progresses. Although tremor is a common clinical feature, 30% of PD patients do not experience any tremor at all (Quinn, 1995).

Rigidity of the muscles on passive movement is characteristic of PD, with passive movement of the joints demonstrating continuous resistance throughout the full range of movement; this is referred to as 'lead-pipe' rigidity. When the rigidity has a ratchet-like feel it is described as 'cogwheel' rigidity. Rigidity is initially present in the limbs, but later spreads axially (Brooks, 2002).

Bradykinesia is the core disabling feature of parkinsonism and is a complex of symptoms that includes slowness of movement, hypokinesia (poverty of movement), difficulty initiating and maintaining the rhythm of movement, and a loss of normal automatic movements such as emotional expression (Marsden, 1987; Quinn, 1995).

Postural instability is a result of the impairment of postural or righting reflexes and is usually seen late in PD. It results in an unsteadiness or lack of balance and leads to a reduced ability of PD patients to right themselves following a trip or stumble and, owing to falls, can lead to an increased rate of injury among PD patients.

A number of secondary symptoms may also be present in PD, including masked facies (paucity of facial expression), reduced arm swing, stooped posture, and shuffling gait. Writing may also become micrographic. Dementia also occurs in a substantial proportion of patients and increases in prevalence amongst PD patients with increasing age.

2.2.3 Pathology

The major pathological feature of PD is the profound loss of pigmented neurons, mainly in the pars compacta of the substantia nigra. Associated with this is the presence of large eosinophilic inclusions, called Lewy bodies, within the remaining pigmented neurons (Perl, 1998). No mechanism responsible for the death of the dopaminergic neurons has yet been established (Barzilai & Melamed, 2003). The first clinical signs of PD only become apparent after the loss of about 70–80% of dopaminergic neurons (although estimates vary from as low as about 50% to up to 85%; Schapira, 1999). The number of dopaminergic neurons declines naturally with age. It is possible that many people who have developed the pathology of PD do not develop the clinical features of the condition because cell loss has not reached a particular threshold (Barzilai & Melamed, 2003). It is suggested that the threshold for clinical expression of PD is reached by an acceleration, precipitated by genetic or environmental factors, in the normal rate of neuronal loss (Schapira, 1999). However, it is also theoretically feasible that some individuals may be born with smaller numbers of nigral neurons, hence making them more susceptible to reaching the critical level of neuronal loss (Barzilai & Melamed, 2003). This latter hypothesis is supported by the discovery of specific transcription factors that regulate dopaminergic neurogenesis during brain development (Barzilai & Melamed, 2003).

The Lewy bodies seen in PD are a collection of protein filaments, including α-synuclein and ubiquitin. The existence of these Lewy bodies has led to the suggestion that PD may be caused by a fault in intracellular protein degradation that results in protein accumulation; however, it is not known how such a defect in protein handling would lead to cell death (Schapira, 1999). Lewy bodies are also seen, usually in the substantia nigra, in about 5–10% of brains from asymptomatic individuals dying of other causes, (Pearce, 2001). These could potentially be subjects with subclinical PD (Marsden, 1987).

2.2.4 Diagnosis

The diagnosis of PD is entirely clinical, made by examination without recourse to the laboratory (Quinn, 1995). However, histopathology on autopsy is the only way to confirm diagnosis definitively (Guttman *et al*., 2003). Despite this, there are no universally accepted neuropathological criteria for PD and, as a result, the assessment of the validity of clinical diagnostic criteria for PD is difficult (Litvan *et al*., 2003).

Several sets of clinical diagnostic criteria have been proposed, although most have not been fully evaluated for their validity or reliability (Litvan *et al*., 2003). Probably the most widely used and accepted are the clinical diagnostic criteria developed by the UK Parkinson's Disease Society Brain Bank (Box 2.1; Hughes *et al*., 1992a). This is a three-step diagnostic process. Step 1 involves the diagnosis of a parkinsonian syndrome. To make this diagnosis, the subject must have bradykinesia, defined as slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions. Also, at least one of the following must be present: muscular rigidity; 4–6 Hz resting tremor; or postural instability, not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction. The second step is to exclude specific causes of the parkinsonian syndrome: for example, history of repeated strokes with stepwise progression of parkinsonian symptoms; history of repeated head injury; history of encephalitis; or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

exposure. Finally, Step 3 involves looking for supportive prospective positive criteria for PD: for example, unilateral onset; resting tremor; progressive disorder; persistent asymmetry affecting side of onset most; or excellent response (70–100%) to L-dopa. Under Step 3, three or more criteria are required for a definite diagnosis of PD.

Box 2.1 UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria

Step 1 Diagnosis of Parkinsonian syndrome

- Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions); and
- At least one of the following:
  muscular rigidity, 4–6 Hz rest tremor, postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction.

Step 2 Exclusion criteria for Parkinson's disease

- History of repeated strokes with stepwise progression of parkinsonian features
- History of repeated head injury
- History of definite encephalitis
- Oculogyric crises
- Neuroleptic treatment at onset of symptoms
- More than one affected relative
- Sustained remission
- Strictly unilateral features after 3 years
- Supranuclear gaze palsy
- Cerebellar signs
- Early severe autonomic involvement
- Early severe dementia with disturbances of memory, language, and praxis
- Babinski sign
- Presence of cerebral tumour or communicating hydrocephalus on computer tomography scan
- Negative response to large doses of L-dopa (if malabsorption excluded)
- 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure

Step 3 Supportive prospective positive criteria for Parkinson's disease

- Unilateral onset
- Rest tremor present
- Progressive disorder
- Persistent asymmetry affecting side of onset most
- Excellent response (70–100%) to L-dopa
- Severe L-dopa induced chorea
- L-dopa response for 5 years or more
- Clinical course of 10 years or more

From Hughes *et al.* (1992a)

However, a number of different conditions may present with similar clinical symptoms to PD. Differentiation of parkinsonism from other conditions with similar clinical symptoms, such as essential tremor, multiple system atrophy and progressive supranuclear palsy, is facilitated by a thorough clinical examination. Neurological imaging techniques, such as computerised tomography or magnetic resonance imaging, do not reveal any specific changes related to PD, but can be used to exclude other conditions, such as normal pressure hydrocephalus, that would require different management (Guttman *et al*., 2003). The use of [^{18}F]-dopa positron emission tomography can provide

information on dopa-decarboxylase activity and therefore assist in diagnosis and identification of pre-symptomatic patients. However, the production of [^{18}F]-dopa requires a cyclotron and, hence, its use as a diagnostic tool is limited.

The accuracy of a clinical diagnosis of PD has been examined in several clinicopathological studies. Two studies found that of patients diagnosed as having idiopathic PD only 76% had pathological findings, at autopsy, consistent with the clinical diagnosis (Rajput *et al*., 1991; Hughes *et al*., 1992a). In a follow-up to the study by Hughes *et al.* (1992a), which looked at 100 more recent cases of PD, the accuracy of the clinical diagnosis of PD amongst clinicians was found to have increased to 90% (Hughes *et al*., 2001). This study also retrospectively assessed the clinical features according to four different sets of clinical diagnostic criteria: the UK PD Society Brain Bank diagnostic criteria; those proposed by Calne *et al*. (1992); those proposed by Gelb *et al.* (1999); and a selected set of three clinical features, which have been suggested as having a high positive predictive value. All sets of diagnostic criteria had a positive predictive value of between 90–93%. However, the sensitivity (i.e. the proportion of cases of PD detected by the criteria) varied considerably from 67% for the three selected clinical features to 90% for the UK PD Society Brain Bank diagnostic criteria, which, overall, were the most reliable diagnostic criteria. The authors suggested that the failure to improve the diagnostic accuracy with retrospective application of diagnostic criteria may mean that a diagnostic accuracy of 90% may be the highest that can be expected (Hughes *et al*., 2001).

2.2.5 Conditions similar to parkinsonism

Other neurodegenerative conditions may also include some features of parkinsonism in addition to other neurological symptoms defining the specific condition. These include progressive supranuclear palsy, multiple system atrophy (which involves multiple neuronal areas of the brain and spinal cord), Alzheimer's disease, and diffuse Lewy body disease (Adler, 1999).

2.2.6 Causes of parkinsonism

A number of causative factors are known to induce a clinical picture of parkinsonism, and it is important to understand these when undertaking epidemiological studies. Some well established causative factors are outlined below. However, the most common cause of true parkinsonism is PD (Quinn, 1995).

Drug-induced parkinsonism

A number of drugs can induce parkinsonism, usually within 3 months of starting treatment. In particular, neuroleptics (major tranquillisers), as a class, are the drugs that most commonly induce parkinsonism. The mechanism by which this occurs is unclear, but neuroleptics have the ability to block D-2 and D-3 dopamine receptors and also to inhibit Complex I of the mitochondrial electron transport chain, *in vitro* (Adler, 1999). The frequency of clinically significant parkinsonism symptoms in patients taking neuroleptics is estimated to be 20–40%. In addition to neuroleptics, an antiemetic (metoclopramide) is also known to induce symptoms of parkinsonism. In most cases of drug-induced parkinsonism the effects are reversible if the drug is discontinued, although recovery may be slow. In cases where recovery does not occur it is often not clear whether the resulting parkinsonism is drug induced or the result of idiopathic PD.

Toxin-induced parkinsonism

Exposure to several toxins (including pesticides, although these are not considered here) has been associated with parkinsonism. One of the most well known incidences of toxin-induced parkinsonism occurred among a small group of drug users in northern California who were exposed to MPTP, which was inadvertently synthesised as a by-product of 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP), a synthetic heroin, and injected intravenously. This resulted in clinical symptoms of parkinsonism, within 4 to 14 days of the initial use of the substance and, on autopsy, one case showed degeneration of the substantia nigra and the presence of Lewy bodies (Langston *et al*., 1983). Cases

were responsive to L-dopa therapy; five months after onset, none of the patients showed signs of remission and all continued to require medication (Langston *et al*., 1983). Although a very uncommon cause of parkinsonism, the effect of MPTP is important, as it demonstrates that a toxic insult can lead to clinical parkinsonism and provides the basis for an animal model of parkinsonism.

Exposure to manganese is another well-described toxic cause of parkinsonism and manganese toxicity has been observed in exposed miners and industrial workers. Clinical symptoms include akinesia and rigidity and a peculiar plantar flexion action dystonia and a flexed upper limb posture, resulting in a characteristic cock-walk gait, although resting tremor is rare (Riley, 1998; Adler, 1999). Other toxins reported to produce features of parkinsonism include carbon disulphide, carbon monoxide (following acute poisoning), cyanide, n-hexane and methanol (following acute intoxication; Tanner, 1992).

Infection and parkinsonism

Postencephalitic parkinsonism was a common aftermath of the epidemic of encephalitis lethargica that occurred in parallel with the epidemic of influenza between 1915 and 1928. However, postencephalitic parkinsonism is now rare (Adler, 1999).

Several studies investigating infectious agents as potential risk factors in PD have been reviewed by Goldman and Tanner (1998), who cited one study that found increased cerebrospinal fluid antibody titres to corona viruses in PD patients. Two other studies cited by the same authors found increased *Nocardia asteroides* (a common soil bacterium) titres in PD patients (although this was not corroborated by a subsequent case–control study; Hubble *et al.,* 1995). *N. asteroides* is of interest as it has been shown to produce degradation of the substantia nigra and a L-dopa-responsive movement disorder in animal models. However, overall, Goldman and Tanner (1998) concluded that infection is unlikely to play a major role in PD.

Other causes of parkinsonism

Symptoms of parkinsonism may appear following vascular insults to the brain, such as an infarct in the caudate, putamen, globus pallidus, or brain stem (Adler, 1999). Parkinsonism may also result from structural lesions in the brain, although this is rare. Such lesions include brain tumours, infectious masses and aneurysms (Adler, 1999). Other causes of Parkinson-like symptoms include hydrocephalus, metabolic disorders, such as hypothyroidism, which can result in general slowness, ataxia and tremor, and hyperthyroidism, which can result in tremor. Hypoparathyroidism can also result in basal ganglia calcification and signs of parkinsonism (Adler, 1999).

Head trauma has also been associated with parkinsonism. Dementia puglistica is a well-established traumatic cause of parkinsonism amongst boxers, as a result of repeated injury to nerve cells from blows to the head (Tanner, 1992; Riley, 1998). Single severe traumatic head injury is, however, rarely associated with parkinsonism (Adler, 1999).

Certain hereditary disorders are also associated with parkinsonism, including Wilson's disease, a rare autosomal recessive disorder of copper metabolism, the clinical features of which can include such parkinsonian symptoms as tremor, rigidity and bradykinesia. Huntington's disease, an autosomal dominant condition, may include features such as bradykinesia and dementia among its clinical presentations (Adler, 1999).

2.2.7 Epidemiology and risk factors

This section briefly reviews the general epidemiology and risk factors associated with PD. Where possible UK or European data have been cited.

General epidemiology

The overall prevalence of PD has been estimated in several UK and European Union populations. In a prospective community-based study in the London area, the overall lifetime prevalence of PD, from three General Practices (representing 27 658 patients) over an 18-month period, was estimated to be 2 per 1000 population (95% confidence interval (CI) 1–3; MacDonald *et al.*, 2000). In the Medical Research Council (MRC) Cognitive Function and Aging Study the overall prevalence was estimated (from a population of over 15 000 subjects aged 65 and over) to be 0.7% and 0.6% in males and females, respectively, aged 65–74, and 1.8% and 1.2% in males and females, respectively, aged over 75 (Table 2.1; Parker *et al*., 1997). A study of parkinsonism and PD in five European community surveys found the overall prevalence of parkinsonism was 2.3% and that of PD was 1.6% in persons aged 65 years or older (de Rijk *et al*., 1997). In a similar prevalence study the overall prevalence of PD was estimated from seven European population-based cohorts and found to be 1.8% in persons 65 years or older (de Rijk *et al*., 2000).

Table 2.1 Prevalence (%) of self-reported Parkinson's disease in the MRC Cognitive Function and Ageing Study

Centre	Cambridge-shire		Gwynedd		Liverpool		Newcastle		Nottingham		Oxford		Overall	
Age group	65–74	75+	65–74	75+	65–74	75+	65–74	75+	65–74	75+	65–74	75+	65–74	75+
M	0.5	2.2	0.1	1.4	0.7	0.7	0.5	1.6	1.0	2.5	1.1	1.2	0.7	1.8
F	1.0	0.4	0.8	1.3	0.2	0.6	0.0	1.7	0.8	1.1	0.5	1.7	0.6	1.2

Adapted from Parker *et al.* (1997)
F, female; M, male; MRC, Medical Research Council

The incidence of PD has recently been the subject of a systematic review (Twelves *et al*., 2003). Of five studies that were sufficiently similar to allow comparison (four from Europe and one from the USA), all gave similar standardised incidences of approximately 16 to 19 per 100 000 per year, except one study in Italy, which gave a much lower incidence (the authors suggested that this may be a result of a high level of undiagnosed PD in Italy). Overall, the authors suggested these incidence rates might be an underestimate because, without population screening, a significant number of patients remain undiagnosed (Twelves *et al*., 2003).

Geography

Geographical variation of PD prevalence and/or incidence can provide insights into potential risk factors. In the MRC Cognitive Function and Ageing Study, the prevalence of PD was estimated in six centres across England and Wales, but showed no between-centre variation (Table 2.1; Parker *et al*., 1997). Similarly, in a joint analysis of five European Community prevalence studies of parkinsonism and PD, the prevalence did not differ significantly between studies, except in France where the prevalence was lower (de Rijk *et al*., 1997). However, regional differences in prevalence have been observed in several North American studies, with a suggestion of a northwest to southeast gradient (Goldman & Tanner, 1998). This could reflect differential access to healthcare or, alternatively, differential distributions of risk factors.

Temporal trends

Investigation of the incidence of a disease over time can provide important aetiological information if, for example, changes in the disease can be related to a change in the environment (e.g. the influence of industrialisation, or the introduction of, and general population exposure to, a specific chemical). However, measurement of temporal changes in the incidence of chronic diseases, such as PD, can be very difficult. As a result there have been few studies of the change in incidence of PD over time, and none in the UK. Several studies have used the Mayo Clinic database from Olmsted County, Minnesota, USA to study temporal trends in PD. The most recent study looked at the incidence of PD

and parkinsonism between 1976 and 1990 in three 5-year periods (Rocca *et al*., 2001). The study found that the incidence of parkinsonism remained stable over the three 5-year periods in the age groups 0–36, 40–59 and 60–69; there was an increase in parkinsonism in the 70–99 year age group, but this was attributed to an increased incidence of drug-induced parkinsonism. The incidence of PD also remained stable over all three 5-year periods and in all age groups. Comparison of the results with previous studies (looking back to 1935) in the same population did not identify any long-term trends in the incidence of parkinsonism (the data relating to PD were not sufficiently comparable). The authors concluded that no major environmental risk factors for PD were introduced or removed from the study population during the study period.

Age

The mean age of onset of PD is typically between 60 and 65 years (Twelves *et al*., 2003). Age is unequivocally associated with increasing risk for PD, although the underlying process of PD is distinct from the natural ageing process (compared with normal ageing there is a marked microglial reaction to neuronal damage in PD and the distribution of loss of nigral neurons in PD is different; Goldman & Tanner, 1998). Incidence increases from fewer than 10 per 100 000 before the age of 50 to a peak incidence generally between the ages of 70–79 years, although the difficulties in identifying and including very elderly patients in incidence studies may mean that the true incidence may increase beyond 80 years of age (Twelves *et al*., 2003). Two UK studies have estimated the incidence between 70 and 79 years to be 65 and 161 per 100 000 (Twelves *et al*., 2003).

Young onset PD (YOPD), in which the onset of PD symptoms occurs before the age of 40, is considered to be no different clinically or pathologically from PD that manifests after the age of 40. Based on hospital data YOPD has been estimated to account for 3–10% of total PD patients (Tsai *et al*., 2002). However, this is likely to be an overestimate of the true proportion of YOPD as a greater proportion of YOPD cases, compared with normal onset PD cases, are likely to be referred to a hospital.

Sex

Most studies have found a modest increase in age-adjusted PD prevalence in men relative to women, irrespective of geographical location and ethnicity (Goldman & Tanner, 1998). A cross-sectional prevalence study of PD in London found consistently higher prevalence rates among men than among women, in all age groups (Schrag *et al*., 2000). However, the evidence is conflicting. For example, the MRC Cognitive Function and Ageing Study found no sex difference in the prevalence of PD across six study centres (Parker *et al*., 1997), and two pan-European studies also found no sex differences in the prevalence of PD (de Rijk *et al.,* 1997, 2000).

The incidence of PD is also thought to be higher among men. In their review of incidence studies, Twelves *et al.* (2003) found that five of nine studies in which age-standardised sex ratios were available reported a significantly greater incidence in men (ratio 1.5 to 2.0). However, the largest study identified by Twelves and colleagues found no difference in incidence rates between men and women.

Ethnicity

Prevalence of PD is generally considered to be highest in nations with predominantly white populations (Goldman & Tanner, 1998). However, this does not necessarily equate to a higher PD risk amongst whites. A recent incidence study of PD in northern California, USA found that age- and sex-adjusted PD incidence was highest amongst Hispanics, followed by non-Hispanic whites, Asians, and blacks (Van den Eeden *et al*., 2003). However the differences between individual groups were either not statistically significant ($p \geq 0.05$) or only of borderline significance. Other incidence studies have also failed to find a difference between ethnic groups. For example, Morens *et al.* (1996) found that men of Japanese or Okinawan ancestry residing in Hawaii, USA experienced PD risks of the same pattern and magnitude as Caucasian men in Europe and the USA, and higher than Asian men living in

Asian nations. Additionally, PD prevalence amongst blacks in Mississippi, USA (many of whom descended from populations in West Africa) has been found to be similar to that of the white population in Mississippi (341 and 347 per 100 000, respectively), and substantially higher than blacks in Nigeria, West Africa (67 per 100 000; Schoenberg *et al.,* 1988). This suggests that ethnicity may not be a risk factor in PD, but rather that environmental and/or lifestyle factors may have a greater influence on the risk of developing PD.

Genetics

Gowers first suggested the possibility of a genetic component to the risk of PD in 1888, when he observed that 15% of his patients had a family history of the disease (Foltynie *et al*., 2002). Since then a number of studies have been undertaken to understand better the potential genetic determinants of PD.

A number of studies have examined the frequency of a family history of PD. A recent review of the genetic basis of PD identified nine modern case–control studies that explored the frequency of family history of PD (Foltynie *et al*., 2002). Of these, seven were clinic-based studies and therefore possibly subject to bias. However, all nine studies found higher rates of the disease in relatives of those affected compared with controls. Crude familial relative risks (RRs) ranged from 1.8 to 9.7 in the clinic-based studies and were 1.3 and 2.9 in the two population-based studies.

Twin studies have also been used to examine the genetics of PD. These have consistently shown low rates of concordance (5–8%) in monozygotic and dizygotic twins (Goldman & Tanner, 1998; Foltynie *et al*., 2002). Were a genetic factor involved, concordance rates would be expected to be much higher in monozygotic than dizygotic twins. A more recent large study of twins also found similar concordance rates between monozygotic (16%) and dizygotic twins (11%) for any-age onset PD (Tanner *et al*., 1999). However, for PD with onset before 50 years of age concordance was 16% in dizygotic twins and 100% in monozygotic twins; this suggests that YOPD may have a primarily genetic basis.

A number of specific loci for several genetic forms of PD have also been reported. In particular, two mis-sense mutations in the α-synuclein gene (PARK 1) have been identified in an Italian–American family (the Contursi kindred), which is inherited in an autosomal dominant fashion (Goldman & Tanner, 1998; Guttman *et al*., 2003). Another important mutation is that of the PARK 2 or parkin gene, inherited in an autosomal recessive fashion. This gene has been found to be responsible for 77% of parkinsonism in patients with an onset of 20 years of age or younger, and codes for a ubiquitin protein ligase thought to be involved in the degradation of abnormal proteins in the proteasome (Foltynie *et al*., 2002). Other mutations have also been observed in the genes PARK 3 to 8 (Foltynie *et al*., 2002). However, these genetic forms of PD, whilst helpful in the development of understanding of the disease, are rare and usually display atypical features of the disease, such as young onset or absence of Lewy bodies.

A large number of genetic association studies have been conducted on up to 30 different gene polymorphisms and the risk of PD (Tan *et al*., 2000). In particular, two recent meta-analyses looked at genetic association studies and PD (Rostami-Hodjegan *et al*., 1998; Tan *et al*., 2000). The first investigated parkinsonism and CYP2D6 polymorphisms (an enzyme involved in the metabolism of xenobiotics) and found an increased risk in poor metabolisers (odds ratio (OR) 1.32, 95% CI 0.98–1.78; Rostami-Hodjegan *et al*., 1998). However, this was of borderline statistical significance ($p < 0.074$) and the analysis became non-significant ($p > 0.489$) if the only statistically significant study was excluded. The second study looked at 84 studies of 14 genes; a meta-analysis was conducted for polymorphisms for which there were a minimum of four studies (Tan *et al*., 2000). Significant associations with PD were identifiable for only four polymorphisms (Table 2.2). However, the authors cautioned that a significant association does not imply a causal relationship between the presence of the polymorphism and PD pathogenesis. This is particularly so as some polymorphisms may interact

with environmental factors or may only be of significance combined with other polymorphisms and so may be important only in a small subset of a population (Tan *et al*., 2000).

Table 2.2 Summary of meta-analysis association studies

Gene	Gene product	Odds ratio (95% CI)
DRD2	Dopamine receptor 2	<4 Comparable studies
DRD4	Dopamine receptor 4	1.28 (0.93–1.77)
DAT	Dopamine transporter	Repeat 9: 0.89 (0.70–1.12) Repeat 10: 1.03 (0.83–1.28) Repeat 11: 1.71 (0.80–3.63)
MAOA	Monoamine oxidase A	<4 Comparable studies
MAOB	Monoamine oxidase B	2.58 (1.38–4.82)
COMT	Catechol-*o*-methyl-transferase	1.05 (0.89–1.25)
NAT2	*N*-Acetyl transferase 2 detoxification enzyme	1.33 (1.08–1.62)
APOE	Apo-lipoprotein E	1.04 (0.90–1.20)
GSTT1	Glutathione transferase detoxification enzyme T1	1.34 (CI 1.00–1.79)
GSTM1	Glutathione transferase detoxification enzyme M1	1.13 (CI 0.90–1.41)
GSTP1	Glutathione transferase detoxification enzyme P1	<4 Comparable studies
GSTZ1	Glutathione transferase detoxification enzyme Z1	<4 Comparable studies
TRNA Glu	tRNA Glu mitochondrial gene	3.0 (1.1–8.2)[a]
ND2	Complex I mitochondrial gene	Not significant (odds ratio not reported)

Adapted from Tan *et al. (*2000); Foltynie *et al*. (2002)
CI, confidence interval
[a] If new data from a recent large study were included in the analysis, the result became non-significant

A number of studies on xenobiotic metabolism in neurodegenerative diseases have been carried out (reviewed by Steventon *et al.,* 2001). A defect in an unidentified cytosolic oxidase enzyme catalysing S-oxidation of the mucoactive drug, carbocysteine, was observed in 38–39% of patients with PD or motor neuron disease and only 7% of controls. The OR for the development of PD with this defect was calculated as 10.50 (Steventon *et al*., 2001).

The role of mitochondrial inheritance has also been considered. A deficiency in the activity of Complex I in the mitochondrial respiratory chain in the nigrostriatal system has been described in some patients (Foltynie *et al*., 2002). Mitochondrial DNA encodes some of the subunits of Complex I, and a high rate of mutation has been observed in the mitochondrial DNA of PD patients in comparison with controls, although no specific mutation has been found. Abnormal mitochondrial functioning may lead to increased production of reactive oxygen species, which could explain the oxidative stress seen in PD tissues. However, the genetic risk for PD is not confined to maternal mitochondrial DNA inheritance. Environmental factors such as MPTP, rotenone and neuroleptic drugs have also been implicated in the production of Complex I deficiency. Therefore, the mechanism of Complex I deficiency may vary between PD patients (Foltynie *et al*., 2002).

Dietary factors

The potential role of diet in the development of PD has been examined in a number of studies. These have been based largely on the hypothesis that oxidative mechanisms may play a role in PD and hence intake of antioxidant vitamins may have a protective effect. However, results of dietary studies have generally been inconsistent. Consumption of foods high in α-tocopherol was associated with a decreased risk of PD in two case–control studies, and consumption of vitamin E and a number of other vitamins and supplements, such as vitamin A, β-carotene and cod liver oil, has also been, inconsistently, associated with a decreased risk of PD (Goldman & Tanner, 1998).

A recent prospective cohort study has provided further information on dietary risk factors for PD (Chen *et al*., 2002). This large US based study found a significant positive association between total dairy product intake and PD risk in men (relative risk (RR) comparing highest and lowest intake categories, 1.8, trend p = 0.004), but not among women (RR 1.1, trend p = 0.9). No other food groups (meat, red meat, chicken, fish, fruits or vegetables) were associated with PD risk in either men or women.

A recent population based case–control study amongst newly diagnosed PD cases (n = 250) examined the association of PD with dietary nutrients (Powers *et al*., 2003). The study found that subjects with an iron intake in the highest quartile, compared with subjects in the lowest quartile, had an increased risk of PD (OR 1.7, 95% CI 1.0–2.7, trend for all quartiles p = 0.016). The study also identified an apparent joint effect of iron and manganese. Dietary intakes above median levels of both nutrients resulted in a significantly higher risk compared with lower intakes of each nutrient (OR 1.9, 95% CI 1.2–2.9). No other strong association was found for a range of other nutrients (including manganese alone), antioxidants or fats. The results are of particular interest, as iron and manganese are known sources of oxidative stress; thus there is a plausible biological mechanism to support the findings.

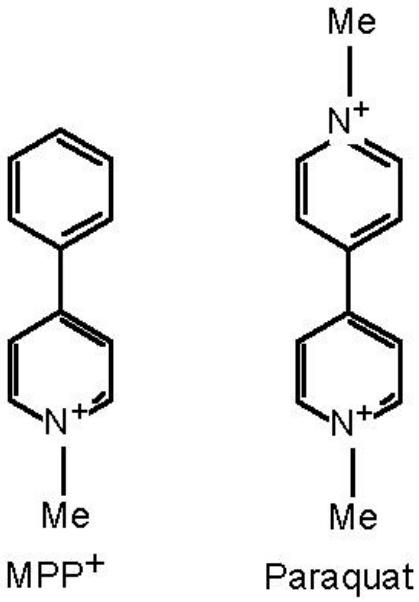
Smoking and caffeine intake

Both caffeine intake and smoking have been associated with a decreased risk of PD in many epidemiological studies. Coffee drinking and cigarette smoking and the risk of PD have been the subject of a recent meta-analysis by Hernàn *et al.* (2002). Case–control and cohort studies that reported the RR of PD (confirmed by a doctor) by coffee intake or smoking status were included. The analysis identified eight case–control studies and five cohort studies reporting risks by coffee intake. Compared with non-coffee drinkers, the RR of PD amongst coffee drinkers was 0.69 (95% CI 0.59–0.80). For smoking, 44 case–control studies and 4 cohort studies were included. These showed that, compared with never smokers, the RR of PD was 0.59 (95% CI 0.54–0.63) for ever smokers, 0.80 (95% CI 0.69–0.93) for past smokers, and 0.39 (95% CI 0.32–0.47) for current smokers. Overall, the risk of PD was 30% lower amongst coffee drinkers and 60% lower amongst smokers, suggesting that coffee drinking and smoking are protective against PD. It has been suggested that the relationship between coffee drinking or smoking and PD may in fact be acting in the opposite direction; that is those with PD may have personality traits that make them less likely to drink coffee or smoke (Martyn & Gale, 2003). However, a recent twin study (not included in the meta-analysis of Hernàn and colleagues) found that the risk of PD was inversely correlated with smoking dose (in pack years; Tanner *et al.*, 2002). This effect was most pronounced in monozygotic twins, suggesting smoking has a true biological protective effect.

Environmental and occupational exposures

The identification of MPTP as the causative agent that resulted in an outbreak of parkinsonism that was almost identical to PD, in drug addicts, has resulted in much interest in the idea that exposure to xenobiotics might play a role in the development of PD. In particular, interest has been directed at pesticides because of the structural similarities between a metabolite of MPTP, 1-methyl-4-phenylpryridine (MPP^+), and the pesticide paraquat (Figure 2.1). Ecological studies have found correlations between high PD prevalence and vegetable farming, wood pulp mills, and steel alloy industries; paper, chemical, iron and copper industries have also been correlated with higher prevalence of PD (Goldman & Tanner, 1998). Overall, these studies suggest that PD may be associated with exposure to agricultural or industrial toxicants. However, the studies have a high potential for confounding and misinterpretation and so should be viewed with caution. Case–control studies from several areas of the world have identified rural living, farming, gardening, pesticide use and well-water use as risk factors for PD (see Section 4). Other potential exposures associated with an increased risk of PD may include prolonged exposure to metals. Gorell *et al*. (1997) found an increased risk of PD with greater than 20 years occupational exposure to copper or manganese and more than 20 years exposure to combinations of copper and lead, copper and iron, and iron and lead. However, epidemiological studies of metal exposures and PD risk are generally inconsistent and, so far, such studies are considered insufficient to establish a causal link between occupational metal exposure and PD (Gorell *et al*., 1999a).

Figure 2.1 Structural similarities between MPP+, a metabolite of MPTP, and paraquat



MPP^+, 1-methyl-4-phenylpyridine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

2.2.8 Management and prognosis

The current treatment options for PD are largely based on drug therapy, which aims to improve the symptoms associated with PD. L-dopa, a dopamine precursor, which crosses the blood–brain barrier (BBB), is the most commonly used treatment for PD. It is combined with a dopa-decarboxylase inhibitor and a serotonin uptake inhibitor to reduce peripheral side effects. The dopa-decarboxylase inhibitor acts to prevent the peripheral breakdown of dopamine but does not cross the BBB. There are many side effects of L-dopa, including nausea, vomiting, dizziness and abnormal involuntary movements. Further, the duration of action of L-dopa decreases as the disease progresses, and patients need higher more frequent doses to achieve the same effect. Other drug treatments for PD include amantidine, a dopamine releaser, *N*-methyl-D-aspartate antagonist, which may be of use in the earlier stages of the disease, as it has a mild antiparkinsonian effect, and selegiline, a monoamine-oxidase-B inhibitor, which can be used alone in the early stages of the condition or in conjunction with L-dopa in more advanced stages, to reduce end of dose effects by reducing dopamine metabolism.

Surgical procedures may be indicated for patients who do not respond to drug therapy. Procedures used include ablative surgery (thalamotomy, pallidotomy), restorative surgery (embryonic dopaminergic tissue transplantation), and deep brain stimulation (thalamic, subthalamic or pallidal; Mazzoni & Rowland, 2001). Implants of fetal dopaminergic tissue may improve the symptoms of PD significantly (Schapira, 1999). However, treatment by surgical brain stimulation and fetal transplantation are not neuroprotective and do not halt the loss of further dopaminergic neurons (Gill *et al*., 2003).

In addition to the above treatments, supportive therapies, such as physiotherapy and speech therapy, and specialist nurses play an important role in maintaining the quality of life and independence of people with PD and parkinsonism.

2.3 Pesticides

2.3.1 Introduction

This section aims to give a brief overview of pesticides, their use and the sources of pesticide exposure in the UK. Where possible a historical perspective is given, to provide a picture of how exposures may have varied over time and hence, potentially, some insight as to whether exposure may be related to disease incidence.

Pesticides are defined as '...any substance, preparation or organism prepared or used for destroying any pest...' (HMSO, 1985). Pesticide products typically consist of numerous ingredients including the active ingredient(s), which provide the pesticidal activity, and a variety of other ingredients to enhance the activity, persistence and/or delivery of the active ingredient.

Pesticides have a wide variety of uses in the UK:

- to prevent damage to crops by controlling insects and weeds;
- to improve animal welfare by controlling parasitic diseases (e.g. sheep-dip);
- to control weed growth on non-agricultural land (e.g. railway tracks, roads, paths, etc.);
- to moth-proof wool and protect some cloths from fungal damage;
- to preserve timber or masonry; and
- to treat the hulls of boats to prevent fouling by marine organisms[1].

Overall, pesticides have important economic and health benefits, preventing loss of crops and damage to other commodities, and controlling vectors of human and animal disease. Often pesticides are most effective when used in combination with other pest control methods as part of an integrated pest management strategy.

2.3.2 Pesticide use

The use of substances for the control of pests goes back many hundreds of years. Homer, in about 1000 BC, refers to pest-averting sulphur; and reports from the 1600s refer to the use of arsenic as an insecticide, by the Chinese, and of derris root (containing rotenone) as a fish poison, in South America. However, it was not until the mid-1800s that the discovery and development of pesticides became more widespread. In particular, in about 1850, rotenone and pyrethrum were introduced and, in 1882, Millardet recognised the pesticidal properties of Bordeaux mixture (a mixture of copper sulphate, lime and water) against mildew. This led to the discovery of the pesticidal properties of a number of other inorganic compounds, such as iron sulphate and lead arsenate, and the development of equipment to apply these compounds to crops effectively (Cremlyn, 1991).

It was not until the 1930s that the development of synthetic organic pesticides began. During the 1930s the pesticidal properties of compounds such as dithiocarbamate fungicides (1934) and DDT (1939), amongst others, were discovered. The large-scale pesticide industry, however, mainly dates from the end of World War II (Cremlyn, 1991). The 1940s and early 1950s saw the commercial production of pesticides, such as the organophosphates and phenoxyacetic acids, and the discovery of carbamate esters and cyclodiene compounds, such as aldrin and dieldrin (1949). During the 1950s the use of cyclodiene compounds became widespread and other compounds were introduced, such as malathion (1950), the fungicide captan (1951), and the bipyridinium herbicides, diquat and paraquat (1958). The discovery and introduction of many compounds with pesticidal activities has since

[1] Environment Agency (2003) *Pesticides*, available [February 2004] at http://www.environment-agency.gov.uk/yourenv/eff/business_industry/agri/pests/

continued, including the introduction of systemic fungicides in the 1960s and the widely used pesticides, chlorpyrifos (1965), glyphosate (1971) and permethrin (1973).

Currently there are approximately 450 active ingredients approved for use as a pesticide in the UK. The use of pesticides in the UK has generally increased as it has become more economic and profitable. In particular, the mid-1970s saw an increase in pesticide use owing to the profitability of cereal growing, which, in turn, was a result of the UK joining the European Community and low world stocks of grain (MAFF, 1999). During the late 1980s there was a decrease in the amount of pesticide active ingredients used in the UK. Data for England and Wales indicate that the amount of pesticide ingredients used in the past 14 years has fluctuated slightly, but remained fairly steady over the past six years. In 2001 19 090 tonnes of pesticide were used in England and Wales[2]. Also over the past 14 years there has been a steady increase in the total area treated with pesticide, in England and Wales, with 41.41 million hectares treated in 2001. The increase reflects the introduction and use of newer pesticides, applied at lower rates of active substance per hectare over larger areas. Approximately 40% (by weight) of the active ingredients used in England and Wales in 2001 were used in the Eastern region[3]; the total area treated with pesticides in the Eastern region represented about 41% of the total area treated in England and Wales.

2.3.3 Sources of pesticide exposure

Exposure to pesticides can occur from numerous sources and can principally be divided into occupational and non-occupational sources of exposure. Occupational exposure to pesticides can occur during the manufacture, mixing and loading, and application of pesticides, during re-entry to inspect a treated crop, during harvest and post-harvest handling, and during disposal of pesticides. Principally pesticides are used in agriculture and horticulture; other important areas of use requiring professional applications include the control of pests of public health or economic importance (e.g. rats or cockroaches), the protection of timber or masonry, and the treatment of certain natural products to prevent pest infestations. Occupational pesticide exposure is controlled by a number of mechanisms. These include the assessment of occupational exposure at the product registration stage to ensure operator exposure will not result in adverse health effects, the provision of on-label instructions on the safe use of the product, and the use, if necessary, of engineering solutions and/or personal protective equipment to minimise exposure. Furthermore, legal requirements, under the Control of Substances Hazardous to Health Regulations 2002, ensure that exposure is prevented or adequately controlled in order to prevent ill-health to both occupationally and non-occupationally exposed individuals.

Non-occupational sources of pesticide exposure are more diffuse and include exposure from the application of pesticide products (either professionally or by an amateur user) in the home and garden, the ingestion of pesticide residues on food or in water, and exposure to environmental levels of pesticides (e.g. from spray drift). Current data suggest, however, that total non-occupational pesticide exposure in the UK is low. The UK Government conducts routine monitoring of residues of pesticides on food. In 2002, 4015 samples of food were tested; of these, residues were only detected in 30% of the samples, and only 1% of samples exceeded the maximum residue level[4]. Similarly, drinking water is routinely monitored and, in surveys carried out during 2002, over 53 812 samples of drinking water were monitored for pesticides, of which only 10 samples exceeded the 0.1 µg/l limit. This indicates that over 99.98% of drinking water in the UK is effectively pesticide free[5]. Less information is

[2] Environment Agency (2003) *Pesticides*, available [February 2004] at http://www.environment-agency.gov.uk/yourenv/eff/business_industry/agri/pests/

[3] The Eastern Region, as defined by the Department for the Environment, Food and Rural Affairs, comprises Bedfordshire, Cambridgeshire, Essex, Greater London (East), Hertfordshire, Lincolnshire, Norfolk, Northamptonshire and Suffolk

[4] PRC (2003) *Pesticides Residue Committee Annual Report 2002*, available [February 2004] at http://www.pesticides.gov.uk/committees/PRC/2002.htm

[5] DWI (2003) *Drinking Water Inspectorate Report 2002*. London, UK, Drinking Water Inspectorate, available [February 2004] at http://www.dwi.gov.uk/pubs/annrep02/mainindex.htm

available on the exposure of the general population to pesticides from applications in the home and garden or environmental sources of exposure, particularly as the level of exposure from these sources is dependent on how the pesticide product is used. However, consideration of the exposure to pesticides of applicators and bystanders forms part of the approval process for all pesticide products; and use according to label instructions is designed to minimise exposure. Other sources of pesticide exposure may include exposure to pesticides from human or veterinary medicines or from products pre-treated with pesticides to prevent damage or economic loss.

3 Methodology

3.1 Literature search

A literature search of ten on-line databases of published literature was conducted in December 2002 and updated in April 2003, to identify references pertaining to the role of pesticides in the development of Parkinson's disease (PD) and parkinsonism. The terms used in the literature search were selected based on terms used in the titles and abstracts of published literature identified from initial (pilot) literature searches and with reference to the relevant indexing terms used in Embase and Medline (Table 3.1). A full list of the indexing terms and sub-terms searched is presented in Annex 1.

Table 3.1 Literature search strategy

Database	Search terms[a]
Embase	(**dopamine$4** or **essential tremor** or **lewy bod$3** or **substantia nigra** or **parkinson$3** or **striatonigral degeneration**).ab.de.ti. and (**pesticide#**).de. or (**DDT** or **dieldrin** or **diquat** or **heptachlor** or **malathion** or **maneb** or **paraquat** or **rotenone** or **well water** or **rural** or **farm$3**).ab.de.ti.
CancerLit, Medline and ToxFile	**(dopamine$4** or **essential tremor** or **lewy bod$3** or **substantia nigra** or **parkinsonian-disorders#** or **striatonigral degeneration**).ab.de.ti. and (**pesticides#**).de. or (**DDT** or **dieldrin** or **diquat** or **heptachlor** or **malathion** or **maneb** or **paraquat** or **rotenone** or **well water** or **rural** or **farm$3**).ab.de.ti.
Biosis, CAB Abstracts, Japanese Science and Technology (JIST), National Technology Information Service (NTIS), Pascal and SciSearch	(**dopamine$**4 or **parkinson$3** or **lewy bod$3** or **striatonigral degeneration** or **substantia nigra** or **essential tremor**).ab.de.ti. and (**pesticide$1** or **herbicide$1** or **insecticide$1** or **fungicide$1** or **DDT** or **dieldrin** or **diquat** or **heptachlor** or **malathion** or **maneb** or **paraquat** or **rotenone** or **well water** or **rural** or **farm$3)**.ab.de.ti.

ab.de.ti., abstract, descriptor and title, respectively. 'ab', 'de', and/or 'ti' after a search term indicate whether the term was searched for in the abstract, descriptor and/or title field of the literature database
$, a wild card term allowing the words in question to have variable endings; a number after the '$' indicates restricts the number of wild card letters after the word stem
#, explosion term, this means that all sub-headings indexed under the main descriptor were included in the search
[a] Specific pesticide terms were included in the literature search as the indexing of Medline does not list individual pesticide chemicals under the descriptor term 'pesticides', but rather under their chemical grouping (e.g. heterocyclic compounds); the pesticide terms used were identified from the initial (pilot) literature searches

The literature searches were limited to papers published from 1983 onwards, as this was when the first case reports of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine poisoning were published (Langston *et al*., 1983). This paper largely initiated the interest in pesticides as a potential risk factor in the development of PD and parkinsonism. No language limit was applied to the searches. However, as the search terms were in English, only papers for which an English title and/or abstract were available would have been identified. Additional papers were identified from the reference lists of those papers obtained, from ad hoc searches of the Internet, from current issues of journals, and from recommendations made by professionals in the fields of PD and pesticides that were consulted.

3.2 Criteria for selecting papers for review

The literature searches identified over 5000 references. Of these 336 papers were obtained for further review. The remainder were judged, on the basis of their titles and abstract, not to be relevant to the present review, as they concerned the clinical management of PD or were concerned with other human disorders involving dopamine or the effects of pesticides on insects.

Papers for inclusion in this review were selected on the basis that they met the following criteria:

- English language; and
- the subject matter directly concerned the role of a pesticide or pesticides in an aspect of PD or parkinsonism.

Restricting the review to English language papers might mean that some relevant papers have been excluded from the review. Three non-English language papers of potential relevance were not obtained. Consideration of the titles and abstracts of a further six non-English language papers indicated that their exclusion would not compromise the integrity of the review.

An additional 109 references were identified from other sources (e.g. reference lists, current journal issues, etc.) and obtained for review. The majority of these papers concerned issues not directly related to pesticides and PD (e.g. accuracy of diagnosis of PD) and so would not have been expected to be identified in the main literature search.

4 Epidemiological review

This chapter reviews epidemiological studies published since 1983 that investigate Parkinson's disease (PD) in relation to pesticide exposure. Case studies, descriptive studies (including case series, observational studies, and cross-sectional studies), cohort and case–control studies that specifically investigate pesticides are summarised, together with any comments or opinions from the authors of the studies. Studies that have considered other risk factors for PD (e.g. well-water consumption, rural living, etc.) together with pesticides are reviewed at the end of each section.

4.1 Case studies

Case studies are anecdotal reports of patients with specific symptoms that appear to be related to a particular cause. On their own they are simple descriptions but, if other similar reports occur, they may lead to the development of testable hypotheses.

Seven papers were identified that described a number of case studies of individual patients exhibiting a number of the cardinal signs (resting tremor, rigidity, bradykinesia and postural instability) of PD after being exposed to pesticides. Two cases of PD-like symptoms following attempted suicide with the organophosphate (OP) insecticide, chlorpyrifos, have been reported (Bhatt *et al*., 1999; Shahar & Andraws, 2001). Both cases recovered with treatment. Stefano *et al.* (1989) described the case of a greenhouse farmer, continually exposed to insecticides, fungicides, and herbicides, who developed bradykinesia and rigidity; the symptoms spontaneously improved after relocating to another job not involving the handling of chemicals. Sechi *et al.* (1992) described the occurrence of persistent PD in an elderly farmer dermally exposed to an aqueous solution of 10% diquat bromide. Bocchetta and Corsini (1986) described a young farmer (age 41) who had first experienced symptoms 8 years earlier, after he had been using pesticides extensively for several years. The compounds handled included large amounts of insecticides (including chlorinated cyclodienes and carbofuran), fungicides and herbicides (including chlorophenoxy compounds). Lazzarino de Lorenzo (2000) described a farmer diagnosed with PD and responding to L-dopa therapy. He had no family history of PD, but reported being exposed to OP pesticides for more than 15 years.

Bhatt *et al.* (1999) also describe the cases of two elderly women who developed PD features after fumigating their apartments with an aerosol OP pesticide. Two relatives of one of the women also developed similar features after visiting the apartment post-fumigation. All recovered from their symptoms but one of the elderly women relapsed after returning to her fumigated apartment and eventually had to relocate. PD signs are rare in OP poisoning, and a genetic susceptibility to OP pesticide-induced PD may account for the three family members developing this syndrome (Arima *et al*., 2003).

These case studies demonstrate that acute exposure to pesticides can apparently give rise to symptoms similar to those experienced in PD. However, in some cases these symptoms seem to disappear with treatment and/or removal from the apparently relevant exposure.

4.2 Descriptive studies

4.2.1 Pesticide-related studies

Ten descriptive studies of PD with information on pesticide exposure were identified, including two studies of case series, three studies of mortality patterns, four of prevalence and one of incidence.

Case series

Rajput *et al.* (1986) examined the life histories of 21 patients diagnosed with PD before the age of 40 years in Saskatchewan, Canada. Of these patients, 19 were born and lived for their first 15 years exclusively in a rural community (population of 169 people or less), an association that was statistically significant. This could suggest that rural environmental factors play a role in the development of young onset PD (YOPD) or, as they were such a small communities, that genetics played a role. In a later study, Rajput *et al.* (1987) examined these people and one additional patient in more detail, to evaluate the use of paraquat and other herbicides and pesticides in agriculture and the historical incidence of YOPD. Six of the patients developed PD after paraquat was first used locally and two of these were farmers; YOPD incidence was not associated with the use of any agricultural chemical in the province.

Incidence

As reported in a conference abstract, Bennett *et al.* (1988) undertook a survey of the use, since 1968 (to an unspecified date), of agricultural chemicals and incidence of PD in Saskatchewan, Canada. A total of 552 PD cases were seen at a Parkinsonian clinic; these included the cases examined by Rajput *et al.* (1986, 1987). A significant number of the patients were born and raised in rural communities. However, when the incidence of PD was examined in relation to the chronology of major herbicide and pesticide usage in the province, incidence patterns did not correspond to the use of any chemical. Although the data confirmed the association of rural living and PD incidence, the authors did not feel this indicated that farm chemicals were aetiological agents for PD.

Prevalence

In a study by Barbeau *et al.* (1987) in nine rural regions of Quebec, Canada, cases of PD were identified by three different methods — medical records, sales of medication and death certificates for 1980–1984. The number of cases identified by each method was 5270, 4233 and 388, respectively. Two additional sources (movement disorder clinics and a survey of physicians) were used as ascertainment checks. Regional differences were then calculated using each method and compared with data collected on agriculture and pesticide sales. A marked variation in prevalence rates for PD was seen between the various regions with all three methods of ascertainment. Overall, the methods estimated prevalence to be 70 per 100 000 (medical records), 47 per 100 000 (sale of medication) and 105 per 100 000 (death certificates). The region (southwest) with the highest prevalence of PD was the same for all the methods. This region produces 70–80% of the province's vegetables and also had a large number of apple orchards. Consequently, the region was also the largest user of pesticides (carbamates, triazines and triazoles, and OPs). A significant correlation was found between pesticide use and the prevalence of PD in the nine rural regions. However, the authors stated that this should not be interpreted as proof of causal relationship, because any number of factors associated with rich agricultural land (soil composition, water content, fertilisers, sprays of various kinds, manure, water acidity, etc.) should also be considered. It was also noted by the authors that farmers in the region had long been utilising potent arsenate herbicides; these are known to affect catalase and peroxidase enzymes that are important in the defence against free radicals and, thereby, the prevention of damage to the substantia nigra.

Sala *et al.* (1999) examined the health status of inhabitants of a rural village who had ever worked in the local electrochemical factory, which produced volatile chlorinated solvents for several decades and was characterised by high levels of hexachlorobenzene (which is also used as a fungicide) in the air. PD risk was not significantly increased in factory workers exposed to hexachlorobenzene although four PD cases were seen in male workers and none among inhabitants who did not work at the factory.

A study by Herishanu *et al.* (1989) reviewed medical records of patients attending a local neurology outpatient clinic in Israel, and identified 156 PD patients, of whom 13 lived in three adjacent Kibbutzim in the Negev, Southern Israel. Six of these patients were involved in agriculture and were

probably exposed to various pesticides, herbicides and fungicides (mainly maneb), which were usually spread from light airplanes; no statistics were presented. An increased PD risk was found among those who were born and had lived in rural communities for at least 15 years and had consumed well-water (11 of the 13 PD patients).

Engel *et al.* (2001a) carried out a cross-sectional study of 310 subjects from a cohort of men occupationally exposed to pesticides (orchardists, professional applicators, pesticide formulation plant workers, other farm or agricultural workers) between 1972 and 1976, in Washington State, USA. Information was gathered by a self-completed questionnaire; examination of pesticide exposure established 238 subjects with some occupational exposure to pesticides and 72 with none. Comparison of ever versus never having experience of other potential risk factors found no significant associations between PD and use of well-water, farm employment, or exposure to any specific pesticides or pesticide classes. The prevalence of PD in the second and third tertiles of years of exposure to pesticides was double that of the lowest tertile, but there was no statistically significant exposure–response relation. Prevalence was not associated with acre-years of any category of pesticide used. Significant exposure–response relations, as assessed by trend tests, were found only for crude analyses of exposure to pesticides, insecticides and lead arsenate. Recall bias may have been a problem in the study, but it was stated that it was unlikely to have occurred for two reasons: an increased risk of PD was only found for general use of pesticides and not for any specific ones; and most of the subjects with PD showed only slight signs of PD and did not seem to be substantially impaired by it. Any recall error was therefore likely to be non-differential, given the many pesticides reported, the complex temporal pattern of their use and the fact that subjects were not informed of the study hypothesis. In the original cohort there were 1300 men, of whom 573 had died, were lost to follow-up or had moved outside the study area; 245 could not be contacted and 13 had unknown exposure. A total of 159 declined to participate. These non-participants were similar in age to participants, but were less likely to have reported occupational use of pesticides in the original study. Their health status was also unknown.

Mortality

Strickland *et al.* (1996) examined PD mortality (PD mentioned anywhere on the death certificate) in Nebraska, USA, between 1984 and 1993, and sales of anti-PD medication between 1988 and 1990. These data were compared with agricultural data (fertiliser usage, irrigation, crops harvested, and use of pesticides and herbicides) for the corresponding counties. Significant correlations were observed between sales of anti-PD medication and acres of fertiliser used, acres of weeds sprayed and acres of hay insecticides sprayed. The average annual rate of PD was 10.7 per 100 000 per year, based on 1693 deaths over 10 years. However, age-adjusted mortality rates showed a low degree of association with sales of medication and agricultural variables. In the study, mortality and sales of medication were used as proxy measures for incidence and prevalence, respectively. However, there are problems with the use of such variables. Firstly, the study could only measure sales of medication and not prescriptions of anti-PD medication. Since sales would depend on the location of neurology practices and the number of pharmacies and physicians within the area, counties with small populations would have less reliable estimates of anti-PD medication sales than larger counties. Also, some individuals might have travelled outside the study area to purchase medication. It is also possible that death certificates might not have reflected the true burden of PD in the region. Mortality rates based on PD as first cause of death on the death certificate were 0.82 per 100 000 per year; based on PD as the underlying cause of death, rates were 3.5 per 100 000 per year; and for both combined rates were 4.3 per 100 000 per year. These figures are less than half the overall estimate based on any mention of PD on the death certificate. These limitations were acknowledged but the authors considered that the techniques used nevertheless provided a useful tool for delineating possible differences in incidence and prevalence.

Ritz and Yu (2000) examined mortality between 1984 and 1993 in California, USA. Mortality figures were compared with agricultural activities; counties within the state were classified according to their use of restricted pesticides in farming activities. PD mortality was 19–47% higher in counties

reporting use of pesticides than in counties reporting no such use. Adjusting for sex, race, age, place of birth and education did not reduce the effect. Relative to counties using no restricted pesticides, PD mortality was 2.5 times higher if insecticides were applied to more than 37% of the county's total surface area, and 1.5 times greater when 5–37% of the county's land was treated. Crop specific analysis showed an increased risk for PD in counties in which more than 10% of agricultural land was used for vegetable farming. Although this study was large, with PD reported as the underlying cause of death in 7516 cases and as a contributory cause in 15 222 cases, the study may have suffered from exposure misclassification. Bias may have been introduced when attributing the same exposure level to all county residents; that is, the assumption was made that living in certain counties increased the likelihood of pesticide exposure for all residents homogeneously. The authors stated that the resulting exposure misclassification was most likely non-differential with respect to disease status, biasing the effect estimates towards the null value of no association. In addition, they conceded that their pesticide use measure might have been a surrogate for another countywide risk factor for PD mortality. The authors also comment that because PD is not, *per se*, a fatal disease, it would have been listed as the underlying cause of death only for the most severe cases and for those who had not concurrently suffered from other fatal diseases, such as cancer or ischaemic heart disease.

As reported in a conference abstract, Vanacore *et al.* (1991) examined mortality in Italy between 1969 and 1987 in relation to the use of herbicides and paraquat, specifically (data obtained from Government sources). A positive temporal correlation was found between use of herbicides and PD rates for the country as a whole and in all regions, apart from the Northwest. However, the correlation for paraquat was negative with the exception of the Northeast region.

4.2.2 Other descriptive studies

A number of other descriptive studies were identified that described PD risk in relation to other risk factors, particularly rural living and agricultural occupations. They are briefly summarised below.

Ben-Shlomo *et al.* (1993) examined admission rates for PD in the Republic of Ireland and found them to be greater in rural than semi-rural areas, where rates were, in turn, greater than in urban areas. However, the trend was only significant for men. Tandberg *et al.* (1995) observed the prevalence of PD in Norway to be higher in urban than rural areas. However, when the data were adjusted for age the prevalence was higher in rural areas. In rural Spain PD prevalence was observed to be comparable with other European rural regions and lower than European centres of population (Errea *et al*., 1999). Svenson *et al.* (1993) also observed a significantly increased PD risk in four primarily rural census divisions of Alberta, Canada, but the risk was significantly decreased in the two divisions with the largest cities in the state. Imaizumi (1995) found PD mortality rates to be significantly greater in rural prefectures of Japan. In the rural province of Kinmen, Republic of China, where agriculture is the main occupation, prevalence was considerably higher than those reported from other regions of the country although rates were similar to those found in Western countries (Wang *et al*., 1994a).

Ferraz *et al.* (1996) studied 118 PD patients who had attended outpatient clinics in Sao Paulo, Brazil, between 1987 and 1992. The majority of these (60.2%) were from a rural community; no difference between the urban and rural groups was seen in the age of onset of PD. Gérard *et al.* (2002) recruited 103 PD patients between 1995 and 1998 from hospital wards and outpatient clinics in France to examine the relationship between the CYP2D6 allele and PD. About 30% had some form of 'rural' exposure, that is history of farm residence, drinking well-water or agricultural pesticide and herbicide exposures, although this association was not tested statistically.

In contrast to the above studies, Sethi *et al.* (1989) found no difference in the mortality from PD between urban and rural areas of Georgia, USA. Kuopio *et al.* (1999b) found no difference in the prevalence of PD among people living in rural and urban areas of Finland, although after the age of 60 years the prevalence in the former was significantly greater than in the latter. In further contrast, PD mortality rates were lower in rural than urban areas of Estonia, for both men and women (Taba & Asser, 2002), and in Michigan, USA (Rybicki *et al*., 1993).

Rybicki *et al.* (1993) observed a significant positive correlation between PD mortality and farming density in Michigan, USA, but a negative (inverse) correlation with well-water consumption. As reported in an abstract, La Bella *et al.* (1990) found a high prevalence of PD amongst Sicilian farm workers. In another study in north-western Italy, the prevalence was also found to be significantly higher among agricultural workers compared with other industries (Granieri *et al*., 1991). In studies of Kibbutzim workers in Israel, PD prevalence was observed to be higher compared with the rest of the region (Goldsmith *et al.,* 1990, 1997). However, a more recent study found the prevalence to be similar to that reported in most other population-based studies, although there was an older age at symptom onset (Anca *et al*., 2002). The latter study also observed large (but not statistically significant) differences in age-adjusted prevalence in different Israeli climatic zones (inland > arid > coastal > mountain).

In a mortality study of 27 US states between 1982 and 1991, a clustering of PD cases among agricultural workers was reported (Schulte *et al*., 1996) although no data were presented. In a similar study in 26 states between 1984 and 1993, PD mortality was found to be significantly increased in white livestock farmers, especially among those over the age of 65 and crop farmers aged 16–64 years (Lee *et al*., 2002).

4.3 Cohort studies

Five cohort studies were reviewed and their findings are summarised in Table 4.1. Two of the studies were from the USA (Yesalis, III *et al*., 1985; Petrovitch *et al*., 2002) and three from Europe (Tüchsen & Jensen, 2000; Vanacore *et al*., 2002; Baldi *et al*., 2003).

The study by Yesalis *et al.* (1985) identified a cohort of 3097 rural residents in Iowa, USA, aged 65 years and over between December 1981 and July 1982. The target population was all people in this age group living in two rural counties adjacent to the county in which the University of Iowa is located. The study was designed to explore a broad range of physical, mental and social characteristics of the elderly. The paper does not state the follow-up period. Information was gathered by interview and respondents were only asked whether they had ever worked on a farm and the duration of farm work. No information on pesticide exposure was collected. Men who had ever worked on a farm of at least 10 acres in size had a significantly lower prevalence of PD compared with non-farm workers ($p < 0.001$), both whilst still working and after retirement. Amongst women the prevalence was slightly greater (non-significant) in farm workers (both whilst still working and after retirement). However, both working on a farm and health status were self-reported. Some patients were also too ill to participate. The authors stated that case ascertainment could have been compromised by lack of medical examination, age and work restrictions, lack of accounting for subjects who moved out of the area, and bias introduced by the healthy-worker effect.

Tüchsen and Jensen (2000) identified a cohort of over 2 million men and women aged 20–59 years on 1 January 1981 through the Danish Central Population Register, and followed them up to the end of 1993. All first time hospitalisations with PD as the primary diagnosis were identified through the national inpatient register. Occupations were classified using the Employment Classification Module at Denmark's Central Bureau of Statistics. In the 13-year follow-up period, 611 men and 338 women were admitted to hospital with a primary diagnosis of PD. Standardised Hospitalisation Ratios (SHR) were calculated by dividing the observed number of hospitalisations by an expected number based on incidence ratios for economically active individuals on 1 January 1981. A statistically significant higher risk of PD was found for male self-employed farmers (SHR 130, 95% confidence interval (CI) 103–163). In addition, a non-significant higher risk was found in all other groups known to handle pesticides: self-employed men employed in horticulture and fruit growing (SHR 160, 95% CI 44–410); self-employed male landscape gardeners (SHR 448, 95% CI 54–1617). A similar pattern was found for female self-employed farmers (SHR 149, 95% CI 31–435) and wives assisting farmers (SHR 120, 95% CI 77–179). The SHRs for all men and women who may have been occupationally exposed to pesticides because they worked in agriculture or horticulture were 134 (95% CI 109–162)

and 118 (95% CI 78–172), respectively. However, other occupations also showed statistically significant high risk of hospitalisation for PD. Among men elevated risks were found for paint/wallpaper dealers and pharmacists (SHR 752, 95% CI 244–1754), psychologists and welfare staff with local authorities (SHR 440, 95% CI 143–1027), lawyers (SHR 503, 95% CI 104–1460), railway and transport staff (SHR 316, 95% CI 103–739), and bus drivers (SHR 677, 95% CI 140–1979). For women, occupations showing a statistically significant increased risk included self-employed, laundry and dry-cleaning (SHR 677, 95% CI 140–1979), and cleaners (SHR 381, 95% CI 104–974). The authors stated that occupation and industry were used as proxy measures for individual pesticide exposure and that their classification was crude and may have diluted the risk estimates. In addition, there were no data on duration of exposure. The authors stated that a risk was demonstrated in spite of the general belief that the handling of pesticides in Denmark is and has been safe owing to regulations and the training of workers.

Petrovitch *et al.* (2002) studied a cohort of 7986 Japanese/American men born between 1900 and 1919, enrolled in 1965 into the longitudinal Honolulu Heart Program, Hawaii, USA and followed-up to the end of 1996. During this period 116 were diagnosed with PD by neurologists. After adjustment for age, the incidence of PD increased significantly with increasing number of years of plantation work, with the risk of developing PD in those working for more than 20 years double that of those who had never worked on plantations. The age-adjusted incidence also tended to increase with the number of years of self-reported exposure to pesticides but the trend was not statistically significant. No documentation was available on the historical use of specific pesticides, although it is known that pineapple growers used large amounts of insecticides and fumigants whereas herbicides were used on sugar-cane plantations. No breakdown of incidence by plantation type was given. The authors felt that sample size may have played a role in the statistical findings because data on pesticide use were unavailable for about 15% of the cohort, follow-up was less than 25 years, and the population incidence of PD was low. Recall bias may have affected the self-reporting of pesticide exposure since it depended on the recall of cumulative exposure episodes. In addition regular exposure on plantations may have been more common than perceived, and the authors stated that many who reported no exposure to pesticides might have had high levels of exposure. The authors also suggested that infectious agents, such as *Nocardia asteroides*, or exposure to heavy metals (copper, manganese, lead and iron) in the soil and dust could have contributed to the degradation of dopaminergic neurons. However, the authors did not consider the microorganism to be a risk factor, citing a case–control study in humans (Hubble *et al*., 1995) that found no association between *N. asteroides* in serologic test results and PD.

As reported in a conference abstract, Vanacore *et al.* (2002) assembled a cohort of 5575 Italian licensed pesticide users during 1971–1973, and followed them up to the end of 2000. In this period 757 individuals died and 28 were lost to follow-up, but only two of the deaths were confirmed as PD. No further analysis was presented but the cohort was in the process of being contacted to evaluate the prevalence of PD.

Baldi *et al.* (2003) assembled a cohort of 1507 elderly people (mean age 78.6 years) from electoral rolls of 75 districts in two administrative areas of south-western France, living at home or in an institution in 1987, and examined the incidence of PD between 1992 and 1998, when information on pesticide exposure was collected. The cohort was originally assembled to study cerebral and functional ageing. A job–exposure matrix for pesticide exposure was developed by a panel of experts from a list of job codes, based on the likelihood of and level of exposure to pesticides. The experts considered that owners of and workers on small farms (compared with those on larger ones) had experienced higher exposure, since they had mixed and sprayed pesticides themselves using older and less efficient equipment than was used on larger farms. Between 1992 and 1998, 24 cases of PD were diagnosed, resulting in an incidence of 500 per 100 000 person-years. Eight cases were amongst those occupationally exposed to pesticides, giving an incidence of 890 per 100 000 person-years, which was not significantly different from the incidence of 410 per 100 000 person-years amongst non-exposed workers. In men, a significant association was found between PD and occupational exposure to pesticides as determined by the job exposure matrix; this remained significant after adjustment for

smoking and educational level (age was not adjusted for). The adjusted relative risk of PD also increased with cumulative occupational exposure to pesticides, until the highest exposure quartile (no levels given), in which no cases were observed. No analysis of specific pesticides was undertaken, although the reported number of different pesticides used ranged from 3–23. Dithiocarbamates accounted for 37% (by weight) of all organic substances applied and folpet for 26%. The main weakness in this study was the criteria used for the diagnosis of PD. At baseline screening and 5-year follow-up, diagnosis was ascertained by a two-phase design. Firstly, two validated questions were used to screen for PD: "Do your arms or legs shake at rest?" and "Do you experience slowness or stiffness in your movements?" All subjects who gave positive answers to both questions and/or were taking anti-PD drugs were visited at home by a trained neurologist for confirmation or exclusion of a diagnosis of PD. However, at 8 and 10 years follow-up, the possibility of PD was explored solely by the question, "Do you have Parkinson's disease?" The diagnosis was not clinically confirmed, which could have induced a differential bias. However, it was felt that such misclassification was not related to pesticide exposure because the study hypothesis was not disclosed to the participants.

Table 4.1 Summary of cohort studies

Reference	Country	Cohort description, recruitment and follow-up period	Exposure measure	Number of PD cases	Risk estimate (95% CI)	Comments
Yesalis *et al.* (1985)	Iowa, USA	3097 rural residents 1981–82 Unknown follow-up	Occupation	Not reported	Prevalence (per 100) Men Retired: Farmer 0.9, Non-farmer 5.8 Men Working: Farmer 0.4, Non-farmer 2.8 Women Retired: Farmer 1.0, Non-farmer 0.7 Women Working: Farmer 1.1, Non-farmer 0.8	Significantly lower risk of PD amongst male farmers compared with non-farmers
Tüchsen & Jensen (2000)	Denmark	2 273 872 Danes 1981 Up to 1993	Occupation	949 Total 611 Men 338 Women	Standardised hospitalisation ratio Agriculture/horticulture workers Men: 134 (109–162) Women: 118 (78–172) All: 132 (11–156) Farmers Men: 130 (103–163)	
Petrovitch *et al.* (2002)	Hawaii, USA	7986 Japanese/Americans 1965 Up to 1996	Occupation as plantation worker and self-reported pesticide exposure	116 men	Incidence (per 10 000, age-adjusted) Plantation workers Number of years worked 0: 5.8 1–10: 5.4 11–20: 9.2 >20: 10.3 Self-reported pesticide exposure Number of years 0: 7.8 1–10: 6.5 11–20: 8.2 >20: 12.7 Relative risk (workers/non-workers) Number of years 0 1–10: 1.0 (0.6–1.6) 11–20: 1.7 (0.8–3.7) >20: 1.9 (1.0–3.5) Trend p = 0.006, adjusted for age, smoking and coffee intake	Dose–response trend with duration of plantation work but not duration of pesticide exposure

Reference	Country	Cohort description, recruitment and follow-up period	Exposure measure	Number of PD cases	Risk estimate (95% CI)	Comments
Vanacore *et al.* (2002)	Italy	5575 Farmers 1971–73 Up to 2000	Occupation as licensed pesticide user	2 (deaths)	None	Incomplete investigation at this stage
Baldi *et al.* (2003)	France	1507 Elderly 1987 1992–98	Occupational exposure to pesticides, main job in agriculture, and rural living	24	Incidence (per 1000) Overall 5.0 Pesticide Exposed 8.9 Not exposed 4.1 Relative risk Occupational exposure Men 5.63 (1.47–21.58) Women 1.02 (0.22–4.82) Main job in agriculture Men 1.62 (0.31–8.63) Women 0.81 (0.10–6.40) Rural residency Men 1.45 (0.38–5.49) Women 1.31 (0.40-4.30) Adjusted for smoking and education	

CI, confidence interval; PD, Parkinson's disease

4.4 Case–control studies

4.4.1 Introduction

Forty reports of case–control studies, published after 1983, that examined the association between exposure to pesticides and PD were identified. Two reports (Semchuk *et al*., 1993; Semchuk & Love, 1995) were excluded from the review because data were included in another study report (Semchuk *et al*., 1992) and the later reports did not offer any new data. Of the 38 separate studies, 11 were carried out in Europe (Jiménez-Jiménez *et al*., 1992; Morano *et al*., 1994; Seidler *et al*., 1996; Smargiassi *et al*., 1998; Fall *et al*., 1999; Kuopio *et al*., 1999a; Preux *et al*., 2000; Herishanu *et al*., 2001; Zorzon *et al*., 2002; Vidal *et al*., 2002; Duzcan *et al*., 2003), 13 in the USA (Golbe *et al*., 1990; Koller *et al*., 1990; Wechsler *et al*., 1991; Wong *et al*., 1991; Stern *et al*., 1991; Butterfield *et al*., 1993; Hubble *et al*., 1993; Fleming *et al*., 1994; Gorell *et al*., 1998; Taylor *et al*., 1999; Nelson *et al*., 2000; Kamel *et al*., 2001; Kirkey *et al*., 2001; Firestone *et al*., 2002), five in Canada (Hertzman *et al*., 1990; Zayed *et al*., 1990; Semchuk *et al*., 1992; Hertzman *et al*., 1994; Chaturvedi *et al*., 1995), five in Asia (Ho *et al*., 1989; Tanner *et al*., 1989; Liou *et al*., 1997; Chan *et al*., 1998; Behari *et al*., 2001), two in Australia (Menegon *et al*., 1998; McCann *et al*., 1998), one in South America (dos Santos Werneck & Alvarenga, 1999), and one was from Nigeria (Falope *et al*., 1992). No study from the UK that examined the relationship between PD risk and pesticide exposure was identified. The number of cases in the studies ranged from 34 to 496 and the number of controls ranged from 25 to 2070, although the nested US case–control study by Kamel *et al.* (2001) used the rest of the cohort (22 286) as their controls. The mean age of the cases ranged from less than 50 years to 72 years. In addition, three autopsy studies (1 USA, 2 UK) of PD were identified that had 20 (Fleming *et al*, 1994), 8 (Corrigan *et al*., 1998) and 10 (Corrigan *et al*., 2000) cases, with 14, 7 and 6 controls, respectively.

A brief summary of each study follows (Section 4.4.2), including the main findings and comments by the authors about the limitations of their study. In each summary the authors' specific wording describing pesticide exposure has been used. Therefore, ‘use of herbicides and pesticides’ and ‘exposure to herbicide or pesticide’ probably means use of or exposure to herbicide and other pesticides. General limitations of case–control studies are discussed in Section 4.6. Table 4.2 gives a brief description of each study, including the country of origin, time span over which the cases and controls were selected, source of cases and controls, diagnostic criteria, case numbers and ages, and whether controls were matched to cases in any way. Table 4.3 summarises the results of each study, including type of exposure measure, potential confounders examined, crude or unadjusted odds ratios (ORs) and adjusted ORs.

4.4.2 Case–control studies on pesticide exposure

Original reports of case–control studies are summarised herein. Meta-analyses of case–control studies are described in Section 4.5.

One study published before 1983 (Ohlson & Hodstedt, 1981) was identified. Cases were male inpatients treated for PD and controls were male patients with sub-arachnoid haemorrhage. Of 97 cases located, six did not respond and six did not complete their questionnaires, giving a response rate of 87.6%. Similarly, for the controls 83 were contacted, eight did not respond and three failed to complete the questionnaire, giving a response rate of 86.7%. Information was gathered via a postal questionnaire, and occupational exposure to agricultural chemicals was defined as working with the chemicals for more than 15 days (unclear over what time period). Exposure to agricultural chemicals in this study was not found to be a risk for PD; however, very few of the cases (3/85) and controls (7/72) were exposed to them. There was a small, non-significant association between PD risk and occupational exposure to solvents (OR 1.1, 95% CI 0.4–2.9) and/or mercury (OR 2.4, 95% CI 0.5–5.0). Although the study was small the authors stated the probability of detecting a threefold increased risk for PD for agricultural chemicals was 71%.

Ho *et al.* (1989) recruited cases (N = 35) and controls (N = 105) from geriatric day hospitals and an old people's home in Hong Kong. Information was obtained by face-to-face interview using a structured questionnaire, and details of exposure to pesticides before age 20, age 20 to 40, and after age 40 were collected. Risk of PD increased with use of herbicides and pesticides at any age, but the study was small and confidence intervals were large. Very few people were exposed to pesticides (7/35 cases, 7/105 controls). PD risk was significantly increased in those with more than 40 years of rural living (OR 4.9, 95% CI 1.4–18.2) or more than 20 years of farming (OR 5.2, 95% CI 1.6–17.9), and also among those who consumed raw vegetables (OR 10.8, 95% CI 2.4–40.0). The latter finding, it was suggested, could have been the result of exposure to pesticides, and paraquat in particular, which are sprayed on the crops shortly before harvesting, followed by incomplete washing of the vegetables before consumption. However, this needs further investigation as no other study identified has shown an association between raw vegetable consumption and PD.

Tanner *et al.* (1989) selected cases (N = 100) from outpatient clinics from two centres in China and age- and sex-matched controls (N = 200) from the same centres. Information was obtained by an interview-based questionnaire. Although no results were given, the authors reported that an association was observed between exposure to herbicide/pesticide manufacturing and PD risk. However, questions only concerned categories of risk and not specific chemicals, so that a single agent or class of chemicals could not be identified. There was no difference in mean age at PD onset or mean duration of disease between PD cases with exposure to herbicide or pesticide industries and those with no such exposure. An increased risk for PD was associated with occupational or residential exposure to industrial chemical and printing plants and quarries. A decreased risk for PD was associated with living in villages, exposure to pig and chicken raising, and wheat growing. Information was primarily obtained from the patient; however, on occasion, accompanying family members were allowed to remind patients of events, possibly leading to recall bias. Many risk factors were analysed, thereby increasing the possibility of a chance finding.

Golbe *et al.* (1990) selected cases (N = 106) from the Movement Disorder Clinic of a New Jersey hospital in the USA; spouses were used as controls (N = 106). No diagnostic criteria were given other than that cases qualified if they had 'typical idiopathic PD'. Simple exclusion criteria were given (no dementia, unmarried before onset of PD and no first degree relative with PD). Exposure was assessed via a telephone interview. The spouse control was interviewed first and instructed not to repeat questions aloud to prevent the patient influencing their response or formulating his or her own answers in advance. Patients were also requested not to solicit advice from the spouse during questioning. Individuals were asked if they had sprayed pesticides or insecticides at least once a year for 5 years (not necessarily consecutively) before marriage. The risk of PD was statistically significantly increased with exposure to sprayed pesticides or insecticides sprayed at least once a year for 5 years. The study also found a positive association between PD and rural experience (not defined; OR 2.00, 95% CI 1.04–4.00), exposure to farm animals (OR 1.33, 95% CI 0.65–2.80), and use of well-water (OR 1.14, 95% CI 0.52–2.53); the latter two findings were not significant. An association was also found with the consumption of spicy and fried foods, radishes and apple juice.

Hertzman *et al.* (1990) recruited patients (N = 57) from a large area of British Columbia, Canada, through local physicians. Controls (N = 122) were randomly selected from regional electoral rolls (but did not undergo neurological examination to eliminate PD or other disorders). Of the 78 cases originally contacted, six chose not to complete the questionnaire, giving a response rate of 92%. One person had moved out of the area and could not be contacted and two patients had died. A total of 55 were stated to have met generally accepted diagnostic criteria, although these were not specified. However, the analysis included 57 cases, and it is not clear where the two extra cases came from. Among the controls the response rate was 78%. Information, including past handling of chemicals, including pesticides, was obtained via a postal questionnaire. An association with PD was found for those who ever worked in an orchard or were involved in chemical spraying. However, no association was found among those who ever handled any of certain chemicals, including glyphosate, picloram, formaldehyde, malathion, 2,4-dichlorophenoxyacetic acid, tebuthiuron, diazinon, atrazine, pyrethrum, diquat and bromacil; however, this is not unexpected with so few cases. Four PD patients and no

controls reported paraquat contact. Thus, an OR could not be calculated, but a Fisher's exact test gave a significant probability of 0.01. Adjusting for age and sex, and age and smoking, reduced the ORs slightly, but the association with PD remained significant. Four PD patients and no controls reported handling paraquat; the probability of this occurring was significant — a Fisher's exact test gave a probability of 0.01. A similar result was also obtained for familial tremor (Fisher's exact test 0.03). Logistic regression modelling indicated a statistically significant relationship between the development of PD and having worked in an orchard as well as a borderline significant relationship with having worked in a planer mill, where industrial chemicals, including pesticides, insecticides and anti-sapstain, are used. Chemical spraying appeared to be dependent on orchard work.

Koller *et al.* (1990) selected cases (N = 150) randomly from the Movement Disorder clinic of a Kansas medical centre in the USA. Controls (N = 150) were from other outpatient clinics but no indication was given about the size of the base population. Interviews were carried out with the subject and family but there was no indication of how much influence the family had in answering the questions on exposure. Quantification of exposure included the type of exposure, for example the number of years exposed, number of acres to which herbicides were applied and type of crops treated. A sub-sample of the original group was re-interviewed 4–13 months after the initial interview, and this re-testing showed an average reliability of 91% for the factors studied. It is not clear whether the interviewer or interviewee was blind to the study hypothesis. A non-significant increase in risk for those exposed to herbicides or pesticides was found, including a weak association for those cases that applied herbicides/pesticides to corn. PD risk was significantly increased with regard to living in a rural environment (OR 1.88, 95% CI 1.13–3.19) and drinking well-water (OR 1.67, 95% CI 1.01–2.79). However, multivariate analysis indicated drinking well-water was dependent on rural living.

Zayed *et al.* (1990) identified cases (N = 42) through general practitioners (GPs) and consultant neurologists in Quebec, Canada; controls (N = 84) were identified by random calling of people in the telephone directory living in the same area as the cases. Information was obtained through face-to-face interview using a structured questionnaire. A spouse or close relative was present during the interview with cases and was asked to clarify responses if required. For ten cases who were unable to speak, the spouse/relative completed the questionnaire for the patient. The questionnaire was validated on a small sample of cases/controls. A non-significant association was observed between PD risk and pesticide exposure, especially for those with over 30 years exposure (OR 1.92, 95% CI 0.56–6.57). PD risk also increased with occupational exposure to manganese, iron and aluminium (OR 2.28, 95% CI 0.85–2.77), again especially when duration of exposure was greater than 30 years (OR 13.64, 95% CI 1.52–76.28). PD risk was decreased for those living in rural areas (OR 0.31, 95% CI 0.11–0.91) and near industry and mining (OR 0.85, 95% CI 0.04–0.55). No association was found with farm work (OR 0.05, 95% CI 0.25–1.69), industrial work (OR 2.00, 95% CI 0.62–6.36), or well-water consumption (OR 1.40, 95% CI 0.48–4.02).

Stern *et al.* (1991) selected cases (N = 149) from two hospitals (Philadelphia and New Jersey, USA) and divided them into YOPD (onset before age 40; N = 69) and old-age onset PD (OOPD; onset on or after age 60; N = 80). Controls (N = 149) were selected from three nominees by the cases; they were matched for age, sex and race, and they did not include spouses or relatives. If no control was found then a patient or volunteer from one of the hospitals was selected. Both types of controls had to meet criteria for the study (not specified). No information was given about the response rates for the various groups. Exposure to pesticides or insecticides was recorded as positive if an individual reported the use of agents within the home, in the yard/garden and in the neighbourhood, either by members of the household or by professionals. Exposure to insecticides or herbicides showed no association with PD. Adjustment for smoking, head trauma and previous rural residence did not appreciably alter the estimate. The association between PD risk (YOPD and OOPD combined) and head trauma was statistically significant (OR 2.9, 95% CI 1.5–5.8), whereas those for rural living (not defined; OR 1.7, 95% CI 0.9–3.1) and well-water consumption (OR 0.8, 95% CI 0.4–1.6) were not statistically elevated. However, for those who lived in rural areas for less than 10 years there was an association with PD.

Wechsler *et al.* (1991) recruited a small number of cases (N = 34) and controls (N = 25) from a neurology clinic and local PD support groups in Washington State, USA. Controls were diagnosed with neurological disorders other than PD and tested for dementia. Information was gathered via a postal questionnaire, which requested details of occupational and home pesticide exposures, with an overall response rate of 49%. The analysis of occupational exposure to pesticides was limited to males (19 cases, 9 controls) because most females did not report having been employed in any of the occupations of interest; only one male used pesticides occupationally. Only results for the use of selected home pesticides were presented. Three products, KLEENUP® (glyphosate) grass and weed killer, Ortho TRIOX® (pentachlorophenol), and PESTKIL® (bendiocarb), were more frequently used by cases than controls. However, the OR for Ortho-TRIOX® was the only one significantly elevated. The OR was also significantly increased for those who used ROUND-UP (glyphosate). However, with such small numbers, these findings might have occurred by chance.

Wong *et al.* (1991) selected a small number of cases (N = 38) from a hospital PD centre in Kansas, USA. Age- and sex-matched controls (N = 38) were randomly selected from the general neurology and medical clinics. Common diagnoses included headache, back pain, arthritis, and heart disease. Sibling pairs with the diagnosis of familial essential tremor were randomly selected from the Movement Disorder clinic also to act as a further set of controls. No difference was seen between cases and controls in their exposure to herbicides/pesticides; however, cases were more likely to live in a rural area (OR 4.33, 95% CI 1.19–23.70) and drink well-water (OR 2.75, 95% CI 0.82–11.9).

Falope *et al.* (1992) selected consecutive patients (N = 50) attending the local neurology outpatient clinic in Ibadan, Nigeria and age-and sex-matched controls (N = 50), with a variety of diseases, from other medical outpatient clinics. Information was gathered by interview, identifying exposure as ever/never exposed to pesticides. No association was observed between PD and exposure to pesticides or any other risk factor studied, except occupation as a blacksmith (OR 7.98, 95% CI 1.25–50.71). This was a small study, and very few people were exposed to pesticides (6 cases, 5 controls).

Jiménez-Jiménez *et al.* (1992) recruited cases (N = 128) from a Movement Disorder outpatient clinic in Madrid, Spain, during routine follow-up visits; 128 patients were recruited although the total sample size from which the cases were selected was not given. Controls (N = 256) were selected from emergency admissions instead of from other outpatient clinics, but no indication of the 'non-neurological ailments' from which they suffered was given. No data were given about the dates cases were selected or the diagnostic and exclusion selection criteria specified. Two categories of pesticide exposure were defined: direct — subjects who had applied pesticides themselves; and indirect — subjects who resided and ate vegetables in an area where pesticides were employed. A non-significant positive association between PD and exposure to pesticides for at least one year was observed. No risk was observed with rural living (OR 1.07, 95% CI 0.70–1.63) but significantly more PD cases than controls drank well-water for more than 30 years (OR 1.76, 95% CI 1.09–2.84).

Semchuk *et al.* (1992) recruited cases (N = 130) from a population-based case register and controls (N = 260) from the general population in Calgary, Canada. Information was gathered by an interview-based questionnaire, and exposure to pesticides coded for each 10-year age interval between age 16 and 65. An overall increased risk of PD with exposure to herbicides and insecticides (OR 2.25, 95% CI 1.27–3.99) was observed, but there was no relationship with duration of exposure. No dose–response relationship with cumulative lifetime occupational herbicide use was demonstrated. The authors attributed this to lack of adequate statistical power (no data given), owing to the small number of respondents with a history of occupational use of herbicides, the sharp decline in the number of respondents exposed after age 25, and the small number with more than 10 years continuous use of herbicides. However, it could also have been that no relationship existed. There was sufficient power to detect an overall increased risk of PD with herbicide exposure and also increased risk between specific ages. Significant associations were seen in individuals exposed to pesticides between the ages of 26 and 35 and insecticides between 46 and 55 years. Exposure to herbicides over the age of 26 also increased the risk of PD, as did exposure to agricultural work and field crop and grain farming. Multivariate analysis consistently showed that only previous occupational herbicide use was

associated with a statistically significant increased PD risk estimate, regardless of which other variables were included in the logistic regression model; the risk was approximately threefold. After adjusting for previous occupational herbicide use, there was no significant increase in PD risk associated with any other exposure. Among those cases that could recall specific herbicides used (41%), all but one had used compounds in the chlorophenoxy and thiocarbamate chemical groups exclusively. Only one case reported having worked with paraquat between the ages of 26 and 31 years, and this was the only herbicide-exposure case where the onset of symptoms occurred before the age of 40. An increased risk of PD was also seen in those with a previous history of agricultural work in general, and this risk increased concomitantly with each 10-year increment in cumulative exposure, but not with specific type of work. An increased risk was also observed when the cumulative lifetime exposure to field crop farming or to grain farming was considered.

Butterfield *et al.* (1993) recruited cases (N = 63) of YOPD (i.e. diagnosed before the age of 50) from the community, in Oregon and Washington State, USA. Controls (N = 68) were patients with a diagnosis of rheumatoid arthritis; they were recruited from the same catchment area but were not matched during the selection process. Information was gathered through a postal questionnaire and validated at a later date by administering an abridged version on a small sample of patients. The questionnaire was originally mailed to 100 cases but 28 either did not respond or returned it blank; a further nine were not included because they were too ill, did not speak English, were diagnosed after age 50 years or did not fit the diagnostic criteria. Similarly, for the controls, 186 were mailed: seven were non-contactable; 11 were diagnosed after age 50 years; and a further 100 did not return the questionnaire. The final response rate for the potential PD cases was 69.2% and for the potential controls was 40.4%. The study showed an increased risk of YOPD in those exposed to herbicides and insecticides more than ten times per year. In addition, YOPD cases were also more likely to have a relative with PD (OR 2.37, 95% CI 0.86–6.54). Multivariate analysis of demographic and family predictors of PD showed ethnicity (OR 10.29, 95% CI 1.21–87.17) and educational level (OR 2.44, 95% CI 1.01–5.87) to be significant factors in the first model (also includes sex, age, age at diagnosis, and family history of PD). This model successfully predicted the disease status of 67.2% of subjects/controls. In a second model insecticide exposure and smoking were added to the variables in the first model and while race (OR 27.84, 95% CI 2.70–287.17) remained a significant factor education did not. Insecticide exposure was a significant factor (OR 7.24, 95% CI 2.29–22.92). The predictive success rose to 76.3%. The addition of other variables did not improve the predictive power of the second model; however, the eating of nuts and seeds (OR 1.50, 95% CI 1.03–2.18) was a significant factor in other models. The OR for rural residence was non-significantly increased (OR 2.35, 95% CI 0.57–6.34) when included in the model. These results are unusual in that race is not a factor examined by other studies, and education is an unlikely risk factor unless it was a surrogate for reporting bias, access to diagnosis or a lifestyle factor.

Hubble *et al.* (1993) selected rural (defined as <2500 population) cases (N = 31) from a PD outreach clinic and controls (N = 32) from media releases and a senior citizens luncheon program in Kansas, USA. Cases (N = 45) and controls (N = 31) in an urban area were selected from hospital clinics and non-family friends of cases. Although the latter were not age- and sex-matched, analysis showed no difference for these factors. Information was collected by means of a self-administered postal questionnaire but no response rate was reported for either the cases or controls. Pesticide exposure was assessed as either 'used for more than 20 days during any one year' or 'used for more than 20 days a year for more than 5 years'. As the number of cases in each town was small, principal factor analysis was carried out. Three factors of variables were identified: rural living factor (having lived in a rural area, used ground water, farmed as an occupation, or ever vaccinated animals); lifestyle factor (male, smoking, alcohol abuse, occupational history pertaining to agriculture); and pesticide factor. The rural living factor was the only variable that differed significantly among the subjects (cases and controls) based on residence. No difference was observed between the urban and rural areas for these factors. Logistic regression analysis of the cases and controls from both areas of residence combined showed the pesticide factor to be the strongest predictor of PD, but the confidence intervals were large (OR 3.42, 95% CI 1.27–7.32). The pesticide factor was a stronger predictor than a family history of neurological problems (OR 3.18, 95% CI 1.22–7.05) and a personal history of depression (OR 2.74,

95% CI 1.07–7.57), variables that were not loaded into the principal factor analysis. When all three predictors were positive, the probability for PD in the subject was 92.3%; when all three predictors were negative, the probability of PD was 28.7%. This suggests these are significant risk factors for PD. Depression, which is observed in about 50% of all PD patients (Dubois & Pillon, 1998), could in fact be an early sign of PD, rather than a predisposing factor.

Hertzman *et al.* (1994) obtained cases for their study (N = 127) by contacting GPs, local neurologists and internal medicine specialists in British Columbia, Canada. A total of 159 patients were contacted and 79.9% responded. Two sets of controls (N = 245) were selected; the first was obtained randomly from community electoral rolls and the second from patients with chronic cardiac disease. Rates of participation were 61% for voters and 79% for cardiac disease patients. Exposure to known factors was assessed by interview, during which chemical exposure was assessed using cue cards that listed trade names and all common names of 79 pesticides, five per card, grouped into insecticide, herbicide, fungicide, acaricide, and plant growth regulator. The risk of PD was statistically significantly increased in men exposed to pesticides when compared with cardiac disease patients and controls from the electoral register; the risk was not increased for women. However, there was no association with PD for any of the pesticide groups, with the exception of insecticides, for which the ORs were statistically decreased for men and women compared with controls from the electoral register. However, male PD patients were significantly more exposed to phosalone (Fisher's exact test 0.03) and ferbam (Fisher's exact test 0.03) when compared with cardiac disease controls, but not controls from the electoral register. PD patients were also more likely to be exposed to paraquat (OR 1.11, 95% CI 0.32–3.87) and azinphos-methyl (OR 7.17, 95% CI 0.77–66.98). No differences were seen between cases and controls when examining selected occupations and other environmental factors (including farming and other agricultural work).

Morano *et al.* (1994) recruited patients (N = 74) making their first visit to an outpatient clinic in Cáceras, Spain; controls (N = 148) were recruited from the emergency room (patients with minor, non-neurological ailments) or from the neurology clinic (patients with functional central nervous system pathology, such as tension headache, sciatica, etc.). A questionnaire was administered by face-to-face interview, and direct or indirect exposure to pesticide for at least one year was used to define exposure. The risk of PD was non-significantly raised in those exposed to pesticides, but no difference was seen between cases and controls for the duration of exposure; agricultural work as the main occupation did not increase the risk of PD. Significantly more patients than controls lived in rural areas, defined as populations of fewer than 2000 individuals for more than 50 years; this was also the case for those living in populations of less then 500 (OR 2.50, 95% CI 1.41–4.42). Significantly more patients had also been exposed to well-water for longer than 40 years (OR 2.77, 95% CI 1.51–5.08). Both these factors were highly correlated with disease status. PD patients also reported a significantly higher frequency of a family history of postural tremor (OR 3.52, 95% CI 1.30–9.49), presumed to be essential tremor, and of PD (OR 3.90, 95% CI 1.46–10.40).

Chaturvedi *et al.* (1995) selected cases (N = 87) and controls (N = 2070) from a large cohort of elderly Canadians in Ontario, but no diagnostic or exclusion criteria were reported. Information was gathered via an interview with the patients or their caregivers. It was not clear whether the latter were relatives or not, or whether their knowledge of historical exposures was accurate. However, because PD was not the primary endpoint of the original study the problem of recall bias and proxy reporting of exposure information was believed to be diminished. Exposure to pesticides was of a semi-quantitative nature; that is exposure was considered positive only if pesticides were frequently used. A non-significant positive association was found between PD and exposure to pesticides and fertilisers, and also to defoliants and fumigants. PD risk was also significantly associated with exposure to solvents, especially in aerosol/spray paints (OR 3.89, 95% CI 1.42–9.18), plastic cement/glues (OR 4.26, 95% CI 1.76–9.26), plastic and epoxy resins, and gasoline or petroleum fuels, when used during sporting and hobby activities (OR 2.30, 95% CI 1.12–4.43).

Seidler *et al.* (1996) recruited cases (N = 380) aged 65 years or less from nine neurology clinics throughout Germany. Controls (N = 755) were selected randomly, one from the patient's immediate

neighbourhood and one from the same urban/rural region using address lists. A detailed structured interview was used to assess exposure, and pesticide exposure was categorised according to the number of years of pesticide application weighted by the frequency of usage ('dose-years': rarely, factor 1; for special indications, factor 2; and regularly (seasonally), factor 3). A toxicologist then categorised all pesticides named by subjects who used pesticides regularly. Products fell into five groups — organochlorines, alkylated phosphates, carbamates, cellular metabolism inhibitors and others. Cases were more likely to report herbicide or insecticide use than either neighbourhood or regional controls. A positive trend was seen in those reporting herbicide and insecticide use with the number of years used, although the trends were significant only in comparison with regional controls. Cases provided a higher absolute number of specific product names than controls. However, the proportion of 'dose-years' for which specific names could be given was higher for controls. Cases were more likely to have used organochlorines and alkylated phosphates/carbamates, but only significantly so for the comparison with regional control subjects. There was no difference in the frequency of previous farming activity or employment in agricultural work between cases and controls. Similar results were seen for rural living and well-water consumption. PD risk was also significantly elevated in those with wood panelling in their home (OR 1.36, 95% CI 1.01–1.82), contact with wood preservatives (OR 1.83, 95% CI 1.36–2.46), occupational exposure to lead (OR 1.9, 95% CI 1.1–3.1), mercury (OR 2.0, 95% CI 0.9–4.2) and zinc (OR 1.5, 95% CI 0.9–2.4), gas and vapours (OR 1.48, 95% CI 1.11–1.97), solvents (OR 1.77, 95% CI 1.28–2.44), glues, paints and lacquers (OR 1.39, 95% CI 1.04–1.85), exhaust fumes (OR 1.74, 95% CI 1.30–2.33) and carbon monoxide (OR 1.50, 95% CI 1.08–2.09). The authors calculated that their study had a power of 90% to detect an OR of about 2 for an exposure rate of 10% in controls.

Liou *et al.* (1997) studied patients (N = 120) from the Movement Disorder clinic of the local hospital in Taiwan; controls (N = 240) were recruited from neurological and medical outpatient clinics at the same hospital. Information was gathered using a structured open-ended questionnaire by trained interviewers. Subjects were asked for details of residential and occupational exposure to herbicides/pesticides, and if possible to identify specific herbicides/pesticides used. There were significant associations between PD risk and occupational or residential exposure to herbicides/pesticides, and more specifically to paraquat. There were also significant associations between PD risk and the duration of cumulative lifetime exposure to herbicides/pesticides and again specifically paraquat. Similar findings were also found for rural residence (OR 2.04, 95% CI 1.23–3.38) and farming (OR 1.81, 95% CI 1.25–2.64), especially those involved in growing rice (OR 1.70, 95% CI 1.13–2.58). No association was seen with well-water consumption, or exposure to other chemicals and heavy metals. The associations with PD risk for previous use of herbicides/pesticides and for previous use of paraquat remained statistically significant, after adjustment for other environmental factors (duration of living in rural residence, farming, consuming well-water, smoking), when controlling each for the other.

Chan *et al.* (1998) recruited patients (N = 215) with PD and controls (N = 313), matched by age-group and sex, from two hospitals in Hong Kong. Controls were excluded if they exhibited signs of PD. All participants were interviewed blind to the specific hypothesis being investigated. Exposure to pesticides was assessed as use during farming, including duration. Univariate analysis showed pesticide exposure during farming was not significantly related to PD for the patient group as a whole, although the relationship was significantly elevated for women but not for men; however, only a small number of patients and controls were exposed to pesticides. Duration of pesticide exposure (in years) was found to increase the risk of PD marginally; how many years duration was not clearly reported. Rural residence, farming as an occupation, drinking well-water and consumption of fruits and leafy green vegetables were found not to have a significant relationship with PD. However, a significant positive relationship was found with the presence of a family history of PD (OR 5.21, 95% CI 1.07–25.3). Multivariate analysis showed no variable to be a significant risk factor for PD.

Gorell *et al.* (1998) recruited cases (N = 144) and controls (N = 464) from Detroit, USA, who were receiving primary medical care from a health care provider. Information was gathered by face-to-face interview, and ever/never pesticide exposure was categorised according to type (insecticide, herbicide

and fungicide) in the workplace, on a farm or while gardening. Exposure to pesticides/herbicides whilst at home, gardening as a hobby or as a resident or worker on a farm showed no association with PD. However, contact with herbicides and insecticides at work, including agricultural work, was significantly greater for PD patients than control subjects. Moreover, the association with PD was greater in those with more than 10 years of occupational exposure to insecticides (adjusted OR 5.81, 95% CI 1.99–16.97) compared with those with less than 10 years of such exposure (adjusted OR 2.39, 95% CI 0.89–6.40). Farming as an occupation after age 18 was also significantly associated with PD risk. The significant association of occupational exposure to herbicides (OR 3.36, 95% CI 1.09–10.33) or insecticides (OR 3.15, 95% CI 1.54–6.49) with PD was maintained despite adjustment for farming. Farming as an occupation also appeared to be an independent risk factor for PD; the OR for farming was relatively unchanged after adjusting for any of the three pesticide classes (herbicides, insecticides, fungicides).

McCann *et al.* (1998) recruited cases (N = 224) from a variety of sources that included hospitals, residential care centres and community groups in Queensland and New South Wales, Australia. Controls (N = 310) were matched for age, sex, ethnicity, residential area and source. Exposure was assessed by a self-administered postal questionnaire, but no response rates were given. Regular exposure was classed as daily or weekly exposure to industrial herbicides and pesticides for a cumulative period of greater than 6 months. Exposure to herbicides and pesticides was not a statistically significant risk factor for PD, although the OR was greater than 1. Rural residency (OR 1.8, 95% CI 1.7–2.5), defined as living in an area with less than 10 000 population, and a family history of PD (OR 3.35, 95% CI 1.90–5.88) were both statistically significant risk factors for PD, whilst previous head trauma (OR 1.1, 95% CI 0.7–1.9) and ingestion of well/spring-water for longer than 12 months (OR 0.7, 95% CI 0.4–1.0) was not. In multivariate analysis, rural dwelling (OR 1.7, 95% CI 1.17–2.57) appeared to be confounded by a history of hypertension, and no longer exhibited such a marked risk for PD. The authors suggest that their finding of a highly significant inverse association between hypertension and PD development supports the hypothesis that there is a protective effect of hypertension for PD development. However, they note that the biological mechanism for this association is unclear, although one theory may be an advantageous effect of persistent elevation of brain perfusion. However, they suggest a more likely explanation is the higher mortality rate in hypertensive individuals at an earlier age, hence, a survivor effect.

Menegon *et al.* (1998) selected cases (N = 95), and age- and sex-matched controls (N = 95) from a variety of sources (community groups, hospital inpatients and outpatients) in south-east Queensland and New South Wales, Australia. Information was gathered via a structured questionnaire (no indication given of whether interview-based or postal). Pesticide exposure was defined as more than once weekly exposure for more than 6 months before the onset of PD and, when possible, details such as type of pesticide, duration of exposure, and whether exposure was through farming, industrial exposure, gardening or other mechanisms were recorded. All subjects were also screened for four glutathione *S*-transferase (GST) polymorphisms (GSTM1, GSTT1, GSTP1 and GSTZ1T). Logistic regression analysis showed family history (OR 4.2, 95% CI 1.3–14.0) and pesticide exposure were significant risk factors for PD. The distribution of genotypes for any of the polymorphisms did not differ significantly between cases and controls. However, because GSTs are involved in the detoxification of many xenobiotics (including some pesticides), only those cases/controls reporting exposure to pesticides were examined. The distribution of the GSTP1 polymorphism genotype differed significantly between cases and controls exposed to pesticides. GSTs metabolise various pesticides, and the authors propose that this difference in genotype frequencies might explain susceptibility to PD after pesticide exposure. The methodology, especially case/control sources, was similar to that of McCann *et al.* (1998), and there may have been some overlap in the cases, but no time period was given and only one author appeared on both papers.

Smargiassi *et al.* (1998) selected consecutive patients (N = 86) attending outpatient clinics in Parma, Italy, representing 97.7% of the eligible cases (two declined to participate). Controls (N = 86) were selected from other specialist outpatient clinics at the same hospital (cardiology, ophthalmology, dermatology and nephrology). A trained interviewer using a structured questionnaire collected

information, and an industrial hygienist who was blind to the status of the interviewees' record classified exposure. Exposure to pesticides was established as contact for at least 10 consecutive years prior to the onset of PD. Exposure to pesticides and herbicides was similar in PD cases and controls. However, PD cases more often reported exposure to well-water for at least 10 years (OR 2.78, 95% CI 1.46–5.28) and also occupational exposure to chemicals (OR 2.13, 95% CI 1.16–3.91), especially organic solvents (OR 2.78, 95% CI 1.23–6.26). The authors stated that the limited sample size and restrictive criteria used to define occupational exposure did not allow the role of specific agents to be investigated.

Fall *et al.* (1999) identified cases (N = 113) through records of all prescriptions for anti-parkinsonism drugs and doctor's patient lists in south-eastern Sweden. Of the 170 cases identified, 14 did not return the questionnaire, a further 11 were omitted because age of onset of PD was not given, 4 were less than 40 years of age and 28 were greater than 75 years. Of those eligible, the response rate was 89.0% (113/127). The control group (N = 263) were randomly drawn from the Regional Population Register, and of the initial 321 who lived in the same district as the cases, 81.9% responded. Information was gathered through a postal questionnaire, previously used in other studies, and pesticide exposure was stated to have been assessed, although no criteria were given. Individuals who had handled pesticides as part of their occupation were found to have an increased risk of PD, although the confidence interval included unity. Similar results were observed for those handling pesticides or insecticides within agricultural work. Female cleaners (OR 6.7, 95% CI 1.76–30.0) and male carpenters (OR 3.9, 95% CI 1.0–15.7) were also found to have a significantly increased risk of PD.

Kuopio *et al.* (1999a) recruited cases (N = 123) from a community survey in 1992 in south-western Finland. The survey identified 282 prevalent cases of PD but the study only included patients with onset after 1984 (N = 161), who performed well in the Mini-Mental State Examination (N = 132), and were in adequate physical condition (N = 125) to perform the interview. Control subjects (N = 246), matched for age, sex and case municipality, were selected randomly from a population register. Information was gathered by face-to-face interview and included data on exposure to toxic agents such as DDT, herbicides, pesticides and other chemicals. Use of pesticides, herbicides, or DDT was not associated with a significantly increased risk of PD, and when use was categorised as regular or occasional, the OR for the latter group was greater. No other environmental, occupational, lifestyle or medical factor was associated with an increased risk of PD. However, having had domestic animals at home during their lifetime, including cows, sheep, pigs, chickens, cats, dogs, horses or rabbits was associated with a reduced risk of PD. The reason for this is unclear and this factor may be a marker of other environmental conditions or lifestyles.

Taylor *et al.* (1999) recruited cases (N = 140) from Movement Disorder clinics in Boston, USA and controls (N = 147) through the cases (in-laws or friends of the same age), to obtain a sample of non-diseased people of similar age, sex, and socioeconomic status. In an initial phase, 163 potential controls were contacted of whom 12 declined to participate and four did not return their consent form (response rate 90.2%). Information was gathered by a telephone interview with the controls but for the cases it was not clear whether the same method was used or whether interviews were face-to-face. Exposure to pesticides was recorded as total days of reported lifetime exposure, converted to years. Subjects who indicated that they had been exposed to pesticides or herbicides were asked the frequency (1/year, 1/month, 2/month, 1/week, 3/week or 1/day), duration (<1 year, 1–4 years, 5–9 years, 10–19 years or 20+ years) and age at initial exposure. No association was found between the risk of PD and pesticide and/or herbicide exposure. Multiple logistic regression analysis revealed that four factors were associated with increased risk for PD. They were head injury (OR 6.23, 95% CI 2.58–15.07), family history of PD (OR 6.08, 95% CI 2.35–15.69), family history of tremor (OR 3.97, CI 1.17–13.50), and depression (OR 3.01, 95% CI 1.32–6.88).

Dos Santos Werneck and Alvarenga (1999) randomly selected PD subjects (N = 92) and matched controls (N = 110) from the local neurology department in Rio de Janeiro, Brazil. Controls were excluded if they showed signs of PD or dementia. Information was gathered by interview, and pesticide exposure was assessed as inhaling and/or handling herbicides and/or pesticides for a

minimum period of 15 years. The use of herbicides/pesticides was not significantly associated with the risk of PD, but a family history of PD (OR 14.05, 95% CI 2.98–91.38), use of chemical agents (methanol, toluene, cyanide, mercury, petroleum products; OR 5.87, 95% CI 1.48–27.23), and previous use of drugs with parkinsonism effects (OR 11.01, 95% CI 3.41–39.41) were significantly associated. Multivariate analysis indicated the factors being significantly associated with PD were previous use of drugs (X^2 27.58, p = 0.0001), family history of PD (X^2 4.4, p = 0.006) and use of chemical agents (X^2 10.19, p = 0.014), in order of decreasing importance. No significant risk was associated with rural residency, drinking well-water, herbicides and pesticides or head trauma. The authors stated that a possible reason why no association with pesticide exposure was noted could be the field study area, which had low agricultural productivity and, hence, few cases handling pesticides.

Preux *et al.* (2000) recruited cases (N = 140) from neurology inpatient and outpatient lists at the local hospital, and controls (N = 280) from other specialities in Limousin, France. Exposure to pesticides at work or during leisure time was assessed by an interview-based questionnaire. No significant association was found between PD risk and exposure to pesticides and/or herbicides, but a positive association was found with family history of PD (OR 10.1, 95% CI 2.9–35.0) and the consumption of tea (OR 1.7, 95% CI 1.1–2.9). Multivariate analysis showed that after adjusting for confounding variables neither association changed significantly. Although the measurement of exposure to environmental factors, such as period of stay in an urban area, length of stay on a farm and professional status, was similar to that used in other studies, the authors noted that this was not very accurate in this study but they could serve as surrogates.

Behari *et al.* (2001) recruited consecutive patients (N = 377) attending a Movement Disorder clinic in New Delhi, India; controls (N = 377), matched for age and sex, were outpatients attending neurology clinics at the same hospital for various non-parkinsonism-like disorders. A trained investigator collected information by face-to-face interview. No association was seen between PD risk and exposure to rodenticides and insecticides, and significantly more controls were exposed to herbicides than cases. Rural residency (<2500 people) for at least 1 year and farming as an occupation also showed no association. A family history of PD (OR 7.00, 95% CI 2.09–36.7) was significantly associated with PD, even after adjusting for other potential risk factors (OR 9.98, 95% CI 2.63–37.07), as was the consumption of well-water for more than 10 years (OR 1.94, 95% CI 1.33–2.80).

Herishanu *et al.* (2001) recruited consecutive urban patients (N = 93) attending an outpatient PD clinic in Beer-Sheva, Israel. Age- and sex-matched controls (N = 93) were recruited from outpatient dermatology, neurology and internal medicine clinics at the same hospital. Information was gathered via a questionnaire completed in the presence of one of the research team; it included details of occupational or household use of pesticides, herbicides, fungicides and other chemicals. PD patients showed a higher frequency of pesticide exposure than controls, although this was not significant. An association was also seen with working in the construction industry (OR 2.36, 95% CI 0.92–6.1). Multivariate analysis showed that this occupation was the strongest predictor of PD risk, followed by exposure to pesticides. This was a small study and only 6.5% of cases (1.1% of controls) were exposed to pesticides.

Kirkey *et al.* (2001) re-analysed the work of Gorell *et al.* (1998), where cases (N = 144) and controls (N = 464) were selected from patients receiving primary medical care from a health care provider in Detroit, USA. They examined the possible associations between PD and the spectrum of occupational and industrial categories defined by the US Department of Labor. The crude ORs suggested an increased risk for PD with ever working in the agricultural, fishery and forestry occupational group and in the corresponding industrial category. After adjustment for age, sex and race, ORs remained similar in magnitude and direction. The study was limited to an urban/suburban area so very few subjects had ever worked in an agricultural occupation or industry. The authors stated this might have reduced the statistical power of the study, prohibiting the observed difference from attaining statistical significance. The possibility of occupational miscoding was minimised by using an industrial hygienist, blinded to the subjects' case–control status, to check the coding.

Zorzon *et al.* (2002) recruited consecutive patients (N = 136) referred to the centre for PD and movement disorders of the neurology clinic of the local teaching hospital in Trieste, Italy. Matched controls (N = 272) were randomly selected from the same clinics and excluded if they exhibited PD or were cognitively impaired. A face-to-face interview was used to obtain information, including exposure to pesticides. Exposure to pesticides was significantly associated with the risk of PD and remained significant after adjusting for smoking. Rural living showed a similar pattern (unadjusted OR 1.9, 95% CI 1.1–3.5; adjusted OR 1.5, 95% CI 1.4–2.4), but the significance disappeared after adjustment for smoking, for well-water drinking (unadjusted OR 2.3, 95% CI 1.3–3.8) and rural birth (unadjusted OR 1.5, 95% CI 1.0–2.3). The mean length of exposure to pesticides was also significantly different between cases and controls. A family history of PD was strongly associated with PD (OR 34.8, 95% CI 10.5–115.2), as was previous exposure to general anaesthesia (OR 2.1, 95% CI 1.4–33). Multiple logistic regression showed that a family history of PD (OR 41.7, 95% CI 12.2–142.5) and/or essential tremor (OR 10.8, 95% CI 2.5–43.7) were the strongest predictors of PD. Farming as an occupation (OR 7.7, 95% CI 1.4–44.1) and well-water use (OR 2.0, 95% CI 1.1–3.6) were also positively and independently associated with PD. It is not clear whether pesticide exposure was a predictor of PD or not, or whether the variable was included in the modelling process.

Duzcan *et al.* (2003) selected prevalent cases (N = 36) from a small village in south-western Turkey, and matched controls (N = 36) from healthy members of the village. Exposure was assessed by questionnaire, and exposure to pesticides was defined as more than 20 days during a year for at least 10 years. A significant association was observed between pesticide exposure and PD risk. Those exposed to insecticides and fungicides were especially at an increased risk. Risk was also associated with a family history of PD, especially among first-degree relatives.

Conference abstracts

Four relevant additional case–control studies on PD and pesticide exposure, reported only in conference abstracts are summarised below.

Nelson *et al.* (2000) identified newly diagnosed (no criteria given) cases of PD (N = 496) through a medical care program in California, USA. Controls (N = 541) from the same population were randomly selected and frequency matched by age, sex and respondent type (self versus proxy) to the PD cases. Information on home pesticide exposure was collected by 'in-person' structured interview. Subjects who had ever been exposed to home or garden pesticides were at an increased risk of PD. After excluding pesticide use in the period immediately preceding diagnosis, both past low-dose and high-dose exposure were associated with PD in a dose-related manner. Past use of in-home insecticides was associated with PD, and past herbicide exposure was associated with a modestly increased risk of PD in the high-dose group, only. Garden insecticide and fungicide uses were not associated with PD. This study had a large sample size (cases 496, controls 541), but no diagnostic criteria were given and the meaning of 'lifetime history of exposure to home pesticides' was unclear.

Kamel *et al.* (2001) conducted a nested case–control study of PD in the US Agricultural Health Study, a cohort of licensed pesticide applicators and spouses in Iowa and North Carolina. Cases (N = 55) were individuals with self-reported PD. These cases, compared with the remaining cohort (N = 22 286), were more likely to have ever used insecticides, particularly organochlorines, and less likely to have used fungicides. Greater risk was seen among those using high-exposure methods to apply crop insecticides than among those using low-exposure methods. In addition the use of the pesticides paraquat, maneb, rotenone and dieldrin was more common among the cases. The small number of cases and possible self-misdiagnosis limited this study. However, the authors reported plans to increase the sample size, confirm the diagnosis of PD and use direct measurements of exposure (Personal Communication).

Firestone *et al.* (2002) recruited cases (N = 220) and controls (N = 352) from the local health insurance company in Washington State, USA. Exposure data were obtained by face-to-face interviews, using a structured questionnaire. Subjects identified specific brand-name products and

general pesticide use categories. No association was shown between PD risk for all specific pesticides and general pesticide use categories. Conversely, surrogate indicators for rural living suggested an increased risk from 'ever living on a farm' (OR 1.2, 95% CI 0.8–1.8), 'residence on a farm for more than 5 years during childhood' (OR 1.6, 95% CI 1.0–2.4), and 'well-water consumption' (OR 1.3, 95% CI 0.9–2.0). This study gave no indication of the criteria by which the cases and controls were selected. However, it was relatively large and work is ongoing (Personal Communication).

Vidal *et al.* (2002) recruited cases (N = 227) and matched controls (N = 562) through the French health insurance system for farmers and other agricultural workers. A neurologist confirmed PD diagnosis, although the criteria were not given, and controls were PD free. Pesticide exposure was classified into three groups—never used, used for gardening exclusively, and professionally used. Gardening and professional pesticide use were significantly associated with PD. The association was modified by the age at onset of PD. A positive association was observed for cases over 68 years who had used pesticides in the garden or professionally.

Autopsy studies

Three studies were identified that examined the levels of pesticides and their metabolites in the brains of deceased PD patients and compared them with various controls. Fleming *et al.* (1994) assayed organochlorine pesticides and their residues in post-mortem brain samples of deceased US patients with PD (N = 20), patients with Alzheimer's disease (N = 7) and non-neurological controls (N = 14). Of 16 pesticide residues screened only dieldrin and DDT and its metabolites, *p,p'*-DDE and *p,p'*-DDT, were detected. The majority of brain samples (39 of 41) had some *p,p'*-DDE; the levels were very low, the majority being less than 50 µg/kg. However, despite the small sample size dieldrin was detected in significantly more brains with a diagnosis of PD (6 of 20 compared with 0 of 21). A second study of brain samples by Corrigan *et al.* (1998) in the UK found significantly higher concentrations of dieldrin and PCB congener 153 in the PD tissue of brains. DDE, PCB congener 180, and total PCBs also tended to be higher in PD tissue. A later study by the same authors (Corrigan *et al*., 2000) found significantly higher concentrations of lindane and dieldrin in the brains of PD patients (N = 10) than those of Alzheimer disease patients or non-neurological controls (N = 6). In addition, non-significantly higher concentrations of *p,p'*-DDE and polychlorinated biphenyls were found in brains of PD patients than in those of non-neurological controls.

Table 4.2 Summary of case–control studies on pesticide exposure

Reference	Country	Time period	Cases				Controls			
			Source	Definition	Number	Age (years)	Source	Definition	Number	Matching
Ho *et al.* (1989)	Hong Kong	Not reported	Geriatric day hospital and residents of old people's home	Cardinal signs[a], exclusion criteria applied	35	Range: 65–87	As case	No disease specified	105	Age/sex and source of case
Tanner *et al.* (1989)	China	Not reported	Neurology clinic	≥ 2 Cardinal signs, exclusion criteria applied	100	Mean: 57.2 Range: 31–77	As case	Non-PD diagnosis	200	Age/sex/institution
Golbe *et al.* (1990)	USA	1986–88	Movement disorder clinic	None given, exclusion criteria applied	106	Not reported	Cases	Spouse	106	None
Hertzman *et al.* (1990)	Canada	1988	Physician lists	General accepted criteria	57	Range: 50–79	Regional electoral roll	None	122	Age/sex
Koller *et al.* (1990)	USA	Not reported	Movement disorder clinic	≥ 2 Cardinal signs, exclusion criteria applied	150	Mean: 66	Neurology and medical clinics	Controls were excluded if signs of parkinsonism, had exposure to neuroleptics, or had severe dementia	150	Age/sex
Zayed *et al.* (1990) (in French)	Canada	1987	GPs and neurology consultants	≥ 3 Cardinal signs, exclusion criteria applied	42	Mean: 57.8 SD: 11.1	Randomly from telephone directory	PD excluded	84	Age/sex
Stern *et al.* (1991)	USA	Not reported	Neurology clinic	None given, exclusion criteria applied YOPD OOPD	149	 <40 >60	Case nominations or hospital patient/volunteer	Non-parkinsonism	149	Similar sex/race/age
Wechsler *et al.* (1991)	USA	Not reported	Neurology clinic PD support groups	None given	34	Mean: 68.4	Neurology clinic	Non-parkinsonism	25	Yes
Wong *et al.* (1991)	USA	Not reported	Hospital PD centre	≥ 2 Cardinal signs, exclusion criteria applied	38	Mean: 68.4	Neurology and general medicine clinics Sibling pairs	Various diseases Familial essential tremor	38	Yes
Falope *et al.* (1992)	Nigeria	1987–88	Neurology clinic	≥ 3 Cardinal signs	50	Mean: 65.9	Medical clinics	Spectrum of diseases	50	Age/sex
Jiménez-Jiménez *et al.* (1992)	Spain	Not reported	Movement disorder clinic	None given	128	Mean: 66.8 SD: 9.1	Hospital emergency room	Non-neurological ailments	256	Age/sex
Semchuk *et al.* (1992)	Canada	1984–87	Hospital case register	≥ 2 Cardinal signs	130	Mean: 68.5 SD: 11.5	Community	Non-parkinsonism Non-demented	260	Age/sex

Reference	Country	Time period	Cases				Controls			
			Source	Definition	Number	Age (years)	Source	Definition	Number	Matching
Butterfield *et al.* (1993)	USA	Not reported	YOPD support groups	≥2 Cardinal signs, exclusion criteria applied	63	All <50	Catchment area of cases	Diagnosed with rheumatoid arthritis	68	Age/sex, year of birth and diagnosis frequency matched
Hubble *et al.* (1993)	USA	1990	Rural (PD outreach clinic)	≥ 2 Cardinal signs, exclusion criteria applied	31	Mean: 69 SD: 10.2	Rural (news media and senior citizens' lunch program)	Any signs of PD or other neurodegenerative disease excluded	45	Not reported
			Urban (PD clinic and PD support groups)		32		Urban (neurology and case contacts (not family))		31	
Hertzman *et al.* (1994)	Canada	Not reported	GPs, neurologists, and internal medicine clinics	≥ 2 Cardinal signs, exclusion criteria applied	127	Mean: 70.5	Electoral rolls, Physicians	Community cardiac disease patients	245	No
Morano *et al.* (1994)	Spain	1989–90	Hospital	Staging criteria (Hoehn & Yahr, 1998)	74	Mean: 65.4 SD: 1.1	Accident and Emergency, and Neurology outpatients	Non-neurological and functional CNS pathology	148	Not reported
Chaturvedi *et al.* (1995)	Canada	1991–92	Cohort of Canadians ≥ 65 years	Not reported	87	≥ 65	Same as cases	None given	2070	Not reported
Seidler *et al.* (1996)	Germany	1987–unknown	Neurology clinics	UK PD Brain Bank criteria	380	Mean: 52.5 SD: 6.6	Random addresses	Neighbourhood region	755	No
Liou *et al.* (1997)	Taiwan	1993–95	Movement disorder clinic	≥ 2 Cardinal signs	120	Mean: 58.3	Outpatient clinics	Neurology medical patients	240	Yes
Chan *et al.* (1998)	Hong Kong	Not reported	Accident and Emergency, Neurology outpatients	≥ 2 Cardinal signs, plus others and family history	215	<60–80+	Hospital	No disease given	313	Yes
Gorell *et al.* (1998)	USA	1991–95	Health care provider	Not given, exclusion criteria applied	144	>50	As case	No disease	464	Age/sex frequency
McCann *et al.* (1998)	Australia	Not reported	Hospital, residential care home, and community groups	≥ 2 Cardinal signs	224	Mean: 70.3 SD: 0.6	As case	No disease	310	Yes
Menegon *et al.* (1998)	Australia	Not reported	Community groups, hospital wards and outpatients	Cardinal signs, exclusion criteria applied	95	Mean: 72 SD: 9	As case	Healthy	95	Not reported
Smargiassi *et al.* (1998)	Italy	Not reported	Outpatients	UK PD Brain Bank criteria	86	Mean: 66.4 SD: 9.7	Hospital outpatients	Cardiology, nephrology, ophthalmology, and dermatology patients	86	Not reported

Reference	Country	Time period	Cases				Controls			
			Source	Definition	Number	Age (years)	Source	Definition	Number	Matching
Fall *et al.* (1999)	Sweden	1989	GP lists and prescription records	≥ 1 Cardinal sign, plus progression, exclusion criteria applied	113	Mean: 62.9 SD: 8.9	Regional population register	No disease	263	Not reported
Kuopio *et al.* (1999a)	Finland	1992	Community	UK PD Brain Bank criteria and no dementia	123	Mean: 68.7 SD: 8.9	Population register	None	246	Age/sex & municipality
Taylor *et al.* (1999)	USA	Not reported	Movement disorder clinic	≥ 2 Cardinal signs, L-dopa responsive, plus other signs	140	Range: 31–88	Cases	In-laws and friends	147	Yes
Dos Santos Werneck & Alvarenga (1999)	Brazil	1996–97	Neurology clinic	UK PD Brain Bank criteria	92	Mean: 70.6	Neurology clinic	Non-parkinsonism	110	Age/sex
Preux *et al.* (2000)	France	1995–96	Inpatient and outpatients	UK PD Society criteria	140	Mean: 71.1 SD: 7.5	As cases	Ophthalmology, ENT, endocrinology, rheumatology patients	280	Age/sex
Behari *et al.* (2001)	India	1994–98	Movement disorder clinic	≥ 2 Cardinal signs, plus progression, and responsive to L-dopa	377	Mean: 56.8 SD: 11.1	Neurology clinic	Non-parkinsonism	377	Age/sex
Herishanu *et al.* (2001)	Israel	1989–95	Neurology clinic	≥ 2 Cardinal signs, plus progression, and responsive to L-dopa	93	Not reported	Hospital outpatient	Spectrum of diseases	93	Age/sex
Kirkey *et al.* (2001)	USA	1991–95	Health care provider	Not reported	144	>50	As cases	Disease free	464	Age/sex frequency
Zorzon *et al.* (2002)	Italy	1998	PD Centre & movement disorder clinic	≥ 2 Cardinal signs, asymmetry, clinical progression, exclusion criteria applied	136	Mean: 70 SD: 9.2	Neurology clinic	Non-parkinsonism	272	Age/sex
Duzcan *et al.* (2003)	Turkey	2000	Village prevalence	≥ 2 Cardinal signs or advice of relatives of deceased	36	Not reported	As cases	Non-neurological	108	Age/sex

Reference	Country	Time period	Cases				Controls			
			Source	Definition	Number	Age (years)	Source	Definition	Number	Matching
Conference abstracts										
Nelson *et al.* (2000)	USA	Not reported	Medical care program	Not reported	496	Not reported	As cases	Healthy	541	Age/sex frequency matched
Kamel *et al.* (2001)	USA	Not reported	Cohort of licensed pesticide applicators	Self-reported	55	Not reported	As cases	Non-PD	22 286	Not reported
Firestone *et al.* (2002)	USA	Not reported	Not reported	Not reported	220	Not reported	As cases	Healthy	352	Not reported
Vidal *et al.* (2002)	France	Not reported	Insurance service	Neurologist confirmed	227	Not reported	As cases	PD free	562	Age/sex & place of residence
Autopsy studies										
Fleming *et al.* (1994)	USA	Not reported	Autopsy	Pathological criteria	20	Not reported	Autopsy	Non-neurological	14	Not reported
Corrigan *et al.* (1998)	UK	Not reported	Brain bank	UK PD Brain Bank	8	70–85	Brain bank	Non-neurological	7	Not reported
Corrigan *et al.* (2000)	UK	Not reported	Autopsy	UK PD Brain Bank	10	Not reported	Autopsy	Non-neurological	6	Not reported

CNS, central nervous system; ENT, ear, nose & throat; GP, General practitioner; OOPD, old-age onset Parkinson's disease; PD, Parkinson's disease; SD, standard deviation; YOPD, young onset Parkinson's disease
[a] Cardinal signs: tremor, rigidity, bradykinesia, and postural instability

Table 4.3 Findings from case–control studies on pesticide exposure

Reference	Exposure	Confounders	Pesticide analysis			
			Unadjusted odds ratio (95% confidence intervals)		Adjusted odds ratio (95% confidence intervals)	
Ho *et al.* (1989)	Pesticides/herbicides	Age, diet, farming, medical history, and rural living	Herbicides and pesticides	3.60 (1.00–12.90)		
Tanner *et al.* (1989)	Pesticides	Animal farming, crops, occupation, other chemicals, and rural living	No association, no data given			
Golbe *et al.* (1990)	Pesticides/herbicides	Diet, rural living, and well-water use	Pesticides	7.00 (5.80–8.50)		
Hertzman *et al.* (1990)	Pesticide	Age, occupation, orchard work, other chemicals and sex	Pesticides Paraquat	6.64 (1.17–30.7) p = 0.01 (Fisher's exact test)	Age/sex Age/smoking	6.62 (p = 0.03) 2.23 (p = 0.03)
Koller *et al.* (1990)	Pesticides/herbicides	Farming, rural living, and well-water use	Herbicides and pesticides	1.05 (0.66–1.70)		
Zayed *et al.* (1990)	Pesticides	Farming, other metals, resident near mine or industry, rural living, smoking, and well-water use	Pesticides <u>Duration of exposure</u> 1–10 yrs 11–20 yrs 21–30 yrs >30 yrs	1.23 (0.46–3.29) 0.81 (0.24–2.68) 1.08 (0.24–4.66) 1.08 (0.24–4.12) 1.92 (0.56–6.57)		
Stern *et al.* (1991)	Pesticides/herbicides	Age, education, head trauma, rural living, smoking, and well-water use	Pesticides overall Insecticides Herbicides	0.79 (0.53–1.18) 0.7 (0.3–1.4) 1.1 (0.7–1.7)	<u>Insecticides</u> Adjusted YOPD OOPD <u>Herbicides</u> Adjusted YOPD OOPD Adjusted for smoking, head trauma and rural living	 0.5 (0.2–1.1) 0.6 (0.2–1.7) 0.8 (0.3–2.1) 0.9 (0.6–1.5) 0.9 (0.5–1.7) 1.3 (0.7–2.4)
Wechsler *et al.* (1991)	Pesticides/herbicides (home use only)	Farming, occupation, and other metals	<u>Home use of:</u> Round-Up Kleenup Ortho Triox Pestkil	 4.04 (1.07–15.3) 4.62 (0.81–26.5) 5.00 (1.22–20.5) 7.50 (0.79–71.2)		
Wong *et al.* (1991)	Pesticides/herbicides	Age, farming, rural living, and well-water use	Herbicides and pesticides	1.00 (0.33–3.06)		
Falope *et al.* (1992)	Pesticides	Head trauma, medical history, and smoking	Pesticides	1.23 (0.25–4.34)		

Reference	Exposure	Confounders	Pesticide analysis	
			Unadjusted odds ratio (95% confidence intervals)	Adjusted odds ratio (95% confidence intervals)
Jiménez-Jiménez *et al.* (1992)	Pesticides	Rural living, and well-water use	Pesticides 1.34 (0.85–2.12) Duration of exposure <20 yrs 1.18 (0.69–2.02) >20 yrs 1.75 (0.86–3.55)	
Semchuk *et al.* (1992)	Pesticides/herbicides	Farming	Pesticides 2.25 (1.27–3.99) Herbicides 3.06 (1.34–7.00) Insecticides 2.05 (1.03–4.07) Fungicides 1.63 (0.81–3.29) Age exposed: Pesticide / Herbicide / Insecticide 16–25: 1.41 (0.73–2.73) / 1.40 (0.46–4.30) / 1.49 (0.58–3.81) 26–35: 2.27 (1.08–4.76) / 4.82 (1.51–15.35) / 2.33 (0.78–6.94) 36–45: 2.21 (0.99–4.94) / 3.84 (1.16–12.70) / 1.75 (0.63–4.83) 46–55: 2.07 (0.91–4.72) / 4.88 (1.28–18.60) / 3.50 (1.03–11.96)	Herbicide use adjusted for: Insecticide use 2.60 (1.07–6.32) Fungicide use 3.72 (1.43–9.65) Ins. & fung. use 3.22 (1.19–8.74) Agricultural work 2.48 (1.03–5.94) Ins/fung use & agric work 2.91 (1.06–8.01)
Butterfield *et al.* (1993)	Pesticide/herbicide	Age, diet, education, ethnicity, family history, rural living and smoking	Herbicide 3.46 (1.33–10.1) Insecticide 4.04 (1.68–10.6)	Herbicides 3.22 (2.51–4.12) Insecticides 5.75 (p <0.001) Adjusted for age, age at diagnosis, race sex, education and family history of PD
Hubble *et al.* (1993)	Pesticide/herbicide	Age, family history, farming, history of depression, medical history, rural living, smoking, and well-water use	Pesticides 4.97 (2.22–11.1)	Pesticides 3.42 (1.27–7.32) Adjusted for family history, ethnicity, history of CNS infection, head trauma, depression, age <65 years and diet
Hertzman *et al.* (1994)	Pesticide/herbicide	Age, farming, occupation, other chemicals, smoking, and well-water use	Pesticides (men/women overall) 1.75 (0.95–3.23) Cases vs chronic disease controls Men 2.03 (1.0–4.12) Women 1.11 (0.32–3.80) Cases vs community controls Men 2.32 (1.10–4.88) paraquat 1.25 (0.34–4.63) insecticide 0.33 (0.12–0.90) herbicide 1.19 (0.57–2.45) fungicide 0.52 (0.25–1.08) acaricide 0.61 (0.15–2.39) Women 1.36 (0.48–3.85)	
Morano *et al.* (1994)	Pesticide	Alcohol, head trauma, medical history, rural living, smoking, and well-water use	Pesticides 1.73 (0.98–3.02)	
Chaturvedi *et al.* (1995)	Pesticide/herbicide	Occupation, and other chemicals	Pesticide/fertiliser 1.81 (0.92–3.36) Defoliant/fumigant 1.40 (No CIs)	

Reference	Exposure	Confounders	Pesticide analysis	
			Unadjusted odds ratio (95% confidence intervals)	**Adjusted odds ratio (95% confidence intervals)**
Seidler *et al.* (1996)	Pesticide/herbicide	Farming, occupation, other chemicals, smoking, and well-water consumption	Overall 2.06 (1.62–2.62) <u>Cases vs neighbourhood controls</u> herbicide 1.56 (1.12–2.18) insecticide 1.34 (0.94–1.90) organochlorines 1.59 (1.11–2.26) alkaline phosphates & carbamates 1.54 (1.12–2.11) <u>Cases vs regional controls</u> herbicide 1.97 (1.46–2.79) insecticide 1.77 (1.28–2.43) organochlorines 2.31 (1.57–3.40) alkaline phosphates & carbamates 2.21 (1.57–3.09)	<u>Cases vs neighbourhood controls</u> *Dose/yrs* *Herbicides* *Insecticides* Never 1.0 1.0 1–40 1.7 (1.0–2.7) 1.4 (0.9–2.1) 41–80 1.4 (0.8–2.5) 1.5 (0.9–2.5) >80 2.2 (0.9–5.2) 1.6 (0.1–3.4) <u>Cases vs regional controls</u> *Dose/yrs* *Herbicides* *Insecticides* Never 1.0 1.0 1–40 1.7 (1.0–2.0) 1.8 (1.1–2.7) 41–80 3.0 (1.5–6.0) 2.5 (1.4–4.5) >80 2.4 (1.0–6.0)* 2.1 (0.9–4.8)* Adjusted for education and smoking * Significant trend
Liou *et al.* (1997)	Pesticide/herbicide	Alcohol, farming, other chemicals, rural living, smoking, and well-water consumption	Pesticides and herbicides 2.89 (2.28–3.66) Paraquat 3.22 (2.41–4.31) <u>Duration (years)</u> 0 1.00 1–19 1.48 (0.64–3.43) >20 4.50 (2.33–8.98)	<u>Duration (years)</u> <u>Adjusted</u> 0 1.00 1–19 1.41 (0.52–3.85) ≥ 20 6.72 (2.62–17.21) Adjusted for duration of rural living, farming, well-water consumption and smoking
Chan *et al.* (1998)	Pesticide	Age, diet, family history, farming, rural living, sex, and well-water consumption	Overall 1.80 (0.90–3.58) Men 0.68 (0.25–1.88) Women 6.84 (1.90–24.7) Duration (number of years exposed, not specified) Univariate 1.05 (1.01–1.09)	Duration (number of years exposed) Adjusted 1.05 (0.99–1.11) Adjusted for smoking, family history, rural living and diet
Gorell *et al.* (1998)	Pesticide/herbicide	Farming, rural living, and well-water consumption	<u>All occupations</u> Herbicides 3.31 (1.14–9.61) Insecticides 3.11 (1.56–6.15) Fungicides 1.44 (0.44–4.75) <u>Living or working on a farm</u> Herbicides 1.76 (0.77–4.04) Insecticides 1.40 (0.76–2.59) Fungicides 1.19 (0.37–2.79) <u>Gardening as a hobby</u> Herbicides 1.31 (0.81–2.12)	<u>All occupations</u> Herbicides 4.10 (1.37–12.24) Insecticides 3.55 (1.75–7.18) Fungicides 1.60 (0.47–5.45) <u>Living or working on a farm</u> Herbicides 1.64 (0.70–3.82) Insecticides 1.28 (0.69–2.40) Fungicides 0.96 (0.29–3.12) <u>Gardening as a hobby</u> Herbicides 1.39 (0.84–2.28) Adjusted for age, race, sex and smoking

Reference	Exposure	Confounders	Pesticide analysis	
			Unadjusted odds ratio (95% confidence intervals)	Adjusted odds ratio (95% confidence intervals)
McCann *et al.* (1998)	Pesticide/herbicide	Family history, head trauma, history of depression, stroke of hypertension, rural living, smoking, and well-water use	Herbicides and pesticides 1.20 (0.80–1.50)	
Menegon *et al.* (1998)	Pesticide	Genetics		Pesticides 2.3 (1.2–4.4) Adjusted for age, sex and family history
Smargiassi *et al.* (1998)	Pesticide/herbicide	Alcohol, head trauma, other chemicals, rural living, smoking, and well-water use	Herbicides and pesticides 1.15 (0.56–2.36)	
Fall *et al.* (1999)	Pesticide/herbicide	Diet, occupation, and smoking	Agriculture as occupation 1.4 (0.68–2.9) Pesticide handling within agriculture 1.9 (0.46–7.3) Insecticide handling within agriculture 2.2 (0.48–9.0) Handling pesticides within any occupation 2.80 (0.89–8.70)	
Kuopio *et al.* (1999a)	Pesticides/herbicides	Age, animal exposure, education, farming, head trauma, infections, mercury containing chemicals, rural living, smoking, and well-water consumption	<u>Pesticide use</u> Overall 1.02 (0.63–1.65) Regular use 0.65 (0.33–1.29) Occasional use 1.23 (0.74–2.04) <u>Herbicide use</u> Overall 1.40 (0.79–2.48) Regular use 0.79 (0.38–1.66) Occasional use 1.71 (0.90–3.23) DDT 1.04 (0.68–1.60)	
Taylor *et al.* (1999)	Pesticides/herbicides	Education, family history, farming, head trauma, history of depression, rural living, smoking, and well-water consumption	None presented	Pesticides 1.04 (0.93–1.17) Herbicides 1.03 (0.76–1.40) Adjusted for birth cohort and sex Pesticides 1.02 (0.90–1.17) Herbicides 1.06 (0.68–1.65) Adjusted for birth cohort, sex, head trauma, family history, depression, education, rural living, well-water and smoking
Dos Santos Werneck & Alvarenga (1999)	Pesticides/herbicides	Drug use, family history, head trauma, other chemicals, rural living, smoking, and well-water consumption	Herbicides and insecticides 2.49 (0.53–13.14)	
Preux *et al.* (2000)	Pesticides/herbicides	Family history, farming, rural living, smoking, and well-water consumption	Herbicides and pesticides 1.34 (0.85–2.10)	

Reference	Exposure	Confounders	Pesticide analysis		
			Unadjusted odds ratio (95% confidence intervals)		Adjusted odds ratio (95% confidence intervals)
Behari *et al.* (2001)	Pesticide/herbicide	Alcohol, diet, ethnicity, family history, farming, prior depression, rural living, sex, smoking, and well-water consumption	Insecticide Herbicide Rodenticide	0.73 (0.45–1.17) 0.50 (0.28–0.88) 0.88 (0.47–1.63)	
Herishanu *et al.* (2001)	Pesticide	Country of birth, health status, occupation, and smoking	Pesticides	6.34 (0.75–53.8)	
Kirkey *et al.* (2001)	Agricultural work	Age, and occupation	Agriculture, fishery, forestry (Dictionary of occupational titles) 1.74 (0.85–3.60) Agriculture, fishery, forestry (Standard Industrial Classification) 1.75 (0.76–4.02)		
Zorzon *et al.* (2002)	Pesticides	Family history, farming, head trauma, rural living, smoking, and well-water consumption	Pesticides	1.96 (1.09–3.52)	Pesticides 1.6 (1.0–2.4) Adjusted for smoking
Duzcan *et al.* (2003)	Pesticide	Age, alcohol, education, family history, head trauma, occupation, and smoking	Pesticides Insecticides and fungicides Insecticides only	2.96 (1.31–6.69) 4.52 (1.83–11.2) Fisher's exact not significant	
Conference abstracts					
Nelson *et al.* (2000)	Home or garden pesticides	None reported	Overall low dose high dose Insecticides low dose high dose Herbicides high dose	1.9 (1.3–2.9) 3.1 (1.8–5.2) 4.5 (2.4–8.8) 2.1 (1.4–3.2) 2.6 (1.4–4.8) 1.9 (1.05–3.4)	
Kamel *et al.* (2001)	Pesticides	Not given	Insecticides Organochlorines Fungicides high exposure methods low exposure methods Paraquat Maneb Rotenone Dieldrin	1.6 (0.5–5.2) 1.8 (0.9–3.2) 0.4 (0.2–0.8) 1.5 (0.6–3.4) 1.1 (0.5–2.7) 1.5 (0.7–3.0) 1.6 (0.7–4.1) 1.2 (0.4–3.4) 1.8 (0.9–3.6)	
Firestone *et al.* (2002)	Residential pesticide use	Farming, rural living, smoking and well-water consumption	No effect of specific pesticide or general pesticide use		

Reference	Exposure	Confounders	Pesticide analysis	
			Unadjusted odds ratio (95% confidence intervals)	Adjusted odds ratio (95% confidence intervals)
Vidal *et al.* (2002)	Pesticides	Age of PD onset, rural living, and smoking	Professional use 1.7 (1.1–2.8) Garden use 1.5 (0.9–2.5) Age onset / Garden use / Professional use <62 1.2 (0.5–2.7) 1.3 (0.6–2.8) 62–68 1.2 (0.5–2.6) 1.6 (0.7–3.9) >68 3.0 (1.1–7.7) 2.5 (1.0–6.0)	
Autopsy studies				
Fleming *et al.* (1994)	Pesticides	Head trauma and occupation	Dieldrin p = 0.03 (Fisher's exact)	
Corrigan *et al.* (1998)	Pesticides		Dieldrin p = 0.005 (Mann-Whitney U test) PCB congener 153 p <0.05 (Mann-Whitney U test)	
Corrigan *et al.* (2000)	Pesticides		Lindane PD vs AD p <0.003 (Mann-Whitney U test) PD vs NN p <0.04 Dieldrin PD vs AD p <0.03 PD vs NN p <0.04	

AD, Alzheimer's disease; CI, confidence interval; CNS, central nervous system; NN, non-neurological controls; OOPD, old-age onset Parkinson's disease; PCB, polychlorinated biphenyl; PD, Parkinson's disease, YOPD, young onset Parkinson's disease; yrs, years
* Significant trend

4.4.3 Case–control studies on other risk factors

A number of other case–control studies of PD patients examined PD risk in relation to other risk factors related to pesticide exposure. Five investigated the association between farming and PD with variable results. One found a negative association (OR 0.6, 95% CI 0.3–1.3; Rocca *et al.,* 1996), two found no association (Semchuk *et al*., 1991; Tsui *et al*., 1999) and two found positive associations (Tanner *et al*., 1990; Marder *et al*., 1998). There were also varying results for the association of PD and rural living: three studies showed no increased risk (Semchuk *et al*., 1991; Wang *et al*., 1993; Vieregge *et al*., 1994) and two showed a positive risk (Vieregge *et al*., 1992; Marder *et al*., 1998).

Similarly, studies of well-water consumption showed contrasting results: three had no association (Tanner *et al*., 1990; Semchuk *et al*., 1991; Vieregge *et al*., 1994) and three had a positive association (De Michele *et al*., 1996; Marder *et al*., 1998; Tsai *et al*., 2002). An increase in PD risk has been associated with drinking river water (Wang *et al*., 1993).

Other factors that have been found to be associated with an increased risk of PD include: a family history of the disease (Semchuk *et al*., 1993; Wang *et al*., 1993; De Michele *et al*., 1996), which is suggestive of some genetic role in the disease; a history of head trauma, requiring hospital admission (Factor & Weiner, 1991; Stern *et al*., 1991; De Michele *et al*., 1996; Seidler *et al*., 1996; Taylor *et al*., 1999; Tsai *et al*., 2002); exposure to heavy metals (Zayed *et al*., 1990; Seidler *et al*., 1996; Gorell *et al*., 1997; Gorell *et al*., 1999b; Rybicki *et al*., 1999); and diet (including total fat, cholesterol, lutein and iron intake; Johnson *et al.,* 1999).

4.5 Meta-analyses

Two meta-analysis studies were identified. The first (Priyadarshi *et al*., 2000) was performed on 19 case–control studies published between 1989 and 1999. Articles were excluded for any of the following reasons: (i) they were not in English; (ii) they did not include pesticide as a risk factor; (iii) there was duplication of studies with the same cohort; (iv) insufficient data were published to allow an estimate of relative risk or a confidence interval to be determined; and (v) the disease studied was not specifically designated as PD. Table 4.4 summarises the findings of the studies included in this analysis.

Significant heterogeneity was detected among the studies and the combined OR was calculated using the random effect model. The combined OR for PD risk and pesticide exposure was 1.94 (95% CI 1.49–2.53). One possible source of heterogeneity, the geographical location of the study, was examined by analysing the data with regards to this variable. The combined estimate for the studies conducted in the USA (N = 7) was 2.15 (95% CI 1.14–4.05). The combined estimate for the studies in Asia (N = 3) was 2.53 (95% CI 1.58–4.05). The combined estimate for the European studies (N = 5) was 1.76 (95% CI 1.41–2.21). The combined estimate for the studies carried out in Canada (N = 3) was 1.94 (85% CI 1.37–2.76). The significant finding for each place of study supports an association between pesticide exposure and PD, and excludes the possibility of bias due to place of study. However, the authors included a study from Australia in the calculation of the combined OR for Europe, without explanation.

The majority of the ORs used in the analysis were the crude OR and related to general pesticide exposure. However, two ORs were adjusted; one was adjusted for age at diagnosis, race, sex, education and family history (Butterfield *et al*., 1993), and the other was adjusted for family history, ethnicity, history of central nervous system infection, head trauma, depression, age less than 65 years and diet (Hubble *et al*., 1993). A third OR used was for herbicides only (Gorell *et al*., 1998). In a final study, it was not clear how the OR used in the meta-analysis was derived from the original data (Liou *et al*., 1997). The association between duration of pesticide exposure and risk of PD was examined in some studies. Most of these showed a positive correlation, although no significant relationships were

reported. However, no study evaluated the dose–response relationship with respect to cumulative exposure to pesticides.

Table 4.4 Summary of findings of Parkinson's disease risk and pesticide exposure in the meta-analysis of Priyadarshi *et al.* (2000)

Reference	Number of cases/controls	Odds ratio	95% Confidence intervals
Studies included in meta-analysis:			
Ho *et al.* (1989)	35/105	3.6	1.0–12.9
Golbe *et al.* (1990)	106/106	7.0	5.8–8.5
Koller *et al.* (1990)	150/150	1.1	0.94–1.3
Stern *et al*. (1991)	149/149	0.79	0.53–1.18
Wong *et al.* (1991)	38/38	1.0	0.73–1.4
Jiménez-Jiménez *et al*. (1992)	128/256	1.34	0.85–2.12
Semchuk *et al.* (1992)	130/260	2.25	1.27–3.99
Butterfield *et al.* (1993)	63/68	3.22	2.51–4.12
Hubble *et al*. (1993)	32/44	3.42	1.27–7.32
Hertzman *et al*. (1994)	127/245	1.75	0.95–3.23
Morano *et al.* (1994)	74/148	1.73	0.98–3.02
Chaturvedi *et al*. (1995)	87/2070	1.81	0.92–3.36
Seidler *et al.* (1996)	380/755	2.06	1.62–2.62
Liou *et al.* (1997)	120/240	3.32	1.59–6.94
Chan *et al*. (1998)	215/313	1.80	0.9–3.58
Gorell *et al*. (1998)	144/464	4.10	1.37–12.24
McCann *et al*. (1998)	224/310	1.2	0.8–1.5
Smargiassi *et al*. (1998)	86/86	1.15	0.56–2.36
Fall *et al*. (1999)	113/263	2.8	0.89–8.7

Adapted from Priyadarshi *et al*. (2000)

The second meta-analysis by the same authors (Priyadarshi *et al*., 2001) examined the association between PD and environmental factors such as rural living, well-water use, farming, exposure to farm animals or living on a farm, and pesticide exposure. Table 4.5 summarises the findings of the studies included in the analysis.

Sixteen case–control studies that examined rural living as a risk factor yielded a combined OR of 1.56 (95% CI 1.17–2.07) and an estimate for studies conducted in the USA of 2.17 (95% CI 1.54–3.06). Some of the studies reviewed demonstrated a dose–response relationship with duration of exposure (Ho *et al*., 1989; Liou *et al*., 1997). In one study this relationship was also seen up to age 20 after which the ORs decreased (Semchuk *et al*., 1991). In another study the relationship was found to be negative (Stern *et al*., 1991).

Eighteen case–control studies examining the association between exposure to well-water and PD were identified. The random effect model including all studies yielded a combined OR of 1.26 (95% CI 0.96–1.64). The combined estimate for studies conducted in the USA was 1.44 (95% CI 0.92–2.24). No exposure–response relationship was observed between duration of well-water consumption and the risk of PD.

Table 4.5 Summary of findings (odds ratios, 95% confidence intervals) of Parkinson's disease risk and exposure to pesticides, rural living, farming and well-water consumption

Reference	Risk factor OR (95% Confidence intervals)								
	Number of cases/ controls	Farming		Pesticide exposure		Rural living		Well-water	
Studies included in meta-analysis:									
Ho *et al.* (1989)	35/105	5.2	(1.6–17.7)	3.6	(1.0–12.9)	4.9	(1.4–18.2)	-	
Tanner *et al.* (1989)	100/200	-		-		0.57	(0.33–0.98)	0.74	(0.41–1.32)
Golbe *et al.* (1990)	106/106	1.3	(1.1–1.6)	7.0	(5.8–8.5)	2.0	(1.7–2.4)	1.1	(0.91–1.33)
Koller *et al.* (1990)	150/150	1.3	(1.1–1.53)	1.1	(0.94–1.3)	1.9	(1.61–2.22)	1.7	(1.45–2.0)
Semchuk *et al.* (1991)	130/260	0.90	(0.58–1.37)	-		0.78	(0.51–1.21)	1.07	(0.57–2.02)
Stern *et al.* (1991)	149/149	-		0.79	(0.53–1.18)	1.7	(0.9–3.1)	0.8	(0.4–1.6)
Wechsler *et al*. (1991)	34/25	3.1	(0.3–35.2)	-		-		1.27	(0.44–3.63)
Wong *et al.* (1991)	38/38	2.7	(1.96–3.7)	1.0	(0.73–1.4)	4.3	(3.13–5.91)	2.8	(2.04–3.85)
Jiménez-Jiménez *et al.* (1992)	128/256	-		1.34	(0.85–2.12)	1.1	(0.7–1.63)	1.22	(0.77–1.94)
Butterfield *et al*. (1993)	63/68	-		3.22	(2.51–4.12)	2.72	(2.12–3.48)	-	
Wang *et al*. (1993)	93/186	-		-		0.76	(0.49–1.18)	0.59	(0.36–0.95)
Hertzman *et al*. (1994)	127/245	1.16	(0.62–2.18)	1.75	(0.95–3.23)	-		0.89	(0.48–1.66)
Morano *et al.* (1994)	74/148	-		1.73	(0.98–3.02)	2.5	(1.41–4.42)	2.77	(1.51–5.08)
Martyn & Osmond (1995)	172/343	-		-		1.4	(0.82–2.49)[a]	-	
De Michele *et al.* (1996)	100/200	-		-		-		2.35	(1.32–4.18)
Rocca *et al*. (1996)	62/124	0.6	(0.3–1.3)	-		-		-	
Liou *et al.* (1997)	120/240	1.81	(1.25–2.64)	3.32	(1.59–6.94)	2.04	(1.23–3.38)	1.07	(0.19–5.98)
Chan *et al.* (1998)	215/313	0.92	(0.59–1.43)	1.80	(0.90–3.58)	1.0	(0.995–1.01)	1.04	(0.70–1.54)
Gorell *et al.* (1998)	144/464	1.30	(0.88–1.93)	4.10	(1.37–12.24)	1.19	(0.73–1.93)	0.97	(0.65–1.40)
Marder *et al.* (1998)	89/188	-		-		-		15.3	(1.0–224.8)
McCann *et al.* (1998)	224/310	-		1.2	(0.8–1.5)	1.7	(1.17–2.57)	0.6	(0.38–0.92)
Smargiassi *et al.* (1998)	86/86	1.25	(0.65–2.43)	1.15	(0.56–2.36)	-		2.78	(1.46-5.28)
Studies reviewed but not included in meta-analysis:									
Zayed *et al.* (1990)	42/84	0.65	(0.25–1.69)	1.23	(1.46–3.29)	0.31	(0.11–0.91)	1.40	(0.48–4.02)
Kuopio *et al.* (1999a)	123/246	1.45	(0.88–2.41)	1.02	(0.63–1.65)			1.48	(0.44–4.95)
Taylor *et al.* (1999)	140/147					1.01	(0.98–1.03)	0.96	(0.96–0.99)
Dos Santos Werneck & Alvarenga (1999)	92/110			2.49	(0.53–13.14)	1.00	(0.52–1.95)	1.49	(0.74–3.01)
Preux *et al.* (2000)	140/280	1.06	(0.71–1.59)	1.34	(0.85–2.10)	1.38	(0.89–2.13)	1.19	(0.77–1.84)
Behari *et al.* (2001)	377/377	1.35	(0.91–2.02)	0.50	(0.28–0.88)	0.88	(0.66–1.18)	1.20	(0.88–1.64)
Kirkey *et al.* (2001)	144/464	1.74	(0.85–3.60)						
Firestone *et al.* (2002)	220/352	1.2	(0.8–1.8)					1.3	(0.9–2.0)
Zorzon *et al.* (2002)	136/272	5.2	(0.9–2.69)	1.96	(1.09–3.52)	1.9	(1.1–3.5)	2.3	(1.3–3.8)
Duzcan *et al.* (2003)	36/108	1.69	(0.78–3.65)						

Adapted from Priyadarshi *et al.* (2001)
[a] Reported for African-Americans only
-, not investigated as a risk factor

Twelve studies examined the association between PD and exposure to farming, living on a farm or exposure to farm animals. Analysis yielded a combined OR of 1.42 (95% CI 1.05–1.91). The combined OR estimate for the US studies was 1.72 (95% CI 1.20–2.46). Studies that examined

duration of exposure to farming and risk of getting PD were inconsistent. Fourteen of the studies that examined these environmental factors also explored the relationship between PD risk and pesticide exposure. All of these were included in the first meta-analysis. The combined OR was 1.85 (95% CI 1.31–2.00).

Significant heterogeneity among the studies was found in both meta-analyses. Stratifying the analysis by place of study (one possible source of the heterogeneity) showed combined ORs for each country of origin to be similar to the total combined OR. The authors believed that publication bias due to preferential publication of large studies with positive findings did not appear to have occurred, because the rank correlation test of the natural logarithm of the OR versus the inverse of the standard error revealed no relationship, and the funnel plot of these two variables was inverted.

4.6 Discussion of epidemiological findings

There are a number of general design issues that need consideration when interpreting the results of the epidemiological studies. These include case ascertainment, control selection, case definition (diagnostic and exclusion criteria), assessment of pesticide exposure and exposure to other risk factors, statistical analysis and study size.

4.6.1 Case ascertainment

For case–control studies it is important to identify as many cases as possible so that the power of the study is sufficient and the conclusions can be generalised to future populations. To this end, it may be necessary to access a variety of sources including hospital lists, residential care homes, and disease registries to obtain a representative number of cases. Very frequently, case series for case–control studies are selected from one or more hospitals, rather than from a community. For example, neurology outpatient departments and inpatient patient lists (and movement disorder clinics) have been used in several of the studies reviewed herein. In general, this is appropriate if it can be established that a very high proportion of those developing the disease, PD in this case, will come into hospital for diagnosis and treatment. However, patients not under follow-up include both those with particularly bad outcomes (some may have died or been admitted elsewhere, or were not able to access the hospital easily) and, sometimes, those with particularly good outcomes who need not return for further care. Other sources used have included lists of patients receiving anti-PD drugs, residential care centres, old people's homes, community groups, support groups, door-to-door surveys, etc. However, it is not known whether hospital-based cases of PD differ substantially from those in the community.

Ideally, cases should be newly incident rather than prevalent (e.g. all cases being currently seen in a clinic or existing in a community) PD cases, because the latter excludes those subjects who developed the disease and then left the area, or died.

4.6.2 Study size and power

The size of the available studies varies considerably. Power calculations were given in only two case–control studies, and the sample size used was sufficient to detect a difference in one of the studies (Seidler *et al*., 1996) but not the other (Kirkey *et al*., 2001). A third study had enough power to detect an overall difference, but not in relation to sub-classifications of pesticides (Semchuk *et al*., 1992). No power calculation was reported in any of the other studies. An inspection of the confidence intervals for the ORs can assist in assessing whether the power of the study was adequate. A wide confidence interval suggests poor power and can result from a small sample and/or data with substantial variability.

The sample size needed for an unmatched case–control study to detect an OR of 2.0 with a significance level of 0.05 and 90% power, assuming an estimated exposure rate (proportion exposed) among the controls of 0.10 would be just under 400 for each group (cases and controls). For ORs

higher than 2.0 the sample size needed would be smaller and for an increased proportion exposed among the controls the sample size required would be larger. In matched case–control studies the power and sample size depend on the expected number of discordant pairs (i.e. pairs in which the case and control have different exposures; Schlesselman, 1982). In several unmatched studies (e.g. Stern *et al.*, 1991; Morano *et al.*, 1994) the proportion of controls exposed was near or above 0.50 requiring a much larger sample size than was actually used to detect an OR of 2.0. Both Stern and colleagues and Morano and colleagues found ORs of less than 2.0 for exposure to pesticides. In addition, the ORs for most of the studies were less than 2.0 with various proportions of exposed controls, giving powers of much less than 90%, many estimated to be as low as 10%.

Multiple comparisons were carried out in all the case–control studies, therefore, some of the associations observed may have occurred by chance alone. Only a few studies adjusted PD risk from pesticide exposure for other factors or carried out multivariate logistic regression of the data. Some of the studies that did undertake multivariate analysis did not include pesticide exposure in the predictive models (Wong *et al*., 1991; McCann *et al*., 1998; Preux *et al*., 2000; Behari *et al*., 2001; Zorzon *et al*., 2002). A few studies found pesticide exposure not to be a significant risk factor after adjustment for confounding (Stern *et al*., 1991; Chan *et al*., 1998; Taylor *et al*., 1999; dos Santos Werneck & Alvarenga, 1999). Nevertheless, nine case–control studies showed pesticide exposure to be a significant risk factor after adjustment (Hertzman *et al*., 1990; Semchuk *et al*., 1992; Butterfield *et al*., 1993; Hubble *et al*., 1993; Seidler *et al*., 1996; Liou *et al*., 1997; Gorell *et al*., 1998; Menegon *et al*., 1998; Zorzon *et al*., 2002). These studies were not consistent in the variables used to adjust risk and some did not include the other risk factors related to pesticide exposure (e.g. rural living, well-water consumption, farming). In addition, although these nine studies found pesticide exposure to be a significant predictor for PD, other factors, including a family history of PD, well-water consumption, rural living and occupation, were also significantly associated with PD, independent of pesticide exposure.

The findings of a number of these case–control studies were combined into a meta-analysis, which showed a significant association between PD risk and pesticide exposure (Priyadarshi *et al*., 2000). This association was not related to study location, as similar findings were observed in the USA, Canada, Europe and Asia. However, significant heterogeneity remained between the studies; this could be because of the different inclusion/exclusion criteria used to define a case or the various methods used to assess pesticide exposure. An additional 19 studies to those examined by the meta-analysis were identified for this review (see Tables 4.2, 4.3). Of these 19, only one showed a decreased association between pesticide exposure (specifically herbicides) and the risk of PD (Behari *et al*., 2001) and two showed no association (Tanner *et al*., 1989; Firestone *et al*., 2002). The remainder showed an increased risk of PD with pesticide exposure.

4.6.3 Control selection

The designation of the type, number, and size of the control group or groups, and the selection of the specific control subjects are perhaps the most important and most difficult tasks in planning a case–control study (IARC, 1980). In designing a case–control study the aim is to select controls that give a representative picture of relevant exposure in the population at risk of becoming cases. For example, controls could be matched to the cases for age and sex and, if hospital cases are used, then time of hospitalisation. They should be selected independently of their exposure status; that is they should be representative of the same population (the source population or study base) with respect to exposure of the cases, which would minimise selection bias. As mentioned above, in some studies, hospital outpatient lists were used to select cases but controls were from other sources. For example, Jiménez-Jiménez *et al.* (1992) used the hospital emergency room as their source of controls; Semchuk *et al.* (1992) randomly selected controls from the general population; Golbe *et al.* (1990) used the spouses of their cases; Hertzman *et al.* (1990; 1994) used electoral rolls; Stern *et al.* (1991) used subjects suggested by their cases; Seidler *et al.* (1996) randomly selected addresses in the study area; and Taylor *et al.* (1999) used friends and relatives of the cases. In the study by Jiménez-Jiménez *et al.*

(1992), the Movement Disorder clinic was used as a referral centre for the entire region surrounding Madrid, whereas the emergency room would primarily serve only the city.

It is often acceptable to select controls from the general population, although they may not be as motivated to take part in a study and response rates may, therefore, be poorer among controls than cases. The use of neighbourhood controls may ensure that cases and controls are from similar social backgrounds; however, this could lead to over-matching and result in the exposure prevalence in the controls becoming similar to that of the cases. This would have the effect of driving the estimate of risk towards the null.

4.6.4 Case definition

Clinical diagnostic criteria for Parkinson's disease

There are no biological markers for the ante-mortem diagnosis of degenerative parkinsonian disorders, and diagnosis currently relies upon the presence and progression of clinical features. The confirmation of diagnosis depends on neuropathology. Clinicopathological studies have shown significant false-positive and false-negative rates for diagnosing these disorders, especially PD itself (Litvan *et al*., 2003). Misdiagnosis is especially common during the early stages of the disease, even among movement disorder specialists (Rajput *et al*., 1991; Litvan *et al*., 1996). The Movement Disorder Society Scientific Issues Committee suggested that this limitation could strongly affect epidemiological studies and clinical trials (Litvan *et al*., 2003), by classifying cases when they should not be so classified, which would affect the power of the study and the size of the risk estimate. Ideally, for every disease, there should be a set of widely accepted diagnostic criteria, including well-established reference standard tests that may be applied and reproduced in a blinded manner (Litvan *et al*., 2003).

Several sets of clinical diagnostic criteria for PD have been proposed (Gibb & Lees, 1988; Ward & Gibb, 1990; Hughes *et al*., 1992b; Gelb *et al*., 1999) and this is reflected in the variety of criteria used in the case–control studies reviewed. Several did not give the diagnostic criteria used to define cases, simply stating it was confirmed by a neurologist (Golbe *et al*., 1990; Wechsler *et al*., 1991; Stern *et al*., 1991; Jiménez-Jiménez *et al*., 1992; Butterfield *et al*., 1993; Hertzman *et al*., 1994; Gorell *et al*., 1998; Chan *et al*., 1998; Nelson *et al*., 2000; Kirkey *et al*., 2001; Firestone *et al*., 2002). The majority of studies (N = 17) defined a case on the basis of the presence of two or more of the 'cardinal signs' of PD. One study used a more stringent definition of at least three of these signs, whilst five others also included responsiveness to L-dopa therapy and/or progressive disorder as additional criteria. This raises the question of whether the results from studies that used a more stringent definition of PD are more meaningful than the others. Another study used the criteria given in Box 4.1 (Ho *et al*., 1989), which are similar to the cardinal signs.

Box 4.1 Diagnostic criteria used by Ho *et al.* (1989)

Part A
- Bradykinesia or akinesia
- Resting tremor
- Rigidity – cogwheel, leadpipe
- Stooped posture with generalised flexion of limb, neck, and trunk ± postural instability
- Shuffling and/or festinating gait

Part B
- No arm swinging during walking
- Glabellar tap ±
- Mask face ± infrequent blinking
- Micrographia or voice diminished and monotonous

Diagnostic criteria
- At least: either 3 out of 5 in Part A or 2 from Part A and 2 from Part B

A cross-sectional study used the Unified Parkinson's Disease Rating Scale (Fahn & Elton, 1987) to define cases of PD (Engel *et al*., 2001a). Another (Morano *et al*., 1994) did not specify the criteria used but evaluated their cases according to the staging scale developed by Hoehn and Yahr in 1967 (Hoehn & Yahr, 1998: Box 4.2).

Box 4.2 Hoehn and Yahr Parkinson's disease staging scale

Stage I	Unilateral involvement only, usually with minimal or no functional impairment
Stage II	Bilateral or midline involvement, without impairment of balance
Stage III	First sign of impaired righting reflexes. This is evident by unsteadiness as the patient turns or is demonstrated when he is pushed from standing equilibrium with the feet together and eyes closed. Functionally the patient is somewhat restricted in his activities but may have some work potential depending upon the type of employment. Patients are physically capable of leading independent lives, and their disability is mild to moderate.
Stage IV	Fully developed, severely disabling disease; the patient is still able to walk and stand unassisted but is markedly incapacitated.
Stage V	Confinement to bed or wheelchair unless aided.

Lastly, a number of studies used the UK Parkinson's Disease Society Brain Bank clinical diagnosis criteria as described in Section 2.2.4 (Hughes *et al*., 1992b). However, most of these criteria were based on the experience of the scientists who developed them and have not been evaluated for their validity and reliability (Litvan *et al*., 2003); one set of criteria were based on a literature review (Gelb *et al*., 1999).

Two studies that used at least two of the cardinal signs to define their cases assessed the accuracy of this definition with long-term follow-up and pathological diagnosis (Ward & Gibb, 1990; Rajput *et al*., 1991). Ward and Gibb (1990) found that 69–75% of patients with autopsy-confirmed diagnosis of PD had at least two of the cardinal signs. However, 20–25% of those with two of the cardinal signs had a pathological diagnosis other than PD, and 13–19% of those that had three of the signs had another diagnosis. Rajput *et al.* (1991), after long-term follow-up of PD cases, found that 69.5% retained their diagnosis, and only 52.5% showed histopathological signs of PD at autopsy. Three other studies have found a significant proportion of cases to be misdiagnosed. Hughes *et al.* (1992a) found 76% of those individuals originally diagnosed with PD (using poorly defined criteria) were confirmed at autopsy. When they applied the UK Parkinson's Disease Society Brain Bank criteria retrospectively to the original records, 89% were diagnosed as having had PD; of these 82% were confirmed at diagnosis. A subsequent study suggested an improvement to 84% (Ansorge *et al*., 1997). Follow-up of a further 100 consecutive clinically diagnosed cases showed 90% fulfilled pathologic criteria for PD (Hughes *et al*., 2001). Ten were misdiagnosed and were in fact cases of multiple system atrophy (six), progressive supranuclear palsy (two), post-encephalitic parkinsonism (one) and vascular parkinsonism (one). Jankovic *et al.* (2000) followed up 800 patients diagnosed with PD for an average of 6 years and, after reassessing the original diagnosis by various means, found that 65 (8.1%) did not have PD. Schrag *et al.* (2002) found that among patients originally clinically diagnosed with PD, the diagnosis was unequivocally rejected using more stringent criteria in 15% of the cases.

Benito-León *et al.* (2002) examined the prevalence of PD in three regions of Spain and used five sets of diagnostic criteria to define their cases to compare prevalence estimates. They found that if two or more of the cardinal signs or bradykinesia plus two of the other cardinal signs were used the same number of patients were identified. However, if more stringent criteria were used, the numbers were reduced significantly (three or more cardinal signs, 18.5%; two or more cardinal signs plus 5 years duration, 48.1%; two or more cardinal signs plus asymmetry, 25.9%).

Finally, Litvan *et al.* (1998) assessed the diagnostic ability of six neurologists. They found a low positive predictive value for PD and a relatively high sensitivity, suggesting over-diagnosis of the number of cases.

These studies highlight the problem that, clinically, PD is often confused with other disorders. The main areas of diagnostic difficulty concern the distinction from other types of isolated, late onset tremor, vascular parkinsonism, and atypical types of parkinsonism, which are mistakenly diagnosed as PD (Schrag *et al*., 2002). Therefore, in the case–control studies some of the cases may have been misdiagnosed, which could impact on the power of the studies and have some effect on the risk estimates, tending to bias them towards the null value. On the other hand, patients with PD are sometimes not recognised as having the disorder, particularly those with mild disease or relatively isolated tremor. So, in the cohort studies, there is the problem of missing undiagnosed patients in the community, which has previously been estimated to range from 12% to 60% (Schoenberg *et al*., 1985; de Rijk *et al*., 1995). This would lead to underestimation of the risk estimates.

Exclusion criteria

Some of the studies, in addition to applying diagnostic criteria, excluded subjects on the basis of one or more of the criteria listed in Box 4.3. The use of these criteria would eliminate those individuals whose PD had a known aetiology; their use would also eliminate other risk factors from the analysis.

Box 4.3 Exclusion criteria used to define cases

Clinical features characteristic of alternative diagnosis
Significant cognitive impairment
Aetiology known to cause secondary parkinsonism
History of cerebrovascular accidents and/or head trauma
Receiving neuroleptic therapy
Receiving reserpine
Severe dementia
Atypical features suggesting multiple system atrophy of postencephalitic or other forms of secondary parkinsonism
Essential tremor
Alzheimer's disease
Brain tumour
Family history

4.6.5 Exposure assessment

To a large extent the quality of exposure measurement will determine the validity of an environmental epidemiology study (Rothman & Greenland, 1998). All the case–control studies in this review used a questionnaire to assess exposure. The majority were administered through face-to-face interviews. A few were sent out by post to the subject for self-completion and return. Four of the studies did not give response rates for the questionnaires (Wechsler *et al*., 1991; Butterfield *et al*., 1993; Hubble *et al*., 1993; McCann *et al*., 1998); in the other studies the response rates were good, ranging from 90% to 94% for cases and 78% to 90% for controls (Ohlson & Hodstedt, 1981; Hertzman *et al*., 1990; Fall *et al*., 1999). One study that interviewed cases face-to-face, interviewed controls by telephone, and had a response rate of 90% for the latter (Taylor *et al*., 1999); this may have created a systematic bias, because the collection of information by telephone may affect the manner in which study participants responded to the inquiry. There was also one study in which both cases and controls were interviewed by telephone (Golbe *et al*., 1990). Some of the studies stated the interviewer and respondent were blinded to the study hypothesis, but the majority made no mention of this. Face-to-face interviews have their advantages because the interviewer can prompt and probe and can observe the respondents reaction. However, in general, this is the most expensive form of interviewing, and takes a long time to arrange and conduct. Interviewer bias can be high unless the interviewer is blinded to the study hypothesis, which was not the case in approximately half of the studies. Self-administered questionnaires, unlike face-to-face interviews, would allow subjects more time to think about their

past exposures and would avoid a possibility of interviewer bias. In addition, some of the interviews were conducted in the presence of a relative/carer of the case, who was asked to correct any mistakes. This may have helped the subject in detailing their exposure history but may have also introduced recall bias. Akin to this, subjects sent a postal questionnaire were asked to complete it themselves, but in some instances a proxy respondent, without the knowledge of the study researchers, may have filled it in. However, findings support the use, if necessary, of spouses and adult children as surrogate respondents in case–control studies of rural environmental and occupational exposures and PD, if cases are unable to provide an exposure history (Wang *et al*., 1994b).

All the exposure information relied upon subjects recalling their lifetime exposures. This could potentially lead to recall bias, particularly if cases might be more aware of possible risk factors for their disease, consider more carefully the questions they are asked, and remember in more detail any exposure to these risk factors than the controls. This may occur even when subjects are blinded to the study hypothesis, which in some of the studies they were. For individuals occupationally exposed to pesticides, the accuracy of their historical self-reported pesticide exposure is high for broad categories of pesticides and commonly used pesticides but not for specific pesticides (Engel *et al*., 2001b; Hoppin *et al*., 2002). While the accuracy of recall about pesticide exposure is high among individuals occupationally exposed, accuracy of recall for non-occupational or residential exposure is questionable (Teitelbaum, 2002). Apart from the study of Wang *et al.* (1994a), which crosschecked answers given by subjects and surrogates, only a few studies re-tested the answers to the administered questionnaire on a small sample of subjects. Butterfield *et al.* (1993) administered an abridged version of their questionnaire to about 13% of their cases to test/re-test reliability. Just fewer than 28% of the categorical variables showed no variation in their answers, and 52% had kappa values greater than 0.75, which was interpreted to represent excellent agreement beyond chance. Similarly, Koller *et al.* (1990) re-interviewed a sub-sample of their original group of cases and demonstrated an average reliability of 91% for the factors studied. A lack of independent assessment of exposure could lead to recall bias in each study.

The questions relating to pesticide exposure varied from study to study and in some instances were unclear. A number of studies simply asked the question ‘Have you ever been exposed to pesticides?’ without any further detail (Tanner *et al*., 1989; Wechsler *et al*., 1991; Smargiassi *et al*., 1998; Kuopio *et al*., 1999a; Preux *et al*., 2000; Behari *et al*., 2001; Zorzon *et al*., 2002). Others asked detailed questions, such as ‘Have you sprayed pesticides or insecticides at least once a year for 5 years (not necessarily consecutively)?’ (Golbe *et al*., 1990) and ‘Have you been exposed to pesticides for more than 20 days during a year for at least 10 years?’ (Duzcan *et al*., 2003). Other questions asked are given in Box 4.4. In each instance, if a positive answer was given to the question then that subject was deemed to have been ‘exposed’ to pesticides. It is clear from this that a person who responds positively to the first question above would not have the same amount of exposure as someone answering yes to the last two questions. So, strictly speaking the majority of studies will not be examining PD risk to the same level of exposure.

Box 4.4 Categories used to assess pesticide exposure in studies reviewed

- Direct (subjects applied pesticides themselves) and indirect (subjects lived and ate vegetables in area where pesticides were employed)
- Used pesticides for more than 20 days during any year; used pesticides for more than 20 days a year for more than 5 years
- Type of exposure (e.g. aerial, direct application, sprayer), number of years exposed, the type of crop on which chemicals were used (corn, wheat, sorghum or pastureland), and type of herbicides/pesticides employed, and specific chemicals
- Previous exposure before age 20, age 20 to 40, and after age 40
- Yes, only if frequently used
- Sprayed pesticides or insecticide at least once a year for 5 years, but not necessarily consecutive
- Used cue cards of specific chemicals giving trade names; chemicals were grouped by insecticides, herbicides, fungicides, acaricides and plant growth regulators.
- Use within home, garden, neighbourhood, professional
- Exposure for at least 10 consecutive years prior to onset of PD
- Yes/No, and regular exposure (daily or weekly for cumulative period of greater than 6 months)
- Number of years of application weighted by frequency of usage ('dose-years': rarely, factor 1; for special indications, factor 2; and regularly (seasonally), factor 3).

Some studies attempted to examine associations with PD and groupings of chemicals, for example herbicides, insecticides, fungicide or acaricides (Stern *et al*., 1991; Semchuk *et al*., 1992; Butterfield *et al*., 1993; Hertzman *et al*., 1994; Seidler *et al*., 1996; Gorell *et al*., 1998; Kuopio *et al*., 1999a; Behari *et al*., 2001; Kamel *et al*., 2002; Duzcan *et al*., 2003). A few presented evidence for individual chemicals (Wechsler *et al*., 1991; Hertzman *et al*., 1994; Seidler *et al*., 1996; Liou *et al*., 1997; Kuopio *et al*., 1999a; Kamel *et al*., 2002). However, the authors of a majority of the studies stated that they were unable to identify specific pesticides used, owing to the subjects' lack of knowledge about their exposures.

The majority of studies were only able to examine the risk in relation to pesticides as a whole, with some able to differentiate between herbicides and insecticides. However, seven case–control studies were able to examine the risk of exposure to specific pesticides. Wechsler *et al.* (1991) found an increased risk of PD among individuals who applied the garden pesticides ROUND-UP (glyphosate), KLEENUP® (glyphosate), Ortho TRIOX® (pentachlorophenol) and PESTKIL® (bendiocarb). Lindane was also observed in significantly higher concentrations in the brains of PD patients at autopsy (Corrigan *et al*., 2000). Two case–control studies observed a non-significant increased risk with exposure to paraquat (Hertzman *et al*., 1994; Liou *et al*., 1997; Kamel *et al*., 2002) and two a significant risk (Hertzman *et al*., 1990; Liou *et al*., 1997). Other studies found an excess PD risk in individuals exposed to organochlorines, alkaline phosphates and carbamates (Seidler *et al*., 1996), maneb, rotenone and dieldrin (Kamel *et al*., 2002). Dieldrin has also been found in significantly higher concentrations in the brains of PD patients at autopsy (Fleming *et al.,* 1994; Corrigan *et al.,* 1998, 2000). An increased risk has also been seen in PD cases exposed to DDT (Kuopio *et al*., 1999a), and its metabolites were found in brain autopsies in more PD cases compared with non-neurological controls (Fleming *et al.,* 1994; Corrigan *et al.,* 1998, 2000). In contrast, a cross-sectional study by Engel *et al.* (2001a) found no increase in the risk of PD in those exposed to DDT and its metabolites or other pesticides, including mancozeb, methyl parathion, thiram, zineb and zircam, among PD cases.

A number of other environmental risk factors may also be associated with an increased risk of PD. A recent review (Lai *et al*., 2002) identified rural living, farming activity, well-water drinking, animal exposure, exposure to metals, dietary factors, trauma and head injury, and infection as risk factors. The majority of studies have investigated PD risk with respect to these factors in addition to pesticides. However, in the pesticide studies the method of assessment is often variable and not all were examined in multivariate analysis.

Studies that have investigated rural living, well-water consumption and farming in relation to PD have found ORs to be generally of the same order and direction, although the magnitude of the effect varies. Many studies have postulated that these factors and exposure to pesticides are closely linked and interrelated. The increased risk with farming or living on a farm could be because pesticides may be used more in farming. The association of rural living with PD may be related to exposure to pesticides because they are used in greater quantities in these regions. However, a large population range has been used to define rural living, 'living in areas with populations of less than 200 from birth' to 'living in areas with populations of less than 20 000 for more than 20 years' (Table 4.7). Others relate rural living to particular time periods during a lifetime.

Table 4.6 Definition of rural living used by various studies

Reference	Definition	
	Community size	**Residence time**
Rajput *et al.*(1986)	<169	
Koller *et al.* (1990)	<2500	
Wong *et al.* (1991)	<2000	
Jiménez-Jiménez *et al.* (1992)	<2000	From birth
Butterfield *et al.* (1993)	<10 000	From birth; 20, 15, 10, 5 years before and time of diagnosis
Morano *et al.* (1994)	<2000	From birth
McCann *et al.* (1998)	<10 000	>12 months
Smargiassi *et al.* (1998)	<20 000	>10 years
Taylor *et al.* (1999)	Undeveloped farmland nearby	
Preux *et al.* (2000)	<2000	<15 and >15 years of age
Behari *et al.* (2001)	≤ 2500	≥ 1 year; <10 years; >10 years

In the studies identified, the use of well-water for drinking is highly correlated with rural living; the majority of urban households receive their water from large municipal supplies. In this situation the risk of PD is assumed to be due to some form of water contamination (Butterfield *et al*., 1993).

4.7 Summary of epidemiology studies

This review identified a large number of studies published since 1983 that examined the relationship between pesticide exposure and the risk of PD. Most of these studies used 'pesticide' or 'pesticide and herbicide' as exposure categories, although some studies categorised pesticides into other subgroups, such as herbicide, insecticide, fungicide or other specific agents. A total of ten observational, five cohort and 38 case–control studies were identified and reviewed. The majority of these studies (42) found a positive association between pesticide exposure and PD risk, and in 20 of these the association was statistically significant.

An association between PD and pesticide exposure was actually tested for in eight of the ten pesticide-related descriptive studies (two case-series studies, three mortality studies, four prevalence studies and one incidence study). Of these eight, all the mortality studies (one of which was reported only as an abstract) and one prevalence study found a significant association between PD and a marker of pesticide use. One case-series study, two prevalence studies, one looking at hexachlorobenzene exposure, and an incidence study (reported only as an abstract) found no association between PD and pesticide use/exposure. However, given that it now appears that YOPD has a primary genetic cause (Tanner, 2003), the case-series study (which looked at YOPD patients) would not necessarily be expected to find an association between pesticides and PD. There is also a general association with other environmental factors; the majority of studies identified an association between PD and rural living, and all the studies looking at agriculture as an occupation found a higher PD prevalence or mortality amongst agricultural workers. However, descriptive studies are mainly

confined to the presentation of routinely collected data. These data, by themselves, cannot usually be used as critical evidence in evaluating a causal association between a particular factor or agent and a disease or as the basis for change in health related behaviour. Because data on individuals are not available, the relationship between exposure and disease is indirect. Nevertheless, such studies are important in identifying possible patterns in the data that can be used to generate hypotheses for testing in well-designed studies.

Of the five cohort studies identified (including one reported only as an abstract), three showed an increased risk of PD amongst farming occupations of which two inferred an increased risk of PD from pesticide exposure. One study also identified several other occupational groups with an increased risk of PD. However, these studies should be treated with caution as none were designed to investigate PD risk specifically and the numbers of PD cases in each study were small. There was also no independent measure of pesticide exposure; rather, all the studies used occupation as the exposure variable.

This review identified 38 separate case–control studies on pesticide exposure and three autopsy studies. An increased risk of PD following pesticide exposure was observed in the majority of the 38 case–control studies identified, with a majority of those showing a significantly increased risk. The excess risks reported in these studies varied from 1.01 to 7.00, although confidence intervals were wide in many studies, partly due to small numbers. Whilst most of the studies considered 'pesticides' as an exposure category, several studies looked at more detailed exposure categories, and significant associations with PD were identified with exposure to herbicides and insecticides as major classes of pesticides. Based on five studies (including one abstract), findings for an association with fungicide exposure were inconclusive. A few studies that looked at specific pesticide compounds were identified. One study identified exposures to organochlorines, alkaline phosphates and carbamates as significant risk factors for PD; paraquat was shown to be significantly associated with PD in two studies but not a third; and one study found a weak non-significant positive association with PD and exposure to DDT. The three autopsy studies demonstrated significantly high levels of dieldrin and lindane in the brains of PD patients.

4.8 Conclusions from epidemiology studies

The findings of the case–control studies and the meta-analyses, in combination with the descriptive and cohort studies suggest a fairly consistent association between exposure to pesticides and an increased risk of developing PD; this relationship appears to be relatively consistent in different populations and countries. The level of increased risk is variable between studies, although a meta-analysis focusing on pesticide exposure as a risk factor in PD reported a combined odds ratio of 1.94 (Priyadarshi *et al*., 2000). However, only 19 of the 34 fully published case–control studies identified in this review were included; hence, the limited nature of the dataset may have influenced the overall risk estimate. Nevertheless, some studies present conflicting results and must be interpreted with care in view of the various issues raised, including the variability and inaccuracy of the clinical diagnostic criteria used, the source and ascertainment of cases and controls, and the assessment of exposure to pesticides and other confounding factors. These are difficulties that arise in most epidemiological studies, especially case–control studies, which have been the main method used to assess the association between PD and pesticide exposure. The limitations on exposure assessment also include the limited information on the type of pesticide and level of exposure. The exposure category 'pesticides' represents many hundreds of chemicals and, as a result, one cohort exposed to 'pesticides' may be exposed to a different group of chemicals to another cohort said to be exposed to 'pesticides'. It may be that exposure to only a few pesticide compounds results in an increased risk of developing PD; however, differences in exposure to these compounds would be masked by the use of broad 'pesticide' exposure categories in the studies, possibly resulting in the heterogeneity observed.

In all the studies reviewed pesticide exposure history was collected retrospectively. In all of the case–control studies and some of the cohort studies this was done using a questionnaire, thus introducing

the potential for recall bias to occur, which could impact on the internal validity of a study. This bias may be differential in that cases may recall more exposure or different types of exposure than controls although this is difficult to assess. However, in studies of PD, the disease process may have affected the memory of some subjects. In some studies, therefore (Tanner *et al*., 1989; Golbe *et al*., 1990; Koller *et al*., 1990; Chaturvedi *et al*., 1995; Liou *et al*., 1997), family members and/or carers were involved in detailing exposure history and reducing recall bias. However, this might also have introduced information bias. Recall bias may have also been reduced in those studies that checked the reliability of the answers given to the questionnaire using re-test methods (Hertzman *et al*., 1990; Koller *et al*., 1990; Butterfield *et al*., 1993; Hubble *et al*., 1993). Thus, it is unclear how either recall or information bias have affected the risk estimates in all the studies reviewed. For example, if the biases were non-differential between cases and controls then this would tend to bias the risk estimates towards the null. However, differential bias could underestimate or overestimate the risk estimate.

Most case–control studies identified looked at 'pesticides' as an exposure category, employed more detailed exposure categories, and significant associations with exposure to herbicides and insecticides as major classes of pesticides and PD risk were identified. In one study, exposure to pesticides was a significant risk factor independent of insecticide exposure (Semchuk *et al*., 1992) but the converse was not found. Taken together, studies on fungicides were inconclusive. A few studies looked at specific pesticide compounds. Seidler *et al.* (1996) identified organochlorines, alkaline phosphates and carbamates as significant risk factors for PD; however, although the study had a large number of subjects, relatively few could recall using the specific chemicals. This highlights the general problem of recalling the use of specific products and chemicals retrospectively. Paraquat has been shown to be significantly associated with PD in two studies (Hertzman *et al*., 1990; Liou *et al*., 1997) and in one study a significant association was not found (Hertzman *et al*., 1994). Again only a few subjects reported being exposed. One study also found a weak non-significant positive association with PD and exposure to DDT (Kuopio *et al*., 1999a).

The relationship between pesticide exposure duration and PD risk has been investigated in six case–control studies and one cross-sectional study. Four case–control studies and the cross-sectional study found a significant association between increasing exposure duration and PD risk (Seidler *et al*., 1996; Liou *et al*., 1997; Gorell *et al*., 1998; Chan *et al*., 1998; Engel *et al*., 2001a), although the significance was lost in one case–control study after adjusting for smoking, family history, rural living and diet (Chan *et al*., 1998). The studies suggest that PD risk increases significantly when exposure duration exceeds a particular threshold (e.g. >10 or >20 years). One case–control study and the cross-sectional study examined PD risk in relation to duration of exposure to herbicides and insecticides. The case–control study found a significant positive trend between PD risk and herbicide exposure (Seidler *et al*., 1996), whilst the cross-sectional study did not find a significant relationship with herbicides or insecticides, after adjustment, although risk estimates were elevated in the highest exposure categories (Engel *et al*., 2001a). One case–control study that examined the association between the duration of exposure to paraquat and PD risk observed a significant association with greater than 20 years exposure (Liou *et al*., 1997).

As stated previously there is a very clear relationship between PD incidence and age. It is important, therefore, to adjust for age in the calculation of any risk estimate. In the case–control studies, controls were matched for age and other factors but age was not included in the analysis, with the exception of the calculation of adjusted OR in six studies (Hertzman *et al*., 1990; Butterfield *et al*., 1993; Hubble *et al*., 1993; Gorell *et al*., 1998; Menegon *et al*., 1998; Taylor *et al*., 1999). Thus in those studies that made no adjustments for age (at onset of PD or first exposure), some residual confounding would remain. As a consequence, it is difficult to determine at what levels of exposure to pesticides PD may develop.

Residual confounding may also be the result of other factors that were not adjusted for in the analysis. Well-water consumption, farming and rural life have all been found to be associated with an increased risk of PD in a number of studies. Multivariate analysis indicated well-water consumption to be both independently associated with PD (Zorzon *et al*., 2002) and dependent on rural living (Koller *et al*.,

1990). Meta-analysis indicated the overall risk estimate for well-water use to be 1.26 (95% CI 0.96–1.64; Priyadarshi *et al*., 2001). Several studies have also shown farming to be an independent risk factor, in addition to pesticide exposure (Gorell *et al*., 1998; Zorzon *et al*., 2002). Meta-analysis of these two variables and also rural living showed combined ORs greater than unity (Priyadarshi *et al*., 2001) but despite this there still remains uncertainty as to the exact nature of the relationship between them, pesticide exposure and their relationship to PD risk.

While multivariate analysis was undertaken in a number of the case–control studies (Table 4.3), different variables and combinations of variables were used to calculate the adjusted ORs. Some studies on pesticides did not include any of the variables associated with farming or rural life (Golbe *et al*., 1990; Hertzman *et al*., 1990; Butterfield *et al*., 1993; Hubble *et al*., 1993; Menegon *et al*., 1998; Zorzon *et al*., 2002). Thus, in these studies and those that showed a significant association between PD and pesticide exposure but did not adjust their data (Ho *et al*., 1989; Golbe *et al*., 1990; Hertzman *et al*., 1990; Hertzman *et al*., 1994; Morano *et al*., 1994), the risk estimates may have been overestimated, although in those studies that did adjust a change did not always occur.

Of all the studies reviewed, the two most reliable studies were large case–control studies that attempted to investigate exposure to pesticide groups and not pesticides as a whole (Semchuk *et al*., 1992; Seidler *et al*., 1996). Both studies also undertook multivariate analysis, adjusting the risk for known and potential risk factors. Univariate analysis of herbicide and insecticide use by Semchuk *et al*. (1992) resulted in a significantly increased crude estimate of the PD risk, and suggested that the risk was significantly increased in those aged 26–55 years with herbicide exposure and in those aged 46–55 years with insecticide exposure. In multivariate analysis, as noted above, previous occupational herbicide use was consistently the only significant predictor of PD risk. Seidler *et al*. (1996) observed an increased PD risk with pesticide use and, in particular, as mentioned above, with organochlorines, alkylated phosphates and carbamates, but no association with other rural factors. They also observed a dose–response relationship for years of herbicide and insecticide use (versus regional controls), as they did for alkylated phosphates and carbamates.

Overall, it seems unlikely that the relatively consistent association between PD and reported exposure to pesticides could be explained wholly by a combination of chance, bias and confounding and selective reporting. Based on the available data, extended exposures to classes of pesticides, such as herbicides and insecticides, especially possibly paraquat, appear to be risk factors in the development of PD. However, larger studies are required with more detailed pesticide exposure information and standardised diagnostic (inclusion and exclusion) criteria.

5 Toxicological review

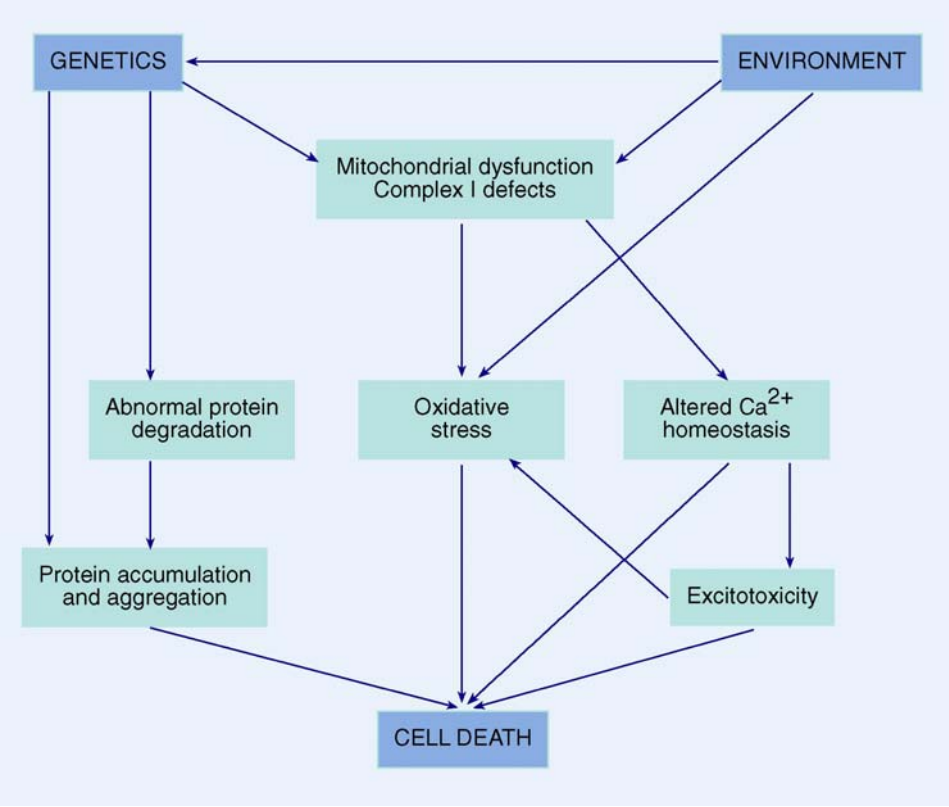
This section reviews the toxicological evidence for a link between pesticides and Parkinson's disease (PD). In Section 5.1, the underlying processes thought to be involved in the aetiology of PD are outlined. Evidence concerning the role of these mechanisms is continually being published but, at present, the temporal and spatial interactions of these various processes are unclear. In Section 5.2, the neurotoxic effects of a number of pesticides are assessed. These pesticides have been identified from literature searches as causing changes that show some similarity with the mechanisms thought to be involved in the aetiology of PD. As the precise role and importance of the proposed mechanisms are uncertain and the data on the effects of pesticides are incomplete, the assessment of the role of pesticides in PD can, at present, only be restricted to identifying plausible biological hypotheses.

5.1 Potential mechanisms of Parkinson's disease

5.1.1 Introduction

One of the pathological hallmarks of PD is the degeneration of the pigmented melanin-containing neurons of the substantia nigra pars compacta area of the brain. These neurons send their projections from the midbrain to two forebrain nuclei, the caudate and putamen, which together make up the striatum. These neurons use dopamine as a neurotransmitter and so degeneration of the substantia nigra eventually leads to severe depletion of dopamine in the striatum. Figure 5.1 outlines potential mechanisms involved in the development of PD. It also indicates potential targets for interactions with environmental factors.

Figure 5.1 Potential mechanisms involved in the development of PD

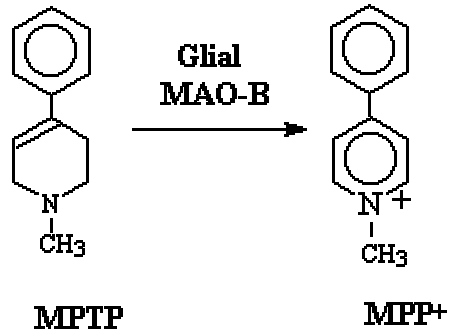


Adapted from Betarbet *et al.* (2002b)

5.1.2 Mitochondrial dysfunction

The inadvertent discovery of the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its metabolite, 1-methyl-4-phenylpyridine (MPP^+; see Figure 5.2) gave the first lead as to possible mechanisms in the aetiology of PD. The discovery that a single chemical could initiate a chain of consequences leading to parkinsonism led to the generation of the hypothesis that 'environmental toxins' could also be involved in PD (see Section 5.2). MPTP is metabolised by the enzyme, monoamine oxidase B, to the active MPP^+, which acts as a substrate for the dopamine transporter expressed in dopaminergic neurons (Figure 5.2; Barc *et al*., 2002).

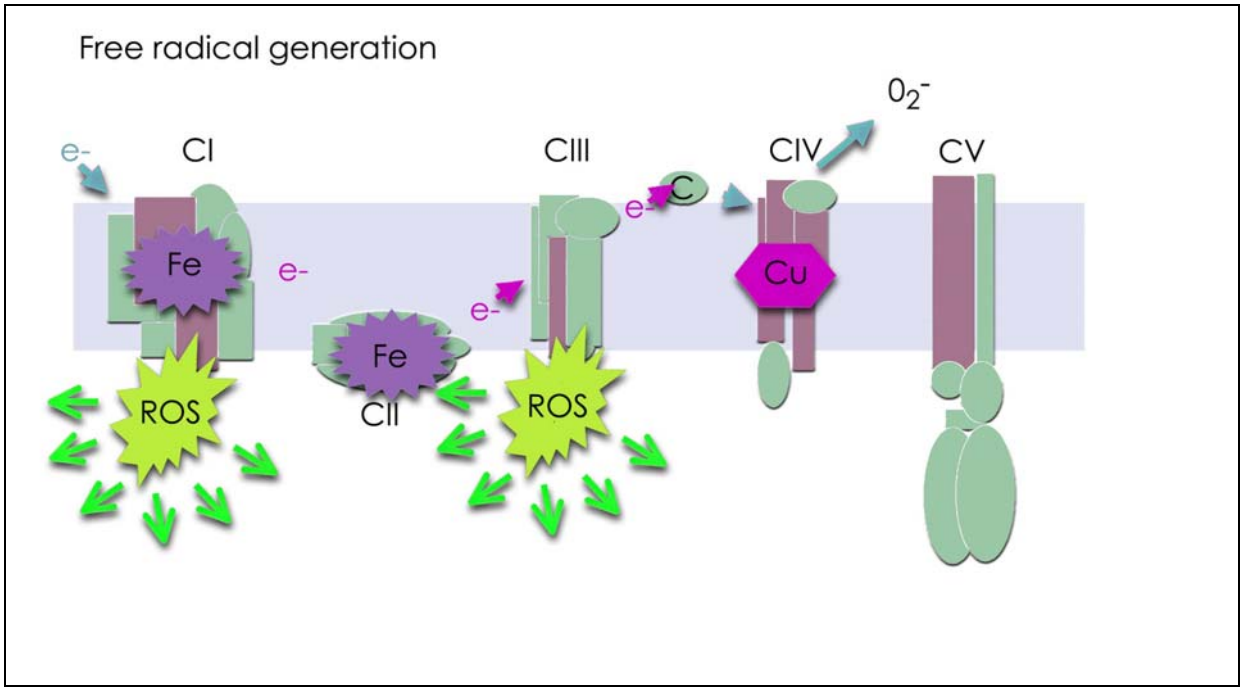
Figure 5.2 Activation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)



MAO-B, monoamine oxidase B; MPP^+, 1-methyl-4-phenylpyridine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

MPP^+ is thus able to accumulate in the mitochondria of these neurons and is believed to exert its toxicity by inhibition of Complex I (NADH dehydrogenase; Figure 5.3) of the mitochondrial electron transport chain (Nicklas *et al*., 1992; Greenamyre *et al*., 1999; Foley & Riederer, 2000; Greenamyre *et al*., 2001; Sherer *et al*., 2002a). Complex I oxidises NADH and transfers electrons to ubiquinone via several complexes and cofactors. It also transfers protons from the matrix of the mitochondria to the inner membrane space, helping to establish the electrochemical gradient used to fuel the production of cellular energy in the form of adenosine triphosphate (ATP). The depression of ATP synthesis resulting from Complex I inhibition may lead to mitochondria being unable to handle calcium transport efficiently and also to the production of reactive oxygen species (ROS; Box 5.1). At several sites along the electron transport chain there are sites of 'electron leak'; a site within Complex I is believed to be relevant to PD. The electrons thus produced may combine with molecular oxygen to form ROS (Betarbet *et al*., 2002a). These two processes may, potentially, lead to cell death (Sherer *et al*., 2002a). A decrease of about 30% in Complex I activity has been reported in the substantia nigra, striatum, skeletal muscle and platelets of idiopathic PD patients without detectable structural or mitochondrial DNA changes (Foley & Riederer, 2000).

Figure 5.3 Schematic diagram of the mitochondrial electron transport chain showing the five complexes and how reactive oxygen species may be generated



Based on a figure supplied from K. Morten, University of Oxford
C, complex; Cu, copper; e-, electron; Fe, iron; ROS, reactive oxygen species

Box 5.1 Reactive oxygen species

Reactive oxygen species (ROS) include weakly reactive molecules such as hydrogen peroxide and free radicals such as the superoxide ($O_2^{\cdot-}$) anion and the hydroxyl radical ($OH^{\cdot}$). Free radicals consist of a cluster of atoms containing unpaired electrons, an unstable configuration, which means that they rapidly react with other molecules or radicals. These species may be generated normally in tissues or by cellular dysfunction and an imbalance of ROS generation and degradation termed 'oxidative stress'.

5.1.3 Oxidative stress

Compared with other organs of the body, the brain is highly susceptible to oxidative damage, owing to several factors, including its high oxygen utilisation, high iron levels, the presence of high lipid concentrations (targets for peroxidation) and the low levels of detoxifying enzymes such as superoxide dismutase, catalase and glutathione reductase (Bharath *et al*., 2002). The substantia nigra, in particular, has decreased levels of glutathione. This antioxidant plays an important role in removing hydrogen peroxide (H_2O_2) produced during normal metabolism in the adult brain. Glutathione depletion is also the first indication of oxidative stress during PD progression, suggesting that there has been an increase in ROS (Bharath *et al*., 2002).

Besides being generated subsequent to the inhibition of Complex I and the consequential mitochondrial dysfunction, ROS may also be produced during the auto-oxidation of dopamine, through the production of toxic semiquinone species or the production of H_2O_2 during the metabolism of dopamine by monoamine oxidase and tyrosine hydroxylase (Foley & Riederer, 2000). In general, a decrease in dopamine is considered a marker for PD. However, an increase in dopamine turnover may occur in the short-term to compensate for decreased nigrostriatal dopaminergic input. This increase, together with a decrease in levels of the antioxidant glutathione and an excess of H_2O_2 and its conversion to hydroxyl radicals via the iron-mediated Fenton reaction (Equation 1), could lead to

localised cellular oxidative stress (Foley & Riederer, 2000; Bharath *et al*., 2002). There are high iron levels in the substantia nigra that could act as a substrate for this reaction (Bharath *et al*., 2002).

Equation 1 The iron-mediated Fenton reaction

$$H_2O_2 + Fe^{2+} \rightarrow \cdot OH + OH^- + Fe^{3+}$$

Inducible nitric oxide synthase (NOS) is also known to be increased in the substantia nigra in PD. Increased nitric oxide (NO) could also elevate local oxidative stress. Although NO is an effective free radical scavenger, it can react with the superoxide radical to form the peroxynitrite anion, a potent oxidative radical. NO also directly inhibits mitochondrial respiration (mainly at the level of Complex IV, but also at Complex I; Foley & Riederer, 2000).

5.1.4 Calcium and excitotoxicity

Glutamate is the predominant excitatory neurotransmitter in the brain. It does, however, have excitotoxic properties under some conditions (Greenamyre *et al*., 1999). The impaired mitochondrial Complex I activity associated with PD may predispose neurons to excitotoxic cell death. Depletion of cellular ATP level, which is associated with the impairment, can alter cellular homeostasis. The loss of ATP would reduce Na^+/K^+ ATPase activity, resulting in partial neuronal depolarisation. This depolarisation leads to a decrease in the voltage-dependent Mg^{2+} blockade of the *N*-methyl-D-aspartate (NMDA) glutamate receptor. This blockade normally acts to prevent the excitotoxic stimulation of glutamate caused by an abnormal influx of calcium into the cell. Without the blockade even normal cellular levels of glutamate may cause excitotoxic activation of the NMDA receptors and lead to a potentially fatal increase in intracellular calcium concentration (Sherer *et al*., 2002a). The sequestration of calcium in the mitochondria, which may normally mitigate this effect, is decreased when electron transport is impaired (Greenamyre *et al*., 1999).

Although the NMDA subtype of glutamate receptor was initially considered to be primarily involved in excitotoxicity because it possesses a calcium permeable ion channel, it is now apparent that activation of non-NMDA receptors may also play a role, particularly after prolonged exposure to an agonist (Heath & Shaw, 2002).

5.1.5 Aberrant protein aggregation

Lewy bodies are prominent in the substantia nigra of PD patients. They are accumulations of a series of proteins, including neurofilaments, α-synuclein fibrils, ubiquitin, parkin, proteasomal elements plus numerous other proteins. Three different components of Lewy bodies, α-synuclein, parkin and ubiquitin carboxyterminal hydrolase (UCHL1), have been associated with genetic mutations in familial PD, which points to the possibility that altered protein conformation and/or degradation could be a key and a common factor in the degenerative process in sporadic PD. α-Synuclein is the major component of Lewy bodies. Transgenic mouse models in which α-synuclein is overexpressed show features of PD, including loss of dopaminergic nigrostriatal neurons, development of α-synuclein and ubiquitin positive cytoplasmic inclusions and motor impairment (Di Monte *et al*., 2002; Betarbet *et al*., 2002a).

5.1.6 Cell death

The nature of cell death in PD is as yet undetermined. However, there are indications that it involves a combination of apoptosis ('programmed' cell death) and necrotic degeneration (Foley & Riederer, 2000). *In vitro* studies suggest that MPP^+ concentrations, which inhibit mitochondrial function, also induce apoptotic cell death, while higher levels cause necrotic cell death. An increased proportion of apoptotic cells have been observed in the substantia nigra of patients with PD and other Lewy body-associated diseases. It is thus possible that low-level exposure to a toxin causing oxidative stress might initially stimulate apoptotic loss but that prolonged exposure may also induce necrotic

neurodegeneration. It is also possible that the triggering event for neurodegeneration might not necessarily be the factor that determines disease progression (Foley & Riederer, 2000).

5.2 Potential involvement of pesticides

5.2.1 Introduction

The observation that exposure to MPTP led to parkinsonian symptoms established the possibility that environmental factors might be involved in the aetiology of PD.

Given the potential mechanisms for the development of PD outlined above, a number of factors need to be considered in assessing the evidence for a role for pesticides in PD development:

- effects on the striatal dopaminergic system — may include a decrease in dopamine levels and/or an increase in dopamine turnover as a short-term compensatory mechanism; this would be identified by an increase in metabolites or the enzyme, tyrosine hydroxylase;
- effects on the substantia nigra — most dopaminergic neurons are present in the basal ganglia, including the substantia nigra, and changes in the substantia nigra although not necessary specific would be seen with an agent with a role in the development of PD;
- mechanistic effects — on oxidative stress, mitochondrial dysfunction/Complex I inhibition, α-synuclein levels and aggregation; and
- effects with appropriate routes of administration for environmental exposure — oral, inhalation or dermal and evidence of entry into the brain.

Many natural or man-made pesticides and herbicides share a common effect on mitochondrial function by inhibiting Complex I, although many of these effects are at very high concentrations (Betarbet *et al*., 2002b) and some of these are outlined below (Table 5.1 and Box 5.2).

Table 5.1 Naturally occurring compounds known to inhibit Complex I

Compounds	Source
Rotenoids	Leguminosae plants
Piericidins	Streptomyces strains
Acetogenins	Annonacae plants (custard apple, paw-paw)
Antibiotics	Myxobacteria
Rhein	Rhubarb

Adapted from Betarbet *et al*. (2002b)

Box 5.2 Pesticides known to inhibit Complex I

Benzimidazole	Hoe 110779
Bullactacin	Pyridaben
6-Chlorobenzothiadiazole	Pyrimidifen
Cyhalothrin	Sandoz 547A
Fenazaquin	Tebufenpyrad
Fenpyroximate	Thiangazole

Adapted from Betarbet *et al*. (2002b)

The formation of Lewy bodies and the aggregation of proteins, in particular α-synuclein, during PD development have recently generated considerable interest, as mutations in these proteins have been associated with inherited PD. The formation of Lewy bodies may thus be integral to the cause of the disease rather than being an accompanying effect. Recent studies *in vitro* have suggested that a number of pesticides (alone or in synergy with certain metals) may induce a conformational change in

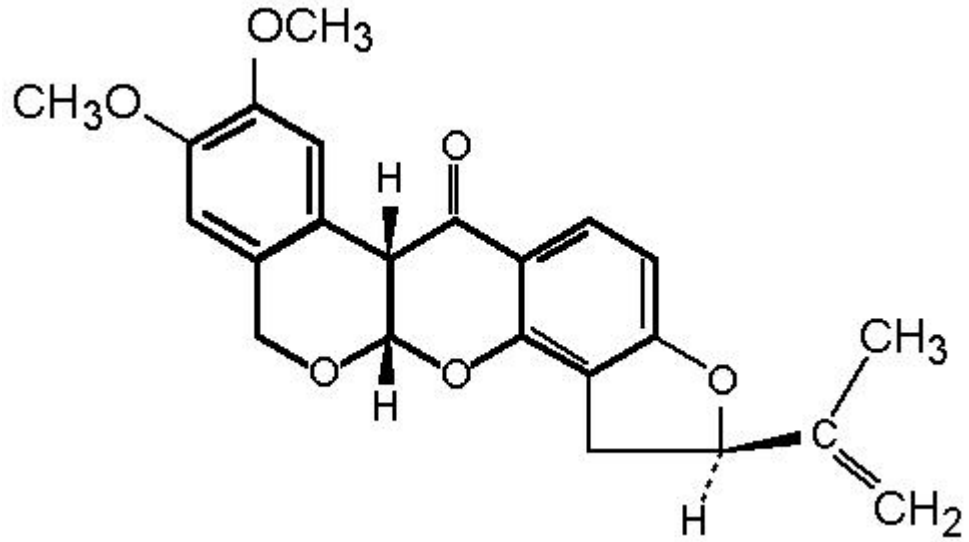
α-synuclein and accelerate the formation of α-synuclein fibrils (Uversky *et al*., 2001; Uversky *et al*., 2002). Such fibrils have been reported as being present in Lewy bodies. Pesticides known to induce this formation are hydrophobic and include rotenone, DDT, 2,4-dichlorophenoxyacetic acid (2,4-D), dieldrin, diethyldithiocarbamate, paraquat, maneb, trifluralin, parathion and imidazolidinethione. Those having no significant effect include iprodione, glyphosate, methomyl, thiuram, mevinphos, carbaryl, alachlor, thiobencarb and also the metabolite of MPTP, MPP^+.

A further means of detecting potential associations with environmental agents is to assess alterations in the metabolism of xenobiotic chemicals in PD patients. Polymorphisms in a number of xenobiotic metabolic enzymes and altered metabolic pathways have been identified in PD patients, including differences in catechol-*o*-methyltransferase, monoamine oxidase A and B, *N*-acetyltransferase 2, glutathione transferase and *S*-oxidation (Section 2.2.7 and Table 2.2). Unfortunately, these enzymes are involved in such widespread mechanisms for the elimination of xenobiotics that specific agents or classes cannot be identified. However, such findings do support the idea of a role for an environmental agent in PD.

5.2.2 Rotenone

Rotenone (Figure 5.4) is a naturally occurring compound derived from the roots of certain plant species. It is commonly used as an insecticide in vegetable gardens and to kill or sample fish populations in lakes and reservoirs. It is widely believed to be a safe, natural alternative to synthetic pesticides. Rotenone is also a well-characterised, high-affinity specific inhibitor of Complex I. It is extremely hydrophobic and crosses biological membranes easily and so, unlike MPTP, does not require a dopamine transporter for access to the cytoplasm. As such, rotenone is likely to produce a systemic inhibition of Complex I (Betarbet *et al*., 2000).

Figure 5.4 Structure of rotenone



In a widely reported and reviewed study, Lewis rats were infused continuously with rotenone by a jugular vein cannula attached to a subcutaneous (sc) osmotic minipump at doses ranging from 1 to 12 mg/kg/day for various lengths of time (from 7 days to 5 weeks). The optimum concentration resulting in minimal systemic toxicity (cardiovascular and non-specific brain lesions) while inducing the pathology of PD was 2–3 mg/kg/day. Specific Complex I binding was reduced by 75%, at a free rotenone concentration in the brain of about 20–30 nmol/l. PD-like symptoms were observed at 2–3 mg/kg/day, a level at which the degree of Complex I inhibition was insufficient significantly to impair oxidative phosphorylation in the brain. This suggests that ATP depletion is not a significant mechanism for the loss of dopaminergic neurons.

In the study, 12 out of 25 animals given rotenone at 2–3 mg/kg/day demonstrated clear nigrostriatal dopaminergic lesions; no control vehicle-treated rats had such lesions. The data suggest that striatal nerve endings were affected earlier and more severely by rotenone than were nigral cell bodies. Rats with these lesions had cytoplasmic inclusions containing α-synuclein in the nigral neurons. These resembled the 'pale body' precursors to Lewy bodies found in humans with PD. Rotenone-treated animals also developed motor and postural deficits characteristic of PD, the severity of which correlated with the extent of the pathological lesions. All the animals with a dopaminergic lesion became hypokinetic and had unsteady movement and hunched posture, even after cessation of the rotenone treatment.

The same features of PD that were observed with the jugular vein cannula infusion of rotenone were also seen when rotenone was infused sc at 2–3 mg/kg/day; the induction of highly selective nigrostriatal dopaminergic lesions and α-synuclein aggregation were also observed (Sherer *et al*., 2003b). However, Betarbet *et al.* (2000) have reported that rotenone seems to have little toxicity when administered orally (based on unpublished data of Sherer and Greenamyre).

In an earlier study on Sprague-Dawley rats using continuous intravenous (iv) infusion of rotenone (7–9 days at a rate of 10–18 mg/kg/day), selective damage was observed in the striatum and the globus pallidus but, unlike the above study of Betarbet and colleagues (2000), the substantia nigra was spared (Ferrante *et al*., 1997).

In a recent study by Alam and Schmidt (2002), rotenone was administered intraperitoneally (ip) to Sprague-Dawley rats at a dose of 1.5 or 2.5 mg/kg/day for 2 months. The treatment caused depletion of dopamine in the posterior striatum (46% loss at the higher dose) and the prefrontal cortex (80% loss at the high dose). The authors suggested that dopaminergic synapses in the substantia nigra pars compacta and in the nigrostriatal pathway were sensitive to the action of rotenone. This is in contrast to the findings of Betarbet and colleagues who found that changes in the substantia nigra were later events. In behavioural tests, the treated animals showed a dose-dependent increase in catalepsy and decrease in locomotion. The authors suggested that this (sub)chronic exposure by ip injection was comparable to chronic environmental exposure and is thus comparable to the 'real life' situation. This would clearly not be the case if orally administered rotenone did indeed have little toxicity.

In a study in mice, animals were treated with a single sc injection of rotenone (5, 10 or 15 mg/kg) or subchronically (1.5 mg/kg, 3 times a week for 3 weeks). No effects on striatal levels of dopamine were observed and there was no dopaminergic damage (Thiffault *et al*., 2000).

Sherer *et al.* (2002b) treated human neuroblastoma cells with 5 nmol/l rotenone for 1–4 weeks. Treatment for 1 week led to elevated α-synuclein levels and chronic exposure increased insoluble α-synuclein and ubiquitin. There was evidence of oxidative stress after 2 weeks with loss of glutathione and increased oxidative DNA and protein damage and increased sensitivity to oxidative caspase-dependent apoptosis, as is observed in PD pathogenesis. After 4 weeks, 5% of cells began to undergo apoptosis.

In *in vitro* studies on primary rat or mice mesencephalic neuron-glial cultures, rotenone induced significant and selective dopaminergic neurodegeneration two days after treatment with 20 nmol/l or eight days after treatment with 1 nmol/l (Gao *et al*., 2002). A non- or minimally toxic concentration of rotenone (0.5 nmol/l) and the inflammatory agent lipopolysaccharide (0.5 ng/ml) 'synergistically' induced dopaminergic degeneration (Gao *et al*., 2003). The authors concluded that this was possibly due to the release of superoxide free radicals mediated by microglial NADPH oxidase. In a recently published letter, Niehaus and Lange (2003) suggested that endotoxin (lipopolysaccharide) might be an environmental factor in the development of PD. Two recent publications have presented data suggesting that microglial activation plays a role in rotenone neurotoxicity (Liu *et al*., 2003; Sherer *et al*., 2003a).

In a further *in vitro* study using human neuroblastoma SH-SY5Y cells, rotenone induced apoptotic cell death via the activation of both mitochondrial and endoplasmic reticulum-dependent caspases (Kitamura *et al*., 2002).

5.2.3 Paraquat

Paraquat (Figure 2.1) is a potent non-selective contact herbicide, which has been widely used in agriculture since its introduction in 1962. It is extremely toxic to the pulmonary system, where it is highly concentrated and induces acute alveolitis, widespread fibrosis and fatal hypoxia. The mechanism of lung toxicity involves cyclic reduction/reoxidation of paraquat, with consequent NADPH consumption and the production of oxygen free radicals (Corasaniti *et al*., 1998). Interest in the neurotoxicity of paraquat came with the discovery of the PD-like syndrome associated with MPP^+, which was marketed as a herbicide under the name Cyperquat® and has a clear chemical analogy to paraquat.

One of the major considerations in relation to the potential neurotoxicity of paraquat, as with other potential neurotoxicants, is the extent to which it can cross the blood–brain barrier (BBB). Paraquat is a charged molecule, which may not cross the BBB, and it is not metabolised to a species more likely to gain access to the brain (Sanchez-Ramos *et al*., 1987). Naylor *et al*. (1995) examined the distribution of 20 mg/kg ^{14}C-labelled paraquat administered sc to rats. A maximum concentration of 0.05% was recorded in the brain within the first hour, decreasing to 13% of that figure after 24 hours (1.6 nmol/g wet weight). After dissection of brain, most of the paraquat was found to be associated with structures lying outside the BBB (pineal gland and linings of the cerebral ventricles) or without a BBB (anterior portions of olfactory bulb, hypothalamus and area postrema). No evidence for neuronal cell necrosis was found, 24 or 48 hours after administration. Overall, paraquat did not appear to pose a major neurotoxicological risk in brain areas with a functional BBB. A further paper from this group reported the entry of paraquat into the brains of neonatal, adult and aged rats using the same dosing procedure (Widdowson *et al*., 1996). Again low levels of paraquat were observed in the brain, again associated with structures outside or with no BBB. There was some accumulation in deeper regions of the brain, particularly in neonatal rats, 24 hours after dosing, which the authors suggested was due to impaired BBB integrity. However, no evidence of neuronal cell damage was noted at histopathological examination. A further study on neonatal mice given paraquat orally, as a single dose (0.07 or 0.36 mg/kg), on gestation days 10 and 11, reported hypoactivity in the treated mice (both doses) at 60 and 120 days, with a reduction in striatal dopamine and dopamine metabolite levels in the higher dose group (Fredriksson *et al*., 1993); this contrasts with the increase in activity and dopaminergic systems associated with PD-like mechanisms. This is the only study that has been reported in which paraquat was administered orally.

Interestingly, early reports describe pathological effects in the brain of patients dying from paraquat poisoning although these were ascribed to hypoxia caused by the characteristic pulmonary fibrosis (Hughes, 1988).

However, there is increasing evidence that paraquat does cross the BBB. Corasaniti *et al*. (1998), using a specific high performance liquid chromatography (HPLC) method, have demonstrated that paraquat administered by ip injection, can cross the BBB, with a differential regional distribution in the brain. However, the low levels of paraquat seen are similar to those that might be expected from plasma trapped within brain blood vessels. A recent study using a brain microdialysis technique (together with HPLC), with freely moving rats, provided evidence of paraquat transportation across the BBB (Shimizu *et al*., 2001). Single sc injections of 5, 10 or 20 mg/kg (15.8, 31.6 and 63.1 μmol/kg) led to the dose-dependent appearance of paraquat in the dialysate. MPP^+ (which does not cross the intact BBB) administered after paraquat did not appear in the dialysate, suggesting that paraquat (or the implantation of the microdialysis probe) had not damaged the endothelial cells of the BBB. Further experiments suggested the involvement of the neutral amino acid transporter in the carriage of paraquat into the brain, followed by transportation into striatal, possibly neuronal, cells, in a Na^+-dependent manner. The authors also suggested that other methods of measuring brain levels of

paraquat have technical disadvantages, which may explain the apparently conflicting reports (Shimizu *et al*., 2001). A recent paper from the same group showed that the uptake of paraquat (given sc 10 mg/kg/day for 5 days) into rat striatal tissue, including dopaminergic terminals, was inhibited by a specific dopamine transport inhibitor. The subchronic treatment significantly decreased dopamine content in the striatum and slightly in the cortex and midbrain (Shimizu *et al*., 2003).

Paraquat has been shown to be neurotoxic following injection into specific areas of the brain (De Gori *et al.*, 1988; Iannone *et al.*, 1988; Calò *et al.*, 1990; Iannone *et al.*, 1991; Bagetta *et al.*, 1992; Corasaniti *et al.*, 1992, 1998). Depending on the brain region into which the paraquat was stereotaxically injected, it produced different behavioural patterns, increased locomotor activity and caused convulsions; these effects were accompanied by neuronal cell death. Pretreatment with superoxide dismutase prevented some of the behaviour patterns suggesting that free radicals and heavy metals (needed for the catalytic function) might play a role (Iannone *et al.*, 1988, 1991). In general, these studies suggest that paraquat neurotoxicity is not specific for the dopaminergic nigrostriatal system, as neurotoxic effects were observed when paraquat was injected into regions of the brain where other neurotransmitter systems are located. In a further study by Liou *et al.* (1996) in rats, single intranigral injections of paraquat (1–5 μg) led to a depletion of striatal dopamine levels, a loss of neurons in the substantia nigra and dose-dependent behavioural changes, which persisted throughout the 16 weeks of the experiment.

In a recent study, paraquat (together with glutamate receptor antagonists) was stereotaxically injected into the left striata of rats, which were then microdialysed. There was a transitory increase in extracellular levels of glutamate and long-lasting elevation of nitrite and nitrate ions and dopamine (Shimizu *et al*., 2003). The authors suggested a mechanism by which paraquat treatment might lead to the death of dopaminergic neurons, through stimulation of glutamate efflux from neural cells or inhibited glutamate uptake and initiation of a cascade of excitotoxic reactions, leading to dopaminergic terminal damage. The proposed mechanism involved glutamate-induced activation of non-NMDA receptors, resulting in activation of NMDA receptors with a subsequent massive influx of Ca^{2+}. Such entry of Ca^{2+} into the cell would stimulate NOS and the release of NO; Ca^{2+} would diffuse to the dopaminergic terminals and induce mitochondrial dysfunction, triggering the production of ROS from mitochondrial electron transport in dopamine neurons. The ROS would react with NO to form peroxynitrite, which, it is considered, could be a major substrate underlying paraquat neurotoxicity. The authors concluded that paraquat could be considered an exogenous neurotoxin involved in the aetiology of PD or, at least, that exposure to low levels of paraquat for a long time would make dopaminergic neurons vulnerable to oxidative stress and cell death (Shimizu *et al*., 2003).

A number of studies have observed neurotoxicity after systemic administration of paraquat. An increase in dopaminergic neuronal death in the substantia nigra pars impacta was observed in mice treated with 1–10 mg/kg paraquat administered by ip injection once a week for three weeks (McCormack *et al*., 2002). The cell loss was dependent on dose and age. This effect appeared to be specific for the substantia nigra pars impacta as there was no decrease in either GABAergic cells in the substantia nigra pars reticulata or in Nissl-stained neurons in the hippocampus. There was no depletion in striatal dopamine but enhanced dopamine synthesis was shown by increased tyrosine hydroxylase activity. The authors suggested that the apparent discrepancy between neurodegeneration and a lack of dopamine loss was probably a reflection of compensatory mechanisms by which neurons that survive damage were capable of restoring neurotransmitter tissue levels.

Single ip injections of paraquat (20 or 100 mg/kg) led to behavioural effects in rats, including shakes, clonus of the forelimbs and rearing (Bagetta *et al*., 1992; Corasaniti *et al*., 1992). These excitatory effects were abolished by prior treatment with atropine. Neuronal cell death in the pyriform cortex was observed 24 hours after ip administration of 20 and 100 mg/kg paraquat but not at 5 mg/kg. Again, atropine reduced the effect, while methylatropine, which does not cross the BBB, offered no protection. This suggested that the paraquat was acting beyond the BBB. Further studies have reported neurobehavioural effects of systemic treatment with paraquat (sc injection of 1, 2 or

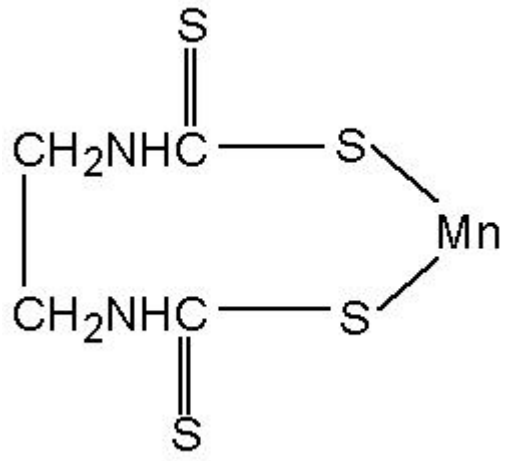
3 mg/kg/day for 8 weeks) in rats (Satayavivad *et al*., 1997) and mice (ip injections of 5 or 10 mg/kg, once a week for 3 weeks; Brooks *et al*., 1999). The effects in the mice were accompanied by dopaminergic neuron loss. Two further studies gave some indication of the mechanisms of effect after systemic administration of paraquat. When rats were treated intravenously with paraquat (100 mg/kg in 5 doses over 10 days), the brains had lower Complex I activity and higher levels of lipid peroxides (indicating free radical activity) and a lower level of dopamine in the striatum (Tawara *et al*., 1996). Mice were treated with 10 mg/kg paraquat once a week for three consecutive weeks and an upregulation and aggregation of α-synuclein was observed after each dose, which returned to normal levels within 7 days (Manning-Bog *et al*., 2002). The studies of Woolley *et al.* (1989) in mice (40 mg/kg given ip and intracranially) and of Naylor *et al*. (1995), in rats (detailed earlier), showed no neurotoxic effects or changes in brain dopamine levels.

The reactivity of paraquat and its cyclic reduction/reoxidation suggest that the generation of oxygen free radicals may be an important effect of paraquat; induction of free radicals has been observed in rat and mouse brain microsomes (Yang & Sun, 1998; Yumino *et al*., 2002) and some of the neurotoxicity of paraquat has been reversed by pretreatment with free radical scavengers (Corasaniti *et al*., 1998) and superoxide dismutase (Iannone *et al*., 1991). However, seizures and neurotoxicity induced by hippocampal infusion of paraquat in rats were prevented by the NMDA receptor antagonist, MK801, suggesting a role for the excitatory amino acid, glutamate (Bagetta *et al*., 1992). Oxygen free radicals generated by the redox action of paraquat may lead to the abnormal release of glutamate, which is excitotoxic, leading to neuronal damage. In addition, several studies, mostly *in vitro,* suggest that apoptotic cell death may be implicated in the neuronal damage caused by paraquat (Corasaniti *et al*., 1998; Yang & Sun, 1998; Li & Sun, 1999).

5.2.4 Combination of paraquat and maneb

Maneb (manganese ethylene bisdithiocarbamate; Figure 5.5) is a dithiocarbamate herbicide, which has been shown to have dopaminergic activity (Section 5.2.5). The areas of use of maneb and paraquat have a marked geographical overlap in the USA (Thiruchelvam *et al*., 2000a,b). There have been a number of experimental studies of the combined effects of paraquat and maneb.

Figure 5.5 Structure of maneb



Mice were exposed to paraquat (5–10 mg/kg) or maneb (15–30 mg/kg) ip, either alone or in combination, once a week for 4–6 weeks (Thiruchelvam *et al.,* 2000a,b). A sustained decrease in motor activity was consistently observed only in the combined exposure groups. In the combined exposure groups, striatal dopamine and dopamine metabolite levels increased immediately post-injection and then decreased after 7 days, and reduced levels of tyrosine hydroxylase and dopamine transporter occurred in the dorsal striatum.

Combined exposure thus resulted in potentiated effects that appear to target the nigrostriatal dopaminergic systems. The authors suggest that mixtures of pesticides could play a role in the aetiology of PD. In a further study on developmental exposure to the combined pesticides, mice were treated daily by ip injection from post-natal days 5–19 with paraquat (0.3 mg/kg), maneb (1 mg/kg) or in combination (Thiruchelvam *et al*., 2002). A subset of mice was rechallenged as adults, with ten times the dose, alone or in combination, twice a week for three weeks. Mice given the combined treatment and re-challenged as adults were most affected; motor activity was reduced by 70% and striatal dopamine levels by 62% (37% reduction in dopamine after combined developmental exposure alone). Although the greatest loss of nigrostriatal dopaminergic cells was seen after combined treatment, there was significant loss with all treatments following rechallenge in adults, suggesting that the production of a state of 'silent toxicity' was unmasked upon adult rechallenge.

In a further recent study by the same group (Thiruchelvam *et al*., 2003), mice of different ages (5 weeks, 5 months and 18 months) were administered paraquat (10 mg/kg), maneb (30 mg/kg) or a combination of the two, three times a week for three weeks. Reduction in locomotor activity and motor co-ordination and reduction in dopamine metabolites and turnover were greatest in the oldest mice (18 months old). Decreases in the number of nigrostriatal dopaminergic neurons were progressive, particularly in the oldest mice treated with paraquat and maneb in combination. The result demonstrates an enhanced sensitivity to toxicity of the ageing dopamine pathway, particularly to paraquat and maneb.

Another report from the same group (Barlow *et al*., 2003) suggested that a number of different dithiocarbamates potentiate the toxicity of both MPTP and paraquat in mouse models of parkinsonism. This included the increased accumulation of dopamine in synaptosomes due to delayed efflux.

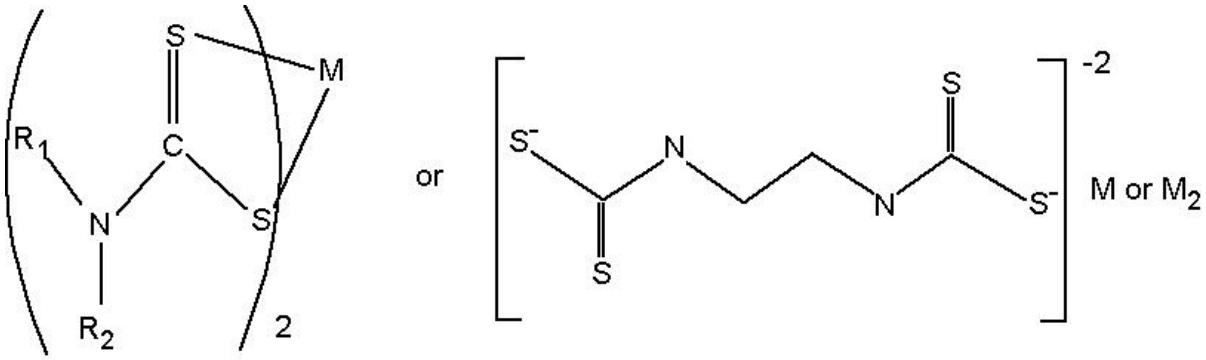
The Acceptable Daily Intake (ADI) for oral paraquat ion exposure is 0.004 mg/kg bw and the Acceptable Operator Exposure Level (AOEL) for systemic exposure is 0.0004 mg/kg bw; for maneb, the ADI and AOEL are 0.03 mg/kg bw. The doses used ip in the animal studies appear to be much higher (the pesticides are much less easily absorbed from the gastrointestinal tract). The specific effects of any combined treatment are not considered when setting these levels.

5.2.5 Dithiocarbamates

There is some evidence for the neurotoxicity of dithiocarbamates (Figure 5.6), including studies on the manganese-containing pesticide, maneb, alone and used in combination with paraquat (as outlined above). While manganese has been shown to cause PD-like effects in workers at high occupational exposure, it affects the globus pallidus rather than the substantia nigra and it is also resistant to the beneficial effects of L-dopa. However, neurotoxic effects have also been observed in studies (outlined below) conducted with the non-manganese-containing parent compound, ethylene bisdithiocarbamate (EBDTC) from which maneb is derived.

Extrapyrimidal effects were observed in a worker following chronic occupational exposure to maneb (exposure for 4 hours/day, 4 days/week, for about 3 years to 45 kg of the fungicide in a closed environment with no personal protection). L-dopa had no beneficial effects, and the authors suggested that both the manganese and the EBDTC components might have had toxic effects (Meco *et al*., 1994). In a brief abstract Ferraz *et al*. (1987) have also described neurobehavioural symptoms (nervousness, fatigue, memory complaints, sleepiness and headache) and some rigidity and tremor, in rural workers exposed to maneb although there was no correlation with estimated degree of exposure. The authors suggested that exposure to manganese might be the source of the effects.

Figure 5.6 Structure of dithiocarbamates



R_1, R_2 = alkyl; M = metal ion

Acute exposure to maneb (30–1000 mg/kg, ip) led to a CNS-depressant-like effect on mice, as measured by locomotor activity and other neurobehavioural tests. The results also suggested the involvement of dopaminergic systems (Morato *et al*., 1989). Maneb was also shown to enhance MPTP neurotoxicity in mice (Takahashi *et al*., 1989). EBDTC (McGrew *et al*., 2000) and diethyldithiocarbamate (DDC; Miller *et al*, 1991) have both been observed to enhance both the neurobehavioural effects and the striatal dopamine depletion of MPTP in mice. Miller *et al.* (1991) did not examine the effects of DDC alone. *In vitro* studies on rat mesencephalic-striatal primary cocultures, using both mancozeb (manganese-zinc-EBDTC) and zineb (zinc-EBDTC), indicated that both compounds had similar inhibitory effects on dopamine and γ-aminobutyric acid (GABA) uptake at 10 and 50 μmol/l (Soleo *et al*., 1996). The authors suggested that EBDTC rather than manganese might have been responsible for the cytotoxic effects on neuronal systems and that the findings were relevant to the pathophysiology of parkinsonism. A recent study showed that metal-dithiocarbamate complexes, such as maneb, have the potential for promoting oxidative stress in catecholaminergic areas of the brain by catalysing catechol oxidation (Fitsanakis *et al*., 2002).

In a recent study by Zhang *et al*. (2003), maneb (20 nmol/day for 14 days) was delivered directly to the lateral ventricles of rats. Dopaminergic neurodegeneration and extensive striatal dopamine efflux were induced, comparable to that induced by MPP^+. *In vitro* experiments on isolated brain mitochondria, showed that maneb preferentially inhibited mitochondrial Complex III (Figure 5.3).

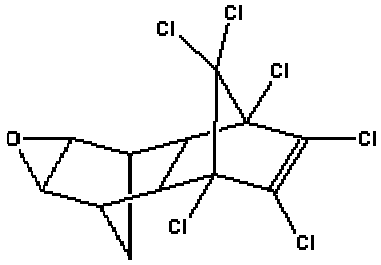
Disulfiram is a drug used in the treatment of alcoholism. It is metabolised to DDC and has been shown to increase the uptake of lead into the brain. In a study, pregnant rats were given lead in their drinking water (0.25%) and disulfiram (peroral (po); 0.01 mmol/kg twice a week) and the offspring also received lead via parental milk and disulfiram po (Oskarsson *et al*., 1986). While neither lead nor disulfiram alone caused any behavioural effects, in combination the two compounds caused an increase in activity and increase in extracellular levels of dopamine and its metabolites in the caudate nucleus (measured by an intracerebral dialysis technique). Interestingly, there were no changes in other neurotransmitter amino acids.

Further experiments, with administration to rats of high doses of disulfiram (500 mg/kg ip) and DDC (290 mg/kg ip), and *in vitro* binding studies with rat striatal membranes and bovine striatal synaptic vesicles, investigated the hypothesis that dopaminergic malfunction was important in disulfiram neurotoxicity (Vaccari *et al*., 1996). Disulfiram antagonised the uptake of dopamine into striatal synaptic vesicles *in vitro* and induced overflow of striatal dopamine *in vivo*. The authors concluded that disulfiram intoxication may acutely disrupt dopamine balance, and that this effect probably underlined some of the central neurotoxic extrapyrimidal symptoms associated with DDC overdose.

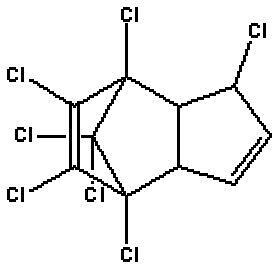
5.2.6 Cyclodienes

Bloomquist and colleagues have carried out a number of studies examining possible effects of the organochlorine cyclodiene pesticides, in particular, dieldrin and heptachlor (Figure 5.7), on possible biomarkers of PD. Although these pesticides are no longer in use, they are still environmentally persistent. Mice were administered heptachlor (3–100 mg/kg) by ip injection, three times a week for two weeks (Bloomquist *et al.*, 1999; Miller *et al*., 1999; Kirby *et al*., 2001). There was an increase in the maximal rate of striatal dopamine uptake, which was attributed to induction of the dopamine transporter (DAT) and a compensatory response to elevated synaptic levels of dopamine. The striatal dopaminergic nerve terminals were found to be differentially sensitive to heptachlor compared with the uptake of 5-hydroxytryptamine into cortical synaptosomes, which was unaltered. There was also an increase in the vesicle monoamine transporter (VMAT2) in the striatum of the mice. The authors suggested that heptachlor and perhaps other organochlorine pesticides exert selective effects on striatal dopaminergic neurons and may play a role in the aetiology of PD (Kirby *et al*., 2001). A further study on heptachlor (0–8.4 mg/kg/day) and dieldrin (3 mg/kg/day) administered po to pregnant rats, while causing some maternal toxicity, resulted in increased activity of striatal DAT in offspring exposed during gestational, perinatal and adolescent periods (Purkerson-Parker *et al*., 2001).

Figure 5.7 Structure of dieldrin and heptachlor



Dieldrin



Heptachlor

There is some evidence that dieldrin may interfere with electron transport and increase the generation of superoxide radicals (Stedeford *et al*., 2001). In proliferating PC12 cells exposed to 100 µmol/l dieldrin, there was evidence for the accumulation of oxidised DNA, which might have arisen owing to increased oxidative stress. In mesencephalic cell cultures (Sanchez-Ramos *et al*., 1998) and PC12 cells (Kitazawa *et al*., 2001), there was a rapid release of dopamine and its metabolite, and this was followed by apoptotic cell death in PC12 cells.

Further studies have been conducted on the organochlorine cyclodiene pesticide, endosulphan. While its convulsant and proconvulsant actions have been attributed to an antagonistic action on GABA, a dopaminergic involvement has been suggested for its induction of hypermotor activity and circling movement (Ansari *et al*., 1987; Paul & Balasubramaniam, 1997). A study on rat development involving repeated administration of endosulphan during gestation and lactation up to 2–3 weeks of age (3 mg/kg ip), produced a significant decrease in the affinity and maximum numbers of striatal dopaminergic receptors without affecting other receptor profiles, suggesting that dopaminergic receptors are unusually sensitive to the action of endosulphan (Seth *et al*., 1986).

A study on rats injected with aldrin (5 mg/kg/day, ip), either for a single day or daily for 12 consecutive days, resulted in enhanced locomotor activity, which was greater after the repeated treatment (Jamaluddin & Poddar, 2001). Subsequent study of the neurotransmitter pathway using agonists and antagonists suggested a decrease in GABAergic activity and an activation of dopaminergic systems via inhibition of cholinergic activity. However, this enhanced activity is not suggestive of a mechanism leading to PD-like effects (Jamaluddin & Poddar, 2001).

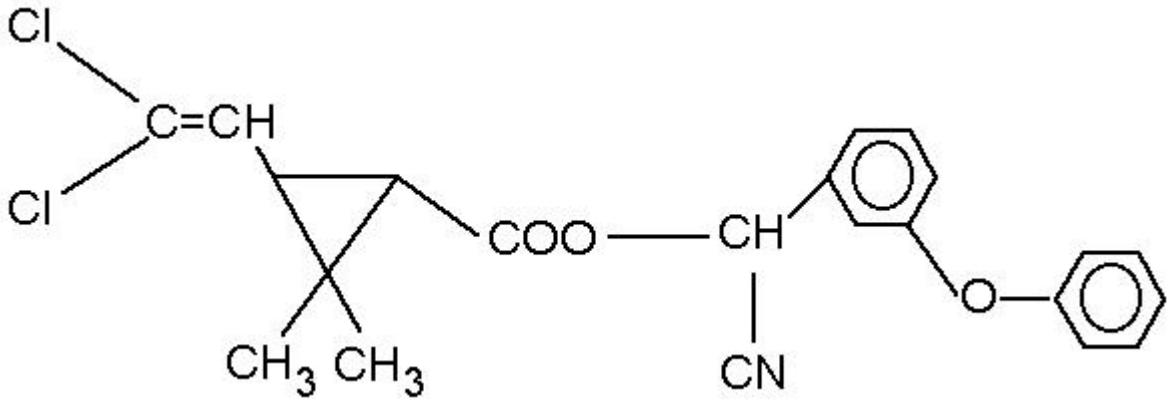
5.2.7 Lindane

The main neurotoxic effect of lindane is the inhibition of $GABA_A$ receptors (Rivera *et al*., 1998). The activities of a number of other neurotransmitters were increased after acute and subchronic high dose treatment of rats with lindane (30 or 60 mg/kg po; Artigas *et al.*, 1988; Rivera *et al.*, 1991; Martinez & Martinez-Conde, 1995); this included increased activity of dopaminergic neurons in the substantia nigra (Artigas *et al*., 1988). A general increase in neurotransmitter turnover and activity was also observed in rat pups treated orally with a single dose of lindane (20 mg/kg) or seven repeated doses of 10 mg/kg (Rivera *et al*., 1998). It is not clear whether the observed changes in other neurotransmitter pathways are due to the release of the inhibitory action of GABA.

5.2.8 Pyrethroids

In a study designed to investigate the possible involvement of the pyrethroid, permethrin (Figure 5.8), and the organophosphate, chlorpyrifos, on the aetiology of PD and 'Gulf War illness', mice were treated with permethrin (three ip doses of 0.2–200 mg/kg over a two week period; Karen *et al*., 2001). At low doses, dopamine uptake was increased (e.g. 134% at 1.5 mg/kg), while at higher doses, dopamine uptake was depressed (e.g. 50% at 25 mg/kg). A reduction in mitochondrial function was observed in *in vivo* synaptosome preparations and, although striatal dopamine levels were not decreased, there was an increased dopamine turnover. The mice showed a decrease in open field behaviour at the highest dose (200 mg/kg). Although frank parkinsonism was not observed, dopaminergic neurotransmission was affected by exposure to permethrin. Overproduction of α-synuclein is increasingly thought to play a role in the development of PD.

Figure 5.8 Structure of permethrin



In a further study by Bloomquist and colleagues, mice were treated with the pyrethroid pesticide, deltamethrin (three times over two weeks with 6 mg/kg; Kirby *et al*., 1999). A 70% increase in maximal dopamine uptake was observed in *ex vivo* synaptosomes suggestive of an upregulation in DAT expression. The dopaminergic nerve terminals of the striatum were more sensitive to deltamethrin than those of other neurotransmitter types. Unlike MPTP, deltamethrin did not decrease dopamine although there was some evidence for an increased turnover. The authors suggested that higher doses or longer exposure times might be expected to yield greater effects on dopamine content in the nigrostriatum.

When the pyrethroid insecticide, fenvalerate, was administered to rats by gavage for 21 days (5, 10, 20 mg/kg/day), there was a pronounced inhibition of dopamine and its metabolites and decreased dopamine binding in several brain regions, including the corpus striatum. However, the effects were not dose related (Husain *et al*., 1991). In a further study, fenvalerate (10 mg/kg) or cypermethrin (15 mg/kg) were given by gavage during gestation and lactation to pregnant and nursing dams (Malaviya *et al*., 1993). With both treatments there was a significant increase in dopamine and muscarinic receptors of striatal membranes in the pups; there were additional effects on

acetylcholinesterase, monoamine oxidase and Na^+, K^+ ATPase. The authors suggested that the findings demonstrated disturbance of the dopaminergic and cholinergic pathways.

5.2.9 Organophosphates

In the study outlined in Section 5.2.8 (Karen *et al*., 2001), chlorpyrifos was administered to mice (three sc doses of 25–100 mg/kg) over a 2 week period. In striatal tissue isolated from the treated mice, a small decrease in dopamine uptake was observed; there was a decrease in mitochondrial function and an increase in dopamine turnover. There was a decrease in open field behaviour at the highest dose tested.

Although the organophosphate, dichlorvos, is a directly acting inhibitor of acetylcholinesterase, a study by Choudhary *et al*. (2002) showed that a single sc dose (200 mg/kg) given to rats (pretreated with 2-pyridinealdoximemetiodide to prevent the anti-cholinergic effects) resulted in marked changes to the dopaminergic neurotransmitter system. Changes included decreased dopamine binding and increased activity of tyrosine hydroxylase and dopamine-β-hydroxylase. The authors suggested that alterations in the dopamine system may be a causative mechanism behind the behavioural and functional changes associated with delayed organophosphate neurotoxicity.

5.2.10 DDT

Several studies have examined the mechanism behind the tremor induced by DDT given orally or iv. Induced tremor is increased by the dopaminergic antagonist haloperidol (Herr & Tilson, 1987; Morio *et al*., 1987). However, a number of other neurotransmitter pathways also seemed to be involved, including glutamate, aspartate and serotonin (Hong *et al*., 1986).

5.2.11 Other pesticides

The pesticide, 2,4-D, has well-established neurotoxic effects. Injection of 2,4-D (100 µg) into one striatum of a rat produced a marked depression in locomotor activity and circling behaviour and an increase in dopamine metabolism (Bortolozzi *et al*., 2001). The result indicated that 2,4-D induced neurotoxicity in the basal ganglia of rats. In a further study, pregnant rats treated by gavage on gestational days 6–15 with a 1:1 mixture of 2,4-D and 2,4,5-trichlorophenoxyacetic acid (50 and 100 mg/kg/day), showed a delayed ontogeny of brain dopamine (but not noradrenaline), together with a delay in the development of certain behaviour (such as self righting reflex, negative geotaxis and swimming performance in pups; Mohammad & St Omer, 1985).

A heightened locomotor and stereotype response was observed in rats receiving the antifungal agent, triadimefon, at very high doses of 100 mg/kg ip on alternate days for 14 days (Hill *et al*., 2000). This was primarily through the potentiation of dopamine activity. There was an increase in dopamine uptake and release in the striatum and nucleus accumbens.

Two studies have examined the effect of carbamate pesticides on dopaminergic behaviour. Rigon *et al*. (1994) administered a single oral dose of carbaryl (10–80 mg/kg) to rats together with dopaminergic agonists and antagonists. At high doses (200 mg/kg) carbaryl has been shown to induce tremor, which can be reduced by prior treatment with L-dopa. Carbaryl potentiated the catalepsy induced by the striatal dopaminergic receptor blocker, haloperidol. However, it also increased the number of yawns induced by the dopamine agonist, apomorphine, except at the highest dose of 80 mg/kg. The observed inhibition of serum acetylcholinesterase led the authors to suggest that the effects of carbaryl involved a disturbance of the balance between cholinergic and dopaminergic systems. A further study involved the treatment of weanling rats for 90 days with carbamate insecticides, aldicarb and methomyl, or a triazide herbicide, metribuzin, either individually or in a mixture with each dose lowered to give equivalent toxicity (Boyd *et al*., 1990). Animals treated with a mixture of all three pesticides were found to have slower speeds in maze-running (motor control) and decreased choline in the neostriatum but no changes in serotonin or dopamine.

5.3 Summary of toxicology studies

Although the processes involved in the development of PD have not been fully defined, there is evidence for the potential involvement of a number of mechanisms. From these, a number of criteria have been identified for assessing the potential of pesticides to play a role in the aetiology of PD. These are:

- effects on the striatal dopaminergic system;
- effects on the substantia nigra region of the basal ganglia;
- mechanistic effects, such as the generation of oxidative stress, mitochondrial dysfunction, inhibition of Complex I and α-synuclein aggregation; and
- evidence of entry into relevant regions of the brain following appropriate routes of administration.

Detailed mechanistic studies show that rotenone, at least when administered parenterally, acts on the appropriate dopaminergic systems in the nigrostriatal region of the brain, which is the target site in PD. Its neurotoxic effects are mediated by the same general mechanisms proposed for the development of PD (e.g. Complex I inhibition, formation of Lewy body-like structures and apoptosis), and exposed animals have developed motor and postural changes characteristic of PD. The dose levels used in experimental studies have clearly been higher than those that would be encountered by anyone, even regular, heavy users of rotenone. The relevant environmental routes of exposure have also not yet been tested. However, the studies do establish the important point that systemic administration of a xenobiotic, with what would otherwise be considered a generalised mode of action, can selectively target the same neurons that degenerate in PD. Both this observation and the other example of MPTP (in man) clearly establish that xenobiotics are a plausible cause of PD.

There is still some question over the degree of entry of paraquat into the brain through the BBB. While paraquat is taken up into the striatal tissue, where it may lead to a depletion of striatal dopamine, a loss of neurons in the substantia nigra and behavioural changes similar to those seen in PD, there is some suggestion that the effects of paraquat are not specific to the nigrostriatal region of the brain. The mechanisms underlying the toxic effects of paraquat are clearly relevant to those proposed in the development of PD, including the generation of oxygen free radicals, effects on glutamate, leading to neuronal damage, inhibition of Complex I, and accumulation of α-synuclein. The studies of systemic administration of paraquat given alone and in combination with maneb suggest that although paraquat may not in itself be highly neurotoxic, it may become so at high doses when combined with dithiocarbamates. The mechanism of such synergy is not at present clear.

There is some evidence that dithiocarbamates alone are neurotoxic via effects on the dopaminergic system, accompanied by reduced locomotor activity such as that proposed for the development of PD. However, there is no clear evidence that the action is specific for the substantia nigra. The results of studies of dithiocarbamates in combination with paraquat suggest that dithiocarbamates may interact with other xenobiotics.

While cyclodiene pesticides do appear to have an effect on the dopaminergic system, in a number of cases this appears to be activation accompanied by increased locomotor activity rather than the inhibitory processes that are proposed in the aetiology of PD.

At present, there is some evidence for an effect of pyrethroids on dopaminergic systems, mitochondrial function, slowing of behavioural activity and accumulation of α-synuclein. At the higher dose levels described in these studies, pyrethroids have acute, reversible actions on motor behaviour, which would be expected to be reflected by changes in brain dopamine.

A number of other pesticides have been shown to have neurotoxic actions, which appear to involve the dopaminergic systems. However, the available evidence is insufficient to make conclusions about their possible role in the aetiology of PD. Although the main action of organophosphate pesticides is the inhibition of acetylcholinesterase, there is some evidence for effects on dopaminergic systems, namely mitochondrial dysfunction and decreased activity. The tremor induced by DDT may involve dopamine, but a number of other neurotransmitter systems appear to be involved and any effects of lindane on dopamine appear to be secondary to the inhibition of GABAergic systems.

5.4 Conclusions from toxicology studies

There are currently insufficient studies to assess fully the potential mechanisms by which pesticides might influence PD development.

However, there are sufficient data to suggest that with certain routes of administration, rotenone and paraquat may have neurotoxic actions that could potentially play a role in the development of PD. These include effects on dopaminergic systems in the substantia nigra, and evidence for the presence of α-synuclein aggregation, which appears to play a specific role in PD. There is also some evidence that the mechanisms of neurotoxicity associated with exposure to pyrethroids are those that would be necessary for a role in the development of PD. There is evidence that dithiocarbamates may interact with other xenobiotic agents to increase neurotoxicity. Studies on lindane, DDT and organophosphates suggest that, while these pesticides have neurotoxic actions, they do not act on systems in the brain of relevance to PD. For other pesticides, there are insufficient data for an association with PD.

Of potentially great importance are the few studies that report dopaminergic neurotoxicity after combined low-level exposure to multiple environmental neurotoxicants, including rotenone and lipopolysaccharide (which may be present due to inflammation or infection) or paraquat and maneb or as a result of the combined effects of pesticides and metals on α-synuclein. Such studies suggest that exposure to multiple low-level environmental neurotoxicants may be a significant aetiological factor in the long-term development of PD.

The examples of PD-like damage induced by MPTP (in man) and rotenone (in animals) make it clear that, at high doses, some xenobiotics can certainly induce parkinsonism. However, many of the studies have been designed to elicit toxic effects in the shortest time in order to study the mechanisms of action. As such, no study has been carried out with administration of pesticides at levels to which the general population, or even pesticides users are exposed. No appropriate studies have been conducted using oral, inhalation or dermal exposure. Therefore, based on experimental studies, it is not possible, at present, to determine whether typical human exposure to pesticides is likely to be involved in the development of PD. However, PD is clearly a multifactorial disease that develops over a long time period thus making it difficult to model.

Thus, while there is some indication of a potential role for some pesticides in the aetiology of PD, there are insufficient data from relevant studies using routes of administration and doses to conclude that exposure to these pesticides at occupational or environmental levels would lead to the neurotoxicity observed.

6 Overall summary

6.1 Introduction

Parkinson's disease (PD) is a clinicopathological entity characterised by parkinsonism due to neuronal loss, with Lewy bodies in the substantia nigra, and has an overall European prevalence of about 1.6-1.8% in people 65 years or older (de Rijk *et al*., 1997; de Rijk *et al*., 2000). A number of epidemiological and toxicological studies have suggested a potential role for pesticides in the development of PD or parkinsonism. The aim of this review was to conduct a critical assessment of the published epidemiological and toxicological literature to assess the potential role of pesticides in the development of PD.

6.2 Epidemiology

The review of the epidemiological literature identified ten descriptive studies, five cohort studies, 38 separate case–control studies and one meta-analysis that investigated the relationship between PD and pesticide exposure. An association between PD and pesticide exposure was actually tested for in eight of the ten pesticide-related descriptive studies (two case-series studies, three mortality studies, four prevalence studies and one incidence study). Of these eight, all the mortality studies (one of which was reported only as an abstract) and one prevalence study found a significant association between PD and a marker of pesticide use. One case-series study, two prevalence studies, one looking at hexachlorobenzene exposure, and an incidence study (reported only as an abstract) found no association between PD and pesticide use/exposure. Of the five cohort studies identified (including one reported only as an abstract), three showed an increased risk of PD amongst farming occupations of which two inferred an increased risk of PD from pesticide exposure. One study also identified several other occupational groups with an increased risk of PD. An increased risk of PD following pesticide exposure was observed in the majority of the 38 case–control studies identified, with a majority of those showing a significantly increased risk. The excess risks reported in these studies varied from 1.01 to 7.00, although confidence intervals were wide in many studies, partly due to small numbers.

From the epidemiological studies reviewed, there appears to be a fairly consistent association between prior pesticide exposure and an increased risk of developing PD; this relationship appears to be reasonably consistent in different populations and countries, although some studies present conflicting results. The heterogeneity between studies may be a result of exposure misclassification. The exposure category, 'pesticides,' represents many hundreds of chemicals and, as a result, one cohort exposed to 'pesticides' may be exposed to a different group of chemicals compared with another cohort said to be exposed to 'pesticides'. It may be that exposure to only a few pesticide compounds results in an increased risk of developing PD; however, differences in exposure to these compounds would be masked by the use of broad 'pesticide' exposure categories in the studies, possibly resulting in the observed heterogeneity. The level of increased risk identified in different studies is variable, although a meta-analysis focusing on pesticide exposure as a risk factor for PD reported a combined odds ratio of 1.94 (95% confidence interval (CI) 1.49–2.53; Priyadarshi *et al*., 2000). However, only 19 of the 34 comparable case–control studies (excluding autopsy studies and conference abstracts) identified were included in the analysis; hence, the limited nature of the dataset might have influenced the overall risk estimate.

In all the studies reviewed, exposure history was collected retrospectively. In all the case–control studies and some of the cohort studies this was done using a questionnaire, thus introducing the potential for recall bias to occur, which could impact on the internal validity of a study. Some studies made efforts to reduce recall bias, either by the involvement of family members and/or carers (Tanner

et al., 1989; Golbe *et al*., 1990; Koller *et al*., 1990; Chaturvedi *et al*., 1995; Liou *et al*., 1997), although this may have introduced information bias, or by using re-test methods to check the reliability of answers given (Hertzman *et al*., 1990; Koller *et al*., 1990; Butterfield *et al*., 1993; Hubble *et al*., 1993). Those studies that used a re-test method found that the original answers were reliable.

Whilst most of the case–control studies identified looked at 'pesticides' as an exposure category, several studies employed more detailed exposure categories, and significant associations with exposure to herbicides and insecticides as classes of pesticides and PD risk were identified. Based on five studies, findings for an association with fungicide exposure were inconclusive. A few studies that looked at specific pesticide compounds were identified. Seidler *et al*. (1996) identified exposures to organochlorines, alkaline phosphates and carbamates as significant risk factors for PD. Paraquat was shown to be significantly associated with PD in two studies (Hertzman *et al*., 1990; Liou *et al*., 1997) but not in a third (Hertzman *et al*., 1994). One study found a weak non-significant positive association with PD and exposure to DDT (Kuopio *et al*., 1999a).

The relationship between exposure duration and PD risk was investigated in six case–control studies and a cross-sectional study. Four case–control studies and the cross-sectional study found a significant association between increasing pesticide exposure duration and PD risk (Seidler *et al*., 1996; Liou *et al*., 1997; Gorell *et al*., 1998; Chan *et al*., 1998; Engel *et al*., 2001a), although the relationship in one case–control study lost significance after adjusting for smoking, family history, rural living and diet (Chan *et al*., 1998). These studies suggest that PD risk is significantly increased when the duration of exposure to pesticides exceeds a particular threshold (e.g. >10 or >20 years). One case–control study and the cross-sectional study examined the relationship between duration of exposure to herbicides and insecticides. The case–control study found a significant positive trend with increasing duration of herbicide exposure, which was the only significant predictor of PD risk in the study (Seidler *et al*., 1996). The cross-sectional study, after adjustment, did not find a significant relationship, but risk estimates were still elevated in the highest exposure categories (Engel *et al*., 2001a). Only one case–control study examined the relationship between duration of exposure to a specific pesticide compound, paraquat, and PD; there was a significant association with greater than 20 years exposure to paraquat (Liou *et al*., 1997). Overall, these studies suggest that herbicides, possibly paraquat especially, may be risk factors for the development of PD, particularly following extended periods of exposure.

A number of potentially confounding exposures, such as well-water consumption, farming and rural living, have also been found to be associated with an increased risk of PD in a number of studies. In a few of these studies multivariate analyses were performed to examine the relationship between the various risk factors. In a study by Koller *et al.* (1990), multivariate analysis indicated that drinking well-water was dependent on rural living, suggesting the risk factors were interrelated. In one study, well-water use was found to be positively and independently associated with PD (Zorzon *et al*., 2002), and meta-analysis indicated the overall risk estimate to be 1.26 (95% CI 0.96–1.64; Priyadarshi *et al.* 2001). Several studies have also found farming to be an independent risk factor, in addition to pesticide exposure (Gorell *et al*., 1998; Zorzon *et al*., 2002), and the meta-analysis of Priyadarshi *et al.* (2001) yielded a combined risk estimate of 1.42 (95% CI 1.05–1.91). Despite these studies, there still remains uncertainty as to the exact nature of the relationship between well-water consumption, farming, rural living and pesticide exposure and their relationship to PD risk. As a result, further work is required to clarify the contribution of each risk factor to the overall risk of developing PD and to determine whether any of these factors are confounding.

Overall, it seems unlikely that the relatively consistent association between PD and reported exposure to pesticides could be wholly explained by a combination of chance and selective reporting. Based on the available data, extended exposure to classes of pesticides, such as herbicides and insecticides, especially possibly paraquat, appear to be risk factors for the development of PD. However, further studies are required to understand better the relationship with other potentially confounding

exposures, such as well-water consumption and farming, and to elucidate more clearly which particular pesticide exposures may result in an increased risk of developing PD.

6.3 Toxicology

Toxicological studies have examined the potential role of a range of pesticides in the development of symptoms of PD in animal models, and potential mechanisms by which the pesticides may act have been investigated. Currently, the available studies are not sufficiently informative to assess fully the potential mechanisms by which pesticides might influence PD development. However, there is some toxicological evidence to support a link between the neurotoxic effects of some pesticides and the mechanisms that are believed to play a particular role in the development of PD.

A number of detailed mechanistic studies have looked at the insecticide rotenone and have shown that, when administered parenterally, rotenone acts on the dopaminergic systems in the nigrostriatal region of the brain that are affected in PD. Its neurotoxic effects are mediated by inhibition of mitochondrial Complex I, formation of Lewy body-like structures and apoptosis, mechanisms that appear to correspond to changes seen during the development of PD; it also results in motor and postural changes.

The herbicide paraquat, in particular, has been studied extensively and, although the evidence is somewhat conflicting, it does appear to be able to cross the blood–brain barrier (BBB) under some circumstances. There is also evidence to suggest that paraquat is taken up into the striatal tissue (Shimizu *et al*., 2003) where it may lead to a depletion of striatal dopamine, a loss of neurons in the substantia nigra and behavioural changes similar to those seen in PD (Liou *et al*., 1996). There are several proposed mechanisms for the neurotoxic effects of paraquat, including the generation of oxygen free radicals and effects on the excitatory amino acid, glutamate, leading to neuronal damage.

There is evidence that dithiocarbamates may interact with other xenobiotic agents to increase neurotoxicity. There is also some evidence that the mechanisms of neurotoxicity associated with exposure to pyrethroids are those that would be applicable to the development of PD. Studies on lindane, DDT and organophosphates suggest that, while these pesticides have neurotoxic actions, they do not act on systems in the brain of relevance to PD. For other pesticides, there are insufficient data to evaluate any possible association with PD.

Of potential importance are a few studies that report dopaminergic neurotoxicity after combined low-level exposure to combinations of factors, such as paraquat and maneb or the combined effects of pesticides and metals on α-synuclein. Such studies suggest that exposure to multiple low-level environmental neurotoxicants may be a factor in the long-term development of PD.

The examples of PD-like damage induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (in man) and rotenone (in animals) provide evidence that, at high doses, some xenobiotics can certainly induce parkinsonism symptoms. However, the toxicology studies identified focused primarily on mechanisms of action after short-term treatments. As a consequence, the routes, doses, and duration of administration employed have not been environmentally or occupationally appropriate. Thus, there still remains considerable uncertainty as to whether the effects seen in animal models, under these treatment regimes, are predictive of changes at lower doses, over longer time periods and via environmentally relevant routes of exposure. However, the difficulties in modelling experimentally the development of a long-term disease, such as PD, must be acknowledged.

Overall, whilst there is some indication of a potential role for some pesticides in the aetiology of PD, there are insufficient data from relevant studies using routes of administration and doses to conclude that exposure to these pesticides at levels likely to be found in the environment would lead to the neurotoxicity observed.

6.4 Conclusions

Taking into account epidemiological evidence and toxicological evidence on specific compounds and mechanisms, there does appear to be evidence of a potential role of pesticides in the development of PD. However, the current body of evidence is insufficient to establish causation for any particular pesticide.

In particular, only one epidemiological study has shown a dose–response relationship between a specific pesticide (the herbicide paraquat) and an increased risk of PD. This finding needs to be confirmed in further epidemiological studies on different populations for it to be considered an established association. Furthermore, additional toxicological research should be undertaken to understand better the potential mechanisms by which paraquat might act, employing environmentally relevant routes of exposure and doses in order for the results to be considered predictive of the situation in humans.

There is some toxicological evidence that rotenone and other insecticides, for example permethrin, could also potentially act by mechanisms relevant to the development of PD. However, there is limited supporting epidemiological evidence other than associations with pesticide exposure and PD. The limited epidemiological and toxicological evidence for an effect of DDT suggest that this insecticide, now banned in the UK, is unlikely to play a role in the development of PD.

There is currently weak evidence for a role of fungicides in the development of PD. Epidemiological studies found no significant associations between fungicide exposure and PD. However, there is some toxicological evidence that dithiocarbamate fungicides are neurotoxic via effects on the dopaminergic system, although the action is not necessarily specific for the substantia nigra. Overall, further research, particularly toxicological studies at levels of exposure comparable to those experienced in the epidemiological studies is needed to ascertain whether exposure to fungicides may be a risk factor in PD.

For other pesticides, limited toxicological and epidemiological evidence prevent any firm conclusions being drawn. However, an active programme of research is currently underway, particularly in the USA, which can be expected to provide further insight.

In conclusion, the weight of evidence is sufficient to conclude that a generic association between pesticide exposure and PD exists, but is insufficient to be able to conclude that this is a causal relationship or that such a relationship exists for any particular pesticide compound or combined pesticide and other exogenous toxin exposure. In addition, the multifactorial aetiology of PD is likely to make it difficult to extablish unequivocally the role of any individual contributory causal factor.

7 Areas for further research

7.1 Epidemiological research

A large body of epidemiological research is currently underway and, as a result, there are few areas in need of research that are not currently being addressed. However, as discussed earlier, the meta-analyses of Priyadarshi and colleagues (2000, 2001) only included a proportion of the studies currently published; an updated meta-analysis, taking into account all of the available data would be beneficial. The lack of temporal data on the change in Parkinson's disease (PD) incidence and prevalence in the UK is disappointing. If sufficient historical data exist, it would be helpful to determine whether PD incidence and prevalence have changed substantially over about the past 50 years in the UK. If such a study were to show little change in incidence since about 1950, it would suggest that there has been no significant change in people's exposure to risk factors during the same period. Finally, no published epidemiological studies on the risk of PD in relation to pesticide exposure in the UK were identified. Given that UK pesticide exposures may be different to those experienced in other countries (such as the USA) owing to agricultural, regulatory and climatic differences, research on the risk experienced by professional users of pesticides in the UK might be needed to provide insight into risks specific to the UK. In order to be of value, such research would need to address the methodological weaknesses of previous studies, identified below.

Besides these specific areas for further research, a number of methodological weaknesses in the published studies were identified. Any future epidemiological studies looking at exposure to pesticides and the risk of PD should attempt to address these. In particular, many studies suffered from inadequate power to detect a true difference between cases and controls, and this has the potential to lead to confusion as to the true relationship between pesticide exposure and PD and parkinsonism. As a result, future studies need to be of adequate size and have large enough differences in exposure between cases and controls to detect any true differences that may be present. Additionally, many studies used broad exposure categories such as 'pesticides', which represents many hundreds of chemical compounds, without taking into account the duration and pattern of exposure. This does not assist in the identification of any particular etiological agent. Hence future research needs to address these weaknesses and, as far as possible, look at exposure to specific pesticides, such as paraquat and/or rotenone, and take into account variables, such as duration and magnitude of exposure. This would allow more informed conclusions to be drawn about which pesticides might potentially be involved in the development of PD and what duration and/or level of exposure might result in the manifestation of such effects. Furthermore, studies need to be appropriately designed to be able to determine the relative influences of well-water consumption, rural living and farming as an occupation and to clearly determine whether these are independent or confounding risk factors.

7.2 Toxicology

A number of specific areas would benefit from further research. In particular, further research is needed to investigate the interaction of pesticides and various other agents, such as heavy metals, and their relevance to mechanisms potentially involved in PD. Furthermore, future research should also consider the potential for other factors, such as endotoxins, to compromise the integrity of the blood–brain barrier and therefore increase an individual's susceptibility to exposure from environmental agents, such as pesticides.

However, as with the published epidemiological research, there were a number of consistent methodological areas that could be improved upon in future toxicological research. In particular, future research should consider including environmentally relevant routes and doses of exposure. In

many of the published studies pesticides have been have administered parentally or intravenously and at relatively high doses. Whereas such studies are useful when trying to elucidate the mechanisms by which pesticides might act, it is very difficult to interpret their significance for humans. As a result, where possible, future studies should include routes of administration of relevance to humans (i.e. oral, dermal and inhalation); studies should also include doses of similar magnitude to those experienced during the normal use of pesticide products.

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Abbreviations

ADI	Acceptable daily intake
AOEL	Acceptable operator exposure level
ATP	Adenosine triphosphate
BAX	Bcl-2 associated x protein
BBB	Blood–brain barrier
CI	Confidence interval
CNS	Central nervous system
CRISP	Computer Retrieval of Information on Scientific Projects
2,4-D	2,4-Dichlorophenoxyacetic acid
DAT	Dopamine transporter
DDC	Diethyldithiocarbamate
DDT	*p,p*'-Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
EBDTC	Ethylene bisdithiocarbamate
GABA	Gamma-aminobutyric acid
GST	Glutathione *S*-transferase
HPLC	High performance liquid chromatography
ip	Intraperitoneal
iv	Intravenous
MAO	Monoamine oxidase
MPP+	1-Methyl-4-phenylpyridine
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NMDA	*N*-Methyl-D-aspartate
NO	Nitric oxide
NOS	Nitric oxide synthase
OOPD	Old-age onset Parkinson's disease
OP	Organophosphate
OR	Odds ratio
PD	Parkinson's disease
PKCδ	Protein kinase Cδ
po	Peroral
ROS	Reactive oxygen species
RR	Relative risk
sc	Subcutaneous
SHR	Standardised hospitalisation ratio
TIQ	1,2,3,4-Tetrahydroisoquinoline
UCHL1	Ubiquitin carboxyterminal hydrolase
VMAT	Vesicular monoamine transporter
YOPD	Young onset Parkinson's disease

Annex 1

Literature database descriptors

Embase disease descriptors

Embase disease descriptors (descriptors used in the literature search are underlined)	
C2.610.150	**. . . brain disease**
	. . . Alzheimer disease
	. . . . brain arachnoid cyst
	. . . . brain atrophy
	. . . . brain calcification
	. . . . brain cortex atrophy
	. . . . brain cortex lesion
	. . . . brain cyst
	. . . . brain degeneration
	. . . . brain dysfunction
	. . . . brain edema
	. . . . brain hernia
	. . . . brain hypoxia
	. . . . brain necrosis
	. . . . brain psuedotumor
	. . . . cerebral blindness
	. . . . chronic brain disease
	. . . . colloid cyst
	. . . . corticobasal degeneration
	. . . . dentatubropallidoluysian atrophy
	. . . . dialysis encephalopathy
	. . . . encephalomalacia
	. . . . Fahr disease
	. . . . Hallervorden Spatz Disease
	. . . . hypertension encephalopathy
	. . . . internuclear ophthalmoplegia
	. . . . intracranial hypertension
	. . . . intracranial hypotension
	. . . . leukoaraiosis
	. . . . leukoencephalopathy
	. . . . leukomalacia
	. . . . Lewy body
	. . . . Marchiafava Bignami disease
	. . . . minimal brain dysfunction
	. . . . neuroaxonal dystrophy
	. . . . neurofibrillary tangle
	. . . . neuropil thread
	. . . . organic brain syndrome
	. . . . paired helical filament
	. . . . pneumocephalus
	. . . . senile plaque
C2.610.150.10.290	**. . . . extrapyramidal syndrome**
	. . . . . Aicardi Gourtieres syndrome
	. . . . . diffuse Lewy body disease
	. . . . . Gilles de la Tourette syndrome
	. . . . . neuroleptic malignant syndrome
	. . . . . on off phenomenon
	. . . . . Parkinson disease

Embase disease descriptors (descriptors used in the literature search are underlined)	
	. . . . . parkinsonism
	. . . . . progressive supranuclear palsy
	. . . . . spasmodic torticollis
	. . . . . striatonigral degeneration
	. . . . . torsion dystonia
	. . . . . Wilson disease
C2.610.150.10.290.160	. . . . . chorea
	. . . . . . chorea minor
	. . . . . . Huntington chorea
	. . . . . . kernicterus
	. . . . . . Lesch Nyhan syndrome
	. . . . . . tardive dyskinesia

Embase pesticide descriptors

Pesticide descriptors (descriptors used in the literature search are underlined)	
D5	**environmental, industrial and domestic chemicals**
D5.30	**. environmental chemical**
D5.30.60	**. . pesticide** . . . aculeximycin . . . aluminium phosphide . . . antifouling agent . . . arsenic trioxide . . . biocide . . . fufural . . . larvicidal agent . . . pesticide residue
D5.30.60.135	**. . . carbamate pesticide** . . . . benomyl . . . . benthiocard . . . . fenobucarb . . . . mancozeb . . . . maneb . . . . pebulate . . . . phenedipham . . . . propineb . . . . triallate . . . . zineb . . . . ziram
D5.30.60.135.440	**. . . . carbamate insecticide** . . . . . aldicarb . . . . . aminocarb . . . . . carbaril . . . . . carbofuran . . . . . ethiofencarb . . . . . fenoxycarb . . . . . formethante . . . . . isoprocarb . . . . . methiocarb . . . . . methomyl . . . . . oxamyl . . . . . pirimicarb . . . . . propoxur
D5.30.60.150	**. . . chemosterilant** . . . . altretamine . . . . apha chlorohydrin . . . . hempa
D5.30.60.320	**. . . fungicide** . . . . anilazine . . . . benomyl . . . . bis(tributyltin) oxide . . . . captafol . . . . carbendazim . . . . carboxin . . . . chlordecone . . . . chlorothalonil . . . . dichlofluanid . . . . dichlorophen . . . . n (3,5 dichlofophenyl)succinimide . . . . dinocap . . . . edifenphos

Pesticide descriptors (descriptors used in the literature search are underlined)	
	. . . . ethylene oxide
	. . . . ethylmercuric chloride
	. . . . fenarimol
	. . . . fenporpidine
	. . . . fenpropimorph
	. . . . flusilazole
	. . . . folpet
	. . . . hexachlorobenzene
	. . . . iprodione
	. . . . mancozeb
	. . . . maneb
	. . . . metalaxyl
	. . . . phenylmercuric acetate
	. . . . prochloraz
	. . . . procymidone
	. . . . propineb
	. . . . quintozene
	. . . . triadimefon
	. . . . triadimenol
	. . . . 2,4,5 trichlorophenol
	. . . . 2,4,6 trichlorophenol
	. . . . tridemorph
	. . . . triphenyltin acetate
	. . . . triphenyltin chloride
	. . . . tirphenyltin fluoride
	. . . . triphenyltin hydroxide
	. . . . vinclozolin
	. . . . zineb
	. . . . ziram
D5.30.60.385	**. . . herbicide**
	. . . . acetochlor
	. . . . alachlor
	. . . . ametryn
	. . . . 2 amino 4 chloro 6 ethylamino 1,3,5 triazine
	. . . . 5 amino 4 cyano 1 [2,6 dichloro 4 (trifluoromethyl)phenyl]pyrazole
	. . . . amitrole
	. . . . atrazine
	. . . . barban
	. . . . benfluralin
	. . . . bentazon
	. . . . benthiocarb
	. . . . bialaphos
	. . . . bromacil
	. . . . bromoxynil
	. . . . bromoxynil octanoate
	. . . . 2 tert butylamino 4 cyclopropylamino 6 methylthio 1,3,5 triazine
	. . . . butylate
	. . . . cacodylic acid
	. . . . chloridazon
	. . . . chlorimuron ethyl
	. . . . chlornitrofen
	. . . . 2 (4 chloro 2 methylphenoxy)acetic acid
	. . . . chlropropham
	. . . . chlorsulfuron
	. . . . chlorthiamid
	. . . . chlortoluron
	. . . . cyanazine
	. . . . cyanuric acid

Pesticide descriptors (descriptors used in the literature search are underlined)

. . . . dacthal
. . . . daminozide
. . . . deethylatrazine
. . . . defoliant agent
. . . . dicamba
. . . . dichlobenil
. . . . 2,4 dichlorophenoxyacetic acid
. . . . dechlorprop
. . . . 2,6 dimethyl n (alpha methylbenzyl) 5 propionylnicotinamide
. . . . dinitro ortho cresol
. . . . dinoseb
. . . . diquat
. . . . diuron
. . . . fenoprop
. . . . flamprop
. . . . fluazifop
. . . . fluazifop butyl
. . . . fluometuron
. . . . fluridone
. . . . fomesafen
. . . . glyphospate
. . . . haloxyfop
. . . . herbicide residue
. . . . hexazinone
. . . . homoalanosine
. . . . isoproturon
. . . . karsil
. . . . linuron
. . . . mecoprop
. . . . metamitron
. . . . methabenthiazuron
. . . . methanearsonate disodium
. . . . methanearsonate sodium
. . . . methanearsonic acid
. . . . metobromuron
. . . . metolachlor
. . . . metribuzin
. . . . molinate
. . . . monolinuron
. . . . monuron
. . . . mapropamide
. . . . nitrofen
. . . . norflurazon
. . . . oxadiazon
. . . . paraquat
. . . . pebulate
. . . . pendimethalin
. . . . pentachlorophenate sodium
. . . . pentachlorophenol
. . . . phenmedipham
. . . . phosphinothricin
. . . . picloram
. . . . prometon
. . . . prometryn
. . . . propachlor
. . . . propanil
. . . . propazine
. . . . simazine
. . . . simetryne

Pesticide descriptors (descriptors used in the literature search are underlined)	
	. . . . sulfometuron methyl
	. . . . tebuthiuron
	. . . . terbacil
	. . . . terbutryn
	. . . . terbutylazine
	. . . . triallate
	. . . . tribenuron methyl
	. . . . 2,4,5 trichlorophenoxyacetic acid
	. . . . tridiphane
	. . . . trifluralin
D5.30.60.440	**. . . insecticide**
	. . . . amitraz
	. . . . anabasine
	. . . . azadirachtin
	. . . . benzyl benzoate
	. . . . bromopropylate
	. . . . chlorphenamidine
	. . . . crotamiton
	. . . . cyromazine
	. . . . diflubenzuron
	. . . . dinitro ortho cresol
	. . . . dinoseb
	. . . . fipronil
	. . . . ibotenic acid
	. . . . imidacloprid
	. . . . methoprene
	. . . . 1 naphthyl isothiocyanate
	. . . . pentachlorophenate sodium
	. . . . pentachlorophenol
	. . . . piperonyl butoxide
	. . . . precocene
	. . . . precocene I
	. . . . precocene II
	. . . . pyriproxyfen
	. . . . rotenone
	. . . . tebufenozide
	. . . . thiodipine
D5.30.60.440.135	**. . . . carbamate insecticide**
	. . . . . aldicarb
	. . . . . aminocarb
	. . . . . bendiocarb
	. . . . . carbaril
	. . . . . carbofuran
	. . . . . carbosulfan
	. . . . . ethiofencarb
	. . . . . fenoxycarb
	. . . . . formetanate
	. . . . . isoprocarb
	. . . . . methiocarb
	. . . . . methomyl
	. . . . . oxamyl
	. . . . . primicarb
	. . . . . propoxur
D5.30.60.440.650	**. . . . organochlorine insecticide**
	. . . . . aldrin
	. . . . . campheclor
	. . . . . chlordane
	. . . . . chlordecone

Pesticide descriptors (descriptors used in the literature search are underlined)	
	. . . . . chlorphenotane
	. . . . . clofentezine
	. . . . . 1,1 dichloro 2,2 bis(4 chlorophenyl)ethane
	. . . . . 1,1 dichloro 2,2 bis(4 chlorophenyl)ethylene
	. . . . . 1,2 dichlorobenzene
	. . . . . 1,4 dichlorobenzene
	. . . . . dieldrin
	. . . . . endosulfan
	. . . . . endrin
	. . . . . heptachlor
	. . . . . heptachlor epoxide
	. . . . . alpha hexanchlorocyclohexane
	. . . . . beta hexanchlorocyclohexane
	. . . . . isobenzan
	. . . . . lindane
	. . . . . methoxychlor
	. . . . . mirex
	. . . . . nonachlor
	. . . . . oxychlordane
	. . . . . photomirex
	. . . . . 1,1,1 trichloro 2 (2 chlorophenyl) 2 (4 chlorophenyl)ethane
D5.30.60.440.655	**. . . . organophosphate insecticide**
	. . . . . acephate
	. . . . . azinphos ethyl
	. . . . . azinphos methyl
	. . . . . chlorpyrifos
	. . . . . chlorpyrifos methyl
	. . . . . coumafos
	. . . . . crufomate
	. . . . . cyanofenphos
	. . . . . cythioate
	. . . . . dichlorvos
	. . . . . dicrotophos
	. . . . . dimethoate
	. . . . . dimpylate
	. . . . . disulfoton
	. . . . . ethion
	. . . . . ethoprop
	. . . . . etrimfos
	. . . . . fenitrothion
	. . . . . fensulfothion
	. . . . . fenthion
	. . . . . fonofos
	. . . . . formothion
	. . . . . isofenphos
	. . . . . leptophos
	. . . . . malaoxon
	. . . . . malathion
	. . . . . methamidophos
	. . . . . methidathion
	. . . . . metrifonate
	. . . . . mevinphos
	. . . . . mipafox
	. . . . . monocrotophos
	. . . . . naled
	. . . . . omethoate
	. . . . . paraoxon
	. . . . . parathion

Pesticide descriptors (descriptors used in the literature search are underlined)	
	. . . . . parathion methyl
	. . . . . phenylphophonothioic acid o ethyl o (4 nitrophenyl) ester
	. . . . . phorate
	. . . . . phosalone
	. . . . . phosmet
	. . . . . phosphamidon
	. . . . . phoxim
	. . . . . profenofos
	. . . . . pyridaphenthion
	. . . . . quinalphos
	. . . . . stirofos
	. . . . . temefos
	. . . . . terbufos
	. . . . . triazophos
	. . . . . tributyl phosphorotrithioite
D5.30.60.440.725	**. . . . pyrethroid**
	. . . . . allethrin
	. . . . . bioallethrin
	. . . . . bioresmethrin
	. . . . . cipermethrin
	. . . . . cismethrin
	. . . . . cyfluthrin
	. . . . . cyhalothrin
	. . . . . cyphenothrin
	. . . . . deltamethrin
	. . . . . fenpropathrin
	. . . . . fenvaleratre
	. . . . . flucythrinate
	. . . . . flumethrin
	. . . . . fluvalerate
	. . . . . permethrin
	. . . . . phenothrin
	. . . . . pyrethrin
	. . . . . resmethrin
	. . . . . tetramethrin
	. . . . . tralomethrin
D5.30.60.560	**. . . molluscacide**
	. . . . aridanin
	. . . . 4 bromo 2,5 dichlorophenol
	. . . . clonitralide
	. . . . metaldehyde
	. . . . phosalone
D5.30.60.650	**. . . organochlorine pesticide**
	. . . . chlornitrofen
	. . . . chlorobenzilate
	. . . . chloropicrin
	. . . . chlorothalonil
	. . . . chlorthiamid
	. . . . dacthal
	. . . . dicofol
	. . . . tetradifon
D5.30.60.650.440	**. . . . organochlorine insecticide**
	. . . . . aldrin
	. . . . . campheclor
	. . . . . chlordane
	. . . . . chlordecone
	. . . . . chlorphenotane
	. . . . . clofentezine

Pesticide descriptors (descriptors used in the literature search are underlined)	
	. . . . . 1,1 dichloro 2,2 bis(4 chlorophenyl)ethane
	. . . . . 1,1 dichloro 2,2 bis(4 chlorophenyl)ethylene
	. . . . . 1,2 dichlorobenzene
	. . . . . 1,4 dichlorobenzene
	. . . . . dieldrin
	. . . . . endosulfan
	. . . . . endrin
	. . . . . heptachlor
	. . . . . heptachlor epoxide
	. . . . . alpha hexanchlorocyclohexane
	. . . . . beta hexanchlorocyclohexane
	. . . . . isobenzan
	. . . . . lindane
	. . . . . methoxychlor
	. . . . . mirex
	. . . . . nonachlor
	. . . . . oxychlordane
	. . . . . photomirex
	. . . . . 1,1,1 trichloro 2 (2 chlorophenyl) 2 (4 chlorophenyl)ethane
D5.30.60.655	**. . . organophosphate pesticide**
	. . . . armin
	. . . . clofenvinfos
	. . . . edifenphos
	. . . . fenamiphos
	. . . . fenclofos
	. . . . phenthoate
	. . . . thiometon
	. . . . vamidothion
D5.30.60.655.440	**. . . . organophosphate insecticide**
	. . . . . acephate
	. . . . . azinphos ethyl
	. . . . . azinphos methyl
	. . . . . chlorpyrifos
	. . . . . chlorpyrifos methyl
	. . . . . coumafos
	. . . . . crufomate
	. . . . . cyanofenphos
	. . . . . cythioate
	. . . . . dichlorvos
	. . . . . dicrotophos
	. . . . . dimethoate
	. . . . . dimpylate
	. . . . . disulfoton
	. . . . . ethion
	. . . . . ethoprop
	. . . . . etrimfos
	. . . . . fenitrothion
	. . . . . fensulfothion
	. . . . . fenthion
	. . . . . fonofos
	. . . . . formothion
	. . . . . isofenphos
	. . . . . leptophos
	. . . . . malaoxon
	. . . . . malathion
	. . . . . methamidophos
	. . . . . methidathion
	. . . . . metrifonate

Pesticide descriptors (descriptors used in the literature search are underlined)	
	. . . . . mevinphos
	. . . . . mipafox
	. . . . . monocrotophos
	. . . . . naled
	. . . . . omethoate
	. . . . . paraoxon
	. . . . . parathion
	. . . . . parathion methyl
	. . . . . phenylphophonothioic acid o ethyl o (4 nitrophenyl) ester
	. . . . . phorate
	. . . . . phosalone
	. . . . . phosmet
	. . . . . phosphamidon
	. . . . . phoxim
	. . . . . profenofos
	. . . . . pyridaphenthion
	. . . . . quinalphos
	. . . . . stirofos
	. . . . . temefos
	. . . . . terbufos
	. . . . . triazophos
	. . . . . tributyl phosphorotrithioite
D5.30.60.770	**. . . rodenticide**
	. . . . ANTU
	. . . . brodifacoum
	. . . . bromadiolone
	. . . . bromethalin
	. . . . chlorophacinone
	. . . . coumatetralyl
	. . . . difenacoum
	. . . . flocoumafen
	. . . . muricide
	. . . . thallium sulfate
	. . . . vacor
	. . . . warfarin

Medline descriptor fields[6]

Medline disease descriptors

Medline disease descriptors (descriptors used in the literature search are underlined)	
C10–DISEASES-NEUROLOGIC	
Nervous System Diseases	C10
Central Nervous System Disease	C10.228
Brain Diseases	C10.228.140
Basal Ganglia Diseases	C10.228.140.79
Basal Ganglia Cerebrovascular Disease	C10.228.140.79.127
Basal Ganglia Hemorrhage	C10.228.140.79.127.500
Putaminal Hemorrhage	C10.228.140.79.127.500.500
Chorea Gravidarum	C10.228.140.79.294
Dystonia Musculorum Deformans	C10.228.140.79.357
Hallervorden-Spatz Syndrome	C10.228.140.79.493
Hepatolenticular Degeneration	C10.228.140.79.501
Hutington Disease	C10.228.140.79.545
Meige Syndrome	C10.228.140.79.590
Multiple System Atrophy	C10.228.140.79.612
Olivopontocerebellar Atrophies	C10.228.140.79.612.600
Shy-Drager Syndrome	C10.228.140.79.612.700
Striatonigral degeneration	C10.228.140.79.612 800
Neuroleptic Malignant Syndrome	C10.228.140.79.737
Parkinsonian Disorders	C10.228.140.79.862
Lewy Body Disease	C10.228.140.79.862.400
Parkinson Disease	C10.228.140.79.862.500
Parkinson Disease, Scondary	C10.228.140.79.862.800
MPTP Poisoning	C10.228.140.79.862.800.300
Parkinson Disease, Postencephalitic	C10.288.140.79.862.800.600
Supranuclear Palsy, Progressive	C10.228.140.79.882
Tourette Syndrome	C10.228.140.79.898
Dementia	C10.228.140.380
AIDS Dementia Complex	C10.228.140.380.70
Alzheimer Disease	C10.228.140.380.100
Creutzfeldt-Jakob Syndrome	C10.228.140.380.132
Dementia, Vacular	C10.228.140.380.230
Dementia Multi-Infarct	C10.228.140.380.230.250
Kluver-Bucy Syndrome	C10.228.140.380.326
Lewy Body Disease	C10.228.140.380.422
Pick Disease of the Brain	C10.228.140.380.615
Movement Disorders	C10.228.662
Angelman Syndrome	C10.228.662.75
Choreatic Disorders	C10.228.662.150
Chorea Gravidarum	C10.228.662.150.500
Huntington Disease	C10.228.662.150.550

[6] Medical Subject Headings (MeSH) created, maintained and provided by US National Library of Medicine (version 2002, 2003, MeSH)

Medline disease descriptors (descriptors used in the literature search are underlined)	
Dystonic Disorders	C10.228.662.300
Dystonia Musculorum Deformans	C10.228.662.300.200
Meige Syndrome	C10.228.662.300.500
Torticollis	C10.228.662.300.750
Essential Tremor	C10.228.662.350
Hallervorden-Spatz Syndrome	C10.228.662.400
Hepatolenticular Degeneration	C10.228.662.425
Multiple System Atrophy	C10.228.662.550
Olivopontocerebellar Atrophies	C10.228.662.550.600
Shy-Drager Syndrome	C10.228.662.550.700
Striatonigral degeneration	C10.228.662.550.800
Parkinsonian Disorders	C10.228.662.600
Lewy Body Disease	C10.228.662.600.200
Parkinson Disease	C10.228.662.600.400
Parkinson Disease, Secondary	C10.228.662.600.700
MPTP Poisoning	C10.228.662.600.700.250
Parkinson Disease, Postencephalitic	C10.228.662.600.700.500
Supranuclear Palsy, Progressive	C10.228.662.700
Tic Disorders	C10.228.662.825
Tourette Syndrome	C10.228.662.825.800
Neurodegenerative Diseases	C10.574
Multiple System Atrophy	C10.574.625
Olivopontocerebellar Atrophies	C10.574.625.600
Shy-Drager Syndrome	C10.574.625.700
Striatonigral Degeneration	C10.574.625.800
Lewy Body Disease	C10.574.531
Parkinson Disease	C10.574.812
F3-MENTAL DISORDERS	
Mental Disorders	F3
Delirium, Dementia, Amnestic, Cognitive Disorders	F3.87
Dementia	F3.87.400
AIDS Dementia Complex	F3.87.400.50
Alzheimer Disease	F3.87.400.100
Aphasia, Primary Progressive	F3.87.400.125
Creutzfeldt-Jakob Syndrome	F3.87.400.300
Dementia, Vacular	F3.87.400.350
Dementia Multi-Infarct	F3.87.400.350.400
Kluver-Bucy Syndrome	F3.87.400.431
Lewy Body Disease	F3.87.400.512
Pick Disease of the Brain	F3.87.400.675

Medline pesticide descriptors

Medline pesticide descriptors (descriptors used in the literature search are underlined)	
D2-Organic Chemicals	
Hydrocarbons	
Hydrocarbons Halogenated	
Hydrocarbons, Chlorinated	D2.455.526.439
Aldrin	D2.455.526.439.42
Carbon Tetrachloride	D2.455.526.439.150
Chlordan	D2.455.526.439.180
Chlordecone	D2.455.526.439.190
Chlorobenzenes	D2.455.526.439.202
Dicofol	D2.455.526.439.202.190
Dinitrochlorobenzene	D2.455.526.439.202.200
Hexachlorobenzene	D2.455.526.439.202.400
Chlorofluorocarbons	D2.455.526.439.220
Chloroflurorcarbons, Methane	D2.455.526.439.220.300
Chloroform	D2.455.526.439.224
Bromotrichloromethane	D2.455.526.439.224.200
DDD	D2.455.526.439.294
DDE	D2.455.526.439.315
DDT	D2.455.526.439.337
Dichloroethylenes	D2.455.526.439.350
Dieldrin	D2.455.526.439.371
Endrin	D2.455.526.439.416
Ethyl Chloride	D2.455.526.439.447
Ethylene Dichlorides	D2.455.526.439.458
Hepatchlor	D2.455.526.439.516
Heptachlor Epoxide	D2.455.526.439.516.350
Lindane	D2.455.526.439.600
Methoxychlor	D2.455.526.439.610
Methyl Chloride	D2.455.526.439.632
Methylene Chloride	D2.455.526.439.642
Mirex	D2.455.526.439.659
Mitotane	D2.455.526.439.681
Picryl Chloride	D2.455.526.439.750
Polychlorinated Biphenyls	D2.455.526.439.773
Aroclors	D2.455.526.439.773.292
Aroclor 1254	D2.455.526.439.773.292.77
Polychloroterphenyl Compounds	D2.455.526.439.785
Aroclors	D2.455.526.439.785.292
Aroclor 1254	D2.455.526.439.785.292.77
Tetrachloroethylene	D2.455.526.439.880
Toxaphene	D2.455.526.439.913
Tichloroepoxypropane	D2.455.526.439.920
Trichloroethanes	D2.455.526.439.927
Trichloroethylene	D2.455.526.439.939
Vinyl Chloride	D2.455.526.439.975
D3-Heterocyclic Compounds	D3

Medline pesticide descriptors (descriptors used in the literature search are underlined)		
Heterocyclic Compounds, 1-Ring	D3.383	
Pyridines	D3.383.725	
Pyridinium Compounds	D3.383.725.762	
Cetylpyridinium	D3.383.725.762.232	
Desmosine	D3.383.725.762.300	
Diquat	D3.383.725.762.352	
Isodesmosine	D3.383.725.762.500	
1-Methyl-4-phenylpridinium	D3.383.725.762.550	
Obidoxime	D3.383.725.762.600	
Paraquat	D3.383.725.762.621	
Pyridostigmine Bromide	D3.383.725.762.740	
Pyrithiamine	D3.383.725.762.760	
Trimedoxine	D3.383.725.762.900	
Viologens	D3.383.725.762.925	
Benzyl Viologen	D3.383.725.762.925.100	
Heterocyclic Compounds with 4 or More Rings	D3.549	
Rotenone	D3.549.937	
Environmental Pollutants, Noxae, and Pesticides		
Pesticides	D5.723	D27.720.
Chemosterilants	D5.723.141	D27.720.
Fungicides, Industrial	D5.723.288	D27.720.
Herbicides	D5.723.366	D27.720.
Defoliants, Chemical	D5.723.366.181	D27.720.
Herbicides, Carbamate	D5.723.366.448	D27.720.
Herbicides, Triazine	D5.723.366.477	D27.720.
Herbicides, Urea	D5.723.366.506	D27.720.
Insect Repellents	D5.723.441	D27.720.
Insecticides	D5.723.491	D27.720.
Insecticides, Botantical	D5.723.491.324	D27.720.
Insecticides, Carbamte	D5.723.491.408	D27.720.
Insecticides, Organochlorine	D5.723.491.491	D27.720.
Insecticides, Organophosphate	D5.723.491.574	D27.720.
Insecticides, Organothiophosphate	D5.723.491.657	D27.720.
Molluscacides	D5.723.596	D27.720.
Pesticide Residues	D5.723.697	G3.850.
Pesticide Synergists	D5.723.748	D27.720.
Rodenticides	D5.723.853	D27.720.