Occupational risk factors for Parkinson disease

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Occupational risk factors for Parkinson disease

Beroepsmatige risicofactoren voor de ziekte van Parkinson

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 9 oktober 2014 des middags te 12.45 uur

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Contents

Chapter 1	General introduction	1
Chapter 2	A case-control study of the protective effect of alcohol, coffee, and cigarette consumption on Parkinson disease risk: time-since-cessation modifies the effect of tobacco smoking	13
Chapter 3	Is pesticide use related to Parkinson disease? Some clues to heterogeneity in study results	29
Chapter 4	Occupational exposure to pesticides and endotoxin and Parkinson disease in the Netherlands	55
Chapter 5	Extremely low-frequency magnetic field exposure, electrical shocks and risk of Parkinson disease	81
Chapter 6	Occupational exposure to solvents, metals and welding fumes and risk of Parkinson disease	99
Chapter 7	General discussion	113
Appendix	Telephone interview	139
	Summary	170
	Nederlandse samenvatting	174
	Affiliations of co-authors	179
	Dankwoord	180
	About the author	182

Chapter 1

General introduction

Parkinson disease

Clinical presentation

Parkinson disease (PD) is named after James Parkinson who in 1817 published his work, An Essay on the Shaking Palsy (1). Based on observations of 6 individuals he gave a detailed description of the disease including descriptions of several symptoms, later considered cardinal signs of the disease. PD is known as a movement disorder and the four cardinal signs are tremor (involuntary movement that is most prominent at rest, mostly present in distal parts of extremities), rigidity (stiffness and resistance of the muscle when limb is being moved), bradykinesia (problems with planning, initiating and executing movements) and postural imbalance (poor posture and balance that may cause falls and walking difficulties) (2). Not all patients have all these cardinal signs, reflecting the heterogeneity of the disease (3). In addition, a large range of non-motor symptoms, such as cognitive impairment, depression, sleeping problems, and impaired sense of smell can appear (2).

The estimated prevalence of the disease in the Netherlands among persons of 55 years and older is around 1.0% to 1.4% (4, 5). Only a small minority of the PD patients is diagnosed before the age of 40, and disease incidence increases steeply after the age of 60. Most studies on disease incidence have observed that the incidence among men is 1.5-2 times higher than among women (6).

The presence of the described cardinal signs of PD is called Parkinsonism. Examples of other diseases that may cause Parkinsonism are progressive supranuclear palsy (PSP), multiple system atrophy (MSA), corticobasal degeneration (CBD) and dementia with Lewy bodies (DLB). Also certain medication and toxins can induce Parkinsonism. Especially during early stages of disease, it may be difficult to distinguish between PD and other causes of Parkinsonism (7).

Pathology

James Parkinson already realized the importance of anatomical examinations of the brain to identify the causes of the Parkinsonian symptoms. However, it took to 1912 when for the first time the neuronal inclusions that are characteristic for PD were described, later named Lewy bodies after its discoverer (8). Lewy bodies and Lewy neurites are aggregates of proteins that can be found in neurons, with the protein alpha-synuclein as the major constituent. In 1919 it was found that neuronal damage in the substantia nigra, an area in the midbrain, is an important characteristic of PD (9). Neuronal loss in the pars compacta of the substantia nigra was related to the characteristic motor symptoms of the disease.

Another breakthrough came in the 1960s when dopamine shortage in PD was discovered (10). Dopamine is a neurotransmitter that can be found, among others in the neurons of the substantia nigra pars compacta that synapse in the corpus striatum, which is part of a major network for movement control. It is estimated that when there is at least 50% neuronal loss in the substantia nigra, the motor symptoms of PD start to appear (11). The development of these motor symptoms leads eventually to the diagnosis of PD, but years or even decades before diagnosis several non-motor and early motor symptoms can already be experienced (12, 13).

Research indicates that the brainstem and the olfactory system are the first affected regions of the brain, while in later stages of the disease, areas throughout the whole brain are affected (14, 15). It is now believed that misfolding and aggregation of alpha-synuclein may play an important role in neurodegeneration. Aggregates of alpha-synuclein not localized in Lewy bodies or Lewy neurites may be transferred from cell to cell contributing to the spread of the disease through the brain (16, 17). Recent research also shows that peripheral areas of the body including the gastrointestinal tract are being affected early in the disease (18). Processes involved in neurodegeneration are mitochondrial dysfunction, oxidative stress and inflammation (19, 20). The reason that neurons in the substantia nigra pars compacta are in particular vulnerable for damage in PD, is possibly related to processes involving cytoplasmic dopamine, high calcium levels in these cells and the presence of alpha-synuclein (21, 22).

Treatment

Discovery of the role of dopamine in the pathogenesis of PD initiated the development of medicines to correct for the shortage of dopamine (10). L-DOPA and dopamine agonists are still widely used in the treatment of PD. A more recent development is the implantation of electrodes for high-frequency deep brain stimulation, which has proven to be successful in mitigating symptoms of PD patients (23). Possible future treatments may be transplantation of dopaminergic cells derived from stem cells (24). While these treatments may increase quality of life of patients and may extend the period in which the patient can function independently, at this moment no treatment that can stop or slow down disease progression is available.

Cause

It is estimated that only a few percent of the patients have a monogenic familial form of the disease. In 1997 the first study was published that showed a mutation in the gene coding for alpha-synuclein in a family with PD (25). Since that time mutations in more than 10 different genes have been identified that cause familial

forms of PD (26, 27). However, for the majority of the PD patients the cause is not clear and the disease is called sporadic or idiopathic Parkinson disease. It is believed that in the majority of cases PD is caused by age related cellular changes that increase vulnerability of neurons combined with genetic susceptibility and exposures to environmental factors (28). More and more gene variants that are associated with increased PD susceptibility are being identified using genome-wide association studies (27). Among those are variants of the gene coding for alpha-synuclein and variants of genes coding for proteins involved in mitochondrial metabolism, synaptic exocytosis and endocytosis, and the removal of abnormal proteins.

One of the most consistently found environmental factors related to PD is smoking. Already in 1959 the first study was published in which an inverse association was observed between smoking and PD (29). This finding has since then been confirmed in many other studies with various study designs, suggesting that smoking may protect against PD (30). However, the mechanism is still unclear and causality of this inverse association is still being debated (31). Associations with other environmental risk factors are less clear. Research on environmental factors was accelerated in the 1980s by the discovery that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was responsible for the Parkinsonism developed in some illegal drug users, where MPTP was produced accidentally as a by-product of a synthetic heroin (32). The active metabolite of MPTP, 1-methyl-4-phenylpyridinium (MPP+) is structural similar to the pesticide paraquat. Although it became clear that paraquat cannot act via the same mechanisms as MPP+, the results of many epidemiological and toxicological studies suggest that an association of PD with pesticide exposure may exist (33, 34). Next to pesticides, also solvents, metals, extremely low-frequency magnetic fields (ELF-MF), viral infections and various dietary items have been suggested to be related to PD risk (35, 36). For none of these environmental exposures the evidence for a relation with PD is conclusive. Furthermore, the lower disease incidence among women, led to the hypothesis that hormones have an influence on PD risk (37).

Research in this thesis

Study objectives

The main focus of this thesis was on studying the association between occupational exposures and PD risk with a particular emphasis on pesticide exposure. Methods of exposure assessment present a challenging issue in occupational epidemiological studies and may be an important reason for the heterogeneous results observed in previous studies (36, 38). For example, pesticides are often analyzed as a single

(non-specific) entity, although a wide range of active ingredients have been on the market with various chemical properties. Also usage of pesticides has changed over time and differs between countries. At this moment for no specific active ingredient, sufficient evidence is available to establish a causal link. Most studies based exposure assessment on self-report by study-subjects. However, it may be difficult to accurately recall pesticide usage, especially for individuals that were not directly involved in selection or application of pesticides. Similar problems apply for estimating exposure to solvents or metals.

We aimed at comprehensively analyzing past occupational exposures to specific pesticides and subclasses of pesticides using methodologies involving both self-reported information on pesticide applications and reported information on jobs performed. Further, we aimed at studying life-time occupational exposure to aromatic and chlorinated solvents, metals, and electricity related exposures, thereby not limiting the analyses to extremely low-frequency magnetic fields but also including electrical shocks.

Study design

The PAGES study is a hospital-based case-control study that was conducted to study risk factors for Parkinson disease and several other Parkinsonian disorders (progressive supranuclear palsy (PSP), multiple system atrophy (MSA), corticobasal degeneration (CBD), dementia with Lewy bodies (DLB), and vascular Parkinsonism). The study was primarily conducted to study environmental pesticide exposure at home addresses, occupational exposures and gene-environment interactions.

Cases and controls were selected from five neurological centers (St Elisabeth Hospital and TweeSteden Hospital in Tilburg, Canisius-Wilhelmina Hospital in Nijmegen, University Medical Center Groningen, and Vlietland Hospital in Schiedam). The included centers were in four different (agricultural) areas in the Netherlands to increase contrast in pesticide use. The goal was to include all patients diagnosed between January 2006 and December 2011 in the participating centers. For the center in Schiedam only patients from 2009-2011 could be invited because before that year the DBC system, the standardized accounting system for hospital care which we used to identify cases and controls, was not yet fully implemented. In each hospital one neurologist reviewed the medical files of all potential cases. For each included case, two controls were selected from individuals who attended the same departments of neurology within the same time-frame for a range of non-neurodegenerative complaints. Controls were further matched to the

cases on sex and age (interquartile range age difference: 6-33 days, max: 512 days).

The questionnaire administered during a telephone interview contained a complete residential and occupational history and questions about potential risk factors for PD (see Appendix). These included detailed questions about pesticide applications while performing farm or gardening jobs. In addition, participants who also provided consent for participating in the genetic analyses received a simple self-collection-kit for saliva collection and were asked to send their saliva sample back by mail.

Participation

Cases and controls were recruited between April 2010 and June 2012. A total of 1330 eligible patients were identified, 1220 of those were still alive at time of recruitment. Ten current addresses were unknown, 530 persons declined participation and 192 did not reply, leaving 488 patients that were interviewed. Of the patients with PD, 448 of the 993 invited patients participated (45%), compared to 40 of the 217 invited patients with another Parkinsonian disorder (18%). Due to the limited number of cases in the study with Parkinsonian disorders other than PD the analyses were restricted to PD. The participation rate among the invited controls was 35%. For 4 PD cases no suitable controls were found, and for 12 PD cases only 1 suitable control was found. Analyses were thus performed on 444 PD cases and 876 matched controls. Because some of the individuals included as control were the best match to more than one case, the 876 controls were 779 unique persons.

This thesis

In **chapter 2** we describe the risk analyses on some known and suspected lifestyle factors associated with PD namely cigarette, coffee and alcohol consumption. These analyses were amongst others conducted to establish internal validity of the case-control study. In addition, we applied a novel modeling approach to study the independent contributions of duration, intensity and time-since-cessation of smoking on PD risk.

In **chapter 3** the results are presented of a systemic review and meta-analysis of previous studies looking to the association between exposure to pesticides and PD risk. Possible sources of heterogeneity of study results are investigated. Subsequently, possible associations of occupational pesticide exposure with PD in our own case-control study were investigated and described in **chapter 4.** This analysis is unique in the comprehensive exposure assessment of pesticides using

different methodologies including self-reported use information, a job-exposure matrix, an exposure algorithm, and a crop-exposure matrix.
Chapter 5 presents the results for exposures to electromagnetic fields. The associations of PD with exposure to extremely low frequency magnetic fields (ELF-MF), electrical shocks and having worked in electrical occupations were evaluated. The results of analyses on occupational solvent and metal exposure and welding activities in relation to PD risk are described in **chapter 6**.
Finally, in **chapter 7** the main findings of this thesis are discussed and directions for further research are given.

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Chapter 2

A case-control study of the protective effect of alcohol, coffee, and cigarette consumption on Parkinson disease risk: time-since-cessation modifies the effect of tobacco smoking

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Abstract

The aim of this study was to investigate the possible reduced risk of Parkinson Disease (PD) due to coffee, alcohol, and/or cigarette consumption. In addition, we explored the potential effect modification by intensity, duration and time-since-cessation of smoking on the association between cumulative pack-years of cigarette smoking (total smoking) and PD risk. Data of a hospital based case-control study was used including 444 PD patients, diagnosed between 2006 and 2011, and 876 matched controls from 5 hospitals in the Netherlands. A novel modeling method was applied to derive unbiased estimates of the potential modifying effects of smoking intensity, duration, and time-since-cessation by conditioning on total exposure. We observed no reduced risk of PD by alcohol consumption and only a weak inverse association between coffee consumption and PD risk. However, a strong inverse association of total smoking with PD risk was observed (OR 0.27 (95% CI 0.18-0.42) for never smokers versus highest quartile of tobacco use). The observed protective effect of total smoking was significantly modified by time-since-cessation with a diminishing protective effect after cessation of smoking. No effect modification by intensity or duration of smoking was observed indicating that both intensity and duration have an equal contribution to the reduced PD risk. Understanding the dynamics of the protective effect of smoking on PD risk aids in understanding PD etiology and may contribute to strategies for prevention and treatment.

Introduction

Coffee, alcohol and cigarette consumption are potential protective factors for the development of Parkinson disease (PD) (1). The evidence for an association between cigarette smoking and PD risk is particularly strong with studies consistently showing an exposure dependent reduction in risk with total lifetime exposure to cigarette smoke, the product of (average daily) smoking intensity and duration of smoking (2-4). However, it has been suggested that duration of smoking and time-since-cessation may be more relevant to PD risk than smoking intensity (5,6). Insight into the relative importance of duration, intensity, and time-since-cessation of cigarette smoking on PD risk may offer important clues to PD etiology and to strategies for prevention and treatment (5).

Due to their interrelatedness, modeling independent effects of duration, intensity and time-since-cessation of smoking on disease risk is complex. For example, when modeling the duration of smoking the parameter estimate represents the risk per year of smoking for a fixed intensity. As such the risks modeled at two different durations do not only reflect the difference in duration, but also the difference in total lifetime smoking. To circumvent this problem Lubin et al. described a model in which the modifying effects of intensity or duration can be investigated by conditioning the model on total exposure, allowing a comparison of the risk of low intensity exposures at long duration with risk of high intensity exposures at short duration (7). Recently, this method was extended by including a term for time-since-cessation of smoking (8).

In a recently conducted hospital based case-control study we assessed the potential protective effect of coffee, alcohol and cigarette consumption on PD risk. The protective effect of cigarette smoking on PD risk was further explored by investigating the independent contribution of duration, intensity and time-since-cessation of smoking.

Methods

Ethics statement

The study was approved by the Medical Ethics Committee of St Elisabeth Hospital Tilburg, the Netherlands. All participants gave written informed consent.

Cases and controls

Cases and controls were recruited between April 2010 and June 2012 from 5 hospitals in 4 different areas in the Netherlands. Eligible study subjects were

identified using DBC codes, which is the standardized accounting system for hospital care based on diagnostic groups in The Netherlands (9). In each hospital one neurologist reviewed the medical files of all subjects identified with DBC codes 0501 (PD) or 0502 (other extrapiramidal disorders) between January 2006 to December 2011. Subjects with an initial diagnosis before January 2006 or initially diagnosed elsewhere and referred to one of the participating centers for follow-up care or second opinion were excluded. Diagnoses included were: Parkinson disease, Progressive supranuclear palsy, Multiple system atrophy, Vascular Parkinsonism, Corticobasal degeneration and Dementia with Lewy bodies. For each included case, two matched controls were selected from individuals who attended the same departments of neurology within the same specified time-frame with DBC codes 0801 (median nerve neuropathy; ICD-10 G56.0 and G56.1), 0802 (ulnar nerve neuropathy; ICD-10 G56.2), 1203 (thoracic and lumbar disc disease; ICD 10 G55.1, G54.3 and G54.4) or 1204 (sciatica; ICD-10 M54.3 and M54.4). Controls were incidence density matched to the cases on hospital, visiting date (within 3 years of the cases diagnose year), sex and age (interquartile range age difference: 6-33 days, max: 512 days). As is standard in incidence density matching a control could serve as a control for more than one case (10).

Telephone interview

Cases and controls were contacted via an invitation letter containing study information and a reply form for giving informed consent or to decline study participation. Non-responders were sent a reminder after one month, and one phone call attempt was performed after another month. Cases and controls were informed that the study objective was to study risk factors for neurological disorders, without specification. In a standardized computer-assisted telephone interview, participants were interviewed by one of three trained interviewers. The questionnaire contained a complete residential and occupational history, questions about selected dietary items, anthropometric measures and a medical history. Detailed questions about smoking behavior were asked to those reporting to have smoked more than 100 cigarettes during their lifetime. The questions about smoking covered start and final year of cigarette smoking and estimated amount of cigarettes smoked in 10 year periods. In addition, information on cigar or pipe smoking was obtained. Alcohol and coffee consumption was ascertained as glasses or cups per week at current age and at the age of 20, 40 and 60. For alcohol consumption information on binge drinking was obtained (i.e. 5 or more alcohol consumptions per occasion at least once a month). A typical telephone interview lasted 30 to 45 minutes.

Exposure data
Smoking duration was defined as the number of years from start of smoking until year of diagnosis or year of smoking cessation, corrected for years not smoked in between. For former smokers time-since-cessation was defined as year of diagnosis minus year of smoking cessation. Intensity was defined as the average amount of cigarettes per day in the period of smoking. Total smoking was expressed as pack-years and calculated by dividing the intensity by 20 multiplied by the duration. Duration and intensity of alcohol and coffee consumption were estimated with the information on current consumption and consumption at the ages of 20, 40, and 60 (if relevant). Cumulative alcohol and coffee consumption was calculated as consumption-years by multiplying the average amount of consumptions per day with the estimated number of years of consumption.

Statistical analysis
Due to the limited number of subjects with another Parkinsonism than PD (n=40) we restricted our analyses to confirmed cases of PD (n=444).

We estimated main effects (odds ratios (OR) and 95% confidence intervals (CI)) for total smoking, smoking intensity (cigarettes per day), time since cessation, total alcohol consumption, alcohol intensity (drinks per day), binge drinking (>4 drinks per day), total coffee consumption, and coffee intensity (drinks per day) with a conditional logistic regression model. Never users constituted the reference categories while users were divided based on the quartiles of the exposure distribution among the controls. The number of never users for coffee consumption was low (n=38) and therefore the first quartile of coffee consumption (including the never users) was used as the reference category.

To investigate the potential modification of the relationship between total smoking and PD by intensity, duration and/or time-since-cessation of smoking, we applied an inverse excess OR model for total smoking, including modifying functions for smoking intensity or duration and time-since-cessation. The models described in this manuscript fall within a general framework for flexible modeling of the effects of intensity, duration, and time since exposure (8).

The model can be described as:

$$\frac{1}{\mathrm{OR}} = 1 + f(\mathrm{PCS}) \times \exp(g(\mathrm{ICS}) + h(\mathrm{TSC})) \qquad \text{or}$$

$$\frac{1}{\mathrm{OR}} = 1 + f(\mathrm{PCS}) \times \exp(g(\mathrm{DCS}) + h(\mathrm{TSC}))$$

Where f(PCS) is a function of total pack-years of cigarette smoking modified by a function of smoking intensity g(ICS) or smoking duration g(DCS) and a function of time-since-cessation h(TSC). Because PCS is defined as ICS*DCS, models 1 and 2 yield a similar inference. f(PCS), g(ICS), g(DCS) and h(TSC) were included as linear function or as three-knot restricted cubic spline (knots located at the 20th, 50th, and 80th percentile). To compare the relative importance of duration, intensity, and time-since-cessation of cigarette smoking, model fit was evaluated based on the Akaike information criterion (AIC).

These models were fitted using the NLMIXED procedure in SAS v 9.2 (SAS Institute Inc., Cary, NC). Bootstrapped 95% confidence intervals were estimated via 100 bootstrap replications of the original data and taking the 2.5th and 97.5th percentiles of the resulting distribution. For presentation purposes the 1/OR were back-transformed to the OR.

In ancillary analyses we assessed the variation of ORs jointly by total smoking and time-since-cessation, by conducting unconditional logistic regression adjusted for the matching variables age, sex and center. In these analyses we categorized total smoking and time-since-cessation into quintiles and estimated ORs for crossed categories. All ORs were estimated relative to never smokers. We conducted unconditional rather than conditional logistic regression as case-control sets were broken due to the cross-categorization.

Results

1,330 subjects with an initial diagnosis of Parkinsonism between 2006 and 2011 were identified. 1,220 (92%) of those were still alive at time of recruitment of which 1,001 had a diagnosis of PD. Ten current addresses were unknown, 530 persons declined participation and 192 did not reply. The number of successfully enrolled cases was 488 of which 448 were diagnosed with PD. Participation rate among PD cases was 45%. Among controls the participation rate was 35%. For 12 PD cases only 1 suitable control was found. For 4 PD cases no suitable controls were found and these were consequently excluded from the analysis. Table 1 shows

the demographic characteristics of the 444 PD cases and 876 controls (of which 779 were unique) included in the present analyses.

Cigarette smoking was inversely related with PD. 53% of the cases were ever smoker as compared to 72% of the controls (χ^2=45.7, *P* <0.0001). When including cigar and pipe smoking these numbers changed only slightly to 57% and 75%, respectively. At the moment of interview 4% of the cases and 15% of the controls were still smoking cigarettes.

Table 1. General characteristics of cases and controls

	Cases (n=444)	Controls (n=876)
Men, No (%)	281 (63.3)	557 (63.6)
Age at interview, median (range)	68 (34 - 91)	68 (34 - 90)
Age at diagnosis, median (range)	67 (34 - 90)	-
Higher education[a], No. (%)	268 (60.5)	477 (54.5)
Ever smoking cigarettes, No. (%)	237 (53.4)	633 (72.3)
Ever regular coffee consumption[b], No. (%)	427 (96.2)	855 (97.7)
Ever regular alcohol consumption, No. (%)	340 (76.6)	679 (77.5)

[a]Information on education was missing for one case
[b]Information on coffee consumption was missing for one control.

Conditional logistic regression

We observed an inverse association between total pack-years of smoking, longer smoking duration, and shorter time-since-cessation and PD risk (table 2). For average smoking intensity, we observed reduced ORs among the exposed, but no trend was observed when limiting the analyses to ever smokers (*P*=0.20).
Crude ORs for high total and average daily coffee consumption point towards a lower risk of PD (table 3), but were not statistically significant after adjustment for smoking. Analyses conducted among never smokers showed a similar non-significant trend for coffee consumption (ORs for the three highest quartiles of total coffee consumption among never smokers: OR 1.11 (95% CI 0.55-2.26), OR 0.97 (95% CI 0.43-2.16) and OR 0.75 (95% CI 0.34-1.66)).

No association with total and average alcohol consumption and PD risk was found (table 3). Binge drinking was not associated with PD risk with a possible exception of binge drinking at age 20 for which a significantly elevated OR was found after adjusting for smoking (table 3).

Table 2. Parkinson disease and cigarette smoking: conditional logistic regression analysis on data of patients and hospital controls

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI	Adjusted[a] OR (95% CI)
Total smoking (pack-years)				
Never smokers	207 (46.6)	243 (27.7)	1	1
>0 - 7.8	86 (19.4)	161 (18.4)	0.58 (0.42-0.82)	0.58 (0.42-0.82)
>7.8 - 17.5	67 (15.1)	155 (17.7)	0.45 (0.32-0.65)	0.46 (0.32-0.66)
>17.5 - 29.4	45 (10.1)	160 (18.3)	0.28 (0.18-0.42)	0.28 (0.18-0.42)
>29.4 - 103	39 (8.8)	157 (17.9)	0.26 (0.17-0.40)	0.27 (0.18-0.42)
P value for trend[b]	207 (46.6)	243 (27.7)	<0.0001 /0.0004	<0.0001 /0.0006
Intensity (cigarettes/day)				
Never smokers	207 (46.6)	243 (27.7)	1	1
>0 - 7.0	77 (17.3)	158 (18.0)	0.53 (0.37-0.75)	0.53 (0.38-0.76)
>7.0 - 12.7	54 (12.2)	159 (18.2)	0.37 (0.25-0.53)	0.37 (0.25-0.54)
>12.7 - 19.2	50 (11.3)	158 (18.0)	0.33 (0.23-0.49)	0.34 (0.23-0.51)
>19.2 - 60.0	56 (12.6)	158 (18.0)	0.38 (0.26-0.54)	0.39 (0.27-0.57)
P value for trend[b]			<0.0001 / 0.16	<0.0001 / 0.20
Duration (years)				
Never smokers	207 (46.6)	243 (27.7)	1	1
>0 - 18	98 (22.1)	165 (18.8)	0.66 (0.48-0.91)	0.66 (0.48-0.91)
>18 - 28	56 (12.6)	152 (17.4)	0.37 (0.25-0.54)	0.36 (0.24-0.53)
>28 - 41	48 (10.8)	166 (18.9)	0.29 (0.19-0.43)	0.29 (0.20-0.44)
>41 - 66	35 (7.9)	150 (17.1)	0.24 (0.15-0.37)	0.25 (0.16-0.39)
P value for trend[b]			<0.0001/<0.0001	<0.0001/<0.0001
Time-since-cessation (years)				
Never smokers	207 (46.6)	243 (27.7)	1	1
> 31 - 53	93 (20.9)	158 (18.0)	0.67 (0.47-0.95)	0.65 (0.46-0.93)
> 19 - 31	68 (15.3)	147 (16.8)	0.52 (0.36-0.76)	0.53 (0.36-0.77)
> 0 - 19	54 (12.2)	166 (18.9)	0.35 (0.24-0.51)	0.36 (0.25-0.52)
0	22 (5.0)	162 (18.5)	0.15 (0.09-0.24)	0.15 (0.09-0.25)
P value for trend[b]			n.a. / <0.0001	n.a. / <0.0001

[a]The adjusted model includes coffee consumption (in quartiles).

[b]The first *P* value for trend was based on analyses with the exposure as a continuous variable including the persons from the reference category. The second p value for trend was based on analysis whereby the persons from the reference category are excluded.

Table 3. Parkinson disease and coffee and alcohol consumption: conditional logistic regression analysis on data of patients and hospital controls

	Cases No. (%)	Controls[a] No. (%)	Crude OR (95% CI)	Adjusted[b] OR (95% CI)
Coffee consumption				
Total (Consumption-years)				
0 – 97	128 (28.8)	220 (25.1)	1	1
> 97 - 156	146 (32.9)	221 (25.3)	1.13 (0.84-1.53)	1.30 (0.95-1.77)
> 156 - 214	90 (20.3)	216 (24.7)	0.70 (0.50-0.97)	0.79 (0.56-1.12)
> 214 – 720	80 (18.0)	218 (24.9)	0.61 (0.43-0.87)	0.83 (0.57-1.21)
P value for trend[c]			0.0016 / 0.0060	0.33 / 0.28
Intensity (consumptions/day)				
0 - 2.1	129 (29.1)	223 (25.5)	1	1
> 2.1 – 3.4	134 (30.2)	215 (24.6)	1.07 (0.79-1.45)	1.16 (0.85-1.59)
> 3.4 – 4.7	103 (23.2)	218 (24.9)	0.79 (0.57-1.10)	0.92 (0.65-1.30)
> 4.7 – 17.1	78 (17.6)	219 (25.0)	0.60 (0.42-0.85)	0.80 (0.55-1.16)
P value for trend[c]			0.0002 / 0.0020	0.10 / 0.12
Alcohol consumption				
Total (consumption-years)				
Never drinkers	104 (23.4)	197 (22.5)	1	1
>0 – 21	93 (20.9)	170 (19.4)	1.05 (0.74-1.49)	1.10 (0.75-1.60)
>21 – 48	99 (22.3)	172 (19.6)	1.10 (0.77-1.56)	1.44 (0.99-2.11)
>48 – 87	80 (18.0)	169 (19.3)	0.87 (0.60-1.27)	1.27 (0.84-1.91)
>87 - 457	68 (15.3)	168 (19.2)	0.75 (0.50-1.11)	1.28 (0.82-1.98)
P value for trend[c]			0.14 / 0.18	0.38 / 0.73
Intensity (Consumptions/day)				
Never drinkers	104 (23.4)	197 (22.5)	1	1
>0 – 0.6	98 (22.1)	169 (19.3)	1.09 (0.77-1.55)	1.23 (0.85-1.78)
>0.6 – 1.2	96 (21.6)	169 (19.3)	1.08 (0.76-1.55)	1.37 (0.94-2.01)
>1.2 – 2	80 (18.0)	172 (19.6)	0.87 (0.60-1.26)	1.21 (0.80-1.81)
>2 – 17.1	66 (14.9)	169 (19.3)	0.72 (0.48-1.06)	1.13 (0.74-1.74)
P value for trend[c]			0.021 / 0.042	0.92 / 0.70
Regular binge drinking (>4 drinks/occasion)				
Age 20 (n=1.320)	130 (29.3)	240 (27.4)	1.14 (0.86-1.52)	1.45 (1.07-1.96)
Age 40 (n=1.305)	118 (26.9)	258 (29.8)	0.85 (0.64-1.13)	1.11 (0.82-1.49)
Age 60 (n=888)	53 (18.2)	122 (20.4)	0.84 (0.59-1.22)	1.03 (0.70-1.51)

[a]Coffee consumption information was missing for one control, and was thus excluded in analyses including coffee consumption

[b]The adjusted model includes smoking and/or coffee consumption (in quartiles)

[c]The first *P* value for trend was based on analyses with the exposure as a continuous variable including the persons from the reference category. The second *P* value for trend was based on analysis whereby the persons from the reference category are excluded.

Excess odds ratio models

An excess odds ratio model that included a spline function for total smoking and a linear function for time-since-cessation provided the best fit to our data (AIC: 886.2 compared to an AIC of 900.0 for a model in which the modifying effect of time-since-cessation and duration (or intensity) was set to zero). Including a spline function for time-since-cessation did not further improve model fit (AIC: 888.0). Similar, including an additional modifying function for intensity or duration of smoking did not improve model fit (AIC: 888.2 and 885.5, respectively).

In Figure 1 we show the marginal effect of total smoking and time-since-cessation on PD risk from our 'best' model, maintaining the other factor in the model at the median level. The plot in figure 1A indicates that the risk of PD decreases with increasing total smoking. Figure 1B indicates that the effect of total smoking on the risk of PD decreases with increasing time-since-cessation.

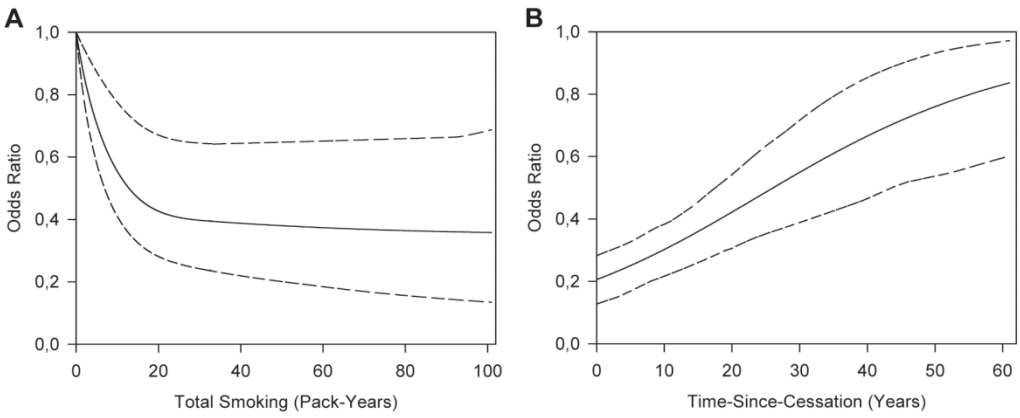

Figure 1. The modifying effects of time-since-cessation on the relation between smoking and PD.The fitted excess OR model with a spline function for total smoking and effect modification by time-since-cessation. 95% confidence intervals were estimated via 100 bootstrap replications. **A:** The OR for different levels of total pack-years, plotted for 21 years-since-cessation**. B:** The OR for different levels of time-since-cessation, plotted for 15 pack-years of total smoking.

Ancillary analyses using standard unconditional logistic regression analyses adjusted for age, sex and center corroborate our findings for time-since-cessation from the excess odds ratio model. ORs for time-since-cessation plotted within categories of total smoking follow a pattern similar to the prediction of our excess odds ratio model with ORs being lowest within categories of higher total smoking and shorter time-since-cessation (Figure 2).

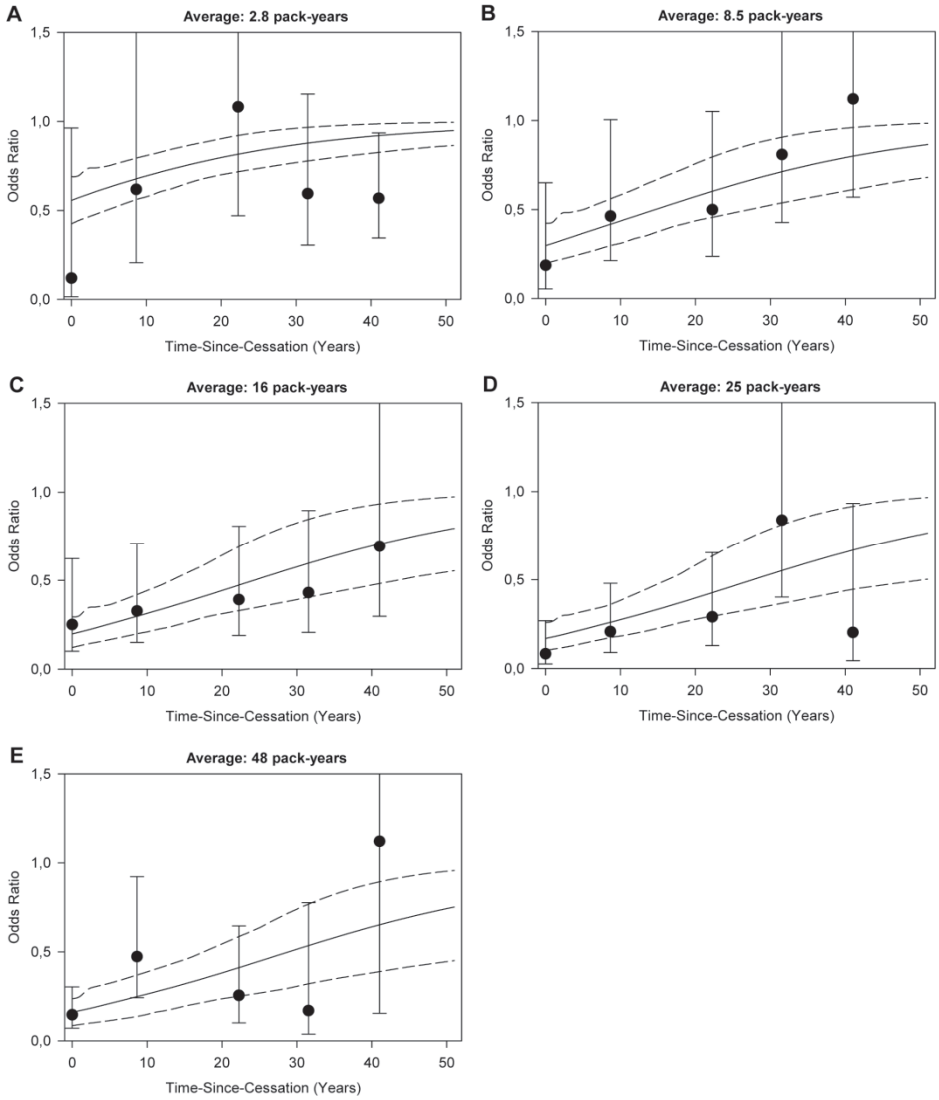

Figure 2. Odds ratios and 95% confidence intervals for time-since-cessation within categories of total pack-years of smoking. **A-E:** Ever smokers were divided according to quintiles of total-smoking and the effect of time-since-cessation within those groups was plotted. ORs were located at the quintile-specific average time-since-cessation and based on unconditional logistic regression relative to never-smokers adjusted for age, sex and center. The lines represent the predicted ORs and 95% confidence intervals for different levels of time-since-cessation based on the excess OR model plotted for the quintile-specific average pack-years of total smoking.

Discussion

In our hospital based case-control study, we found a strong inverse association between cigarette smoking and Parkinson disease, which is in correspondence with earlier studies. We also observed some indication of a potential inverse relation between total and average coffee consumption with PD, but no association with alcohol consumption was found.

Applying a novel modeling method in which the effects of modifying factors are conditioned on total exposure, we found that time-since-cessation has a high impact on the association between total smoking and PD risk. We did not observe effect modification of the effect of total smoking and PD risk by intensity or duration of smoking, indicating that smoking intensity and duration have a similar contribution to reduction of PD risk. The later finding is different from previous findings that smoking duration would be more important than intensity (5, 6).

The applied model in this study is different from previously published models (6, 7) in that it models the inverse OR, rather than the OR itself. This modification accommodates the assessment of a protective effect of total pack-years of smoking on PD by avoiding restriction of the parameter estimation range of function f(PCS) (OR>0). We checked the appropriateness of our model specification by *i)* Inversion of case-control status in the dataset and consequential adjustment of the likelihood function to be able to use a standard excess OR model and *ii*) by fitting a standard excess OR model that included a sufficiently flexible function for f(PCS) to accurately assess the asymptotic protective effect of pack-years of smoking [OR = 1 + f(PCS) x exp (g(ICS) + h (TSC))]. Both analyses yielded essentially similar results as our preferred less constrained inverse OR model.

Biological mechanisms through which PD is linked to smoking include the nicotinic acetylcholine receptors, which trigger downstream signaling molecules, possibly resulting in protection or delay of the development and progression of PD via effects such as decreased apoptosis, enhanced neuronal survival or modified immune responsiveness (11, 12). Other possible non-receptor mediated mechanisms of nicotine include modulation of mitochondrial complex I activity or through its action as an antioxidant (12).

As in any case-control study, we should keep in mind that our results might also be (partly) explained by reverse causality. PD patients have been described as having rigid, introverted and low-tempered, and less novelty-seeking personality traits which may lead to lower (or shorter) cigarette or alcohol consumption (13). By extension, low dopamine levels in individuals long before onset of PD, leading to

premorbid Parkinsonian personality traits (14), might have a similar effect. However, many of the studies investigating premorbid personality traits have methodological deficiencies, which make interpretation difficult (15). Of note, imaging studies have shown nigrostriatal dopaminergic neuronal loss starting to increase less than 10 years before onset of clinical symptoms, suggesting a limited impact on cigarette and alcohol consumption behavior earlier in life (16).

The inverse relationship between smoking and PD could also be the result of a genetic factor related to both smoking behavior and the chance of developing PD, but this hypothesis was not supported by twin studies (17, 18).

Our finding of a relationship between PD risk and time-since-cessation provides some support for a true causal effect, as in the case of reverse causality no direct association with time-since-cessation would be expected. Some of the effect observed for time-since-cessation might in fact be due to smoking duration, as most participants in our study started smoking at a similar age (around 18) and consequently smoking duration and time-since-cessation are inversely correlated (Pearson correlation coefficient: -0.75). However, in a model including modifying factors for both smoking duration and time-since-cessation a strong effect for time-since-cessation, and not for duration, was observed, indicating the observed effect is most likely due to time-since-cessation. In addition, we did not observe a clear increase in the number of cases quitting smoking as a consequence of disease onset in the years before diagnosis (results not shown). As such, results from our analyses of smoking behavior in the aggregate are supportive of a causal protective effect of smoking that diminishes after quitting smoking.

We also observed an inverse exposure dependent association with total coffee consumptions and the average number of consumptions per day and the risk of PD. These associations were however not statistically significant in models adjusted for smoking or in analyses limited to never smokers. Coffee has been shown to be inversely related to PD risk in three meta-analyses (2, 19, 20) and can be explained biologically by the idea that caffeine in coffee is neuroprotective (21). However, given the relatively large impact of the adjustment by smoking on the observed ORs we cannot rule out that the observed effect is due to residual confounding by smoking.

Contrary to what has been suggested in the literature (22), we did not observe an inverse association with alcohol consumption in our study. We did observe a positive association with PD for regular binge drinking at age 20, suggesting that drinking high amounts of alcohol at a young age may increase the risk for PD. Given that this could be a chance finding this result should be interpreted with caution and needs replication.

A limitation of our study is the low participation rate. The participation was lower among women (for cases 40%) than men (49%), and depended on age. Under the age of 70, the participation of cases was 66%. Sensitivity analysis restricted to persons under age 70 showed similar results as when including all participants (data not shown). About 50% of the non-participants provided a reason for their decline. Health related reasons were reported most frequently, but compared to cases, more controls reported to be not interested.

Another possible limitation of our study is that controls were selected from the same neurology departments as the cases and that the underlying disease mechanism for these non-neurodegenerative conditions may share some characteristics with PD. Repeating the analyses leaving out one control group at a time (based on DBC codes), resulted in almost identical results, suggesting that the results are not driven by one specific control group adding to the validity of our results.

In conclusion, in a case-control study of 444 recently diagnosed PD patients and 876 controls, we found an inverse association of cigarette smoking and coffee consumption but not of alcohol consumption with the risk of PD. In the association with smoking, total smoking and time-since-smoking cessation appear to drive PD risk. No effect modification of total smoking and PD risk by either the intensity or the duration of smoking was observed, indicating that both aspects have an equal contribution to the reduction of PD risk.

These results provide some further insights into the etiology of PD and may support the usefulness of randomized controlled trials investigating the possibility that administering nicotine or nicotine-mimicking drugs to PD patients might be effective in delaying disease progression.

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Chapter 3

Is pesticide use related to Parkinson disease? Some clues to heterogeneity in study results

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Abstract

Background: Previous systematic reviews have indicated that pesticide exposure is possibly associated with Parkinson disease (PD). However, considerable heterogeneity has been observed in study results.
Objective: We aimed at providing an update of the literature published on PD and exposure to pesticides by performing a systematic review and meta-analysis. In addition, we investigated whether methodological differences between studies could explain the heterogeneity in study results.
Methods: We identified studies through a systematic literature search. We calculated summary risk ratios (sRRs) for pesticide exposure and subcategories using random effects meta-analyses and investigated sources of heterogeneity by meta-regression and stratified analyses.
Results: Thirty-nine case–control studies, four cohort studies, and three cross-sectional studies were identified. A sRR of 1.62 (95% confidence interval (CI): 1.40-1.88) for pesticide exposure (ever vs. never) was found. Summary estimates for subclasses of pesticides indicated a positive association with herbicides and insecticides, but not with fungicides. Heterogeneity in individual study results was not related to study design, source of control population, adjustment of results for potential confounders, or geographical area. However, results were suggestive for heterogeneity related to differences in the exposure assessment. Job title–based exposure assignment resulted in a higher sRR (2.5 (95% CI 1.5-4.1)) than did assignment based on self-reported exposure (e.g., for self-reported ever/never exposure, sRR 1.5 (95% CI 1.3-1.8).
Conclusions: This review affirms the evidence that exposure to herbicides and insecticides increase the risk of PD. Future studies should focus on more objective and improved methods of pesticide exposure assessment.

Introduction

Parkinson disease (PD) is an idiopathic degenerative disorder of the central nervous system that impairs motor skills, cognitive processes, and other functions. The etiology of PD is largely unknown, although some genetic factors have been identified (1, 2). Based on published epidemiological and toxicological studies, pesticides may be involved in the etiology of PD (3). However, epidemiological evidence is far from conclusive, as considerable heterogeneity has been observed in study results (3-5). Possible methodological causes of heterogeneity in study results have been suggested and include differences in study design, control selection, diagnosis of patients, and statistical analysis (3). Differences in exposure assessment methods could contribute to heterogeneity as well. Most previous studies relied almost exclusively on self-reported exposures, a process that is prone to recall bias, especially in case–control studies, and could lead to false-positive associations. Alternatively, one could speculate that PD patients might underreport pesticide exposure because of cognitive deficits, leading to false-negative associations. Furthermore, differences in the definition of exposure to pesticides (occupational vs. non-occupational use, ever/never vs. regular use) could also result in heterogeneous study results. Lastly, the regions where the studies have been conducted could be of importance as regulation, types, and use of pesticides may differ from region to region.

Several recent studies have been published on pesticide exposure and PD risk, including some prospective (cohort) studies. In the present analysis, we aimed at providing an update of the literature published since the last systematic review on PD (3) and exposure to pesticides and pesticide subcategories by performing a systematic review and meta-analysis. We specifically set out to address the question of whether the previously described heterogeneity in study findings could be explained by differences in study design and exposure assessment methods.

Methods

Data source

We searched the databases Embase (http://www.Embase.com/), starting with 1974, and Medline (http://www.ncbi.nlm.nih.gov/pubmed/), starting with 1950, through November 2010 using the search term "Parkinson" in combination with "pesticide*," "insecticide*," "fungicide*," "herbicide*," "rodenticide*," "organochlorine*," "organophosphate*," "carbamate*," "glyphosate*," "paraquat," "maneb," "lindane," "dieldrin," "rotenone," "DDT," or "environmental factors." The search was limited to publications in English, French, German, or Dutch; to human

studies; and to original publications. We also searched the reference lists of the retrieved publications.

Study selection

We included studies that specifically investigated PD or Parkinsonism. We included cohort studies, case–control studies, and cross-sectional studies. No reviews, case reports, or conference abstracts were included. We excluded studies that summarized results of pesticide exposure only within a broad category of "chemical exposure." Exposure to pesticides was defined as use of pesticides by the subject, thus excluding environmental studies.

Data extraction

Two reviewers (M.M., M.B.) independently extracted reported risk estimates (i.e., odds ratios (ORs), risk ratios (RRs), or prevalence ratios), study designs, exposure assessment methods, and types of source population for the controls. We also evaluated subcategories of pesticides and extracted data about exposure–response relations and individual pesticides. Two other researchers (A.H., R.V.) acted as referees in cases of any differences. If authors reported adjustment for potential confounders, we preferred adjusted risk estimates over crude risk estimates. In cases where no risk estimate or 95% confidence interval (CI) was reported, we calculated crude risk estimates and 95% CIs with the reported numbers. Where risk estimates were reported separately for men and women, we pooled the risk estimates with a within-study meta-analysis (6). Of studies with more than one control group, the results of population controls were preferred above the results of hospital controls because population controls are generally considered to be a more representative comparison group than hospital controls.

Statistical analysis

Because of the observed heterogeneity in study results, we conducted a DerSimonian and Laird (1986) random effects meta-analysis to pool the results of the separate studies for risk for pesticide exposure and the subgroups of herbicides, insecticides, and fungicides (7). We also stratified by whether or not non-occupational exposure (e.g., gardening) was included in the exposed group. This was because of potential differences between occupational and non-occupational exposures in intensity and frequency of exposures. In one publication, results both for occupational and for occupational and/or non-occupational exposure were reported (8). We chose to include risk estimates of the more inclusive exposure definition, although final results did not differ when we included the risk estimates based on only occupational exposure (data not shown).

Subsequently, we explored whether heterogeneity in observed risk estimates could be explained by study and exposure assessment characteristics. We did so by stratification and used meta-regression to explore statistical significance of these characteristics. Given the limited number of studies, we only explored one characteristic at a time. Characteristics explored were the type of exposure assessment (self-reported ever/never pesticide exposure, self-reported regular pesticide exposure, or exposure assessment based on reported job titles by expert judgment and/or applying a job-exposure matrix), source of control population (hospital, general population, or other (studies using family members or case acquaintances as controls, or studies that used a combination of different sources)), geographical area (North America, Europe, or other), and whether adjustments were made for potential confounders. The I^2 measure was used to quantify the heterogeneity between studies; I^2 can be interpreted as a measure of the percentage of the total variation that cannot be explained by chance (9). *p*-Values for heterogeneity are based on the *Q*-statistic. Small study effects were tested with funnel plots and Egger's test (10). All analyses were performed with Stata (version 10; StataCorp, College Station, TX, USA) with the metan, metareg, metafunnel, and metabias commands. All statistical tests were two sided, and a *p*-value of < 0.05 was considered statistically significant.

Results

The search in Embase and Medline yielded 883 publications, of which 52 publications met the inclusion criteria. We excluded 3 publications (11-13) where the study population had been included in subsequent publications (14-16). Lastly, one study (17) was excluded because the reported data showed risk per year of pesticide exposure, which was not comparable with reported risk ratios of other studies. Among the remaining 48 publications, there were two studies for which the relevant results were reported in two separate publications each (18-21). Thus, results of a total of 46 studies were used in the meta-analysis.

An overview of the study characteristics of the included studies can be found in Table 1. There were 39 case-control studies, 4 cohort studies, and 3 cross-sectional studies; 40 publications reported on pesticides, 15 on herbicides, 15 on insecticides, and 9 on fungicides. Three studies included all Parkinsonism (22-24); the rest studied idiopathic PD. Four studies showed only results in men (23, 25-27). One study included only cases with a disease diagnosis before 51 years of age (28), which is much lower than the average age of disease onset in all other studies (generally ~ 60 years of age). Information about participation rates was provided for only 13 of the 39 case-control studies. Studies that reported participation rates

had rates between 69% and 100% for cases and between 41% and 100% for controls.

Figure 1 shows PD relative risk estimates for any pesticide exposure based on studies of occupational and/or non-occupational exposures, and studies of occupational exposures only. The summary risk ratios (sRRs) between these two groups were very similar, with sRRs of 1.69 (95% CI 1.38-2.06) and 1.52 (95% CI 1.23-1.89), respectively, and an overall sRR for all studies combined of 1.62 (95% CI 1.40-1.88). The I^2 for all studies combined was 63.7%. Only three studies estimated effects of non-occupational exposure only (19, 29, 30), with an sRR of 1.18 (95% CI 0.86-1.63).

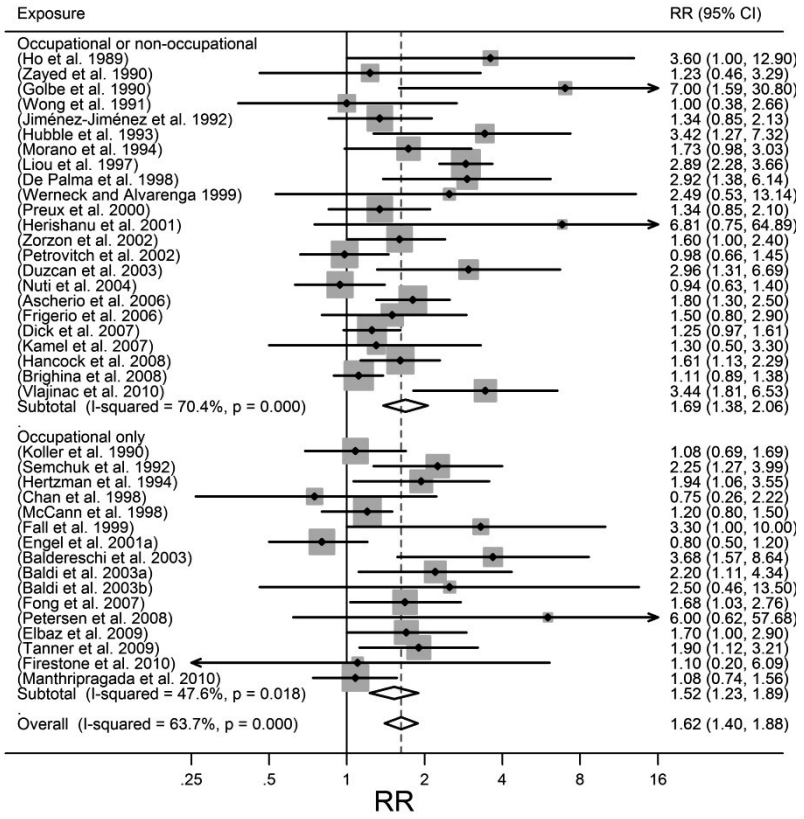

Figure 1. Forest plot for study-specific RRs and sRRs (95% CIs) of PD associated with the use of pesticides. The studies are ordered by publication year and stratified by studies that did or did not include non-occupational exposure in the exposed group. Studies were pooled with the random effects method. The size of the squares reflects the statistical weight of the study in the meta-analyses.

Meta-analyses by herbicide, insecticide, and fungicide exposure are shown in Figure 2. In line with the results for any pesticide exposure, we did not observe noticeable differences between studies of occupational exposures only and studies of non-occupational and occupational exposures combined. The sRR for exposure to fungicides did not indicate an association with PD (overall sRR 0.99 (95% CI 0.71-1.40); Figure 2C), in contrast with positive sRRs for exposure to herbicides (overall sRR 1.40 (95% CI 1.08-1.81); Figure 2A) and insecticides (overall sRR 1.50 (95% CI 1.07-2.11); Figure 2B).

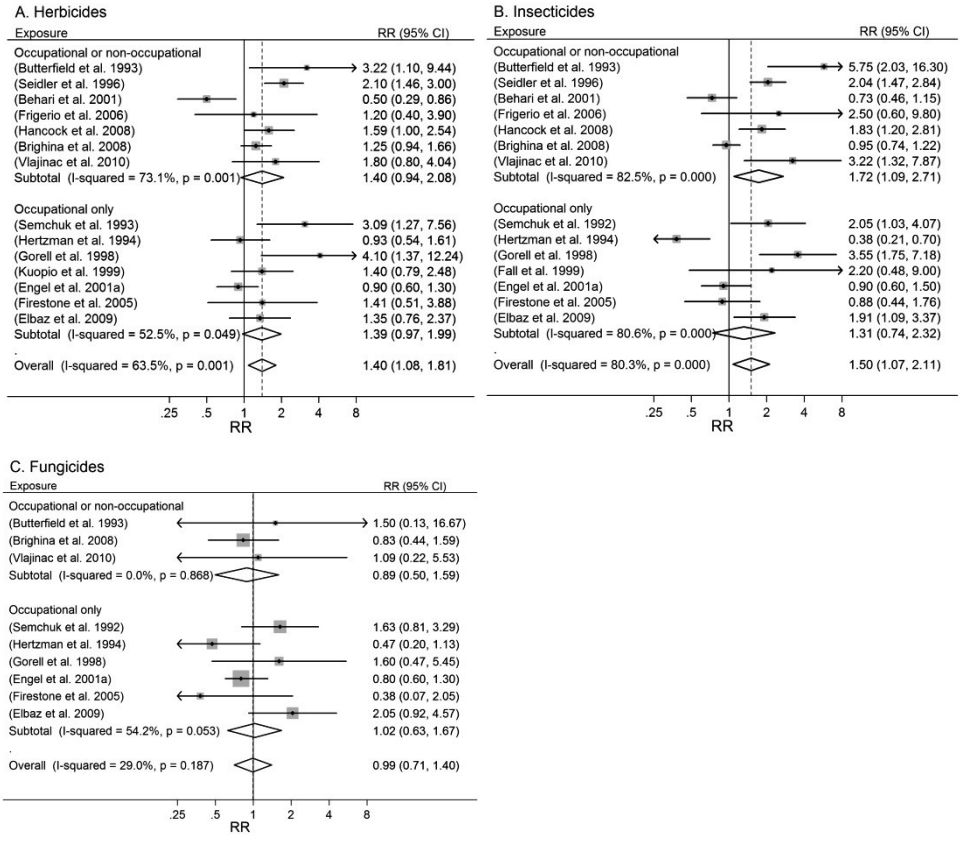

Figure 2. Forest plots for study-specific RRs and sRRs (95% CIs) of PD associated with the use of herbicides (A), insecticides (B), and fungicides (C). The studies are ordered by publication year and stratified by studies that did or did not include non-occupational exposure in the exposed group. Studies were pooled with the random effects method. The size of the squares reflects the statistical weight of the study in the meta-analyses.

Funnel plots of effect estimates for exposure to pesticides and pesticide subcategories were suggestive of small study effects, with a tendency for smaller studies to report higher relative risks compared with larger studies (Figure 3), with Egger's test *p*-values of 0.057, 0.338, 0.208, and 0.680 for pesticide, herbicide, insecticide, and fungicide effect estimates, respectively.

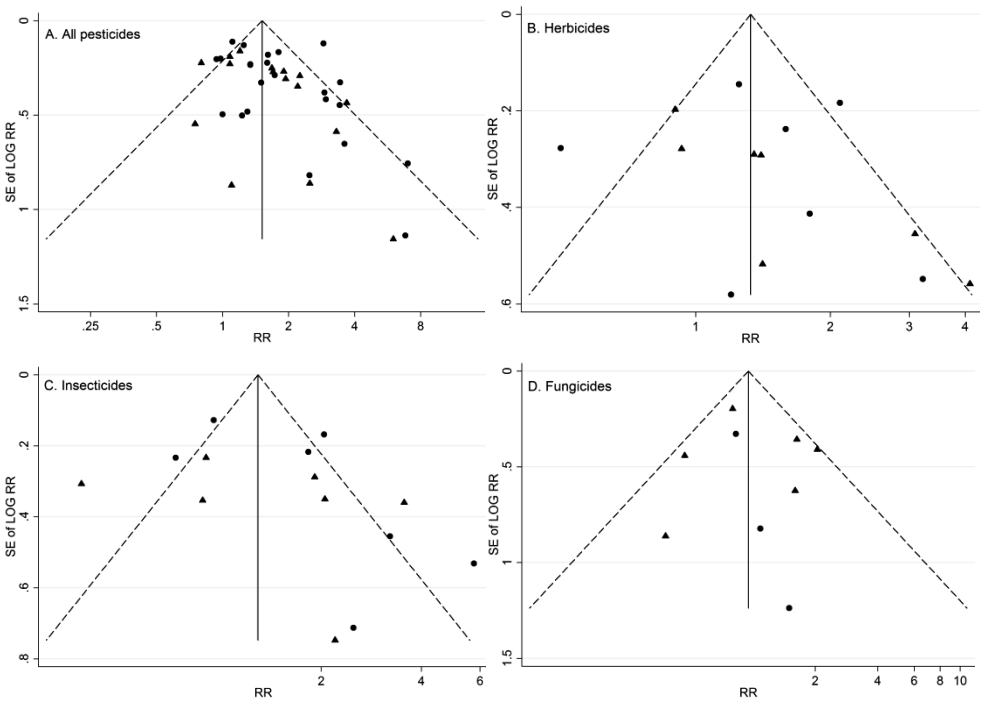

Figure 3. Funnel plots of studies included in the meta-analysis for the risk of PD associated with the use of pesticides (*A*), herbicides (*B*), insecticides (*C*), and fungicides (*D*). Circles represent studies that included non-occupational exposure in the exposed group, and triangles represent studies that were based on occupational exposure only. Egger's test *p*-values were 0.057, 0.338, 0.208, and 0.680 for pesticide, herbicide, insecticide, and fungicide effect estimates, respectively.

Figure 4 presents subgroup sRR estimates for those factors *a priori* hypothesized to be related to the observed heterogeneity in study results. The only study characteristic that was suggestive of contributing to heterogeneity was the exposure assessment method, with the lowest summary estimates observed for self-reported exposures (n = 36) and highest sRR for studies with exposures estimated based on reported job titles (n = 3). However, these differences were not statistically significant (p = 0.30). There was no evidence for a difference in

summary estimates by adjustment of results for potential confounders, type of control population source, geographical area, or by study design. We also investigated whether adjustment for smoking had an effect on the summary risk estimate. Almost identical results were found for studies that did or did not correct for smoking (data not shown). Similar analyses for the subcategories herbicides and insecticides rendered similar results as for all pesticides (data not shown).

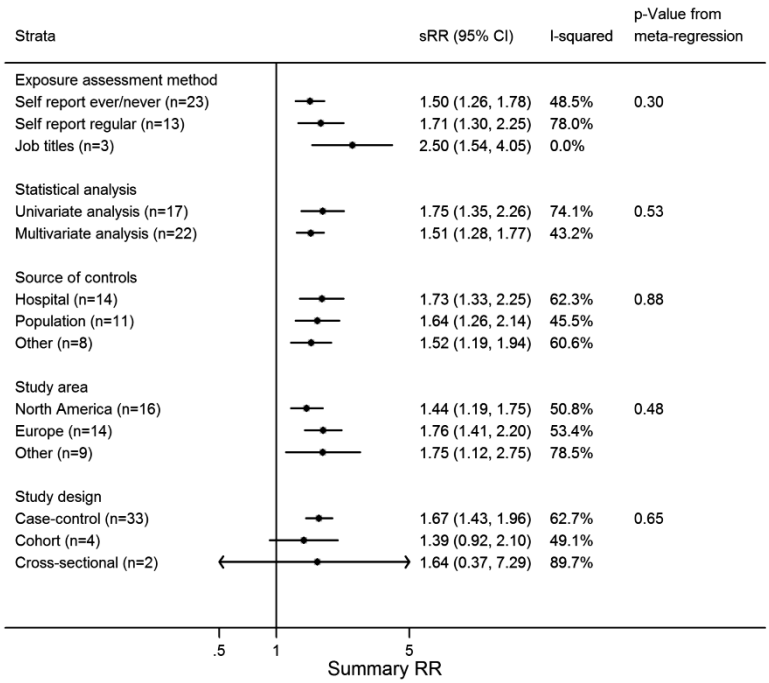

Figure 4. sRRs (95% CIs) for strata of exposure assessment method, statistical analysis, source of controls, study area, and study design. The *p*-value from meta-regression represents the *p*-value of the *F*-test in case of more than two categories, whereas it represents the *p*-value for the *t*-test in the case of the two statistical analysis strata.

Discussion

Our systematic review indicated that PD is related to pesticide exposure with a sRR of 1.62 (95% CI 1.40-1.88). However, there was substantial heterogeneity among individual study estimates (I^2 = 63.7%). Summary estimates also indicated positive associations of PD with herbicides and insecticides, but not with fungicides. We systematically investigated several factors that could explain heterogeneity in study results, but none appeared to be related to the observed heterogeneity, with the possible exception of the method of exposure assessment. Studies that based their exposure assessment on job titles reported somewhat higher risk estimates than studies that used self-reported exposures, but the difference did not reach statistical significance, in part because of low numbers of studies relying on job title and expert judgment.

Including persons who were nonoccupationally exposed to pesticides together with those occupationally exposed resulted in a very similar sRR. Given that occupational pesticide applications are in general more frequent and on larger areas than are non-occupational exposures, one would have anticipated higher RRs for studies focusing only on occupational exposures. On the other hand, use of protective equipment during non-occupational applications may be less. The fact that summary results were similar for both types of studies could indicate that non-occupational and occupational pesticide exposures carry similar risks, or that most of the exposures in the combined studies were occupational. In the three studies that exclusively reported on non-occupational pesticide exposures, only a small increase in relative risk was observed (sRR 1.18 (95% CI 0.86-1.63)), suggesting that risks associated with non-occupational pesticide exposures are lower than from occupational exposures. Nevertheless, non-occupational pesticide exposure cannot be ruled out as a risk factor for PD based on these analyses.

Studies used different methods for exposure assessment and assignment. Most studies (36 of 39) were based on self-reported exposure to pesticides, defined as ever versus never use or as regular versus nonregular use. No difference in sRR was seen between these two definitions of self-reported exposure, although it could have been expected that using a more stringent definition of exposure would have resulted in stronger associations. Studies that used reported job titles and expert judgment, and/or that used a job-exposure matrix to estimate exposures, resulted in a higher sRR compared with studies using self-reported pesticide exposures. This difference cannot be explained by recall bias, because in that case, higher risk ratios would have been expected for studies relying on self-reported exposures. A more likely explanation is that subjects are not able to reliably report exposures to pesticides, resulting in nondifferential exposure misclassification and bias toward the null (31, 32). The fact that some heterogeneity is observed in study results by

exposure assessment method indicates that this may be an important factor that should be taken into consideration when designing or interpreting studies.

A broad range of different pesticides exist with different chemical compositions and working mechanisms. In line with the conclusions of Brown et al. (3), we found that both herbicides and insecticides, but not fungicides, were associated with PD. However, it is difficult to disentangle the effect of herbicides and insecticides given that the use of these two pesticide groups is often highly correlated. This is illustrated by the fact that we observed a correlation coefficient of 0.79 between the study-specific RRs of herbicides and insecticides. Few studies have focused on specific pesticides precluding any meaningful meta-analyses (18, 21, 23, 24, 29, 33-39). However, it is interesting to note that the subgroup of organochlorines was significantly associated with PD in three studies (29, 33, 38). This is also in line with studies on biomarkers in serum (40, 41) and in the brains of deceased patients (42, 43). Organochlorines are mainly insecticides, including DDT (dichlorodiphenyltrichloroethane), dieldrin, and heptachlor.

Funnel plots gave some indication for a small-study effect, such that larger effect estimates appeared to be associated with smaller studies, which suggests that the sRR might be slightly overestimated. In addition, the studies included were generally small, resulting in imprecise effect estimates that could have contributed to the substantial heterogeneity in study results. Meta-regression analyses provided no evidence for a difference in sRRs based on study design, geographical area, adjustment for potential confounders, or type of control population. As such, factors explaining the heterogeneity observed remain largely elusive. We were not able to investigate the effect of differences in criteria used for the diagnosis of PD because there was substantial variation in the exact inclusion criteria among the studies that reported on the criteria used. However, in most of the studies, the diagnosis was made by a physician and included the presence of two or three of the cardinal symptoms of PD, often with some additional inclusion and exclusion criteria. Variations in participation rates could also contribute to study heterogeneity. The ability to investigate this factor was limited because only 13 of the case–control studies reported participation rates. The same is true for differences across sex. Only 8 studies showed separate results for men and women, but the results were not conclusive: RRs were higher for men than for women in 3 studies higher for women than men in 3 other studies (18, 29, 44), and comparable between men and women in the remaining 2 studies (34, 45). Heterogeneity in the results could also arise from both quantitative and qualitative differences in types of agriculture in the study areas. Although we compared large regions (i.e., North America, Europe, and other), this analysis would not have captured regional differences in the types of agriculture and pesticides used. Analyses by time periods might

provide some clues because pesticide use has changed over the decades, but data were insufficient to perform a meaningful analysis of changes over time.

Conclusion

Our overall summary risk estimates strongly suggest that exposure to pesticides, and to herbicides and/or insecticides in particular, increases the risk of developing PD. Heterogeneity among study-specific RRs could not easily be explained by methodological differences, except for a suggestive effect of exposure assessment characteristics. Future studies should therefore focus on using more objective semiquantitative methods for exposure assessment such as job- or crop-exposure matrices, rather than relying solely on self-report. Although classes of pesticides have been linked to PD, it remains important to identify the specific chemicals responsible for this association. Therefore, in new, preferably prospective studies, attention should be given to collecting detailed information on specific pesticide use.

Abbreviations table 1

CC_h, case-control study with hospital controls; CC_o, case-control study with controls from other sources or a combination of sources; CC_p, case-control study with population controls; CNS, central nervous system; Co, cohort study; CS, cross-sectional study; F, fungicides; H, herbicides; I, insecticides; JT, job titles; Non-Occ only, only non-occupational exposure included in the exposed group; NSAIDs, nonsteroidal anti-inflammatory drugs; Occ only, only occupational exposure included in the exposed group; Occ/Non-Occ, non-occupational exposure included in the exposed group; P, pesticides; SR-E/N, self-report ever/never; SR-R, self-report regular.

Table 1. Overview of the studies included in the meta-analyses.

Study	Study design	Location	Cases	Controls	Exposure assessment	Adjustments	Remarks
Ho et al. 1989 (46)	CC_o	Hong Kong	35 PD patients Age range: 65-87	105 age/sex matched	SR-E/N Occ/Non-Occ P	-	-
Koller et al. 1990 (47)	CC_h	USA	150 PD patients Age range: 39-87 Mean age: 66	150 age/sex matched	SR-E/N Occ only P	-	OR calculated from reported numbers
Golbe et al. 1990 (48) (1)	CC_o	USA	106 PD patients No age information	106 spouses	SR-R Occ/Non-Occ P	-	OR calculated from reported numbers
Zayed et al. 1990 (49)	CC_p	Canada	42 PD patients No age information	84 age/sex matched	SR-R Occ/Non-Occ P	Age, sex	-
Wong et al. 1991 (50)	CC_h	USA	38 PD patients Mean age: 70	38 age/sex matched	SR-E/N Occ/Non-Occ P	-	OR calculated from reported numbers
Stern et al. 1991 (51)	CC_o	USA	80 PD patients, diagnosed after 60 years of age No age information	80 age/sex/race/ participating center matched	SR-E/N Non-Occ only H, I	-	-
Jiménez-Jiménez et al. 1992 (52)	CC_h	Spain	128 PD patients Mean age: 66.8	256 age/sex matched	SR-R Occ/Non-Occ P	-	OR calculated from reported numbers
Semchuk et al. 1992, 1993 (20, 21)	CC_p	Canada	130 PD patients Age range: 36-97 Mean age: 68.5 Participation: 88%	260 age/sex matched Participation: 76%	SR-E/N Occ only P, H, I, F	-	Herbicides OR adjusted for PD family history and head trauma

Table 1. Continued

Study	Study design	Location	Cases	Controls	Exposure assessment	Adjustments	Remarks
Hubble et al. 1993 (53)	CC_o	USA	63 PD patients Mean age urban patients: 69.3 rural patients: 69.0	75 with similar mean age	SR-R Occ/Non-Occ P	Age < 65, male lifestyle, ethnicity family history, fresh produce consumption, history of head trauma, depression or CNS infection,	-
Butterfield et al. 1993 (28)	CC_o	USA	63 PD patients, diagnosed before 51 years of age Age range: 35-72 Mean age: 49 Participation: 69%	68 age/sex/ diagnosis-year frequency matched Participation: 41%	SR-R Occ/Non-Occ H, I, F	Age, sex, race, age at diagnosis, education, family history	95%-CIs calculated from ORs and p-values F-OR is not adjusted
Morano et al. 1994 (54)	CC_h	Spain	74 PD patients Mean age: 68.4	148 age/sex matched	SR-R Occ/Non-Occ P	-	OR calculated from reported numbers
Hertzman et al. 1994 (35)	CC_p	Canada	142 PD patients Mean age: 70.4	124 controls 45-80 years of age Participation: 61%	SR-E/N Occ only P, H, I, F	-	Reported results were pooled for men and women A second control group consisting of hospital controls was not used in this meta-analysis

Chaturvedi et al. 1995 (30)	CS	Canada	87 PD patients No age information	2070 controls from cross-sectional study among elderly	SR-R Non-Occ only P	-	-
Seidler et al. 1996 (33)	CC_p	Germany	379 PD patients <66 years of age Mean age: 56.2 Participation: 71%	379 age/sex matched	SR-E/N Occ/Non-Occ H, I	Smoking, education	The reported results for exposure categories were pooled A second control group consisting of neighborhood controls was not used in this meta-analysis
Liou et al. 1997 (36)	CC_h	Taiwan	120 PD patients Age range: 37-91 Mean age: 63.1	240 age/sex matched	SR-R Occ/Non-Occ P	-	-
De Palma et al. 1998 (15)	CC_h	Italy	100 PD patients Mean age: 66.6	200 controls, similar in age and sex	JT Occ/Non-Occ P	-	Substantial leisure activities were also classified for exposure
Chan et al. 1998 (44)	CC_h	Hong Kong	215 PD patients Age < 60: 13.5% Age 60-69: 33.5% Age 70-79: 33.5% Age ≥ 80: 19.5%	313 age/sex/hospital matched	SR-E/N Occ only P	Smoking, family history, rural living, well-water drinking, farming, consumption of tea, fruit vegetables and vitamins/liver oil supplements	Substantial difference between OR from unadjusted and adjusted analysis. Unadjusted OR 1.80 (95% CI 0.90-3.58)

Table 1. Continued

Study	Study design	Location	Cases	Controls	Exposure assessment	Adjustments	Remarks
McCann et al. 1998 (14)	CC_o	Australia	224 PD patients Mean age: 70.3	310 age/sex/ ethnicity/resident ial area/site of collection matched	SR-R Occ only P	-	-
Gorell et al. 1998 (55)	CC_p	USA	144 PD patients 50 years or older Age 50-59: 9.0% Age 60-69: 30.6% Age 70-79: 46.5% Age ≥ 80: 13.9% Participation: 81%	464 ages/sex/race frequency matched Participation: 65%	SR-E/N Occ only, and Non-Occ only H, I, F	Age, sex, race, smoking	-
Werneck and Alvarenga 1999 (56)	CC_h	Brasil	92 PD patients Age range: 55-78 Mean age: 70.6	110 age/sex matched	SR-R Occ/Non-Occ P	-	-
Fall et al. 1999 (25)	CC_p	Sweden	113 PD patients, Age range: 40-75 Mean age: 63.9 Participation: 90%	263 from same age category Participation: 82%	SR-E/N Occ only P, I	Smoking, alcohol, coffee, and fried/broiled meat consumption, carpenters, cabinetmakers	Only results for men are shown I-OR is not adjusted
Kuopio et al. 1999 (57)	CC_p	Finland	123 PD patients Mean age: 69.3	246 age/sex/ municipality matched Participation: 68%	SR-E/N Occ only H	-	The reported results for "pesticides" do not contain herbicides, and are not included in this review

Preux et al. 2000 (58)	CC_h	France	140 PD patients Mean age: 71.1	280 age/sex matched	SR-E/N Occ/Non-Occ P	-	OR calculated from reported numbers
Herishanu et al. 2001 (59)	CC_h	Israel	93 PD patients No age information	93 age/sex matched	SR-E/N Occ/Non-Occ P	Smoking, birth country, peptic disease, work in construction or in mechanical factory	-
Engel et al. 2001 (23)	CS	USA	65 parkinsonism patients No age information	310 of original 1300 men who previously participated in a cohort study	SR-E/N Occ only P, H, I, F	Age, smoking	The study was among men only
Behari et al. 2001 (60)	CC_h	India	377 PD patients Age range: 24-86 Mean age: 56.8 Participation: 100%	377 age matched Participation: 100%	SR-E/N Occ/Non-Occ H, I	-	ORs calculated from reported numbers
Zorzon et al. 2002 (61)	CC_h	Italy	136 PD patients Mean age: 70.0	272 age/sex matched	SR-E/N Occ/Non-Occ P	Smoking	
Petrovitch et al. 2002 (26)	Co	Hawaii	99 PD patients after 30-year follow up Median age at diagnosis: 73.7 Range: 54-89	Baseline: 7986 Japanese men in Hawaii	SR-R Occ/Non-Occ P	-	RR calculated from reported incidence numbers

Table 1. Continued

Study	Study design	Location	Cases	Controls	Exposure assessment	Adjustments	Remarks
Duzcan et al. 2003 (22)	CC_p	Turkey	36 parkinsonism patients, > 50 years of age Age 50-59: 11.1% Age 60-69: 30.6% Age 70-79: 47.2% Age ≥ 80: 11.1%	108 age/sex matched	SR-R Occ/Non-Occ P	-	-
Baldereschi et al. 2003 (62)	CS	Italy	113 PD patients Mean age : 78.1	Study among 4496 randomly selected elderly	SR-E/N Occ only P	Age, sex, education, smoking	Having a pesticide-use license was used as a proxy for pesticide use
Baldi et al. 2003 (63)	CC_p	France	84 PD patients, > 69 years of age Mean age: 75.6	252 age/sex matched	JT Occ only P	Age, sex, smoking, education	-
Baldi et al. 2003 (64)	Co	France	24 PD patients after 5-year follow up No age information	Baseline: 1507 persons who were ≥ 65 years of age in specific area	JT Occ only P	Smoking, education	Reported results for men en women were pooled
Nuti et al. 2004 (65)	CC_p	Italy	190 PD patients Mean age: 63.9	190 age/sex/ sociocultural factors matched	SR-E/N Occ/Non-Occ P	-	OR calculated from reported numbers
Frigerio et al. 2006 (8)	CC_p	USA	149 PD patients Age range: 41-97 Mean age: 70.0 Participation: 76%	129 age/sex matched Participation: 66%	SR-E/N Occ/Non-Occ P, H, I	Age, sex	Also results occupational only (farming)

Ascherio et al. 2006 (45)	Co	USA	413 PD patients after 9-year follow up Mean onset age: 70	Baseline: 184190 persons	SR-R Occ/Non-Occ P	Age, sex, smoking, coffee, NSAID, education, physical activity	-
Kamel et al. 2007 (39)	Co	USA	78 PD patients after 5-year follow up Age ≤ 50: 9% Age 51-60: 40% Age 61-70: 41% Age > 70: 10%	Baseline: 84738 persons (applicants for pesticide use certification and their spouses)	SR-E/N Occ/Non-Occ P	Age, state, applicator or spouse	-
Dick et al. 2007 (66)	CC_o	Scotland Sweden Romania Italy Malta	767 PD patients Mean age: 69.8 Participation: 77%	1989 age/sex/country frequency matched Participation: 59%	SR-E/N (+ JT) Occ/Non-Occ P	Age, sex, country, smoking, family history, ever knocked unconscious	-
Fong et al. 2007 (16)	CC_h	Taiwan	153 PD patients Mean age: 71.7	155 age/sex/ birthplace matched	SR-R Occ only P	Age, sex, smoking	-
Brighina et al. 2008 (34)	CC_o	USA	833 PD patients, Age range: 32-91 Median age: 67.7	361 age/sex/region matched and 472 siblings	SR-R Occ/Non-Occ P, H, I, F	Age, sex	-
Hancock et al. 2008 (38)	CC_o	USA	319 PD patients Age range: 29-94 Mean age: 65.6	296 relatives and spouses	SR-E/N Occ/Non-Occ P, H, I	Age, sex, smoking, caffeine consumption	-
Petersen et al. 2008 (27)	CC_p	Faroe islands	79 PD patients Mean age: 74.4	154 age/sex matched	SR-E/N Occ only P	Smoking	no exposed women in study

Table 1. Continued

Study	Study design	Location	Cases	Controls	Exposure assessment	Adjustments	Remarks
Elbaz et al. 2009 (29)	CC_p	France	224 PD patients < 76 years of age Median age: 69.0 Participation: 83%	557 age/sex/region matched Participation: 75%	SR-E/N Occ only, and Non-Occ only P, H, I, F	Smoking, Mini Mental State Examination score	Reported I-OR, H-OR, and F-OR for men and women were pooled. The OR for Non-Occ only is unadjusted
Tanner et al. 2009 (24)	CC_o	USA	519 parkinsonism patients Age range: 30-88 Median age: 65	511 age/sex/location frequency matched	SR-E/N Occ only P	Age, sex, ethnicity, smoking, alcohol, caffeine, head injury	-
Vlajinac et al. 2010 (37)	CC_h	Serbia	110 PD patients Mean age: 60.8 Participation: 100%	220 age/sex/urban or rural living matched Participation: 100%	SR-E/N Occ/Non-Occ P, H, I, F	I-OR is adjusted for gardening, rural living, well and spring water drinking, dyes or naphta exposure, service-sector work	OR, H-OR and F-OR calculated from reported numbers
Firestone et al. 2005, 2010 (18, 19)	CC_h	USA	404 PD patients Age range: 29-88 Median age: 69 Participation: 70%	526 age/sex frequency matched Participation 60%	SR-E/N Occ only, and Non-Occ only P, H, I, F	Age, ethnicity, smoking	Reported results for all pesticides were pooled for men and women. Only results for men are shown for the subgroups for occ only
Manthripragada et al. 2010 (67)	CC_p	USA	351 PD patients Age ≤ 60: 22% Age > 60: 78%	363 controls from same region	SR-E/N (+ JT) Occ only P	Age, sex, ethnicity, smoking, education, county	-

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Chapter 4

Occupational exposure to pesticides and endotoxin and Parkinson disease in the Netherlands

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Abstract

Objectives: Previous research has indicated that occupational exposure to pesticides and possibly airborne endotoxin may increase the risk of developing Parkinson disease (PD). We studied the associations of PD with occupational exposure to pesticides, specifically to the functional sub-classes insecticides, herbicides and fungicides, and to airborne endotoxin. In addition we evaluated specific pesticides (active ingredients) previously associated with PD.
Methods: We used data from a hospital-based case-control study, including 444 PD patients and 876 age and sex matched controls. Exposures to pesticides from both application and re-entry work were estimated with the ALOHA+ job-exposure matrix and with an exposure algorithm based on self-reported information on pesticide use. To assess exposure to specific active ingredients a crop-exposure matrix was developed. Endotoxin exposure was estimated with the DOM job-exposure matrix.
Results: The results showed almost no significant associations. However, odds ratios were elevated in the higher exposure categories for pesticides in general, insecticides, herbicides and fungicides, and below unity for endotoxin exposure. The analyses on specific active ingredients showed a significant association of PD risk with the fungicide benomyl.
Conclusions: This study did not provide evidence for a relation between pesticide exposure and PD. However, the consistently elevated odds ratios in the higher exposure categories suggest that a positive association may exist. The possible association with the active ingredient benomyl requires follow-up in other studies. This study did not provide support for a possible association between endotoxin exposure and PD.

Introduction

It has been frequently reported in epidemiological and toxicological studies that exposure to pesticides may increase the risk of developing Parkinson disease (PD) (1-3). Pesticides are widely used in agriculture and therefore farm work is an important source of exposure. Exposure occurs during the actual application of pesticides, but also during contact with crops treated with pesticides when carrying out so-called re-entry activities such as weeding or thinning. Exposure from re-entry work could be substantial depending on the crop (4, 5).

Because a wide range of pesticides (active ingredients) have been used in the past, is it unclear which active ingredients are responsible for the reported increase in PD risk in epidemiological studies. The accuracy of self-reported information on performed applications in the past is limited for specific active ingredients (6). Moreover, farm workers who have performed re-entry work but were not involved in the application or purchase of pesticides might not be able to accurately report on the use of pesticides. The use of occupational histories to assign exposure is less affected by recall problems (7). However, only a few of the previous epidemiological studies on PD risk used job titles (8-11) or a combination of job-titles with self-reported information (12, 13) to assess exposure to pesticides or functional sub-classes of pesticides, e.g. insecticides, herbicides and fungicides.

Recently, it has been postulated that not only pesticides may increase PD risk, but also endotoxin exposure, by inducing inflammation-mediated neurodegeneration (11, 14). Endotoxins are the lipopolysaccharide components of gram-negative bacterial cell walls, and exposure is common during agricultural work.

Here we present the results of analyses on the possible associations between occupational exposure to pesticides and endotoxin and PD risk within a recently conducted hospital-based case-control study in the Netherlands. We used the existing general-population ALOHA+ job-exposure matrix (JEM) (15) and self-reported exposure information to estimate exposure to insecticides, herbicides and fungicides through mixing and application work and through re-entry work in treated crops. Furthermore, we constructed a time-dependent crop-exposure matrix to estimate exposure to specific active ingredients based on self-reported cultivation of crops. For exposure to endotoxin we used the recently developed DOM-JEM (16).

Methods

Cases and controls

Details about the study methodology were described previously (17). Briefly, cases and controls were recruited between April 2010 and June 2012 from 5 hospitals in 4 different areas in the Netherlands. We set out to include all patients that had an initial Parkinson disease diagnosis in one of the participating hospitals between January 2006 and December 2011. In the Netherlands, with a universal health care system, all PD patients are seen in a hospital. PD patients included can therefore be regarded to be representative for all PD patients in the service areas of the participating hospitals. In each hospital one neurologist reviewed the medical files of all potential study subjects. For each confirmed PD patient, two matched controls were selected from persons that were seen at the department of neurology between January 2006 and December 2011 for non-neurodegenerative complaints (median nerve neuropathy; ICD-10 G56.0 and G56.1, ulnar nerve neuropathy; ICD-10 G56.2, thoracic and lumbar disc disease; ICD-10 G55.1, G54.3 and G54.4, and sciatica; ICD-10 M54.3 and M54.4) The controls were matched to the cases on hospital, visiting date (within 3 years of the cases diagnose year), sex and age. Cases and controls were initially contacted via an invitation letter from the hospitals' neurology departments together with a reply form for giving informed consent or to decline study participation. The study information explained that the study objective was to study risk factors for neurological disorders, without further specification. Non-responders were sent a reminder after one month, and one phone call attempt was performed after another month.

At recruitment, 1001 (93% of total) eligible PD patients were still alive and of 993 of those we had a valid current address. There were 448 persons that participated (45%), 406 that declined participation and 139 that did not reply. The participation rate for controls was 35%. About 50% of the non-participants provided a reason for their decline. Health related reasons were reported most frequently, but compared to cases, more controls reported to be not interested. For 12 cases only 1 suitable control was found and for 4 cases no controls were found, leaving 444 cases and 876 controls that were included in the analyses. The study was approved by the Medical Ethics Committee of St Elisabeth Hospital, Tilburg, the Netherlands.

Data collection

Participants were interviewed in a standardized computer-assisted telephone interview by one of three trained interviewers. The questionnaire contained an occupational history in which all jobs performed for at least 6 months were included. Study participants reported on years and hours per week worked, job title, type of industry, company name and main tasks. Supplemental questions

about occupational application of insecticides, herbicides and fungicides were asked to those who reported to have worked on a farm or as a gardener. The annual number of days on which applications were performed at the job (<1/year, 1-5/year, 6-20/year, 21-50/year, >50 /year) was asked. Questions about application method and use of protective equipment were asked to participants who had personally applied pesticides. Furthermore, participants who worked on a farm were asked to name the main crop types cultivated at the farm with a maximum of three. Participants reporting having worked at their parents' farm during childhood, were not always asked additional questions on working hours and pesticide applications (n=40). Based on what was reported by other participants with similar jobs, we assumed that those participants helped 8 hours per week at the farm from age 12 to age 18 and in case no farm type was provided we assumed a mixed farm. All jobs were coded according to the International Standard Classification of Occupations 1968 and 1988 (ISCO68 and ISCO88).

Assessment of pesticide exposure

Exposures were estimated from 1955 until the calendar year before diagnosis, as after this year synthetic pesticides became commonly used in the Netherlands. Occupational exposure to pesticides was estimated using three different methods.

First method: JEM approach

The first method estimated pesticide exposure by linking all reported jobs to the ALOHA+ JEM (15). This JEM assigns exposure to pesticides, and to functional sub-classes (i.e. insecticides, herbicides and fungicides) using arbitrary weights of 0, 1 and 4 for no, low and high exposure. For farm and gardener jobs the JEM score was set to 0 if the participant reported that no insecticides (n=40), herbicides (n=41), or fungicides (n=77) had been applied. For jobs coded ISCO88 code 9333 (freight handlers), exposure to insecticides was only assigned to those jobs coded ISCO68 code 97120 (dockers). Cumulative exposures were estimated by multiplying the JEM scores with years worked in a job, summed across all jobs of a participant's occupational history.

Second method: exposure algorithm

The second method estimated more specifically the exposure for participants who held a farm or gardener job. For these jobs, we developed an exposure model (algorithm) for insecticides, herbicides and fungicides, based on the algorithm developed for the US Agricultural Health Study (AHS) for estimating applicator exposure (18). We extended the algorithm by including estimates for exposure due to re-entry work.

Applicator exposure was calculated for participants who reported to have applied insecticides, herbicides or fungicides personally. Exposure intensity was estimated based on the application method and use of personal protective equipment (PPE) in accordance to the AHS algorithm. However, as we had no information on performing maintenance or repair of application equipment, and because essentially all applicators in our study mixed the pesticides before use, these factors included in the AHS algorithm were left out in our study. For the application method, the AHS model uses relative exposure values of 1 for distribution of tablets/granules, 3 for a boom sprayer on a tractor, 8 for a backpack sprayer and 9 for a hand sprayer. Since the European EUROPOEM model shows roughly a factor 2 difference in exposure between manual and tractor spraying, we adjusted the AHS values slightly to 1 for distribution of tablets/granules, 4 for tractor application and 8 for manual application by backpack or hand spray (19, 20). The values for the use of PPE were based on the AHS algorithm and were 1 for individuals not using PPE, 0.8 for individuals using gloves, rubber boots or goggles, and 0.5 for individuals who also used impermeable clothing or facemasks. If a participant reported that the use of PPE had changed over the years of working in a particular job, average values of categories were assigned.

Yearly applicator exposure was calculated by multiplying the intensity level with the number of applications per year using the mid-point of the answer categories and the percentage of applications performed by the participant at the farm (mostly/always: 0.9, sometimes: 0.5, rarely: 0.1).

Yearly applicator exposure = Application method * PPE * applications/year * percentage self-application by participant

Yearly exposure from re-entry work was estimated by multiplying the intensity level for a day of re-entry work with the number days of re-entry work. Based on the EUROPOEM applicator and re-entry worker models we estimated that a day of re-entry work would result in 10% of the exposure of a typical day of application work (19).

Since most applications were conducted by tractor or manually by backpack or hand spray, which in the applicator part of the model have on average an intensity level of 6, we used an intensity level of 0.6 for a day of re-entry work. The yearly number of days with re-entry work was calculated from the reported number of applications per year at the farm, the number of days a pesticide was assumed to be present on the crop after application and an estimated number of days that a participant performed re-entry work after each application. The number of

applications per year at a farm was imputed for participants who did not know if pesticides were applied at a job or at what frequency, based on the most frequently reported answer by other participants for similar jobs. Although the number of days a pesticide is present on the crops after application depends on pesticide type, crop type and weather conditions, we assumed a period of 14 days based on data in the literature (5, 21). Furthermore, we estimated that workers who worked 40 hours per week at farms with horticultural or fruit crops performed 5 days per week re-entry work (ISCO68 job codes: 61230, 62320, 61270, 62720, 62730) and workers at farms with field crops 1 day per week (ISCO68 job codes: 61110, 62105, 61220, 62210). These estimations were adjusted for the number of hours per week a participant had worked.

Cumulative total exposure was calculated by multiplying the yearly exposure from pesticide application plus re-entry work with the number of years in a job, summed across all jobs of a participant's occupational history.

Third method: crop-exposure matrix

The third method assigned exposure to specific active ingredients by linking reported crops cultivated at the participant's farm to a crop-exposure matrix. In this crop-exposure matrix per-decade estimations are given for the percentage of farms that applied a specific active ingredient on a type of crop and the yearly frequency of application. Active ingredients included in the crop-exposure matrix were based on previous studies linking specific pesticides to PD risk and for which we had sufficient data to estimate historical application. The included active ingredients were the insecticides: dichlorvos, lindane, parathion, and permethrin; the herbicides: 2,4-D, atrazine, dinoseb and paraquat; and the fungicides: benomyl and maneb.

Expert judgment on the probabilities and frequencies of application were provided by former extension workers, two per crop type. These experts estimated probability and frequency of use of active ingredients allowed for use on potatoes, cereals, beets, maize, tulip bulbs and fruit back to the year 1960. More details about the expert estimations can be found elsewhere (22). Estimates for other field crops, and vegetables and flowers in green houses which were not covered by the experts were derived from data of Statistics Netherlands that gathered statistics on use of specific active ingredients since 1995. For earlier decades probability and frequency of application for those crops were extrapolated from time trends for crops for which expert estimations were available. Statistics Netherlands also gathered data since 1976 on active ingredient use in public places, which we used to estimate exposure for gardeners (23).

Cumulative exposures to these active ingredients were calculated by summing the yearly probability of application of a specific active ingredient at the farm multiplied with the yearly frequency across all years worked at farms. For farms where exposure to an active ingredient was assigned to more than one crop, the probability and frequency of use for the crop with the highest probability of use were taken for calculating cumulative exposure. As in the approach with the JEM analyses described earlier, no exposure was assigned to a farm job when on that farm according to the participant no insecticides, herbicides, or fungicides had been applied.

Assessment of endotoxin exposure

High, low or no exposure (weights of 4, 1, 0, respectively were assigned to reflect the multiplicative nature of occupational exposure distributions) to endotoxin was assigned by linking the DOM-JEM with the reported jobs (16). Cumulative exposure was estimated by multiplying the JEM scores with years worked in a job, summed across all jobs of a participant's occupational history.

Statistical analysis

Odds ratios (OR) and 95% confidence intervals (CI) were calculated using conditional logistic regression. The exposed participants were categorized in two or three groups based on either median or tertiles of the distribution of the different exposures among controls. In model 1, all analyses were adjusted for pack-years of smoking (5 levels), total coffee consumption (4 levels) and categories for occupational skill and status (high-skilled white-collar worker, low-skilled white-collar worker, high-skilled blue-collar worker, low-skilled blue-collar worker). In model 2, the pesticide analyses were additionally adjusted for cumulative endotoxin exposure (4 levels).

Results

Of the PD patients 63.3% were men with a median age at diagnosis of 67 years (see Table 1). Cases more often had high-skilled white-collar jobs than controls, smoked less and consumed less coffee.

Table 1. General characteristics of cases and controls

	Cases (n=444)	Controls (n=876)
Men, No (%)	281 (63.3)	557 (63.6)
Age at interview, median (range)	68 (34 - 91)	68 (34 - 90)
Age at diagnosis, median (range)	67 (34 - 90)	-
Cigarette smoking[a]		
Never smoked, No (%)	207 (46.6)	243 (27.7)
>0 - 7.8 pack-years, No (%)	86 (19.4)	161 (18.4)
>7.8 – 17.5 pack-years, No (%)	67 (15.1)	155 (17.7)
>17.5 – 29.4 pack-years, No (%)	45 (10.1)	160 (18.3)
>29.4 - 103 pack-years, No (%)	39 (8.8)	157 (17.9)
Coffee consumption[b]		
0 – 97 consumption-years, No (%)	128 (28.8)	220 (25.1)
> 97 - 156 consumption-years, No (%)	146 (32.9)	221 (25.3)
> 156 - 214 consumption-years, No (%)	90 (20.3)	216 (24.7)
> 214 – 720 consumption-years, No (%)	80 (18.0)	218 (24.9)
Occupational skill and status[c]		
High-skilled white-collar worker, No.(%)	198 (44)	335 (38)
Low-skilled white-collar worker, No.(%)	87 (20)	187 (21)
High-skilled blue-collar worker, No.(%)	101 (23)	202 (23)
Low-skilled blue-collar worker, No.(%)	58 (13)	152 (17)

[a]Pack-years of cigarette smoking was calculated by dividing average number of cigarettes per day by 20 multiplied with the number of years of smoking. Pack-years were divided based on the quartiles of the exposure distribution among the controls.

[b]Consumption-years were calculated by multiplying the average amount of coffee consumptions per day (cups per day) with the estimated number of years of coffee consumption. Consumption-years were divided based on the quartiles of the exposure distribution among the controls. Coffee consumption information was missing for one control.

[c]The categories of occupational skill and status were made according to major ISCO88 groups (first digit of the job codes). 1-3: high-skilled white-collar jobs, 4-5: low-skilled white-collar jobs, 6-7: high-skilled blue-collar jobs, and 8-9: low-skilled blue-collar jobs. The participants were categorized according to the group in which they had worked most years during their career.

Prevalence of pesticide exposure was 19.3% for cases and 19.1% for controls as assessed by the JEM approach (see Table 2). The prevalence of exposure to the functional sub-classes insecticides, herbicides and fungicides was slightly higher for cases compared with controls for both the JEM approach and the exposure algorithm. More controls (41.7%) than cases (38.1%) ever had a job with low or high endotoxin exposure. Most participants who held a job with high endotoxin exposure were individuals who worked at a farm with livestock. The most reported jobs with low endotoxin exposure were other farm jobs and cleaning jobs. Correlations between exposure to sub-classes of pesticides as shown in table 2

were high (Spearman correlation coefficients: 0.66-0.87). More moderate correlations (Spearman correlation coefficients: 047-0.60) were observed between endotoxin exposure and exposure to (sub-classes of) pesticides.

Table 2. Prevalence of pesticide, herbicide, insecticide, fungicide, and endotoxin exposure and their correlations

	Cases No. (%)	Controls No. (%)	Correlation insecticides[a]	Correlation herbicides[a]	Correlation fungicides[a]	Correlation endotoxin[a]
JEM approach[b] *Cumulative exposure*						
All pesticides	86 (19.3)	167 (19.1)	0.86	0.82	0.85	0.60
Insecticides	67 (15.1)	123 (14.0)	-	0.83	0.76	0.53
Herbicides	60 (13.5)	109 (12.4)	0.83	-	0.73	0.49
Fungicides	68 (15.3)	130 (14.8)	0.76	0.73	-	0.47
Endotoxin	169 (38.1)	365 (41.7)	0.53	0.49	0.47	-
Exposure algorithm[c] *Cumulative exposure*						
Insecticides	58 (13.1)	108 (12.3)	-	0.87	0.70	-
Herbicides	58 (13.1)	106 (12.1)	0.87	-	0.66	-
Fungicides	32 (7.2)	57 (6.5)	0.70	0.66	-	-

[a]Spearman correlation coefficient

[b]Cumulative exposures were estimated by multiplying the job-exposure matrix (JEM) assigned exposures (0 for no, 1 for low and 4 for high exposure) with years worked in a job, summed across all jobs of a participant's occupational history from 1955. The JEM score was set to 0 for exposure to pesticides, insecticides, herbicides or fungicides if the participant reported that those were not applied.

[c]Cumulative exposures were estimated by multiplying the exposure algorithm assigned exposure from pesticide application and re-entry work with all years worked in a job, summed across all jobs of a participant's occupational history from 1955.

Pesticide exposure as assessed by the JEM approach

In Table 3 the analyses of cumulative pesticide exposure based on the ALOHA+ JEM augmented with self-reported information on actual use of pesticides are presented. The analyses revealed no statistically significant results, but overall, relatively more cases than controls were in the higher exposure tertiles for all groups of pesticides. Increased ORs were most pronounced for exposure to insecticides. Analyses without using augmentation on self-reported actual use of insecticides, herbicides or fungicides within farm or gardener jobs, resulted in ORs closer to 1 (see Supplemental material, table 1).

Table 3. Cumulative exposure to pesticides, specific sub-classes and endotoxin and Parkinson disease risk: JEM approach

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Model 1[b] OR (95% CI)	Model 2[c] OR (95% CI)
Pesticides[a]					
Never exposed	358 (80.6)	709 (80.9)	1	1	1
1-19	26 (5.9)	56 (6.4)	0.92(0.57-1.48)	0.91(0.55-1.52)	0.90(0.52-1.56)
20-32	22 (5.0)	56 (6.4)	0.77(0.46-1.30)	0.74(0.43-1.27)	0.75(0.42-1.36)
33-216	38 (8.6)	55 (6.3)	1.36(0.89-2.09)	1.28(0.79-2.10)	1.56(0.86-2.83)
Insecticides[a]					
Never exposed	377 (84.9)	753 (86.0)	1	1	1
1-20	22 (5.0)	46 (5.3)	0.95(0.56-1.61)	1.05(0.60-1.84)	1.10(0.60-2.03)
21-36	16 (3.6)	38 (4.3)	0.85(0.47-1.55)	0.77(0.40-1.47)	0.83(0.42-1.66)
37-216	29 (6.5)	39 (4.5)	1.48(0.90-2.41)	1.46(0.84-2.53)	1.79(0.95-3.37)
Herbicides[a]					
Never exposed	384 (86.5)	767 (87.6)	1	1	1
1-6	16 (3.6)	37 (4.2)	0.85(0.46-1.58)	0.97(0.51-1.85)	1.00(0.51-1.97)
7-23	23 (5.2)	39 (4.5)	1.19(0.70-2.03)	1.16(0.66-2.04)	1.30(0.70-2.39)
24-168	21 (4.7)	33 (3.8)	1.26(0.71-2.24)	1.13(0.59-2.15)	1.25(0.62-2.53)
Fungicides[a]					
Never exposed	376 (84.7)	746 (85.2)	1	1	1
1-7	19 (4.3)	54 (6.2)	0.70(0.41-1.19)	0.67(0.38-1.18)	0.64(0.35-1.17)
8-25	22 (5.0)	34 (3.9)	1.33(0.75-3.37)	1.35(0.73-2.50)	1.41(0.74-2.71)
26-168	27 (6.1)	42 (4.8)	1.26(0.76-2.07)	1.12(0.65-1.93)	1.24(0.69-2.23)
Endotoxin[a]					
Never exposed	275 (61.9)	511 (58.3)	1	1	
1-7	63 (14.2)	128 (14.6)	0.90(0.64-1.27)	1.09(0.76-1.57)	1.18(0.78-1.79)
8-21	46 (10.4)	117 (13.4)	0.74(0.51-1.07)	0.79(0.52-1.18)	0.76(0.48-1.18)
22-244	60 (13.5)	120 (13.7)	0.93(0.67-1.30)	0.95(0.64-1.40)	0.83(0.51-1.34)

[a]Conditional logistic regression analyses of pesticide and endotoxin exposure as assessed by the job-exposure matrix (JEM) approach. Cumulative exposures were estimated by multiplying the JEM assigned exposures (0 for no, 1 for low and 4 for high exposure) with years worked in a job, summed across all jobs of a participant's occupational history from 1955. The JEM score was set to 0 for exposure to pesticides, insecticides, herbicides or fungicides if the participant reported that those were not applied. The exposed were divided based on the tertiles of the exposure distribution among the controls.

[b]The first adjusted model includes cigarette smoking (5 categories) coffee consumption (4 categories) and occupational skill and status (4 categories).

[c]Additionally mutually adjusted: pesticide exposures to endotoxin and vice versa.

Because information on coffee consumption was missing for 1 control, this participant was excluded from adjusted analyses.

Pesticide exposure as assessed by the exposure algorithm

A total of 94 cases (21%) and 183 controls (21%) stated to have ever worked on a farm or as a gardener and consequently were asked supplemental questions on pesticide applications. The results of the analyses using the adjusted AHS exposure algorithm are shown in Table 4. Relatively more cases than controls were in the third tertile of cumulative exposure for insecticides, herbicides and fungicides, although the elevated ORs did not reach statistical significance. 65% of the insecticide-exposed, 63% of the herbicide-exposed and 55% of the fungicide-exposed had not personally applied pesticides, thus for those participants only re-entry work contributed to the exposure estimates. This was especially the case for women: of the 60 women exposed to insecticides, herbicides and/or fungicides, there were only 2 women who had actually applied pesticides. We also performed analyses on application work only (see Supplemental material, table 2). Ever having performed applications showed higher non-significant elevated ORs for insecticides and herbicides than for fungicides.

Table 4. Cumulative exposure to specific sub-classes of pesticides and Parkinson disease risk: Exposure algorithm

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Model 1[b] OR (95% CI)	Model 2[c] OR (95% CI)
Insecticides[a]					
Never	386 (86.9)	768 (87.7)	1	1	1
>0 -20	18 (4.1)	36 (4.1)	0.99(0.55-1.78)	0.94(0.51-1.75)	0.99(0.51-1.92)
>20-194	17 (3.8)	37 (4.2)	0.92(0.51-1.68)	1.03(0.54-1.96)	1.12(0.57-2.22)
>194-9702	23 (5.2)	35 (4.0)	1.31(0.76-2.26)	1.29(0.71-2.34)	1.46(0.76-2.81)
Herbicides[a]					
Never	386 (86.9)	770 (87.9)	1	1	1
>0 -11	17 (3.8)	35 (4.0)	0.96(0.53-1.74)	0.92(0.50-1.71)	0.98(0.51-1.92)
>11-147	18 (4.1)	37 (4.2)	0.98(0.55-1.76)	1.12(0.60-2.09)	1.21(0.62-2.33)
>147-9702	23 (5.2)	34 (3.9)	1.36(0.78-2.37)	1.33(0.73-2.43)	1.52(0.78-2.97)
Fungicides[a]					
Never	412 (92.8)	819 (93.5)	1	1	1
>0-73	9 (2.0)	19 (2.2)	0.96(0.43-2.15)	1.08(0.47-2.51)	1.17(0.49-2.81)
>73-314	9 (2.0)	19 (2.2)	0.97(0.44-2.14)	1.52(0.64-3.56)	1.66(0.69-4.00)
>314-6684	14 (3.2)	19 (2.2)	1.44(0.72-2.88)	1.23(0.59-2.60)	1.38(0.64-2.99)

[a]Conditional logistic regression analyses of pesticide exposure as assessed by the exposure algorithm. Cumulative exposures were estimated by multiplying the exposure algorithm, assigned exposure from pesticide application and re-entry work with all years worked in a job, summed across all jobs of a participant's occupational history from 1955. The exposed were divided based on the tertiles of the exposure distribution among the controls.

[b]The first adjusted model includes cigarette smoking (5 categories) coffee consumption (4 categories) and occupational skill and status (4 categories).

[c]The second adjusted model additionally includes endotoxin exposure (4 categories).

Exposure to specific pesticides as assessed by the crop-exposure matrix
Table 5 shows the results for specific active ingredients as assessed based on self-reported crops, self-reported actual use of pesticides and applying the active ingredient-specific crop-exposure matrix. For the active ingredient benomyl (a benzimidazole fungicide) a positive association with PD was observed for the highest exposed individuals (OR 2.46 (95% CI 1.16-5.22)), which remained statistically significant after adjustment for potential confounders. Analyses without reclassifying to non-exposed if persons reported that insecticides, herbicides or fungicides had not been applied generally resulted in lower ORs (see Supplemental material, table 3).

Exposure to endotoxin
In Table 3 the results for cumulative exposure to endotoxin and PD risk based on the DOM-JEM are reported. ORs below unity were observed for the highest tertiles, but no trend with cumulative exposure was observed. Previous pesticide and endotoxin exposure in one model resulted in lower ORs for endotoxin and higher ORs for pesticides (see model 2 in Tables 3-5).

Table 5. Exposure to specific active ingredients and Parkinson disease risk: crop-exposure matrix

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Model 1[b] OR (95% CI)	Model 2[c] OR (95% CI)
Paraquat[a]					
Never	411 (92.6)	818 (93.4)	1	1	
>0 – 3.80	18 (4.1)	29 (3.3)	1.27(0.68-2.35)	1.33(0.68-2.60)	1.42(0.71-2.85)
>3.80	15 (3.4)	29 (3.3)	1.03(0.54-1.95)	0.91(0.45-1.84)	1.01(0.48-2.12)
Maneb[a]					
Never	419 (94.4)	835 (95.3)	1	1	1
>0 – 1.38	12 (2.7)	21 (2.4)	1.16(0.56-2.42)	1.33(0.60-2.93)	1.42(0.63-3.21)
>1.38	13 (2.9)	20 (2.3)	1.28(0.64-2.57)	1.16(0.55-2.45)	1.31(0.60-2.85)
Atrazin[a]					
Never	423 (95.3)	845 (96.5)	1	1	1
>0 – 0.35	9 (2.0)	14 (1.6)	1.30(0.56-3.00)	1.03(0.42-2.52)	1.05(0.42-2.61)
>0.35	12 (2.7)	17 (1.9)	1.40(0.66-2.98)	1.08(0.48-2.43)	1.15(0.50-2.65)
Benomyl[a]					
Never	420 (94.6)	841 (96.0)	1	1	1
>0 – 0.27	7 (1.6)	20 (2.3)	0.69(0.29-1.66)	0.80(0.31-2.05)	0.88(0.34-2.27)
>0.27	17 (3.8)	15 (1.7)	2.46(1.16-5.22)	2.23(1.01-4.82)	2.47(1.05-5.78)
Dinoseb[a]					
Never	422	844 (96.3)	1	1	1
>0 – 3.46	11 (2.5)	16 (1.8)	1.38(0.64-2.96)	1.34(0.60-3.03)	1.41(0.61-3.26)
>3.46	11 (2.5)	16 (1.8)	1.36(0.62-2.97)	0.91(0.38-2.18)	0.98(0.39-2.46)
Dichloorvos[a]					
Never	430 (96.8)	850 (97.0)	1	1	1
>0 – 9.6	7 (1.6)	12 (1.4)	1.18(0.45-3.07)	2.04(0.73-5.72)	2.06(0.72-5.90)
>9.6	7 (1.6)	14 (1.6)	0.97(0.38-2.46)	0.87(0.32-2.37)	0.96(0.34-2.66)
Lindane[a]					
Never	407 (91.7)	810 (92.5)	1	1	1
>0 – 0.35	17 (3.8)	35 (4.0)	0.95(0.52-1.74)	0.85(0.45-1.60)	0.89(0.46-1.73)
>0.35	20 (4.5)	31 (3.5)	1.29(0.72-2.31)	1.26(0.67-2.38)	1.39(0.70-2.75)
Parathion[a]					
Never	404 (91.0)	796 (90.9)	1	1	1
>0 – 1.53	17 (3.8)	41 (4.7)	0.81(0.45-1.44)	0.85(0.46-1.57)	0.85(0.45-1.63)
>1.53	23 (5.2)	39 (4.5)	1.16(0.68-1.99)	1.09(0.60-1.98)	1.22(0.64-2.31)
Permethrin[a]					
Never	427 (96.2)	852 (97.3)	1	1	1
>0 – 1.03	7 (1.6)	12 (1.4)	1.18(0.45-3.07)	1.32(0.47-3.74)	1.37(0.47-3.99)
>1.03	10 (2.3)	12 (1.4)	1.62(0.70-3.75)	1.44(0.57-3.67)	1.60(0.60-4.30)

Table 5. continued

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Model 1[b] OR (95% CI)	Model 2[c] OR (95% CI)
2,4-D[a]					
Never	415 (93.5)	832 (95.0)	1	1	1
>0 – 0.25	11 (2.5)	22 (2.5)	1.02(0.48-2.18)	1.05(0.46-2.38)	1.13(0.49-2.64)
>0.25	18 (4.1)	22 (2.5)	1.68(0.87-3.24)	1.51(0.74-3.08)	1.68(0.81-3.49)

[a]Conditional logistic regression analyses of exposure to specific active ingredients as assessed based on self-reported crops and applying the crop-exposure matrix. Exposure was calculated by summing the estimated chance of use at the farm times the frequency of use, summed for all years working on farms. Exposed were divided based on the median of the exposure distribution among the controls. No exposure was assigned to a farm job when on that farm according to the participant no insecticides, herbicides, or fungicides were applied.
[b]The first adjusted model includes cigarette smoking (5 categories) coffee consumption (4 categories) and occupational skill and status (4 categories).
[c]The second adjusted model additionally includes endotoxin exposure (4 categories).
Because information on coffee consumption was missing for 1 control, this participant was excluded from adjusted analyses.

Discussion

We performed a case-control study on Parkinson disease and used complementary methods to assign pesticide exposure. We used i) a job-exposure matrix that accounts for pesticide exposures in all jobs and industries, ii) an exposure algorithm that accounts for exposure during application and re-entry work at farms, and iii) a crop-exposure matrix enabling to estimate exposure to specific active ingredients. The comprehensive evaluation revealed no evidence for an association with pesticides and the functional sub-classes: insecticides, herbicides and fungicides. However, elevated ORs, which were observed in most analyses for the highest exposure categories suggest that an overall effect may exist but that the overall limited number of high exposed cases precluded any statistical significance. Our analyses on specific active ingredients suggest an association with benomyl, a fungicide. In addition, we found no indication for an increased PD risk following occupational exposure to endotoxin.

A strength of our study was the use of a JEM and a crop-exposure matrix to estimate exposure. The results of our recent meta-analysis showed that studies estimating pesticide exposure based on job titles found higher odds ratios than studies using self-reported data only (1). An explanation for this might be that study participants are not able to accurately remember past exposures leading to non-differential exposure misclassification and bias towards the null when solely relying on self-reported data (24). We only downgraded the estimated exposures

from the JEM and crop-exposure matrix for farm workers and gardeners who informed us that insecticides, herbicides or fungicides were not used on the farm where they had worked. We believe that this approach increased the specificity of the exposure assessment, and this is indirectly supported by the analyses that showed regression to the null if this information was not used. However, we cannot exclude that some recall bias was introduced if cases were less likely than controls to report that pesticides had not been applied at the job.

In addition, we analyzed exposure to pesticides for individuals with farm or gardener jobs using an exposure algorithm to estimate exposure in more detail by relying more on self-reported data on exposure determinants. The exposure algorithm was adapted from an existing algorithm for applicator exposure (18). We added re-entry work to the model, because exposures during re-entry work can be substantial, (4, 5) and the majority (57%) of the participants who had worked at farms where pesticides were applied did not personally perform applications but did potentially perform tasks that included re-entry work. We found ORs in the same range as with the JEM approach showing the robustness of our results. In addition, we also analyzed mixing and application work only as to keep the analyses comparable to previous work. These analyses showed non-significant increases in ORs for participants who applied pesticides which seemed to be stronger for exposure to insecticides and herbicides than fungicides. This is in line with our recent meta-analysis that showed increased summary estimates for ever applying insecticides and herbicides but not for fungicides (1). Interestingly, our analyses using a crop-exposure matrix to estimate exposure to specific pesticides found an increased OR for benomyl, which is a fungicide. Exposure to benomyl was only assigned to 1/3 or 1/2 of the participants who were assigned an exposure to fungicides based on the JEM and exposure algorithm, respectively. This points to the necessity of assigning exposure to specific active ingredients as noteworthy observations might be missed when grouped together.

A limitation of the study was the relatively low participation rate, especially among the oldest participants. The participation rate among cases and controls age 70 years or younger was 66% and 39%, respectively. Sensitivity analyses limited to younger participants resulted in higher odds ratios than in the overall analyses (data not shown). The finding of associations in a subgroup with a higher participation rate strengthens the evidence for a relation between pesticides and PD. However, this finding might also reflect a better recall of exposures by younger participants compared with older participants or it could relate to differences in active ingredients used between earlier and later decades.

A limitation of using hospital controls is that the conditions included in the control group might relate to pesticide or endotoxin exposure or may suffer from referral bias and therefore may have influenced results. However, repeating the analyses leaving out one of the 4 categories of neurological conditions from the control group at a time resulted in almost identical results, suggesting that the results were not unduly driven by one specific control group.

A potential source of exposure misclassification in the JEM analyses is that the same exposure is assigned to all participants with the same job code. This was partly solved by adjusting for self-reported non-application of pesticides at the farm, but some non-differential misclassification may still exist because differences in performed tasks within similar jobs were not taken into account. Also the exposure algorithm used to assess pesticide exposure has some limitations. Because no specific questions on job tasks were asked, days with re-entry work had to be estimated from farm type only. In addition, weighting factors in the algorithm were based on the AHS and EUROPOEM exposure models, for which it is uncertain how well those correspond to the actual exposures in our study. These uncertainties most likely resulted in some non-differential exposure misclassification and likely attenuation of results.

The crop-exposure matrix analyses were limited in that exposures to active ingredients were assigned based on estimated probability and frequency of use. Especially when probability of use on a crop was low this could have resulted in incorrect assignment of exposure. For this reason, we categorized those exposed into two exposure groups, and based our conclusions on the highest exposure category for which exposure was most certain. Another limitation is that crops in greenhouses and field crops other than potatoes, beets, cereals, or maize were not covered by the experts and probabilities of exposure were based on information from Statistics Netherlands. Exposure for those crops had to be extrapolated for time periods before 1995, resulting in higher chance of incorrect estimations and non-differential exposure misclassification. Also, for some potentially interesting active ingredients that were withdrawn from the market before 1995 and that were mainly used on crops not covered by the experts (for example some organochlorines like dieldrin and DDT), we could not assign an exposure based on the crop-exposure matrix. Therefore our study is not informative for these active ingredients.

Our crop-specific analyses add evidence for a possible relation between benomyl and PD. Benomyl is a fungicide from the benzimidazole family that has been used for three decades on a wide range of crops. In 2001 it was banned in large parts of the world including the Netherlands. Benomyl has been investigated in two previous epidemiological studies on PD. A non-significant elevated odds ratio of 1.9 for self-reported use of benomyl was found within a nested case-control study in the Agricultural Health Study (25). A case-control study using registration data of pesticide applications showed a trend for increased risk for ambient exposure to benomyl at occupational addresses, however, not at residential addresses (26). Besides these epidemiological studies there is also toxicological evidence supporting a possible association between benomyl and PD risk, through a mechanism where benomyl inhibits microtubule assembly thereby stimulating aggregation of α-synuclein, or by inhibition of aldehyde dehydrogenase activity resulting in accumulation of a toxic dopamine metabolite (26, 27).

Although only active ingredients previously linked to PD were analyzed, the observed association for benomyl might be caused by other factors such as other pesticides related to the crops associated with benomyl exposure. Therefore, we conducted separate analyses on those crop groups. In our study, most individuals in the highest exposure category for benomyl had worked for a large part of their career on farms with field crops, mainly potatoes, cereals and/or beets. Analyses on working 10 or more years after 1955 on a farm with those field crops resulted in non-significant elevated ORs for beets (OR 1.84 (95% CI 0.82-4.12)) and cereals (OR 1.71 (95% CI 0.80-3.66)) but not for potatoes (OR 1.09 (95% CI 0.47-2.56)). In addition, among those participants with the highest benomyl exposure a number of participants had worked on farms cultivating strawberries. Ever working on a farm with strawberries showed a non-significant association with PD (OR 2.87(95% CI 0.87-9.44)). Given that we observe increased risks for most of these crops suggests that the observed effect might be benomyl specific or attributable to a pesticide used in combination with benomyl.

No increase in PD risk was observed after exposure to endotoxin. The fact that more controls than cases had endotoxin exposure and adjusting the pesticide results for endotoxin exposure led to higher ORs even points to a possible protective effect of exposure to endotoxin. Because no exposure response relation for endotoxin exposure was observed, this result should be interpreted with caution and the association between endotoxin exposure and PD should be investigated in more detail, e.g. using quantitative data on endotoxin exposure.

In summary, we studied the relation between exposure to insecticides, herbicides, fungicides and endotoxin and Parkinson disease in a multi-centre case-control study in the Netherlands. The results did not provide evidence for the postulated increase in risk after endotoxin exposure. Also, no evidence for an association with exposure to pesticides was found. However statistically non-significant elevated ORs observed in the higher exposure categories for pesticides, insecticides, herbicides and fungicides are in line with earlier evidence that exposure to pesticides might increase PD risk. Active ingredient-specific analyses revealed a possible association with benomyl, a benzimidazole fungicide that has previously been associated with PD risk.

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Supplemental material

Supplemental material, table 1. Non self-report corrected cumulative exposure to pesticides, specific sub-classes and Parkinson disease risk: JEM approach

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Model 1[b] OR (95% CI)	Model 2[c] OR (95% CI)
Pesticides[a]					
never	351 (79.1)	691 (78.9)	1	1	1
1-19	28 (6.3)	67 (7.6)	0.83(0.52-1.31	0.78(0.48-1.27)	0.78(0.46-1.33)
20-32	24 (5.4)	57 (6.5)	0.83(0.51-1.37)	0.80(0.47-1.35)	0.80(0.45-1.44)
33-216	41 (9.2)	61 (7.0)	1.31(0.87-1.98)	1.23(0.77-1.97)	1.48(0.82-2.67)
Insecticides[a]					
never	364 (82.0)	720 (82.2)	1	1	1
1-20	26 (5.9)	57 (6.5)	0.89(0.55-1.45)	0.92(0.55-1.52)	0.97(0.55-1.72)
21-36	18 (4.1)	43 (4.9)	0.83(0.47-1.48)	0.82(0.44-1.51)	0.88(0.45-1.71)
37-216	36 (8.2)	56 (6.4)	1.27(0.82-1.97)	1.18(0.72-1.94)	1.42(0.78-2.60)
Herbicides[a]					
never	376 (84.7)	739 (84.4)	1	1	1
1-6	18 (4.1)	47 (5.4)	0.74(0.42-1.31)	0.79(0.43-1.43)	0.80(0.43-1.52)
7-23	28 (6.3)	53 (6.1)	1.04(0.65-1.68)	1.08(0.66-1.77)	1.18(0.68-2.06)
24-168	22 (5.0)	37 (4.2)	1.15(0.66-2.00)	1.00(0.54-1.85)	1.07(0.54-2.11)
Fungicides[a]					
never	352 (79.3)	693 (79.1)	1	1	1
1-7	25 (5.6)	65 (7.4)	0.75(0.47-1.22)	0.73(0.44-1.21)	0.72(0.41-1.26)
8-25	31 (7.0)	46 (5.3)	1.37(0.85-2.23)	1.31(0.78-2.19)	1.39(0.78-2.49)
26-168	36 (8.1)	72 (8.2)	0.98(0.64-1.94)	0.87(0.54-1.41)	0.96(0.55-1.69)

[a]Cumulative exposures were estimated by multiplying the job-exposure matrix (JEM) assigned exposures (0 for no, 1 for low and 4 for high exposure) with years worked in a job, summed across all jobs of a participant's occupational history from 1955. The exposed were divided based on the tertiles of the exposure distribution among the controls.
[b]The first adjusted model includes cigarette smoking (5 categories) coffee consumption (4 categories) and occupational skill and status (4 categories).
[c]The second adjusted model additionally includes endotoxin exposure (4 categories).
Because information on coffee consumption was missing for 1 control, this participant was excluded from adjusted analyses.

Supplemental material, table 2. Applicator exposure to specific sub-classes of pesticides and Parkinson disease risk: exposure algorithm

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Model 1[b] OR (95% CI)	Model 2[c] OR (95% CI)
Insecticides					
Never	377 (94.5)	753 (95.4)	1	1	1
Ever	22 (5.5)	36 (4.6)	1.23(0.70-2.14)	1.33(0.73-2.39)	1.60(0.83-3.09)
Cumulative exposure[a]					
1-570	13 (3.3)	19 (2.4)	1.41(0.68-2.90)	1.75(0.82-3.73)	2.12(0.95-4.73)
571-5180	9 (2.3)	17 (2.2)	1.04(0.45-2.37)	0.93(0.39-2.22)	1.10(0.43-2.80)
Herbicides					
Never	383 (94.1)	764 (95.5)	1	1	1
Ever	24 (5.9)	37 (4.6)	1.33(0.77-2.27)	1.38(0.78-2.45)	1.58(0.84-2.97)
Cumulative exposure[a]					
>0-340	12 (3.0)	18 (2.2)	1.35(0.64-2.87)	1.66(0.75-3.66)	1.90(0.83-4.35)
340-5180	12 (3.0)	19 (2.4)	1.30(0.62-2.73)	1.16(0.53-2.55)	1.32(0.58-3.05)
Fungicides					
Never	376 (96.4)	746 (96.6)	1	1	1
Ever	14 (3.6)	26 (3.4)	1.07(0.55-2.10)	1.12(0.55-2.27)	1.30(0.62-2.73)
Cumulative exposure[a]					
>0-449	9 (2.3)	13 (1.7)	1.39(0.58-3.30)	1.69(0.67-4.21)	1.97(0.77-5.06)
450-5180	5 (1.3)	13 (1.7)	0.76(0.27-2.17)	0.67(0.23-1.97)	0.77(0.26-2.34)

[a]Logistic regression analyses of pesticide applicator exposure as assessed by the applicator part of the exposure algorithm. Cumulative exposures were estimated by multiplying the exposure algorithm assigned exposure from application work with all years worked in a job, summed across all jobs of a participant's occupational history from 1955. The exposed were divided based on the median of the exposure distribution among the controls. From the reference category, the persons with a JEM exposure to insecticides, herbicides or fungicides were excluded. Consequently, analyses were performed with unconditional logistic regression.

[b]The first adjusted model includes cigarette smoking (5 categories) coffee consumption (4 categories) and occupational skill and status (4 categories).

[c]The second adjusted model additionally includes endotoxin exposure (4 categories).

Because information on coffee consumption was missing for 1 control, this participant was excluded from adjusted analyses.

Supplemental material, table 3. Non self-report corrected exposure to specific active ingredients and Parkinson disease risk: crop-exposure matrix

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Model 1[b] OR (95% CI)	Model 2[c] OR (95% CI)
Paraquat[a]					
Never	408 (91.9)	803 (91.7)	1	1	1
>0 – 4.38	23 (5.2)	37 (4.2)	1.25(0.73-2.17)	1.36(0.75-2.46)	1.42(0.76-2.66)
> 4.38	13 (2.9)	36 (4.1)	0.71(0.37-1.35)	0.58(0.29-1.16)	0.60(0.29-1.25)
Maneb[a]					
Never	412 (92.8)	814 (92.9)	1	1	1
>0 – 2.7	15 (3.4)	31 (3.5)	0.97(0.51-1.83)	1.04(0.53-2.06)	1.10(0.54-2.21)
> 2.7	17 (3.8)	31 (3.5)	1.08(0.59-1.97)	0.90(0.47-1.73)	0.98(0.49-1.95)
Atrazin[a]					
Never	421 (94.8)	831 (94.9)	1	1	1
>0 – 1.2	15 (3.4)	23 (2.6)	1.30(0.68-2.49)	0.97(0.49-1.94)	0.98(0.48-1.99)
>1.2	8 (1.8)	22 (2.5)	0.70(0.31-1.60)	0.66(0.28-1.57)	0.70(0.29-1.69)
Benomyl[a]					
Never	412 (92.8)	830 (94.7)	1	1	1
>0 – 0.27	10 (2.3)	25 (2.9)	0.79(0.37-1.68)	0.94(0.42-2.09)	1.06(0.47-2.39)
> 0.27	22 (5.0)	21 (2.4)	2.26(1.19-4.30)	2.01(0.98-4.10)	2.37(1.09-5.13)
Dinoseb[a]					
Never	419 (94.4)	830 (94.7)	1	1	1
>0 – 1.53	6 (1.4)	23 (2.6)	0.52(0.21-1.28)	0.50(0.20-1.29)	0.53(0.20-1.37)
> 1.53	19 (4.3)	23 (2.6)	1.65(0.89-3.05)	1.31(0.66-2.59)	1.46(0.70-3.04)
Dichloorvos[a]					
Never	430 (96.8)	848 (96.8)	1	1	1
>0 – 7.8	7 (1.6)	14 (1.6)	1.00(0.39-2.53)	1.62(0.60-4.36)	1.63(0.59-4.51)
> 7.8	7 (1.6)	14 (1.6)	0.96(0.38-2.45)	0.87(0.32-2.36)	0.95(0.34-2.64)
Lindane[a]					
Never	398 (89.6)	793 (90.5)	1	1	1
>0 – 0.35	17 (3.8)	44 (5.0)	0.76(0.42-1.35)	0.68(0.36-1.26)	0.72(0.37-1.37)
> 0.35	29 (6.5)	39 (4.5)	1.48(0.90-2.43)	1.47(0.86-2.54)	1.69(0.93-3.09)
Parathion[a]					
Never	395 (89.0)	776 (88.6)	1	1	1
>0 – 1.34	18 (4.1)	50 (5.7)	0.70(0.41-1.22)	0.71(0.39-1.27)	0.73(0.40-1.36)
> 1.34	31 (7.0)	50 (5.7)	1.22(0.77-1.94)	1.20(0.71-2.01)	1.32(0.74-2.35)
Permethrin[a]					
Never	424 (95.5)	846 (96.6)	1	1	1
>0 – 1.05	11 (2.5)	18 (2.1)	1.24(0.58-2.67)	1.31(0.57-3.02)	1.42(0.59-3.42)
> 1.05	9 (2.0)	12 (1.4)	1.46(0.61-3.47)	1.29(0.49-3.35)	1.39(0.51-3.79)

Supplemental material, table 3. continued

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Model 1[b] OR (95% CI)	Model 2[c] OR (95% CI)
2,4-D[a]					
Never	413 (93.0)	818 (93.4)	1	1	1
>0 – 0.39	18 (4.1)	29 (3.3)	1.27(0.68-2.38)	1.22(0.62-2.38)	1.29(0.64-2.59)
> 0.39	13 (2.9)	29 (3.3)	0.88(0.45-1.72)	0.85(0.42-1.74)	0.94(0.45-1.96)

[a]Conditional logistic regression analyses of exposure to specific active ingredients as assessed based on self-reported crops and applying the crop-exposure matrix. Exposure was calculated by summing the estimated chance of use at the farm times the frequency of use, summed for all years working on farms. Exposed were divided based on the median of the exposure distribution among the controls.

[b]The first adjusted model includes cigarette smoking (5 categories) coffee consumption (4 categories) and occupational skill and status (4 categories).

[c]The second adjusted model additionally includes endotoxin exposure (4 categories).

Because information on coffee consumption was missing for 1 control, this participant was excluded from adjusted analyses.

Chapter 5

Extremely low-frequency magnetic field exposure, electrical shocks and risk of Parkinson disease

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Abstract

Purpose: Previous studies did not provide strong evidence for an increased Parkinson disease (PD) risk after exposure to extremely low-frequency magnetic fields (ELF-MF), but were limited in their scope to address other exposures related to the use of electricity such as electrical shocks. We evaluated the associations of PD with exposure to ELF-MF, electrical shocks and having worked in "electrical occupations".
Methods: We conducted a hospital-based case-control study, including 444 PD patients and 876 age- and sex-matched controls. Occupational histories were collected in telephone interviews and were linked to job-exposure matrices on ELF-MF exposure and on electrical shocks. In addition, questions on use of household appliances involving ELF-MF exposure, experienced electrical shocks and potential confounders were asked.
Results: No association of PD risk with any of the evaluated exposures related to electricity was observed. We did, however, observe quite consistently reduced risk estimates across the majority of the exposure categories explored. Given the results of the previous studies and the absence of any postulated mechanism, this is unlikely to represent a true protective effect of ELF-MF or electrical shocks on the occurrence of PD.
Conclusions: The results of this study suggest that no association exists between PD and exposure to ELF-MF, electrical shocks or having worked in "electrical occupations".

Introduction

Environmental factors probably play an important role in the development of Parkinson Disease (PD) (1). Exposure to extremely low-frequency magnetic fields (ELF-MF), as well as working in an "electrical occupation" has been linked to increased risks of two other neurodegenerative diseases, Alzheimer disease and amyotrophic lateral sclerosis (ALS), and it has been suggested that especially for ALS, electrical shocks rather than ELF-MF might be a more relevant exposure (2, 3). For PD, fewer studies on ELF-MF exposures and only one study on electrical shocks have been published (4). Overall, neither occupational ELF-MF exposure (5-1) nor residential exposure to ELF-MF from power lines (14, 15) has been shown to increase risk of PD. To our knowledge, none of the previous studies investigated ELF-MF exposure from household appliances in relation to PD. The one study on electrical shocks did not find increased risk of PD in a cohort of survivors of electrical accidents, but because only 4 PD cases were identified in this cohort, no conclusion could be drawn (4).

We comprehensively investigated the possible association of PD with exposure to ELF-MF and to electrical shocks from both occupational and non-occupational sources, and in addition evaluated "electrical occupations" using data of a recently conducted hospital-based case-control study.

Methods

Cases and controls

Cases and controls were recruited between April 2010 and June 2012 from 5 hospitals in the Netherlands (St. Elisabeth Hospital Tilburg, TweeSteden Hospital Tilburg, Canisius-Wilhelmina Hospital Nijmegen, UMCG Groningen and Vlietland Hospital Schiedam). Patients with a first diagnosis of PD between January 2006 and December 2011 in one of these hospitals were eligible. Subjects initially diagnosed in another hospital and referred to one of the participating hospitals for follow-up care or for second opinion were excluded. Eligible study subjects were identified using DBC codes, which is the standardized accounting system for hospital care based on diagnostic groups in the Netherlands (16). In each hospital, one neurologist reviewed the medical files of all subjects identified with DBC codes 0501 (PD) or 0502 (other extrapiramidal disorders) to select all PD patients. For each confirmed PD patient, two matched controls were selected from individuals who attended the same departments of neurology within the same specified time-frame with DBC codes 0801 (median nerve neuropathy; ICD-10 G56.0 and G56.1), 0802 (ulnar nerve neuropathy; ICD-10 G56.2), 1203 (thoracic and lumbar disc disease;

ICD 10 G55.1, G54.3 and G54.4) or 1204 (sciatica; ICD-10 M54.3 and M54.4). The controls were matched to the cases on hospital, visiting date (±3 years), sex and age. When one individual was the best match to more cases, he/she was allowed to serve as a control for more than one case.

A total of 1001 subjects with an initial diagnosis of PD between 2006 and 2011 and still alive at the time of recruitment (93% of total) were identified. For 8 subjects no current address was known. Of the 993 invited persons, 448 persons agreed to participate (45%), 406 persons declined participation and 139 did not reply. Among potential controls the participation rate was 35%; for 12 cases only 1 suitable control was found, and for 4 cases no controls were found, leaving 444 cases and 876 controls that were included in the present analyses. The median age difference between case and matching controls was 15 days with a maximum of 512 days. The study was approved by the Medical Ethics Committee of St Elisabeth Hospital, Tilburg, the Netherlands. All participants gave their informed consent prior to their inclusion in the study.

Data collection

Selected cases and controls were contacted via an invitation letter from their neurology department containing study information and a reply form. Non-responders were sent a reminder after one month, and one phone call attempt was performed after another month. Cases and controls were informed that the study objective was to study risk factors for neurological disorders, without specification of which neurological disease or potential risk factors.

Study participants were interviewed in a standardized computer-assisted telephone interview by one of three trained interviewers. The questionnaire contained a complete residential and occupational history; questions about selected dietary items, smoking, anthropometric measures; and a medical history. For each job in the occupational history, the study participants were asked to report on years worked, job title, type of industry, company name and main tasks of participant. Of several household appliances that could substantially contribute to ELF-MF exposure to the head, the use at current age and at the age of 20, 40 and 60 was ascertained. These appliances included weekly use of a hairdryer, use of an electrical shaver plugged into a socket while shaving (men only), sleeping within 1 meter of the head with an electrical alarm clock plugged into a socket, sleeping on a water bed, and sleeping with an electrical blanket turned on while sleeping. For non-work-related electrical welding, the number of years of welding, average number of days per year and average welding duration was asked. Moreover, the estimated lifetime number of experienced electrical shocks was asked.

Exposure assessment
All jobs were coded by one author (MM) according to the International Standard Classification of Occupations 1968 and 1988 (ISCO68 and ISCO88). All job codes were linked to a modified version of an existing measurement-based ELF-MF job-exposure matrix (JEM) containing mean exposure intensity per job, in micro-Tesla (μT) (17). This JEM used 4-digit ISCO88 codes on the job axis except for about 30 electrical jobs where the more detailed 5-digit ISCO68 codes were used. In the modified JEM, jobs were (re-)classified into low, medium and high ELF-MF exposure based on not only intensity of exposure, but also probability of exposure (18). The job histories were also linked to a recently developed electrical shock JEM that categorized 3-digit ISCO88 jobs into low, medium and high potential for electrical shocks (19). This JEM was based on national registry data of accidents resulting in electrical injury at work that had been registered in five European countries, including data from the Netherlands.

We analyzed three exposure metrics for occupational exposure to ELF-MF and electrical shocks: ever exposure (medium and high exposure), duration of exposure and cumulative exposure. Duration was defined as the number of years a participant had worked with medium or high exposure up to the year before diagnosis. Cumulative exposure was calculated summing exposed years in the job history using weights of 0 for low, 1 for medium and 4 for high exposure (18). Furthermore, risk analyses for existing classifications of "electrical occupations" were performed (5, 20) (see Supplemental material for lists of jobs).

To evaluate ELF-MF exposure from household appliances, estimates of exposure intensity, weekly frequency and duration of use were used. Based on exposure values reported in the literature and for usual distance of use from the appliances, we estimated intensity of exposure to be around 0.5 μT for water beds and electrical alarm clocks, 1 μT for electrical blankets, and 10 μT for shavers and hairdryers ((21) and personal communication with Myron Maslanyj, Centre for Radiation, Chemical and Environmental Hazards, Public Health England, Chilton, UK). Using measurement data from the ELF-MF JEM, we estimated an exposure intensity of 5 μT during self-reported non-occupational electrical welding (17). We assumed an exposure frequency and duration for hairdryers and shavers of 3 times a week for 5 minutes, for alarm clocks, blankets and water bed 7 times a week for 8 hours, where for blankets only use during half of the year (during wintertime) was assumed. These assumptions correspond with a yearly exposure of alarm clocks, blankets and water beds that is 10 times higher than exposure from shavers and hairdryers. The number of years of use for each of those appliances was estimated from reported use at age 20, 40 and 60. For non-occupational electrical

welding, the individually reported frequencies, durations and years of use by cases and controls were used to estimate cumulative exposure. Cumulative exposure to these appliances together was expressed in microtesla-years (μT-years), whereby one μT-year is an average exposure of one μT during one year.

Statistical analysis

Duration and cumulative exposure of occupational ELF-MF and electrical shock exposure were analyzed as categorical variables, using the study participants who had only jobs classified in the low category as the reference group and dividing the rest in three categories based on the tertiles of exposed controls. Exposure categories for household electrical appliances, non-occupational welding and self-reported number of electrical shocks were created in a comparable way. Odds ratios (OR) and 95% confidence intervals (CI) for exposure categories were calculated using conditional logistic regression. Models were corrected for education (2 categories), cumulative smoking (5 categories) and cumulative coffee consumption (4 categories). Furthermore, we performed a stratified analysis by median number of years since last job with medium or high exposure to address the timing of exposure as previous studies were inconclusive (10, 11, 13). Finally, in order to investigate the influence of skill and status of jobs, we divided the jobs in 4 categories of occupational skill and status according to major ISCO groups (first digit of the ISCO 88 job codes) (22). 1-3: high-skilled white-collar jobs, 4-5: low-skilled white-collar jobs, 6-7: high-skilled blue-collar jobs, and 8-9: low-skilled blue-collar jobs. The participants were categorized according to the group in which they had worked most years during their career.

Results

Table 1 shows the age and sex distribution of the cases and controls included in the analyses together with the variables included as potential confounders in the adjusted analyses. Of the PD patients, 63.3% were men with a median age at diagnosis of 67. On average, cases were higher educated, smoked less and consumed less coffee.

Table 1. General characteristics of cases and controls

	Cases (n=444)	Controls (n=876)
Men, No (%)	281 (63.3)	557 (63.6)
Age at interview, median (range)	68 (34 - 91)	68 (34 - 90)
Age at diagnosis, median (range)	67 (34 - 90)	-
Higher education, No (%)[a]	268 (60.5)	477 (54.5)
Cigarette smoking[b]		
Never smoked, No (%)	207 (46.6)	243 (27.7)
>0 - 7.8 pack-years, No (%)	86 (19.4)	161 (18.4)
>7.8 – 17.5 pack-years, No (%)	67 (15.1)	155 (17.7)
>17.5 – 29.4 pack-years, No (%)	45 (10.1)	160 (18.3)
>29.4 - 103 pack-years, No (%)	39 (8.8)	157 (17.9)
Coffee consumption[c]		
0 – 97 consumption-years, No (%)	128 (28.8)	220 (25.1)
> 97 - 156 consumption-years, No (%)	146 (32.9)	221 (25.3)
> 156 - 214 consumption-years, No (%)	90 (20.3)	216 (24.7)
> 214 – 720 consumption-years, No (%)	80 (18.0)	218 (24.9)

[a]Information on education was missing for one case

[b]Pack-years of cigarette smoking was calculated by dividing average number of cigarettes per day by 20 multiplied by the number of years of smoking. Ever smokers were divided based on the quartiles of the exposure distribution among the controls. Never smokers constitute a separate category.

[c]Consumption-years were calculated by multiplying the average amount of coffee consumptions per day with the estimated number of years of coffee consumption. The participants were divided based on the quartiles of the exposure distribution among the controls. The number of never coffee drinkers was too low (3%) to constitute a separate group. Coffee consumption information was missing for one control.

With respect to differences in skill and status of jobs performed by cases and controls, Table 2 illustrates that more cases than controls were in the high-skilled white-collar category and more controls than cases were in the low-skilled blue-collar category. Using high-skilled white workers as the reference category, a significant decreased OR for PD was still visible for low-skilled blue-collar workers after adjusting for smoking.

Table 2. Parkinson disease and longest duration category job status

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Smoking adjusted OR (95% CI)
High-skilled white-collar worker (ISO88 1-3)	198 (44)	335 (38)	1	1
Low-skilled white-collar worker (ISCO88 4-5)	87 (20)	187 (21)	0.75 (0.54-1.04)	0.80 (0.57-1.12)
High-skilled blue-collar worker (ISCO88 6-7)	101 (23)	202 (23)	0.84 (0.62-1.14)	0.87 (0.63-1.19)
Low-skilled blue-collar worker (ISCO88 8-9)	58 (13)	152 (17)	0.60 (0.42-0.87)	0.67 (0.46-0.97)

ELF-MF

Cases were less likely to be occupationally exposed to medium or high ELF-MF exposure levels than controls, 57% and 64%, respectively (see Table 3). Only 1.5% of the female study participants had ever worked in a high ELF-MF exposed job compared with 18% of the male participants, while 58% of the women and 45% of the men had ever had a medium ELF-MF-exposed job as highest exposure (data not shown). Odds ratios were consistently below one for most of our exposure categories, although most were not statistically significant (see model 1 in Table 3). Adjusting the ELF-MF analyses for the 4 categories of occupational skill and status instead of educational level resulted in odds ratios closer to unity (see model 2 in Table 3). No trend in PD risk was observed with duration of ELF-MF exposure or cumulative ELF-MF exposure. Results of analyses whereby the medium and high exposed were divided on number of years since last job with ELF-MF exposure did not show differential results (last exposure ≤26 years ago: OR 0.80 (95% CI 0.58-1.10), last exposure >26 years ago: OR 0.80 (95% CI 0.59-1.08)). Risk analyses of ELF-MF exposure from household electrical appliances did not show any associations with PD risk (see Table 3).

Table 3. Parkinson disease and ELF-MF exposure: conditional logistic regression analysis.

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Model 1[a] OR (95% CI)	Model 2[b] OR (95% CI)
JEM ELF-MF					
Only low	190 (43)	319 (36)	1	1	1
Ever exposure					
Medium	209 (47)	447 (51)	0.78 (0.61-1.00)	0.82 (0.63-1.06)	0.85 (0.65-1.12)
High	45 (10)	110 (13)	0.68 (0.46-1.01)	0.72 (0.48-1.10)	0.78 (0.50-1.20)
Duration[c]					
1-8	83 (19)	185 (21)	0.75 (0.55-1.03)	0.81 (0.58-1.14)	0.83 (0.59-1.17)
9-23	82 (18)	197 (22)	0.71 (0.52-0.96)	0.74 (0.53-1.03)	0.78 (0.56-1.10)
24-55	89 (20)	175 (20)	0.85 (0.62-1.17)	0.86 (0.62-1.21)	0.94 (0.65-1.36)
Cumulative exposure[d]					
1-9	91 (20)	187 (21)	0.82 (0.60-1.12)	0.87 (0.63-1.22)	0.90 (0.64-1.25)
10-26	72 (16)	191 (22)	0.63 (0.46-0.88)	0.68 (0.48-0.96)	0.71 (0.50-1.02)
27-188	91 (20)	179 (20)	0.86 (0.62-1.18)	0.86 (0.61-1.20)	0.93 (0.64-1.34)
Household appliance exposure + non-occupational welding[e]					
0	35 (8)	67 (8)	1	1	1
>0-1-2.0	90 (20)	209 (24)	0.83 (0.51-1.35)	0.72 (0.43-1.20)	0.73 (0.43-1.23)
>2.0-3.8	92 (21)	196 (22)	0.90 (0.55-1.47)	0.82 (0.48-1.39)	0.84 (0.49-1.41)
>3.8-5.7	102 (23)	206 (24)	0.96 (0.59-1.57)	0.79 (0.47-1.34)	0.82 (0.48-1.38)
>5.7-14.0	125 (28)	198 (23)	1.26 (0.77-2.06)	1.11 (0.66-1.88)	1.13 (0.67-1.90)

[a]The first adjusted model includes educational level, cigarette smoking and coffee consumption

[b]The second adjusted model includes, collar worker category, cigarette smoking and coffee consumption

[c]Duration was defined as the number of years a participant had jobs with medium or high ELF-MF exposure as assessed with a JEM

[d]Cumulative exposure was calculated summing exposure of all years in the job history using weights (0 for low, 1 for medium and 4 for high exposure)

[e]Cumulative exposure (μT-years) was calculated from reported years of use of several electrical appliances and assumptions about exposure intensity, frequency and duration of use.

Electrical shocks

Pearson's correlation between cumulative ELF-MF exposure and cumulative electrical shocks exposure was 0.51. Table 4 presents the results of the analyses on electrical shocks. Similar to ELF-MF exposure, cases (39%) were less likely to have ever had a job with medium or high risk of electrical shocks than controls (47%). Most of the exposed were men; 61% of the men as compared to 16% of the women had ever had a job with medium or high risk of electrical shocks (data not shown). As with the analyses for ELF-MF, adjusted analyses showed in general risk estimates below unity for all shock exposure metrics, but were not statistically significant after adjustment for confounders (see model 1 in Table 4). Adjusting the analyses for the 4 categories of occupational skill and status instead of educational level resulted, similar as for the analyses on ELF-MF, in odds ratios closer to unity (see model 2 in Table 4). Results of analyses whereby the exposed were divided on number of years since last job with medium or high shock risk were not materially different (last exposure ≤21 years ago: OR 0.79 (95% CI 0.56-1.10), last exposure >21 years ago: OR 0.80 (95% CI 0.57-1.12)). The self-reported number of electrical shocks experienced during life at home, at work or elsewhere was only moderately correlated with the occupational shock exposure estimates as assessed by the JEM (Pearson's correlation coefficient: 0.28). Risk analyses resulted in non-significant odds ratios below unity as well (see Table 4).

Electrical occupations

Analyses by ever having worked in an "electrical occupation" did not indicate any associations with PD. For the list of electrical occupations given in Deapen et al. (20), the OR was 0.90 (95% CI 0.59-1.37) and for the list evaluated in Feychting et al. (5) the OR was 1.01 (95% CI 0.62-1.63).

Table 4. Parkinson disease and electrical shocks: conditional logistic regression analysis.

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Model 1[a] OR (95% CI)	Model 2[b] OR (95% CI)
JEM occupational shocks					
Only low	269 (61)	459 (52)	1	1	1
Ever exposure					
Medium	67 (15)	177 (20)	0.62 (0.44-0.86)	0.75 (0.53-1.05)	0.77 (0.54-1.10)
High	108 (24)	240 (27)	0.71 (0.52-0.96)	0.81 (0.58-1.13)	0.85 (0.59-1.21)
Duration[c]					
1-10	58 (13)	139 (16)	0.68 (0.48-0.96)	0.80 (0.55-1.16)	0.83 (0.57-1.21)
11-30	56 (13)	145 (17)	0.61 (0.43-0.88)	0.74 (0.50-1.08)	0.77 (0.52-1.15)
31-59	61 (14)	133 (15)	0.71 (0.49-1.03)	0.81 (0.54-1.21)	0.83 (0.53-1.29)
Cumulative exposure[d]					
1-17	50 (11)	143 (16)	0.57 (0.39-0.82)	0.69 (0.47-1.01)	0.71 (0.48-1.05)
18-68	67 (15)	143 (16)	0.75 (0.53-1.06)	0.88 (0.61-1.27)	0.93 (0.64-1.37)
69-218	58 (13)	131 (15)	0.69 (0.47-1.01)	0.79 (0.52-1.19)	0.81 (0.51-1.28)
Self-reported number of shocks					
Never	288 (65)	491 (56)	1	1	1
1-2 shocks	74 (17)	188 (21)	0.62 (0.45-0.86)	0.68 (0.48-0.96)	0.69 (0.49-0.97)
3-10 shocks	62 (14)	154 (18)	0.61 (0.42-0.87)	0.63 (0.43-0.92)	0.64 (0.44-0.94)
11-150 shocks	20 (5)	43 (5)	0.71 (0.41-1.25)	0.90 (0.50-1.62)	0.91 (0.50-1.66)

[a]The first adjusted model includes educational level, cigarette smoking and coffee consumption
[b]The second adjusted model includes, collar worker category, cigarette smoking and coffee consumption
[c]Duration was defined as the number of years a participant had jobs with medium or high risk of electrical shocks as assessed with a JEM
[d]Cumulative exposure was calculated summing electrical shock risk of all years in the job history using weights (0 for low, 1 for medium and 4 for high risk)

Discussion

Our analyses did not provide evidence of an increased risk of Parkinson disease in persons exposed to ELF-MF or having experienced electrical shocks. In addition, no association with working in so-called electrical occupations was observed. However, we did observe marginally but consistently reduced risk estimates across the majority of the exposure categories explored here.

No mechanism is known by which electromagnetic fields might cause PD, which makes the choice of the relevant exposure metric difficult. Strength of our study is that we were able to include several sources of exposures (occupational exposure to ELF-MF but also exposure to ELF-MF from household appliances) and several

exposure metrics into our analysis: Evaluating electrical occupations, occupational ELF-MF exposure, exposure to ELF-MF sources (appliances) at home, and occupational and non-occupational exposure to electrical shocks makes our study the most comprehensive study to date on PD risk and electricity-related exposures.

Another strength of our analysis is that we were able to use incident cases confirmed by neurologists. This can be seen as an improvement to the far majority of the previous occupational studies that relied on PD as registered on death certificates (only one study used hospital records (7)). PD is a chronic condition that in itself is non-fatal and is therefore often not mentioned on death certificates (23, 24). Also, the information from death certificates might be inaccurate if also atypical Parkinsonian syndromes such as multiple systems atrophy are listed as PD on death certificates, although this would only be expected to affect a small proportion of the cases (25, 26). In addition, we evaluated the complete occupational history to assess exposure to ELF-MF, enabling us to account for potential preclinical symptoms resulting in job changes that could affect occupational ELF-MF exposure. Particularly, the use of primary occupation on death certificate as done by a few of the previous studies (8, 9, 12) is of limited value for exposure assessment (27). Other previous studies used occupation at baseline (5-7) or job histories within certain companies (10, 11, 13).

It cannot be excluded that a small effect of ELF-MF exposure or electrical shocks on PD risk exists but that results were attenuated by exposure misclassification. Particularly, the analyses on ELF-MF sources at home were limited in that the available data on duration and years of use of household appliances were not very detailed. However, we included the most relevant appliances that may add to ELF-MF exposure to the head, and believe we were thus able to generate a meaningful ranking of individuals' exposure to ELF-MF from residential use of electrical appliances.

Another limitation of our study is a potential bias caused by low participation rates for cases and controls. Health-related reasons were most often brought forward for non-participation, but about 50% of the non-participants did not provide an explanation for non-participation. Participation depended on age: The participation rate among cases and controls age 70 or younger was 66% and 39%, respectively. However, analyses on participants aged 70 or younger did not result in differential results (data not shown). Also, among women (for cases 40%, for controls 32%) the participation was lower than among men (for cases 49%, for controls 38%). Because women in the study were working less frequently in high-exposed jobs, odds ratios as reported in the overall analyses were driven by men. Stratified

analyses by gender provided similar result for men and women (data not shown), but, given the low numbers of exposed women, analyses for women were imprecise.

Furthermore, hospital controls may not be representative for the general population, and some neurological conditions included in the control group might be related to occupations with high ELF-MF or electrical shock exposures. For example, electrical injury may induce peripheral nerve damage (4). Although shock-related peripheral nerve damage would be rare, it would have been covered in the DBC codes 0801 and 0802. Similarly, carpal tunnel syndrome (falling under DBC code 0801) is associated with regular and prolonged use of handheld vibratory tools (28). This could have attenuated any true risk. Sensitivity analyses, however, leaving out one subgroup of the controls at a time (based on DBC codes) or analyses leaving out all controls with DBC codes 0801 and 0802, did not materially affect the reported odds ratios, suggesting that our results were not unduly influenced by characteristics within the subgroups (data not shown).

Our results are in line with previous studies that provided no evidence for increased risks of PD after occupational (5-13) or residential (14, 15) ELF-MF exposure. Odds ratios in our study were also very similar across groups with earlier or more recent exposures. Previous studies have also not provided clear evidence of an effect of timing of the exposure on PD risk: no effect on the risk estimates was observed in two studies when excluding most recent exposures (10, 13), while studies reported either lower (11) or higher (10) odd ratios when evaluating more recent exposure compared with lifetime exposure.

Of note, and although most results were not statistically significant, we observed quite consistently odds ratios below unity for both ELF-MF and electrical shocks exposures. Given the results of the previous studies and the absence of any postulated mechanism, this is unlikely to represent a true protective effect of ELF-MF or electrical shocks on the development of PD. If affected persons with first disease symptoms prior to diagnosis would change occupation to less-exposed jobs, this effect would result in decreased odds ratios. The extent of this reduction would, however be relatively minor as manifestation of the disease is late in life. We adjusted our analyses for cigarette smoking and coffee consumption, for which in agreement with previous studies we observed inverse associations with PD risk (29). However, adjusting for these factors had only a small effect on our reduced odds ratios.

The low odds ratios observed in our study might also relate to the fact that cases were more often highly educated than controls and may have been working more likely in high-skilled white-collar occupations, which represent more often lower-exposed jobs. Our analyses confirmed this and correspond well with literature showing that PD is positively associated with "white-collar jobs" such as teaching or legal professions in some studies (30, 31). Possible explanations for this association include physical activity, more common in low-skilled jobs that may protect against PD (32), or a premorbid Parkinsonian personality that might make affected persons preferentially select for white-collar jobs (33, 34). Adjusting the ELF-MF and electrical shocks analyses for occupational skill and status increased the odds ratios and brought them closer to unity although this effect was not very strong.

In conclusion, our case-control study did not indicate an increase in risk of PD after exposure to ELF-MF or after experiencing electrical shocks. This in combination with the results of earlier studies and the lack of an established mechanism suggests that no relation exists between PD and exposures related to electricity.

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Supplemental material

Supplemental material, table 1. Translation of list of electrical occupations of Deapen et al. into ISCO88 codes used in the present study

Original list of electrical occupations	ISCO88 codes used in present study
electrical and electronic engineers	2143: electrical engineer
electrical and electronic engineering technicians	3113: electrical engineering technicians
electricians, electrician apprentices	7241: electrical mechanics and fitters 7137: building and related electricians
electric power linemen and cablemen	7245: electrical line installers, repairers and cable joiners
air conditioning, heating and refrigeration repairmen	3115: mechanical engineering technicians
data processing machine repairmen	7242: electronics fitters
household appliance and accessory installers and mechanics	7243: electronics mechanics and servicers
office machine repairmen	7243: electronics mechanics and servicers
radio and television repairmen	7241: electrical mechanics and fitters
power station operators	8161: power production plant operators
telephone installers ad repairmen	7244: telegraph and telephone installers and servicers
telephone linemen and splicers	7244: telegraph and telephone installers and servicers
welders and flame-cutters	7212: Welders and flamecutters
conductors and motormen, urban rail transit	8311: locomotive engine drivers
assemblers in following industries: household appliances, radio, television and communication equipment, electrical machinery, equipment and supplies, not elsewhere classified, not specified electrical equipment, machinery and supplies	8282: electrical equipment assemblers 8283: electronic equipment assemblers

Supplemental material, table 2. Translation of list of electrical occupations of Feychting et al. into ISCO88 codes used in the present study

Original list of electrical occupations	ISCO88 codes used in present study
electrical fitters	7241: electrical mechanics and fitters
wiremen	7245: electrical line installers, repairers and cable joiners
radio and television assemblers	8283: electronic equipment assemblers
radio and television repairmen	7243: electronics mechanics and servicers
recording, sound and equipment operators	3132: broadcasting and telecommunications equipment operators
telephone and telegraph installers	7244: telegraph and telephone installers and servicers
telephone and telegraph repairmen	7244: telegraph and telephone installers and servicers

Chapter 6

Occupational exposure to solvents, metals and welding fumes and risk of Parkinson disease

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Abstract

Objectives: The aim of this study was to investigate the potential association between occupational exposure to solvents, metals and/or welding fumes and risk of developing Parkinson disease (PD).
Methods: Data of a hospital-based case-control study including 444 PD patients and 876 age and sex matched controls was used. Occupational histories and lifestyle information of cases and controls were collected in a structured telephone interview. Exposures to aromatic solvents, chlorinated solvents and metals were estimated by linking the ALOHA+ job-exposure matrix to the occupational histories. Exposure to welding fumes was estimated using self-reported information on welding activities.
Results: No statistically significant associations with any of the studied occupational risk factors were found. We observed slightly elevated risk estimates for the highest exposure categories for aromatic solvents (third tertile cumulative exposure: odds ratio (OR) 1.33 (95% confidence interval (CI) 0.86-2.05)). For self-reported welding activities we observed reduced risk estimates (third tertile cumulative exposure: OR 0.51 (95% CI 0.21-1.24)).
Conclusions: The results of our study did not provide strong support for an increased chance on developing PD after occupational exposure to aromatic solvents, chlorinated solvents or exposure to metals. We did observe a reduced risk for welding, which is in line with previous research, but evidence for a plausible mechanism of a true protective effect is lacking.

Introduction

Various occupational exposures have been discussed to possibly increase the risk of developing Parkinson disease (PD) (1). Exposure to pesticides has received much attention, but among the less studied potential occupational risk factors for PD are exposure to organic solvents and (heavy) metals.

Organic solvents are widely used in industry and are present in products as fuels, paints, printing inks, degreasers and cleaning products (2). Accordingly, exposures to these compounds are common in occupational settings. Because of known neurotoxic effects after acute solvent exposure, concerns have been raised that long term exposures might be associated with neurodegenerative diseases, such as PD (2). Previous epidemiological studies that have tried to elucidate the potential association of solvent exposure with PD have produced mixed results: two recent literature reviews reported either no or weak effects (3, 4). Most of the studies evaluated solvent exposure in general, but few evaluated risks of classes of solvents or specific solvents. Stronger associations of PD with exposure to specific chlorinated organic solvents were observed in a twin study (5), suggesting that accounting for specific categories of solvents may be important.

Occupational exposure to metals like manganese, copper, lead, mercury or iron can occur at workplaces in primary metal production or in jobs involving metal working activities like welding, galvanizing, or grinding. Although clinically distinct from PD, a neurological syndrome with Parkinsonian symptoms induced by high occupational exposure to the metal manganese has been described (6), and a possible association of PD with chronic exposure to lower levels of manganese has been proposed (7). The evidence from epidemiological studies that investigated associations of PD with exposure to manganese or other metals is limited and largely inconclusive (1). Since welding fumes may contain low levels of manganese, welding has been implicated as a potential risk factor for PD, but so far, studies were inconclusive and a meta-analysis showed a decreased risk on PD for welders (8).

We set out to study occupational exposures to metals, welding fumes and categories of organic solvents, specifically aromatic and chlorinated solvents, and risk of PD in a large case-control study in the Netherlands.

Methods

Cases and controls

Details about the study design were described previously (9). Briefly, cases and controls were recruited between April 2010 and June 2012 from 5 hospitals in the Netherlands. Eligible patients had an initial Parkinson disease diagnose in one of the participating hospitals between January 2006 and December 2011. For each case, two matched controls were selected from persons that were seen at the department of neurology for non-neurodegenerative complaints (median nerve neuropathy; ICD-10 G56.0 and G56.1, ulnar nerve neuropathy; ICD-10 G56.2, thoracic and lumbar disc disease; ICD 10 G55.1, G54.3 and G54.4, and sciatica; ICD-10 M54.3 and M54.4). The controls were matched to the cases on hospital, visiting date (within 3 years of the cases diagnose year), sex and age (interquartile range age difference: 6-33 days). At recruitment 93% of the eligible patients were still alive and a total of 448 PD patients participated. The participation rate was 45% for cases and 35% for controls. For 12 cases only 1 suitable control was found and for 4 cases no controls were found, leaving 444 PD patients and 876 controls who were included in the analyses. The study was approved by the Medical Ethics Committee of St Elisabeth Hospital Tilburg, the Netherlands, and all participants provided their informed consent.

Data collection

Participants were interviewed in a standardized computer-assisted telephone interview by one of three trained interviewers. During the interview, the complete residential and occupational history was obtained, as was information about electrical welding activities, anthropometric measures, selected dietary items, smoking and a medical history. All jobs a participant had performed for at least 6 months were included in the occupational history. The study participants were asked to report on number of years and hours per week worked, job title, type of industry, company name and main tasks of participant. When a participant reported electrical welding activities during a job, questions about type of electrical welding, frequency (<1/year, <1/month, 1-3/month, 1-3/week, >3/week) and duration were asked. Furthermore, questions about years and frequency of non-work related electrical welding activities were asked. All jobs were coded according to the International Standard Classification of Occupations 1968 (ISCO68) and 1988 (ISCO88).

Exposure assessment

All ISCO88 job codes were linked to the ALOHA+ job-exposure matrix (JEM) (10). This JEM assigns exposure to chlorinated solvents, aromatic solvents and metals as no, low or high using arbitrary weights for intensity and probability of exposure of

0, 1 and 4 respectively. Exposure to welding fumes from electrical welding was assigned when a participant reported to have been welding at least weekly in a particular job. An exposure score of 1 (low) was assigned when a participant welded frequently (1-3 days per week) and an exposure score of 4 (high) was assigned when welding was part of day-to-day work (4 or more days per week). Participants also received an exposure score of 1 (low) for each year with more than 50 days of non-work related welding. Duration of exposure to metals, solvents or welding fumes was defined as number of years with low or high exposure. Cumulative exposure was calculated by summing the exposure score (0, 1 or 4) for all years until the year prior to diagnosis.

Statistical analysis

Odds ratios and 95% confidence intervals were estimated using conditional logistic regression. The exposed subjects were categorized in three groups based on tertiles of the distributions of exposure among controls. Variables included in the adjusted analyses were total pack-years of smoking (5 categories), total coffee consumption (4 categories) and occupational skill and status (4 categories): All jobs in the occupational histories were divided according to major ISCO88 groups (first digit), 1-3: high-skilled white-collar jobs, 4-5: low-skilled white-collar jobs, 6-7: high-skilled blue-collar jobs and 8-9: low-skilled blue-collar jobs. Participants were subsequently categorized according to occupational skill and status based on the job category in which a participant had worked most years.

Results

Table 1 shows the general characteristics of cases and controls. Mean age of the cases was 68 years with 63.3% of the cases being men. Cases smoked on average fewer cigarettes, drank less coffee and were more often high-skilled white-collar workers than controls.

Table 1. General characteristics of cases and controls

	Cases (n=444)	Controls (n=876)
Men, No (%)	281 (63.3)	557 (63.6)
Age at interview, median (range)	68 (34 - 91)	68 (34 - 90)
Age at diagnosis, median (range)	67 (34 - 90)	-
Cigarette smoking[a]		
Never smoked, No (%)	207 (46.6)	243 (27.7)
>0 - 7.8 pack-years, No (%)	86 (19.4)	161 (18.4)
>7.8 – 17.5 pack-years, No (%)	67 (15.1)	155 (17.7)
>17.5 – 29.4 pack-years, No (%)	45 (10.1)	160 (18.3)
>29.4 - 103 pack-years, No (%)	39 (8.8)	157 (17.9)
Coffee consumption[b]		
0 – 97 consumption-years, No (%)	128 (28.8)	220 (25.1)
> 97 - 156 consumption-years, No (%)	146 (32.9)	221 (25.3)
> 156 - 214 consumption-years, No (%)	90 (20.3)	216 (24.7)
> 214 – 720 consumption-years, No (%)	80 (18.0)	218 (24.9)
Occupational skill and status[c]		
High-skilled white-collar worker, No.(%)	198 (44)	335 (38)
Low-skilled white-collar worker, No.(%)	87 (20)	187 (21)
High-skilled blue-collar worker, No.(%)	101 (23)	202 (23)
Low-skilled blue-collar worker, No.(%)	58 (13)	152 (17)

[a]Pack-years of cigarette smoking was calculated by dividing average number of cigarettes per day by 20 multiplied with the number of years of smoking. Pack-years were divided based on the quartiles of the exposure distribution among the controls.
[b]Consumption-years were calculated by multiplying the average amount of coffee consumptions per day (cups per day) with the estimated number of years of coffee consumption. Consumption-years were divided based on the quartiles of the exposure distribution among the controls. Coffee consumption information was missing for one control.
[c]The categories of occupational skill and status were made according to major ISCO88 groups (first digit of the job codes). 1-3: high-skilled white-collar jobs, 4-5: low-skilled white-collar jobs, 6-7: high-skilled blue-collar jobs, and 8-9: low-skilled blue-collar jobs. The participants were categorized according to the group in which they had worked most years during their career.

The results for occupational exposure to aromatic and chlorinated solvent are presented in table 2. Cumulative exposure to aromatic and chlorinated solvents was moderately correlated (Pearson's correlation coefficient: 0.41). For both classes of solvents, slightly more controls than cases ever had a job with high exposure. However, more cases than controls had their exposure to aromatic solvents in the highest tertile for both duration and cumulative exposure, resulting in non-statistically significant elevated odds ratios. For exposure to chlorinated solvents, a non-significant elevated odds ratio was seen for individuals in the highest tertile of duration, but not for cumulative exposure. The majority of individuals exposed to

solvents were men: 82% for aromatic solvents and 88% for chlorinated solvents. Stratified analyses by gender showed similar results for women and men for both types of solvents, but given the low number of occupationally exposed women, risk estimates were imprecise (data not shown).

Table 2. Parkinson disease and exposure to aromatic and chlorinated solvents as assessed by a job-exposure matrix: conditional logistic regression analyses

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Adjusted[c] OR (95% CI)
JEM aromatic solvents				
Never	262 (59.0)	505 (57.6)	1	1
Ever exposure				
low	168 (37.8)	323 (36.9)	0.99(0.77-1.27)	0.97(0.73-1.29)
high	14 (3.2)	48 (5.5)	0.56(0.30-1.03)	0.82(0.43-1.58)
Duration[a]				
1-7	57 (12.8)	132 (15.1)	0.84(0.59-1.18)	0.88(0.61-1.26)
8-24	54 (12.2)	117 (13.4)	0.90(0.63-1.30)	0.91(0.62-1.34)
25-87	71 (16.0)	122 (13.9)	1.13(0.80-1.61)	1.26(0.80-1.97)
Cumulative exposure[b]				
1-8	58 (13.1)	130 (14.8)	0.86(0.61-1.21)	0.89(0.61-1.28)
9-27	52 (11.7)	119 (13.6)	0.86(0.60-1.24)	0.86(0.58-1.27)
28-192	72 (16.2)	122 (13.9)	1.14(0.80-1.62)	1.33(0.86-2.05)
JEM chlorinated solvents				
Never	336 (75.7)	645 (73.6)	1	1
Ever exposure				
low	71 (16.0)	141 (16.1)	0.96(0.70-1.31)	1.04(0.74-1.46)
high	37 (8.3)	90 (10.2)	0.78(0.52-1.18)	0.86(0.55-1.36)
Duration[a]				
1-8	26 (5.9)	85 (9.7)	0.59(0.37-0.94)	0.65(0.40-1.06)
9-24	38 (8.6)	74 (8.4)	0.98(0.65-1.49)	1.01(0.65-1.57)
25-50	44 (9.9)	72 (8.2)	1.10(0.77-1.74)	1.39(0.88-2.20)
Cumulative exposure[b]				
1-11	27 (6.1)	78 (8.9)	0.67(0.43-1.06)	0.74(0.46-1.19)
12-37	43 (9.7)	78 (8.9)	1.05(0.70-1.58)	1.09(0.70-1.69)
38-180	38 (8.6)	75 (8.6)	0.98(0.64-1.49)	1.15(0.72-1.84)

[a]Duration of exposure was defined as all years in the job history with low or high exposure. Exposed were divided based on the tertiles of the exposure distribution among the controls.

[b]Cumulative exposure was calculated summing exposure of all years in the job history using weights (0 for no, 1 for low and 4 for high exposure). Exposed were divided based on the tertiles of the exposure distribution among the controls.

[c]The adjusted model includes cigarette smoking (5 categories), coffee consumption (4 categories) and occupational skill and status (4 categories).

Exposure to metals also occurred slightly more frequently in controls than in cases (see Table 3). The analyses on duration and cumulative exposure did not provide evidence for an increased risk of PD in persons occupationally exposed to metals. There were only 16 women with exposure to metals. Similar as the other exposures more controls than cases reported to have ever had a job involving more than 3 days per week of electrical welding, resulting in a significant decreased odds ratio (see Table 3). The analyses on duration and cumulative welding exposure no longer showed statistically significant reduced odds ratios. Only 2 women reported welding on a weekly basis.

Discussion

Our study did not provide strong support for an increased risk of Parkinson disease among persons occupationally exposed to aromatic solvents, chlorinated solvents or metals. For self-reported electrical welding activities we observed reduced odds ratios.

Strengths of our study were the use of full occupational histories in combination with a JEM to objectively estimate life-time occupational exposures. Furthermore, instead of using a single category of solvent exposure, we studied two classes of organic solvents: aromatic and chlorinated solvents separately. In addition, we had detailed information on lifestyle factors most notably detailed information on smoking which in line with a range of previous reports (11), showed a reduced risk of PD in smokers.

Limitations of the study include the low participation rate potentially hampering generalizability of results. Participation rate was higher among subjects below 70 (participation cases: 66%, controls: 39%) and therefore we repeated the analyses on participants age 70 or younger. Results did not materially change (data not shown). In addition, sensitivity analyses were performed leaving out one of the 4 non-neurodegenerative complaints subgroups of controls at a time. This did not materially affect the reported odds ratios (data not shown), suggesting that our results were not influenced by characteristics of one of the neurological conditions included in the control group.

Table 3. Parkinson disease and exposure to metals as assessed by a job-exposure matrix and self-reported welding: conditional logistic regression analyses

	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	Adjusted[c] OR (95% CI)
JEM metals				
Never	348 (78.4)	671 (76.6)	1	1
Ever exposure				
low	51 (11.5)	99 (11.3)	0.98(0.68-1.42)	1.06(0.72-1.58)
high	45 (10.1)	106 (12.1)	0.81(0.55-1.19)	0.88(0.58-1.33)
Duration[a]				
1-8	31 (7.0)	69 (7.9)	0.86(0.56-1.33)	1.03(0.64-1.65)
9-23	25 (5.6)	69 (7.9)	0.70(0.44-1.13)	0.69(0.42-1.15)
24-53	40 (9.0)	67 (7.6)	1.15(0.75-1.76)	1.27(0.78-2.05)
Cumulative exposure[b]				
1-14	32 (7.2)	68 (7.8)	0.90(0.58-1.34)	0.98(0.62-1.56)
15-44	32 (7.2)	71 (8.1)	0.87(0.55-1.36)	0.93(0.57-1.52)
45-180	32 (7.2)	66 (7.5)	0.93(0.59-1.46)	1.00(0.61-1.64)
Self-reported welding				
Never	419 (94.4)	803 (91.7)	1	1
Ever exposure				
low	19 (4.3)	44 (5.0)	0.84(0.49-1.45)	0.93(0.53-1.68)
high	6 (1.4)	29 (3.3)	0.41(0.17-0.99)	0.41(0.16-1.01)
Duration[a]				
1-7	10 (2.3)	26 (3.0)	0.75(0.35-1.58)	0.82(0.38-1.78)
8-31	5 (1.1)	23 (2.6)	0.43(0.16-1.13)	0.45(0.16-1.25)
32-50	10 (2.3)	24 (2.7)	0.82(0.39-1.71)	0.85(0.39-1.86)
Cumulative exposure[b]				
1-13	11 (2.5)	26 (3.0)	0.83(0.40-1.71)	0.92(0.43-1.96)
14-40	7 (1.6)	23 (2.6)	0.60(0.26-1.40)	0.73(0.30-1.80)
41-200	7 (1.6)	24 (2.7)	0.57(0.25-1.33)	0.51(0.21-1.24)

[a]Duration of exposure was defined as all years in the job history with low or high exposure. Exposed were divided based on the tertiles of the exposure distribution among the controls.
[b]Cumulative exposure was calculated summing exposure of all years in the job history using weights (0 for no, 1 for low and 4 for high exposure). Exposed were divided based on the tertiles of the exposure distribution among the controls.
[c]The adjusted model includes cigarette smoking (5 categories), coffee consumption (4 categories) and occupational skill and status (4 categories). Because information on coffee consumption was missing for 1 participant, this participant was excluded from adjusted analyses.

Recent reviews on solvent exposure and PD risk showed that observed risk estimates of most previous studies were between 1.0-1.5, suggesting that a weak association might exist (3, 4). We also observed a weak association with aromatic solvents and PD risk with slightly elevated odds ratios for the highest tertile of duration and cumulative exposure. No increased risks were observed for cumulative exposure to chlorinated solvents, which is in contrast to a previous study that found strong positive associations for several chlorinated solvents (trichloroethylene, perchloroethylene and carbon tetrachloride) (5). Because these solvents are among the most commonly used chlorinated solvents, it is unlikely that differences in risk estimates from their and our study are caused by the fact that we grouped all chlorinated solvents together. As such the difference between the studies remains unknown.

The result of our analysis on occupational exposure to metals is in line with previous studies that analyzed occupational metal exposure as a single entity and observed no or only slightly increased risks of PD (12-16). Unfortunately, we were not able to analyze specific metals separately. Potential existing effects of single metals may therefore be diluted in the results, especially if the prevalence of exposure to a specific metal is low.

As a proxy for exposure to metals, we also evaluated the self-reported frequency of welding per week as welding fumes contain manganese and other metals. We did not observe higher risks in persons classified to have experienced higher levels of cumulative exposure, but instead, observed reduced odds ratios for frequent welders were observed. Only questions on electrical welding methods were included in the telephone interview because they were originally intended for estimating electromagnetic field exposure. However, because electrical welding constitutes the most frequently used welding application (17), omitting gas welding is unlikely to have strongly affected our results. Several factors determine exposure levels to manganese or other metals due to welding: exposure levels may increase with higher content in the base metal and welding rods, when electrode current densities and arc time is high, and in rooms with poor ventilation (6). Unfortunately, information on these determinants were not collected in our study and could therefore not be explored. Of the exposed, 85% reported shielded metal arc welding; limiting our abilities to perform meaningful analyses stratified by type of electrical welding.

Our finding of decreased odds ratios in welders is consistent with a recent meta-analysis showing a summary risk ratio of 0.86 (95% CI 0.80–0.92) for PD in welders (18). Explanations brought forward for the risk estimates below unity

include subtle, sub-clinical effects that appear long before diagnosis and that might lead to a self-selection of affected persons into specific jobs (19). The time interval between onset of motor symptoms and diagnosis has been described to last about one to two years (20, 21). Non-motor preclinical manifestations (e.g. constipation) of PD may occur much earlier, time frames between a few years and more than two decades prior to diagnosis have been described (22). However, because onset of PD is late in life, this means that in order to fully explain the reduced risk estimates, such non-motor manifestations would have to appear much earlier than previously reported and to have a relatively strong impact on career-choices, which appears unlikely. Also, it has been hypothesized that a premorbid Parkinsonian personality predispose cases to select for white collar jobs associated with low exposures, such as teaching or legal professions, but no clear evidence exists to that effect (20, 23). Further possible explanations for the decreased odds ratios include protective effects of physical activity (24) or smoking (25) which both occur more frequently in jobs with relatively high exposure to solvents, metals or welding. We adjusted our results for smoking behavior and for skill and status of jobs using a relatively crude categorization based on major ISCO88 groups. This generally led to odds ratios closer to unity, but residual confounding by those factors cannot be ruled out.

In conclusion, the results of our hospital-based case-control study did not provide strong support for an association between PD risk and occupational exposure to chlorinated or aromatic solvents. However, the slightly elevated odds ratios for the highest exposure categories for aromatic solvents are in line with previous reports that an association between solvents and PD risk may exist. Our results did not provide any evidence for an increase in PD risk after exposure to metals. However, we did observe, similarly as others, a protective effect of exposure to welding fumes for PD risk. A biological mechanism for this later observation is however missing and therefore should be interpreted with caution.

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Chapter 7

General discussion

The PAGES study was setup to primarily study environmental and genetic risk factors for Parkinson disease (PD). The focus of this thesis is on occupational exposures (pesticides, endotoxin, extremely low-frequency magnetic fields (ELF-MF), electrical shocks, solvents, metals and welding fumes) and their possible association with PD risk. In addition, we studied the impact of selected lifestyle factors (cigarette smoking, coffee and alcohol consumption) on PD risk. In this chapter I will discuss (i) the main findings of this thesis, (ii) possible effects of methodological issues of the study on the reported results and will (iii) give some directions for future studies on occupational risk factors for Parkinson disease.

Main findings

Smoking, coffee and alcohol consumption

Cigarette smoking is the most consistently found environmental factor associated with the occurrence of PD. In correspondence with previous studies (1), we observed a strong inverse association of cigarette smoking with PD risk. Additional analyses showed that time-since-cessation is more important than duration and intensity of smoking in relation with PD risk. This finding suggests that a true protective effect of smoking is responsible for the inverse association between smoking and PD risk and not a premorbid Parkinsonian personality, because in the case of reverse causality an association with duration of smoking but no direct association with time-since-cessation would be expected. Our results are thus supportive for an influence of timing of smoking on PD risk whereby the protective effect of smoking diminishes after quitting smoking.

It has been suggested that also coffee and alcohol consumption are inversely associated with PD risk, but results of previous studies were, especially in the case of alcohol consumption, not consistent (1). We found a weak inverse association between coffee consumption and PD risk and no association with alcohol consumption. As we had less detailed information on coffee consumption of the cases and controls we could not investigate the importance of duration, intensity and time-since-cessation in a similar way as we did for smoking.

Exposure to pesticides

The results of the meta-analysis described in chapter 3 point to an increase in PD risk after exposure to pesticides and more specifically after exposure to insecticides and herbicides. When we stratified by method of exposure assessment, source of control population, geographical area, study design, or adjustment for potential confounders, we observed that only method of exposure assessment had an effect on the reported results. Compared to studies that relied on self-reported

information on pesticides applications, a higher summary risk ratio was observed for studies that based exposure assessment on job-titles. We concluded that future epidemiological studies should not solely rely on self-report for exposure assessment, but (in addition) use more objective and improved methods for exposure assessment such as job- or crop-exposure matrices.

In the present case-control study, we tried to improve upon methods for pesticide exposure assessment by combining information from the occupational histories with self-reported information on frequency and methods of pesticide applications at the job. In this way we were able to include exposures from both application of pesticides and exposures resulting from re-entry work and to include analyses on specific active ingredients. As described in chapter 4 we observed statistically non-significant elevated odds ratios for the highest exposed individuals to pesticides and its functional sub-classes: insecticides, herbicides and fungicides. Furthermore, a significant increased association with the active ingredient benomyl was found, a benzimidazole fungicide that has been removed from the market in large parts of the world since 2001.

The strong association between smoking and PD may introduce some concern that the frequently observed increased risk of PD after exposure to pesticides results from residual confounding by smoking (i.e. reduced prevalence of smoking among exposed). In our study we observed relatively more never smokers among the pesticide exposed (50% and 34% of pesticide exposed cases and controls and 46% and 26% of non-exposed cases and controls were never smokers). To investigate the existence of any residual confounding by smoking, we additionally analyzed the effects of exposure to pesticides among never smokers. These analyses resulted in some reduced odds ratios for risk of PD indicating that smoking might have confounded the results to some extend (for example: the odds ratio for the highest exposure category of pesticide exposure as assessed with the JEM approach decreased from 1.28 (95% CI 0.79-2.10) to 1.13 (95% CI 0.54-2.40) when limiting the analyses to never smokers). However, because of the low number of exposed cases the risk estimates for non-smokers were rather imprecise, making it difficult to draw any firm conclusions. In contrast, two other studies that showed stratified results found stronger elevated odds ratios for pesticide exposed when limiting the analyzes to never smokers (2, 3), making it unlikely that, in the aggregate, smoking is responsible for the frequently reported increased risk of PD as a result of pesticide exposure.

Exposure to endotoxin

It was postulated that besides pesticides also exposure to other agents present in the farming environment such as endotoxin might increase PD risk. Endotoxins are the lipopolysaccharide components of gram-negative bacterial cell walls for which it has been suggested that exposure may lead to inflammation-mediated neurodegeneration (4, 5). The results presented in chapter 4, showed no evidence for an increase in PD risk resulting from exposure to endotoxin. Instead, the results showed a potential protective effect of endotoxin exposure. At this moment no evidence is available that exposure to endotoxin imposes an increase in PD risk. However, because endotoxin exposure has not been examined in earlier epidemiological studies of PD, more studies are needed before we can conclude if endotoxin exposure is associated with PD risk or not.

Exposure to magnetic fields and electrical shocks

The results of previous research indicated that occupational exposure to ELF-MF is likely not associated with PD (6-13). An improvement of our study compared to most of the earlier studies is that we used PD patients as confirmed by neurologists instead of relying on information on death certificates on which PD is often not mentioned (14, 15). We observed no association with ever having worked in so called "electrical occupations" and PD risk (see chapter 5). Analyses on occupational ELF-MF exposure also did not show an association with PD risk. In addition, we also observed no association of PD with ELF-MF exposure resulting from the use of selected electric household appliances. Lastly, we extended our analyses with the inclusion of analyses on electrical shocks and did not find an increased risk of PD after exposure to electrical shocks. In conclusion, the results of our study in combination with the results of previous studies argue against an association between ELF-MF exposure and development of PD. This is supported by the fact that no mechanism is known by which magnetic fields may induce PD.

Exposure to aromatic and chlorinated solvents

In chapter 6 we reported on analyses of occupational exposure to chlorinated and aromatic solvents. Earlier epidemiological studies that investigated exposure to organic solvents generally found no or only weak associations with PD risk (16). We found no clear associations with PD risk, although for aromatic solvents statistically non-significant elevated odds ratios were observed for the highest exposed individuals. However, it might be possible that effects of specific solvents on PD risk exist but that these effects were diluted in the analyses of broad classes of solvents. One previous study investigated some specific chlorinated solvents using detailed occupational histories and found strong positive associations of trichloroethylene, perchloroethylene and carbon tetrachloride with PD risk (17).

Because these solvents are among the most commonly used chlorinated solvents, it is unlikely that differences in risk estimates from their and our study are caused by the fact that we grouped all chlorinated solvents together. The reason for the difference in results between the studies may thus relate to the more extensive method of exposure assessment used by the previous study. In conclusion, the total evidence for an association between exposure to solvents and PD risk is inconclusive. Future studies should focus on using methods of exposure assessment to analyze specific (classes of) solvents.

Exposure to metals and welding (fumes)
It has been proposed that chronic exposure to metals may induce PD, however, the evidence from epidemiological studies is inconclusive (18, 19). We did not observe an association between metal exposure and PD risk. We also analyzed self-reported electrical welding because welders can be exposed to welding fumes consisting of a complex mixture of metals and other substances. The results did not support an increased risk of PD for welders, but showed significantly reduced odds ratios, which is in correspondence with a recent meta-analysis (20). This points to a potential protective effect on PD risk of an agent in welding fumes, but a specific mechanism for this is not known. In conclusion, similar as for pesticides and solvents, studies using improved methods of exposures assessment are needed to further investigate the relation with specific metals. Further, the potential protective effect of welding on PD risk should be investigated in more detail.

In conclusion, the results of our study suggest that the investigated occupational risk factors are not or only weakly associated with an increase in PD risk. Overall, of the occupational exposures studied in this thesis, the strongest evidence for a relation with PD risk is for exposure to pesticides. Although it is thought that environmental factors play an important role in disease etiology, the only well-established environmental factor associated with PD is smoking. Explanations for the lack of identified environmental risk factors for PD risk are not known. This may suggest that environmental factors are less important in disease etiology than previously thought or that the most relevant environmental factors have not yet been identified. The latter may be due to methodological shortcomings in the exposure assessment of our and previous studies.

Methodological issues

In this section I will discuss methodological issues that might have influenced the reported results in this thesis. These include: (i) design of the study, (ii) diagnosis of PD and (iii) applied methods for exposure assessment.

Study design

The fact that we confirmed the results of previous studies by observing a strong inverse association between smoking and risk of PD provides support to the general validity of the study. However, it cannot be excluded that some bias was introduced by issues in study design such as case ascertainment and selection of controls (21). Ideally, a case-control study is conducted as a population-based study, in which all (incident) cases in a defined geographic area are identified and controls are selected randomly from the same population. For practical reasons, we performed a hospital-based case-control study, in which we set out to include all PD patients diagnosed between January 2006 and December 2011 in the five participating hospitals. To establish that cases and controls were selected from the same source population, controls were selected from individuals that were seen in the same hospitals for non-neurodegenerative neurological complaints. Patients that were initially diagnosed elsewhere and referred to one of the participating hospitals were therefore not eligible for inclusion. Figure 1 shows that the addresses of cases and controls at the time of interview are clustered around the participating hospitals with no clear difference in capture area for cases and control diseases.

The neurological disorders included in the control group were chosen because they have not been linked to PD or to the main occupational risk factors studied in this thesis. To check if one of the four diagnosis groups of controls had a major influence on the presented results, we performed sensitivity analyses by leaving out one of the four groups of the controls at a time. This led to similar results, suggesting that the choice of control group did not unduly bias the results. Stratified risk analyses of occupational exposures and PD risk by hospital produced essentially similar results for each hospital, indicating that selection of cases and controls in one hospital was unlikely to be systematically different from the others (for example: meta-analyses on the individual effect estimates of the 5 hospitals from analyzing ever/never exposure to insecticides, herbicides or fungicides analyses showed low heterogeneity (I^2 between 0.0% and 11.4%)).

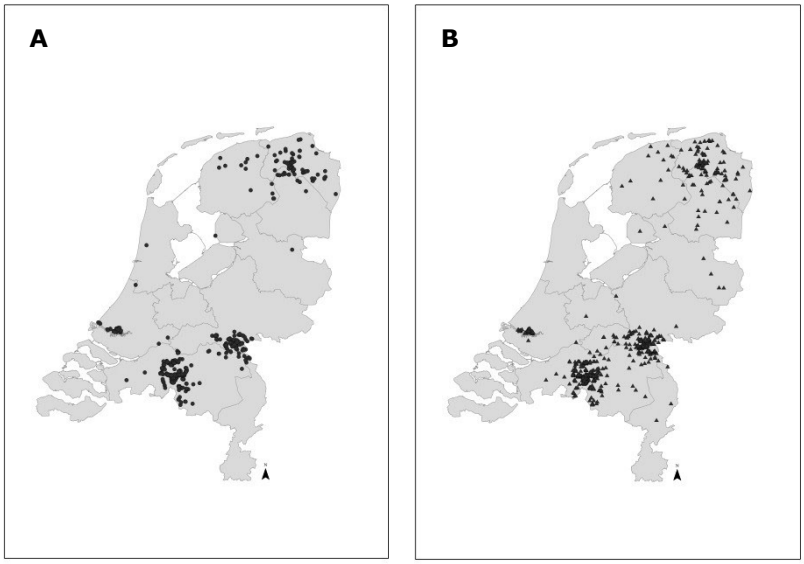

Figure 1. The current (at time of interview) residential locations of the 444 Parkinson disease cases (Figure 1A) and 779 controls (Figure 1B). Addresses are clustered around the participating hospitals in Tilburg, Nijmegen, Groningen and Schiedam.

The participation rate in the study was low (PD patients: 45%, controls: 35%) giving rise to concerns about generalizability and biases due to differential participation by risk profiles. If the chance of participation would have been associated with the studied exposures, low participation rates could have biased the results. No exposure information was available for persons who declined participation to investigate this directly. Because the participation rate among younger subjects (≤70) was somewhat higher (cases: 66%, controls: 39%), we performed additional sensitivity analyses restricted to participants of 70 years of age or younger. For most exposures investigated, results were similar as for the overall analyses, suggesting that for those exposures results were not severely biased by low participation rates. However, for exposure to pesticides, analyzes restricted to this younger age group resulted in somewhat increased odds ratios (for example: the odds ratio for the highest exposure category of pesticide exposure as assessed with the JEM approach increased from 1.28 (95% CI 0.79-2.10) to 1.75 (95% CI 0.96-3.16) when limiting the analyses to younger participants (≤70)). Interpretation is, however, not straightforward as next to participation bias, these stronger associations could also be caused by differences in past exposure to pesticides for different age categories, since pesticide use has changed substantially over time.

The results of the majority of occupational risk factors frequently showed that there were lower numbers of high exposed cases than high exposed controls (endotoxin, ELF-MF, electrical shocks, solvents, metals, welding). The odds ratios below unity are not consistently observed in other epidemiological studies of PD, with the exception of welding for which a recent meta-analysis showed a significant reduced summary risk estimate (20). Although the majority of the observed odds ratios below unity were far from statistically significant this is a notable observation, and may relate to the design of the study:

- *First disease symptoms prevent working in jobs with exposure:* PD can have a long preclinical phase and first disease symptoms may appear years before diagnosis (22). It can be hypothesized that cases because of first disease symptoms were less likely to have performed physical demanding jobs in the years before diagnosis. Since physical demanding jobs are also more often jobs with higher exposure to the occupational risk factors studied in this thesis, this may have resulted in lower occupational exposures in cases. However, because PD is diagnosed for the majority of cases years after retirement and the time interval between onset of motor symptoms and diagnosis has been described to be about one to two years (23, 24), this effect would not be substantial. Also, there was no clear difference between cases and controls in the number of individuals that quitted jobs with exposure in the immediate years before diagnosis. This leads to the conclusion that the reduced odds ratios are most likely not a direct consequence of PD cases leaving exposed jobs earlier because of first disease symptoms.
- *Residual confounding of cigarette smoking or coffee consumption:* Many studies have shown that smoking is strongly inversely associated with PD, which we confirmed in our study. And for the occupational risk factors with odds ratios below unity, we observed that there were more smokers among the exposed. All analyses on occupational risk factors were adjusted for lifetime smoking, which generally resulted in only minor increases in odds ratios. Furthermore, we performed analyses among never smokers only. However, because of low numbers these analyses resulted in imprecise risk estimates, whereby for some exposures the risk estimates increased, while for other exposures the risk estimates were reduced. Similar as for smoking, adjustment for coffee consumption did not have a major effect on results. Thus, it is unlikely that residual confounding by smoking or coffee consumption is the major explanation for the observed reduced odds ratios.

- *Reverse causality:* For at least a century, observations have been published about a premorbid Parkinsonian personality at young age among persons who later develop PD (25). This personality has been described with terms as serious, rigid, cautiousness, law-abiding and lack of novelty seeking and it has been suggested that this might explain the low prevalence of smokers among persons later developing PD. It can be hypothesized that such a personality affects choice of occupation. So called "white-collar jobs" such as jobs in teaching, healthcare, law and justice and religion have been associated with PD with some consistency (26). In chapter 5 of this thesis we showed that cases were higher educated than controls and performed more often high-skilled white-collar jobs based on a crude categorization of jobs performed. Adjustment of the analyses on occupational risk factors for either educational level or these status categories of performed jobs resulted generally in slight increases of the odds ratios. An explanation for such a premorbid personality may be that a shortage of brain dopamine long before disease onset affects personality (27). However, because no well performed prospective studies on personality traits are available (28) and imaging studies have shown nigrostriatal dopaminergic neuronal loss starting to increase less than 10 years before onset of clinical symptoms (29), the evidence for a premorbid personality long before diagnosis is weak. As such it is not expected that a premorbid personality is responsible for the observed reduced odds ratios.
- *Protective effect of physical activity:* A meta-analysis showed an inverse association of moderate to vigorous physical activity with PD risk (30), and results of animal studies suggested that a neuroprotective effect of exercise is plausible by effects on neuroplasticity and neurotrophic factors (31). A protective effect of physical activity might be another explanation for observing that cases more often performed high-skilled white-collar jobs and controls more often performed low-skilled blue-collar jobs which are also more often jobs with occupational exposure. However, the effect of physical activity should be quite strong to be able to explain the observed reduced odds ratios. The telephone interview did not contain questions on physical activities performed during life, making it impossible to investigate the effect of physical activity in this study.
- *Control conditions are related with exposures of interest:* In the control group individuals with non-neurodegenerative neurological disorders were included (based on DBC codes) such as persons with carpal tunnel syndrome or spinal disc herniation. A concern may be that those neurological disorders could have been related to the occupational risk factors studied. For example, electrical shocks have been associated with neuropathy (DBC codes 0801 and 0802) (32), and carpal tunnel syndrome (DBC codes 0801) has been associations with

occupations that involve regular and prolonged use of hand-held vibratory tools that may be associated with for example high ELF-MF exposure (33, 34). However, the results of sensitivity analyses in which one of the four subgroups of controls was left out at a time, did not indicate that the observation of the reduced odds ratios was driven by one particular group of controls.

In conclusion, although the low participation rate and the way controls were selected are reasons for concern, our analyses do not suggest that results were largely influenced by these factors. It remains unclear what the reason is for the observed odds ratios below unity.

Diagnosis of Parkinson disease

The clinical diagnosis of PD is based on manifestation of the cardinal motor symptoms of the disease and differentiation from other Parkinsonian disorders (35). The responsiveness to levodopa, the occurrence of other symptoms and neuroimaging techniques may help in determining if PD is the cause of the Parkinsonian symptoms. For the most definite diagnosis of PD possible, post mortem confirmation of Lewy pathology and neuronal loss in the substantia nigra pars compacta is needed (36). In a pathological study of 100 brains of patients that were diagnosed with PD during life, the diagnosis was confirmed in 90 of these patients, and applying more strict diagnostic criteria increased accuracy of diagnosis (37). However, generalizability of results of such pathological studies is difficult as atypical cases may be more likely to be used for autopsy (37).

For our study it was not feasible to perform clinical examinations of included PD patients using strict specified criteria. Selection of patients was based on what was reported in the patients' medical files. For the majority of the patients the screening of medical files occurred within one year of initial diagnosis. Because sometimes the initial diagnosis changes as disease progresses (38), this increased the chance of misdiagnosis. It is thus possible that a minority of the PD cases included in our study did not have PD and that some eligible PD patients may have been missed.

Regarding diagnosis, another point of attention is that PD is a heterogeneous disease, and different classifications of disease subtypes have been proposed on the basis of age at onset, progression rate and clinical features such as the predominance of rest tremor and the occurrence of dementia (39). A future challenge lies in the elucidation of the biological mechanisms behind the differences in disease appearance between patients and to find out if genetic or environmental factors play a role in this (40).

Exposure assessment

A lack of methods to accurately assess exposure decreases the chance to find an existing effect, especially if few of the study subjects are highly exposed, which is frequently the case for occupational exposures in the general population (41). I will first discuss the methods we used to assess occupational exposures, followed by the discussion of some general issues regarding exposure assessment: metric of exposure, time-frame of exposure and route of exposure.

Methods used for occupational exposures

Exposure to pesticides

Most previous studies used self-reported information about past applications performed by the participants, which especially for specific pesticides (active ingredients) is difficult to recall (42). Pesticides are therefore often analyzed as a single entity or subdivided into functional sub-classes such as insecticides, herbicides or fungicides. However, these sub-classes encompass a large group of active ingredients with different chemical properties, and the usage of specific active ingredients can differ substantially between countries and over time. Furthermore, correlations between exposures to these functional sub-classes are often high, because on many farms more types of pests (insects, weeds and/or fungi) are being controlled at the same time. Another limitation of studies that relied only on self-reported pesticide application information is that exposure occurring during re-entry activities such as weeding or thinning is not taken into account. It has been shown that exposure through re-entry activities could contribute significantly to annual exposure (43, 44).

In our study we focused on improving methods for exposure assessment to overcome these limitations. First, we linked a job-exposure matrix (JEM) to the occupational histories of all participants to estimate lifetime cumulative exposure to pesticides in general and to the functional sub-classes, insecticides, herbicides and fungicides (45). In addition, exposure to specific active ingredients was estimated using a time-dependent crop-exposure matrix that was developed to be linked to crops that were reported to have been cultivated at farms where the participants had worked. A disadvantage of using such exposure matrices is that the same exposure level is assigned to all jobs with the same job code or with the same crops based on estimations of average probability and intensity of exposure, resulting in non-differential misclassification of exposure and consequently attenuation of study results. We used the extra questions on actual application of pesticides asked to participants who held farm or gardener jobs to improve their exposure classification. Participants explicitly stating that (sub-classes of) pesticides had not

been applied at the job were not assigned an exposure. This approach generally resulted in slightly higher odds ratios than when only applying the job-exposure matrix. We believe that this approach increased specificity of our exposure classification resulting in fewer subjects falsely being assigned an exposure.

Second, an exposure algorithm was developed that used exposure values derived from the literature and self-reported information of farm workers and gardeners about type of farm, type of pesticides and application methods used to estimate exposure to insecticides, herbicides and fungicides in more detail. The number of potential exposed participants that were identified more than doubled when re-entry work was included in the algorithm compared to including only exposure from self-reported applicator activities. However, it should be noted that including re-entry work in the algorithm is most important for crops that need a lot of manual work, as is the case with fruit crops and crops in greenhouses. This is especially relevant for assessing exposure to pesticides that are applied many times throughout the season which can be the case for fungicides in fruit growing (44) and for insecticides and fungicides in greenhouses (46). Because the majority of the participants in the present study worked at mixed farm with animals and with crops such as potatoes, beets, grains and maize that require much less re-entry work most of the highest exposed in our study were applicators (only 12-15% of the participants that did not personally apply, but who had worked at farms where insecticides, herbicides or fungicides were used, were classified in the highest exposure category).

Of course, there is always room for further improvements of the questionnaire and the exposure algorithm. For example, questions regarding the frequency of performing tasks with crop contact, the use of personal protective equipment such as gloves during such tasks, and the rules at the farm about re-entry after spraying pesticides could have made exposure estimations more precise. Also, limited research was available on the relative contribution of exposure from a day of performing re-entry work compared to a day of performing applicator work, which we now estimated at 10% based on data from the European EUROPOEM models (47).

There was some agreement in exposure assessment of the JEM-based approach with the exposure algorithm approach: of the exposed in the third tertile of the analyses using the exposure algorithm, 72% (insecticides), 54% (herbicides) and 79% (fungicides) were also in the third exposure tertile using the JEM approach. The fact that we observed similar results with both approaches, strengthens our finding of a potential relation between high pesticide exposure and PD risk.

It cannot be excluded that the elevated odds ratios for the highest exposed individuals to insecticides, herbicides and fungicides were the result of recall bias. Probably more cases than controls were aware that pesticides have been linked to PD risk as reports on this topic have frequently been published in the media and on websites addressing PD. Patients searching for a potential cause of their disease might have spent longer time than controls on remembering past exposures and therefore controls may be more likely to report that pesticides were not used. Another potential cause for differential recall of cases compared to controls is that cognitive problems developing in cases as a result of PD may decrease accuracy of self-reported information and most likely lead to lower odds ratios. Because we included only recent diagnosed patients this may be of limited concern in our study, although mild cognitive impairment can appear already in early stages of the disease (48). For only 0.9% of both cases and controls it was noted by the interviewer that the participant had cognitive problems and difficulties answering (some of) the questions, making it unlikely that cognitive problems had a major effect on the results.

In conclusion, given the retrospective study design, all used exposure assessment methods had their well-known shortcomings for estimating past exposures accurately. However, compared to previous studies, we used comprehensive parallel approaches for exposure assessment and by combining these methods were most likely able to identify reliably the majority of highest exposed participants. The approach of using a crop-exposure matrix to objectively assess exposure to specific pesticides provides a good alternative for using self-reported information on pesticide use and could be used more often in retrospective studies.

Exposure to endotoxin
We used the DOM-JEM to assess occupational exposure to endotoxin (49). Using this method we were most likely able to identify the highest exposed individuals. The fact that we did not find an association between exposure to endotoxin and increased risk of PD suggest that no effect exits, but attenuation of study results because of exposure misclassification cannot be ruled out. More epidemiological studies on PD risk should put effort in estimating endotoxin exposure, possibly by using a JEM or by measuring endotoxin exposure of occupations in industry (farming) based studies, to confirm that no association exists.

Exposure to magnetic fields and shocks
Compared to previous research, we tried to improve upon methods for exposure assessment of ELF-MF and electrical shocks, and performed the most comprehensive analyses on the association between PD and electricity related

exposures to date. We had lifetime occupational histories of participants and estimated lifetime cumulative exposure using JEMs. For ELF-MF exposure, we asked additional questions on electrical welding, working on the high voltage power network (including for example maintenance painting of pylons with life cables), and working with industrial electric ovens for heating metals (see questionnaire in the Appendix). During the study, it appeared that it was not very clear to participants what was meant with the high voltage power network and industrial electric ovens for heating metals, and these questions were often answered for jobs for which these activities would be unlikely such as mechanics of electrical installations at places not related to the high voltage power network. This showed that estimating occupational ELF-MF exposure using self-reported information is questionable and therefore it was decided to estimate occupational exposure solely based on the JEM.

The JEM on electrical shocks is limited in that it is based on registered number of accidents resulting in electrical injury per job and this does not necessarily correlate with the frequency of electrical shocks not resulting in injury (50). We additionally analyzed the association with PD risk using the self-reported number of experienced shocks (not necessarily at work). A difficulty in assessing exposure to electrical shocks is that electrical shocks can vary in severity. It is likely that very light electrical shocks are not able to induce neurological effects. In the telephone interview we asked about experienced major electrical shocks, but because this is not a very specific definition this could have been interpreted differently by participants. The exposure estimated from both approaches was only low to moderately correlated (Pearson's correlation coefficient: 0.28), but both approaches resulted in comparable risk estimates, thereby strengthening the finding of no increased risk of PD after having experienced electrical shocks.

Exposure to solvents

Most previous studies investigated organic solvents as a single entity. In our study we used a JEM that differentiated between exposures to aromatic and chlorinated solvents. A limitation of our study was that we did not include detailed questions on job tasks that are associated with aromatic or chlorinated solvent exposure to make the exposure classification more accurate. Industrial based studies with high quality methods of exposure assessment are needed to further investigate the relation with specific organic solvents.

Exposure to metals and to welding (fumes)
We used a JEM to assess lifetime occupational exposure to metals. These analyses were clearly limited because the JEM did not differentiate between different kinds of metals. Possibly, existing effects of specific metals were therefore diluted in the analyses. This could be improved by using more specific JEMs or adding specific questions on job tasks with potential exposures. We additionally evaluated the effects on PD risk of self-reported frequent welding (at least once per week) as welding fumes contain manganese and other metals. A limitation of the welding analyses was that only questions on electrical welding were included in the telephone interview and not on gas welding, because these questions were originally asked for estimating ELF-MF exposure. This will have resulted in some degree of exposure misclassification (decreased sensitivity). However, because electrical welding techniques became the prominent form of welding in the second half of last century (51), this shortcoming is unlikely to have affected our results substantially.

General issues regarding exposure assessment

Metric of exposure
Occupational exposures typically have a complex time-varying pattern (52). Exposures are often summarized in simple (convenient) exposure measures such as average exposure, duration of exposure, cumulative exposure or peak exposure. Which measure is more appropriate depends on the presumed biological mechanism between exposure and disease. Since the mechanisms by which PD develops are not yet fully understood, it is not clear what kind of (summary) exposure metric should be used. In this thesis the main focus was on estimating cumulative exposures, thereby assuming that each exposure contributes to the total damage that leads eventually to development of PD. However, it may also be that sporadic "peak" exposures are more relevant for some risk factors. For example, it could be that only very high ELF-MF exposures or very strong electrical shocks are able to induce neuronal damage (53). However, we did not have the data to analyze peak exposures.

Time-frame of exposure
There is some evidence that exposures occurring decades before disease onset, even exposures during childhood or before birth, can affect PD risk (54). If a study is large enough, analyses could be performed to identify relevant exposure periods. The analyses on smoking indicated that not only total smoking is relevant for PD risk, but also time-since-cessation. However, we also see relatively more cases among the smokers that quitted smoking before age 40 suggesting that smoking

behavior at young age, decades before diagnosis, is potentially associated with PD risk (see Figure 2). Because almost all smokers started smoking as a teenager, the study is too small to perform detailed analyses on relevant lifetime exposure periods. The same limitation applies for occupational exposures such as pesticides. The majority of participants who worked at farms later in life also did so earlier in life. Furthermore, because PD has a long preclinical phase very recent exposures may not be relevant to include in the exposure estimation. However, because PD is a disease developing in persons that are often retired or in the last years of their professional career, eliminating exposures accrued in recent years will not have a strong effect on the estimated cumulative exposures.

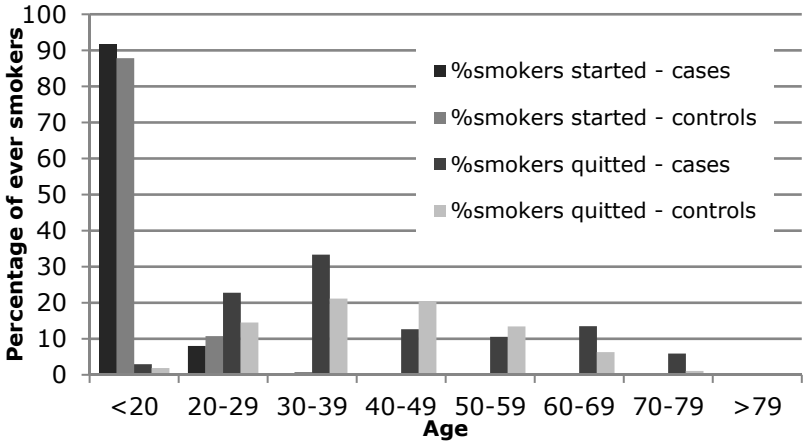

Figure 2. Percentage of smokers that started or quitted smoking by 10-year age categories. The percentages are based on 237 cases (53% of all cases) and 633 controls (72% of all controls) that were ever smokers.

Route of exposure

Exposure assessment for the occupational risk factors that were studied in this thesis is generally based on the assumption that inhalation and dermal uptake are the relevant routes of exposure, whereby the substance, its metabolites or biological effect markers reaches the brain where it induces neuronal damage (55). However, currently there is some debate if a gastrointestinal route for exposures might be more relevant (56, 57). Because the enteric nervous system is affected with neuronal inclusions of alpha-synuclein in an early stage of disease, it has been hypothesized that the disease could start here (58). More and more evidence shows that misfolded alpha-synuclein is able to transfer from cell to cell affecting cellular processes in the recipient cell (59). This behaviour of alpha-synuclein might be responsible for the slow but progressive spread of the disease through the brain. It

has been estimated that the propagation of the disease from the gut to the substantia nigra and the associated onset of motor symptoms can take 20 years (60). Interestingly, chronic intragastric administration of the pesticide rotenone to rats induced progression of alpha-synuclein first appearing in the enteric nervous system and later also in the substantia nigra accompanied with motor dysfunction (61). If future research indeed shows the gastrointestinal route is relevant in disease etiology, then it would be advisable for future studies on occupational risk factors of PD to take this route into account. The inadvertent ingestion of hazardous substances at workplaces has not been studied often in epidemiological studies in the past, but it has been estimated that ingestion of substances including metals and pesticides can occur in substantial amounts at workplaces (55).

Directions for future research

Study design

The case-control design can be a cost- and time- efficient design to study risk factors for relative rare diseases, as is the case for PD. As discussed in the previous sections, a major limitation of case-control studies on occupational risk factors is that there are limited possibilities to gather accurate information on exposure to specific agents such as specific pesticides, solvents or metals retrospectively. A prospective study design has the advantage over case-control studies that exposure information can be collected before the disease manifests itself. More and more large prospective cohort studies are being published that include analyses on PD. However, as with case-control studies, exposure assessment for occupational risk factors in cohort studies is in general not very specific and the prevalence of specific occupational exposures is often low.

Another approach would be to perform retrospective industry based studies where exposures to certain agents are common. In general, exposures can be estimated more precisely in such studies than within general population based case-control studies. Sometimes measurement data is available or it is feasible to conduct measurements of workday exposure for several job types and extrapolate these back to historical levels, as was done in some studies investigating ELF-MF exposure in relation with PD (11, 12, 62). Disadvantage of such studies is that often no information is available about potential important confounders such as smoking behavior and about exposures at jobs outside the participating companies. Also, often there is a need to rely on registry data such as mortality or hospital records for disease information. This may be a limitation because such data is not always accessible and because PD is a chronic condition that in itself often is non-fatal, PD is frequently not mentioned on death certificates (14, 15).

When a prospective cohort study is performed in a specific industry more specific exposure questions can be asked at a moment someone is still working in exposed jobs or even measurements can be performed. Also information on potential confounders can be obtained. An example is the Agricultural Health Study in the United States in which almost 90.000 pesticide applicators and their spouses were questioned about lifetime pesticide use and are followed up over time (63). PD disease cases were identified using self-report and mortality records, which were confirmed by neurologists that performed in-person assessments or checked medical records (64). Disadvantage is that a large group of participants need to be followed for long duration before enough incidence PD cases are identified to have enough power to study risk factors. Also, it may not always be possible to identify all incident PD cases in the cohort, which results in that part of the PD patients are analyzed as controls thereby reducing the power of the study. Thus although prospective studies in which exposures could be assessed in more detail and more accurately are a valuable contribution for research on risk factors for PD, such studies are at the same time much more expensive and time consuming than case-control studies.

Gene-environment interactions

It is thought that genetic susceptibility in combination with environmental exposures plays a role in disease etiology of sporadic PD (65-67). Examining the effect of an environmental risk factor in persons that are genetic susceptible for a certain exposure should result in observing stronger associations between exposure and disease (68).

The relative contribution of genetics and environmental risk factors to risk of PD is not known. Numerous studies have shown that PD patients are more likely to have a first degree relative with PD than persons without PD (69). However, this may be caused either by shared genetics or shared environmental exposures. Two large twin studies in which disease concordance rates between monozygotic and dizygotic twins were compared, provided evidence for a small genetic contribution in PD risk, which was more pronounced in disease with young onset (70, 71). More and more gene variants are being identified that are associated with PD in genome-wide association studies (72). In one recent genome-wide association study the genetic component of PD risk was estimated to be at least 27% (73).

Research on gene-environment interactions has accelerated in the last two decades and has focused on genes and exposures for which it is biological plausible that they interact in the same biological pathways. Challenges in research on

gene-environment interactions include the large number of exposures and gene variations that exists and the sample size needed to detect interaction effects (68). So far, no well-established gene-environment interactions for PD risk have been identified. Regarding pesticide exposure, studies that looked to genetic susceptibility were often small case-control studies that not always focused on specific (classes of) pesticides. To date the current knowledge on gene-pesticide interactions is limited and conflicting (74). Examples of genes for which positive gene-pesticide interactions have been observed, although with inconsistent results, are genes coding for proteins involved in pesticide metabolism (cytochrome P450 gene CYP2D6 and paraoxonase-1 (PON1)), pesticide transport (multidrug resistance gene (MDR1)), oxidative stress (glutathione S-transferase genes (GSTs), mitochondrial manganese superoxide dismutase (mnSOD)), or neuronal toxicity (dopamine transporter gene (DAT)) (74).

In our case-control study saliva samples for future DNA analysis were collected from 76% of the participating cases and 64% of the participating controls. As such, the study is likely too small to detect gene-environment interactions especially if the marginal effects of pesticides and genes are small. Therefore, the best approach would be to pool data from several case-control studies. In such a pooled effort special attention should be given to the exposure assessment as different exposure assessment methods may have been used. Such an approach has been successfully applied in the past, including for example a pooled study on smoking behavior and PD (75).

General conclusion

In this thesis the conduct of a hospital-based case-control study of PD in the Netherlands was described. The main focus of this study was on occupational risk factors, especially occupational exposure to pesticides. We put significant effort in developing methods to improve exposure classification for pesticides. Overall, the results of our study and previous studies indicate a slightly elevated risk of PD for individuals occupationally exposed to pesticides. We observed the strongest associations with benomyl, a fungicide that has been banned from the market in large parts of the world. Future studies should replicate this finding and focus on improving methods of exposure assessment to identify the specific (classes of) pesticides that are affecting PD risk. The identification of specific chemicals related with PD would give more insight in the mechanisms behind the development of the disease. Because self-reporting on the past use of specific pesticides is difficult, a crop-exposure matrix such as we developed is an approach that could be used in more retrospective epidemiological studies.

Furthermore, we performed the most comprehensive analyzes on electromagnetic fields to date by analyzing lifetime occupational and household appliance exposures and including analyses on electrical shocks. No associations with risk of PD were observed, which together with the results of previous studies strongly argues against an association between electricity related exposures and PD.

The analyses on occupational exposure to aromatic and chlorinated solvents and metals were less comprehensive. But the fact that we did not observe associations with PD risk added to the existing literature on risk factors for PD, which shows weak evidence for an association with PD. Similar as for pesticides, studies focusing on improved methods for classifying exposure to specific metals or solvents are needed to determine if a relation exist. Furthermore, the reason for the consistently observed inverse association with welding needs to be elucidated.

It has long been thought that environmental factors play an important role in PD disease etiology. However, to date not many environmental factors have been identified for which unequivocal evidence is available for a relation with PD risk. For occupational risk factors, only for exposure to pesticides enough evidence is available to state that most likely a relation with PD exists. We speculate that the lack in ability to detect environmental risk factors is because of limitations in exposure assessment; both in terms of quantification as timing of exposure. As such, most gain can be achieved in future studies by focusing on developing improved methods for assessing exposure to specific substances and including genetic analyses using the developments in knowledge on which genes variants might increase susceptibility for specific occupational risk factors.

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Appendix

Questions asked in the computer-assisted telephone interview

1. Algemene inleidende vragen

1.1 Wat is uw nationaliteit? Het is mogelijk twee nationaliteiten aan te geven.

- o Nederlands
- o Belgisch
- o Duits
- o Marokkaans
- o Surinaams
- o Turks
- o Anders nl……..
- o Anders nl……..
- o Onbekend
- o Weigering

1.2 Wat was de nationaliteit van uw biologische vader bij zijn geboorte? Het is mogelijk twee nationaliteiten aan te geven.

- o Nederlands
- o Belgisch
- o Duits
- o Marokkaans
- o Surinaams
- o Turks
- o Anders nl……..
- o Anders nl……..
- o Onbekend
- o Weigering

1.3 Wat was de nationaliteit van uw biologische moeder bij haar geboorte? Het is mogelijk om twee antwoorden te geven.

- o Nederlands
- o Belgisch
- o Duits
- o Marokkaans
- o Surinaams
- o Turks
- o Anders nl……..
- o Anders nl……..
- o Onbekend
- o Weigering

1.4 Ik zou graag willen weten wat de hoogste opleiding is die u heeft afgemaakt? Is dat:

- o Lagere school of basisschool
- o Lager beroepsonderwijs (bijv. LTS, Huishoudschool, LEAO, LHNO, lagere agrarische school)
- o Middelbaar Algemeen Onderwijs (bijv. (M)ulo, MAVO, 3 jr HBS)
- o Hoger Algemeen onderwijs (bijv. HBS, MMS, HAVO, VWO, Gymnasium)
- o Middelbaar beroeps onderwijs (bijv. MBO, MTS, MEAO, LO akte)
- o Hoger Beroepsonderwijs of universiteit (HBO, HTS, HEAO, sociale of pedagogische academie)
- o Geen opleiding
- o Onbekend
- o Weigering

1.5 Wat is of was de hoogste opleiding van uw eventuele partner?

- o Geen partner
- o Lagere school of basisschool
- o Lager beroepsonderwijs (bijv. LTS, Huishoudschool, LEAO, LHNO, lagere agrarische school)
- o Middelbaar Algemeen Onderwijs (bijv. (M)ulo, MAVO, 3 jr HBS)
- o Hoger Algemeen onderwijs (bijv. HBS, MMS, HAVO, VWO, Gymnasium)
- o Middelbaar beroeps onderwijs (bijv. MBO, MTS, MEAO, LO akte)
- o Hoger Beroepsonderwijs of universiteit (HBO, HTS, HEAO, sociale of pedagogische academie)
- o Geen opleiding
- o Onbekend
- o Weigering

1. 6 Wat is uw lengte?

Lengte…….cm

1.7 Hoeveel weegt u momenteel?

Gewicht…….kg

1.8 Heeft u ooit meer gewogen dan *(programma berekent bij de opgegeven lengte het aantal kilo dat iemand moet wegen om een BMI van 30 te hebben)* kilo?

o Ja o Nee o Onbekend o Weigering

2. Woongeschiedenis

Ik ga u nu vragen stellen over elke adres waar u in uw leven voor een periode van tenminste 1 jaar gewoond hebt. Als het goed is heeft u hiervoor thuis al wat informatie opgestuurd gekregen. Geef bij elk adres zoveel mogelijk informatie. Op het moment dat u iets niet meer weet, dan kunt u dit gewoon aangeven.
Kunt u beginnen bij het eerste adres waar u een jaar of langer gewoond hebt. Bijvoorbeeld het adres waar u geboren bent.

2.1 In welke stad of dorp was dit adres?

Woonplaats:…….

2.2 Van welk jaar tot welk jaar heeft u op dit adres gewoond. U mag hierbij antwoorden in jaartallen maar ook in leeftijd net wat u makkelijker vindt.

Van jaar:…… Leeftijd:…….

Tot jaar:…… Leeftijd:…….

2.3 Wat waren de straatnaam, huisnummer en postcode van dit adres?

Straatnaam:…… Nummer…… Toevoeging……
Postcodecijfers…… Postcodeletters……

2.4 Op welke verdieping van het gebouw waarin u woonde was de kamer waar u het meeste sliep? De begane grond telt hierbij als 0.

Verdieping nummer:……

Nu gaan we door naar het volgende adres waar u een jaar of langer gewoond heeft.
(zelfde vragen als adres 1 (vragen 2.1-2.4)

Nu gaan we door naar het volgende adres waar u een jaar of langer gewoond heeft.

(zelfde vragen als adres 1 (vragen 2.1-2.4) totdat alle adressen geweest zijn)

2.5 Heeft u ooit in uw leven in een huis gewoond dat niet op het waterleidingnetwerk was aangesloten en waarbij u uw drinkwater uit de grond oppompte?

o Ja o Nee→ 2.7 o Onbekend → 2.7 o Weigering→ 2.7

2.6 **Als ja:** Bij welke adressen woonde u zonder aansluiting op het waterleidingnetwerk en pompte u uw drinkwater uit de grond op?

- o *Vink adressen aan uit lijst straten/woonplaatsen zojuist opgenoemd*
- o Ander adres dan zojuist opgenoemd → 2.1-2.4 en dan deze vraag opnieuw (als korter dan 6 maanden geef dat aan bij woonplaats)

2.7 Heeft u ooit voor een periode van tenminste 1 jaar een huisgenoot gehad die werkte in de land of tuinbouw, de plantsoenendienst, de ongediertebestrijding of als hovenier? Het gaat er hier dus niet om welk werk u zelf heeft gedaan, maar wel om het werk van bijvoorbeeld uw ouders of partner.

o Ja o Nee→ 3.1 o Onbekend → 3.1 o Weigering→ 3.1

2.8 **Als ja:** Bij welke adressen had u voor een periode van tenminste 1 jaar een huisgenoot die werkte in de land of tuinbouw, de plantsoenendienst, de ongediertebestrijding of als hovenier?

- o *Vink adressen aan uit lijst straten/woonplaatsen zojuist opgenoemd*
- o Ander adres dan zojuist opgenoemd → 2.1-2.4 en dan deze vraag opnieuw

3. Beroepsgeschiedenis

Dank u wel voor uw antwoorden. Nu zou ik graag een overzicht willen hebben van al het werk dat u gedaan heeft gedurende uw leven. Ook hierover heeft u als het goed is al informatie thuis ontvangen.

3.1 Heeft u ooit gewerkt? Hierbij bedoelen we zowel betaald als onbetaald werk dat u gedaan heeft voor een periode van tenminste 6 maanden. **Voor mannen:** Ook de eventuele dienstplicht die u vervuld heeft moet u meetellen. Huishoudelijk werk wat u gedaan hebt in uw eigen huis telt niet mee.

o Ja o Nee→ 4.1 o Onbekend → 4.1 o Weigering→ 4.1

Als u een soortgelijke functie voor meerdere werkgevers hebt uitgevoerd vragen we u deze apart te noemen. Ook als u bij dezelfde werkgever in de loop van de tijd van functie bent veranderd en duidelijk andere taken bent gaan uitoefenen, vragen we u deze apart op te noemen. Begin bij uw eerste beroep.

3.2 Wat was uw functie? Bijvoorbeeld leraar/lerares of schilder.

3.3 Van welk jaar tot welk jaar oefende u dit werk uit? U mag hierbij antwoorden in jaartallen maar ook in leeftijd; net wat u makkelijker vindt.

Van jaar:…… Leeftijd:…… Tot jaar:…… Leeftijd:……

3.4 Hoeveel uur per week werkte u gemiddeld?

Aantal uur: ……

3.5 Bij wat voor soort bedrijf of instelling werkte u? (b.v. gemeente, metaalbewerking etc.)

Soort bedrijf/instelling:……

3.6 Wat was de naam van het bedrijf of instelling waar uw werkte? (open antwoord)

Naam bedrijf……

3.7 In welk plaats was dit bedrijf of deze instelling gevestigd?

Naam dorp op stad:……

3.8 Kunt u een korte omschrijving geven van ongeveer 2 zinnen van uw belangrijkste taken?

Belangrijkste taken:……

3.9 Welk vervoersmiddel(en) gebruikte u meestal om bij uw werk te komen? Indien van toepassing, geef het gemiddeld aantal minuten per enkele reis aan voor dat vervoermiddel.

- o Woning bij werk
- o Lopen, aantal minuten……
- o Fiets , aantal minuten……
- o Brommer/Scooter, aantal minuten……
- o Auto, aantal minuten……
- o Motor, aantal minuten……
- o Bus, aantal minuten……
- o Trein, aantal minuten……
- o Tram, aantal minuten……
- o Metro, aantal minuten……
- o Boot, aantal minuten……
- o Anders, namelijk……, aantal minuten……
- o Onbekend
- o Weigering

Dank u wel. Nu gaan we door naar de volgende functie die u 6 maanden of langer hebt uitgevoerd.
(zelfde vragen als beroep 1 (vragen 3.2-3.9) totdat alle beroepen geweest zijn)

Dank u wel, nu wil ik een aantal vragen stellen over een aantal werkzaamheden die u mogelijk gedaan heeft tijdens uw werk.

3.10 Heeft ooit tijdens uw werk metaal gelast?

o Ja o Nee → 3.18 o Onbekend → 3.18 o Weigering → 3.18

3.11 **Als ja**: Bij welke van de zojuist door u opgenoemde functies was dit het geval?

- o *Vink functies aan uit lijst functies zojuist opgenoemd*
- o Andere functie dan zojuist opgenoemd → 3.2-3.9 invullen en dan deze vraag opnieuw (als functie korter dan 6 maanden geef dat aan bij functie)

Per functie waarbij deelnemer gelast heeft vragen:

3.12 Heeft u bij uw werk als *(noem functie en naam bedrijf)* gewerkt met een lastechniek die werkt op elektriciteit? Bijv. booglassen zoals MIG, MAG of TIG lassen, plasmalassen of weerstandlassen zoals puntlassen.

o Ja o Nee → 3.12/3.18 o Onbekend o Weigering→ 3.12/3.18

3.13 **Als ja of onbekend:** Welke (elektrische) lastechniek gebruikte u? U kunt meerdere technieken noemen (maximaal 3). Indien meer dan 3 geef de belangrijkste aan.

o Lastechniek…… o Lastechniek 2…… o Lastechniek 3……

Per lastechniek één voor één vragen:

3.14 Hoeveel dagen per jaar laste u gemiddeld met genoemde lastechniek tijdens de genoemde functie?

- o > 4 dagen per week
- o 1-3 dagen per week
- o 1-3 dagen per maand
- o <1 dag per maand
- o <1 dag per jaar
- o Onbekend
- o Weigering

3.15 Hoeveel uur per dag laste u op dagen dat u laste in genoemde functies met genoemde lastechniek?

- o >4 uur per dag
- o 1-3 uur per dag
- o <1 uur per dag
- o Onbekend
- o Weigering

3.16 Laste u hierbij handmatig bij genoemde functie en lastechniek?

o Ja o Nee→3.14/3.12/3.18 o Onbekend→3.14/3.12/3.18 o Weigering→3.14/3.12/3.18

3.17 **Als ja:** Had u tijdens het lassen met genoemde techniek over het algemeen de kabel over uw schouder hangen?

- o Ja/meestal → 3.14 (volgende techniek) of 3.12 (volgende functie) of 3.18
- o Soms→ 3.14 (volgende techniek) of 3.12 (volgende functie) of 3.18
- o Zelden→ 3.14 (volgende techniek) of 3.12 (volgende functie) of 3.18
- o Nee→ 3.14 (volgende techniek) of 3.12 (volgende functie) of 3.18
- o Onbekend → 3.14 (volgende techniek) of 3.12 (volgende functie) of 3.18
- o Weigering → 3.14 (volgende techniek) of 3.12 (volgende functie) of 3.18

3.18 Heeft u ooit tijdens uw werk, werkzaamheden of onderhoud aan onderdelen van het hoogspanningsnetwerk uitgevoerd, zoals bovengrondse hoogspanningslijnen, opstijgpunten of schakelstations van hoog-naar middenspanning

o Ja o Nee → 3.22 o Onbekend → 3.22 o Weigering → 3.22

3.19 **Als ja**: Bij welke van de genoemde functies heeft u **ooit tijdens uw werk**, werkzaamheden of onderhoud aan onderdelen van het hoogspanningsnetwerk uitgevoerd?

- o *Vink functies aan uit lijst functies zojuist opgenoemd*
- o Andere functie dan zojuist opgenoemd → 3.2-3.9 invullen en dan deze vraag opnieuw (als functie korter dan 6 maanden geeft dat aan bij functie)

Per functie waarbij deelnemer aan hoogspanningsnetwerk gewerkt heeft vragen:

3.20 Tijdens uw werk als *(noem functie en naam bedrijf)*, hoe vaak kwam het voor dat u hieraan gewerkt heeft?

- o > 4 dagen per week
- o 1-3 dagen per week
- o 1-3 dagen per maand
- o <1 dag per maand
- o <1 dag per jaar
- o Onbekend
- o Weigering

3.21 Op dagen dat u hieraan werkte, hoeveel uur was dit?

- o >4 uur per dag → 3.20 (volgende functie) of 3.22
- o 1-3 uur per dag → 3.20 (volgende functie) of 3.22
- o <1 uur per dag → 3.20 (volgende functie) of 3.22
- o Onbekend → 3.20 (volgende functie) of 3.22
- o Weigering →3.20 (volgende functie) of 3.22

3.22 Heeft u ooit tijdens uw werk een elektrisch apparaat bediend dat metaal verhit of smelt? Bijvoorbeeld een industriële inductie verwarmer of industriële elektrische oven?

o Ja o Nee → 3.26 o Onbekend → 3.26 o Weigering → 3.26

3.23 **Als ja**: Bij welke van de zojuist door u opgenoemde functies was dit het geval?

- o *Vink functies aan uit lijst functies zojuist opgenoemd*
- o Andere functie dan zojuist opgenoemd → 3.2-3.9 invullen en dan deze vraag opnieuw (als functie korter dan 6 maanden geef dat aan bij functie)

Per functie waarbij deelnemer deze ovens gebruikt heeft vragen:

3.24 Tijdens uw werk als *(noem functie en naam bedrijf)* hoeveel dagen gemiddeld per jaar heeft u dit soort verwamers bediend?

- o > 4 dagen per week
- o 1-3 dagen per week
- o 1-3 dagen per maand
- o <1 dag per maand
- o < 1 dag per jaar
- o Onbekend
- o Weigering

3.25 Hoeveel uur was dit gemiddeld op een dag dat u met zulke verwarmers werkte?

- o >4 uur per dag → 3.24 (volgende functie) of 3.26
- o 1-3 uur per dag → 3.24 (volgende functie) of 3.26
- o <1 uur per dag → 3.24 (volgende functie) of 3.26
- o Onbekend → 3.24 (volgende functie) of 3.26
- o Weigering →3.24 (volgende functie) of 3.26

3.26 Heeft u ooit gewerkt op een veehouderij?

o Ja o Nee → 3.30 o Onbekend → 3.30 o Weigering → 3.30

3.27 **Als ja**: Bij welke van de zojuist door u opgenoemde functies was dit het geval?

- *Vink functies aan uit lijst functies zojuist opgenoemd*
- Andere functie dan zojuist opgenoemd → 3.2-3.9 invullen en dan deze vraag opnieuw (als functie korter dan 6 maanden geeft dat aan bij functie)

3.28 **Per functie waarbij deelnemer op veehouderij heeft gewerkt:** Binnen uw werk op een veehouderij als *(noem functie en naam bedrijf),* wat voor vee werd hier gehouden? Indien meer dan 3 verschillende soorten; geef de belangrijkste 3

1. …… 2. …… 3. ……

3.29 Hoeveel *(noem diersoorten)* waren er gemiddeld op dit bedrijf? (open antwoord) (per soort)

Aantal:……. → 3.28 (volgende functie) of 3.30

3.30 Heeft u ooit gewerkt in de akkerbouw?

o Ja o Nee → 3.33 o Onbekend → 3.33 o Weigering → 3.33

3.31 **Als ja**: Bij welke van de zojuist door u opgenoemde functies was dit het geval?

- *Vink functies aan uit lijst functies zojuist opgenoemd*
- Andere functie dan zojuist opgenoemd → 3.2-3.9 invullen en dan deze vraag opnieuw (als functie korter dan 6 maanden dat dat invullen bij functie)

3.32 **Per functie waarbij deelnemer in de akkerbouw heeft gewerkt:** Binnen uw werk in de akkerbouw als *(noem functie en naam bedrijf),* wat voor producten werden hier verbouwd? Indien meer dan 3 verschillende soorten; geef de belangrijkste 3 (open antwoord) (meerdere antwoorden mogelijk met een max. van 3)

1. …… 2. …… 3. …… → 3.32 (volgende functie) of 3.33

3.33 Heeft u ooit gewerkt in de tuinbouw?

o Ja o Nee → 3.36 o Onbekend → 3.36 o Weigering → 3.36

3.34 **Als ja**: Bij welke van de zojuist door u opgenoemde functies was dit het geval?

- *Vink functies aan uit lijst functies zojuist opgenoemd*
- Andere functie dan zojuist opgenoemd → 3.2-3.9 invullen en dan deze vraag opnieuw (als functie korter dan 6 maanden geef dat aan bij functie)

3.35 **Per functie waarbij deelnemer in de tuinbouw heeft gewerkt:** Binnen uw werk in de tuinbouw als *(noem functie en naam bedrijf),* wat voor producten werden hier geteeld Indien meer dan 3 verschillende soorten; geef de belangrijkste 3 (open antwoord) (meerdere antwoorden mogelijk met een max. van 3)

1. …… 2. …… 3. ……→ 3.35 (volgende functie) of 3.36

3.36 Heeft u ooit gewerkt binnen een bedrijf dat gespecialiseerd is in ongediertebestrijding?

o Ja o Nee → 3.38 o Onbekend → 3.38 o Weigering → 3.38

3.37 **Als ja**: Bij welke van de zojuist door u opgenoemde functies was dit het geval?

- *Vink functies aan uit lijst functies zojuist opgenoemd*
- Andere functie dan zojuist opgenoemd → 3.2-3.9 invullen en dan deze vraag opnieuw (als functie korter dan 6 maanden geef dat aan bij functie)

3.38 Heeft u ooit gewerkt als hovenier?

o Ja o Nee → 3.40 o Onbekend → 3.40 o Weigering → 3.40

3.39 **Als ja**: Bij welke van de zojuist door u opgenoemde functies was dit het geval?

- *Vink functies aan uit lijst functies zojuist opgenoemd*
- Andere functie dan zojuist opgenoemd → 3.2-3.9 invullen en dan deze vraag opnieuw (als functie korter dan 6 maanden geef dat aan bij functie)

3.40 Heeft u ooit gewerkt in de plantsoenendienst?

o Ja o Nee → 3.42 o Onbekend → 3.42 o Weigering → 3.42

3.41 **Als ja**: Bij welke van de zojuist door u opgenoemde functies was dit het geval?

- *Vink functies aan uit lijst functies zojuist opgenoemd*
- Andere functie dan zojuist opgenoemd → 3.2-3.9 invullen en dan deze vraag opnieuw (als functie korter dan 6 maanden geef dat aan bij functie)

3.42 **Als ja op vraag 3.26, 3.30, 3.33, 3.36, 3.38 of 3.40. Per functie waarbij deelnemer in veehouderij/akkerbouw/tuinbouw/hovenier/ ongediertebestrijding/plantsoenendienst gewerkt heeft:**
Werden er binnen uw werk als *(noem functie en naam bedrijf/instelling),* chemische bestrijdingsmiddelen, ofwel pesticiden, toegepast? Bijvoorbeeld voor de bestrijding van insecten, onkruid, schimmel of knaagdieren.

o Ja o Nee → 3.42/4.1 o Onbekend → 3.42/4.1 o Weigering → 3.42/4.1

Als ja: Werden bestrijdingsmiddelen toegepast tegen:

3.43 Insecten?

o Ja o Nee o Onbekend o Weigering

3.44 Onkruid?

o Ja o Nee o Onbekend o Weigering

3.45 Schimmel?

o Ja o Nee o Onbekend o Weigering

3.46 Muizen of ratten?

o Ja o Nee o Onbekend o Weigering

3.47 Iets anders dan zojuist opgenoemd?

o Ja, namelijk…… o Nee o Onbekend o Weigering

Als ja bij vraag 3.42 (gebruik bestrijdingsmiddelen) per soort waarop ja geantwoord is bij vraag 3.43-3.47 → Vragen 3.48 tot 3.56

3.48 Hoeveel dagen per jaar werden bestrijdingsmiddelen tegen *(noem soort waar ja is op geantwoord)* ongeveer toegepast op uw werk?

- Minder dan 1 dag per jaar
- 1-5 dagen per jaar
- 6-20 dagen per jaar
- 21-50 dagen per jaar
- >50 dagen per jaar
- Onbekend
- Weigering

3.49 Was u degene die deze middelen voor toepassing mengde en klaarzette? Meestal wel, soms, zelden of nooit

- Ja/meestal
- Soms
- Zelden
- Nooit
- Niet van toepassing / niet gemengd
- Onbekend
- Weigering

3.50 Paste u zelf deze middelen toe? Meestal wel, soms, zelden of nooit.

- Ja/meestal
- Soms
- Zelden → 3.59 (volgende soort) of 3.52 (volgende functie) of 4.1
- Nooit → 3.59 (volgende soort) of 3.52 (volgende functie) of 4.1
- Onbekend → 3.59 (volgende soort) of 3.52 (volgende functie) of 4.1
- Weigering → 3.59 (volgende soort) of 3.52 (volgende functie) of 4.1

3.51 **Als ja/meestal of soms:** Welke van de volgende methoden gebruikte u meestal om deze middelen toe te passen? (max 2 antwoorden mogelijk)

- Trekker met spuitmachine
- Rugspuit, waarbij de tank met het middel zich op uw rug bevond
- Handspuit, waarbij de tank met het middel zich niet op uw rug bevond
- Strooien van korrels
- Anders namelijk……..
- Anders namelijk……..
- Onbekend
- Weigering

3.52 Gebruikte u persoonlijke beschermingsmiddelen zoals handschoenen of beschermende kleding wanneer u deze middelen gebruikte? Als dit gedurende de tijd is veranderd kunt u dit ook aangeven.

- Ja
- Ja in de loop van de tijd steeds meer.
- Ja in de loop van de tijd steeds minder
- Nee → 3.48 (volgende soort) of 3.42 (volgende functie) of 4.1
- Onbekend → 3.48 (volgende soort) of 3.42 (volgende functie) of 4.1
- Weigering → 3.48 (volgende soort) of 3.42 (volgende functie) of 4.1

3.53 **Als ja:** Welke van de volgende persoonlijke beschermingsmiddelen gebruikte u? (meerdere antwoorden mogelijk)

- Rubber laarzen
- Handschoenen
- Ondoorlaatbare kleding
- Gezichtsmasker
- Beschermingsbril
- Anders namelijk:……
- Onbekend
- Weigering

3.54 **Vragen als soort is insecten:** Kunt u herinneren of u op uw werk één van de volgende middelen heeft toegepast? (meerdere antwoorden mogelijk)

- Lindaan (ook verkocht onder de merknamen gamma, perfectan fluid, verindal, lirogam, AAmeltex, AAritna en Lindafor)
- Parathion (ook verkocht onder de merknamen AAtiol, Asepthion, Di-Thios, Folidol, fosferno, Jebophos, Dethion, AAparon, Jeboterra, Paratox, Spintex, Condor, Penncap en Thionylc E.
- Dieldrin (ook verkocht onder de merknamen Jebodriel, Dieldrasept en Dieldrex)
- Rotenon

3.55 **Vragen als soort is onkruid:** Kunt u herinneren of u op uw werk één van de volgende middelen heeft toegepast? (meerdere antwoorden mogelijke

- Dinoseb (ook verkocht onder de merknamen, AATox, Brabant Selective Weedkiller, Du-Tox, Jebutox, AAlotox, Liromort en Solamort)
- Paraquat ofwel Gramoxone
- Linuron (ook wel verkocht onder de merknaam Afalon)
- Atrazine (ook wel verkocht onder de merknamen Primatol, Gesaprim, Atranex en Vectal)

3.56 **Vragen als soort is schimmel**: Kunt u herinneren of u op uw werk het volgende middel heeft toegepast?

- Maneb (ook wel verkocht onder de merknamen AAmagan, AAzinam, Duphar-dithane, Mangatex, Trimangol, AAbetam, AAmangan, Dequiman, Manzate D, Manex, Manogil en Vondac)

→ 3.48 (volgende soort) of 3.42 (volgende functie) of 4.1

4. blootstelling buiten beroep

Dank u wel. Nu zou ik graag het een en ander willen weten over activiteiten en omstandigheden buiten uw werk om.

4.1 Heeft u ooit metaal gelast buiten uw werk om?

o Ja o Nee → 4.10 o Onbekend → 4.10 o Weigering → 4.10

4.2 **Als ja:** Heeft u hierbij gewerkt met een lastechniek die werkt op elektriciteit? Bijvoorbeeld booglassen zoals MIG, MAG of TIG lassen.

o Ja o Nee → 4.10 o Onbekend o Weigering → 4.10

4.3 **Als ja of onbekend:** Wat voor (**als ja:** elektrische) lastechniek gebruikte u? (max 3)

o Lastechniek 1…… o Lastechniek 2…… o Lastechniek 3……

4.4 Hoe oud was u toen u voor het eerst elektrisch laste buiten uw werk?

Leeftijd:……of Jaar…….

4.5 Hoe oud was u toen u voor het laatst elektrisch laste buiten uw werk om?

Leeftijd:……of in Jaar……

4.6 Hoeveel jaar ongeveer heeft u in totaal buiten uw werk om gelast?

Aantal jaar:…….

4.7 Hoeveel dagen per jaar laste u gemiddeld met een elektrische lastechniek buiten uw werk om?

- 1-5 dagen per jaar
- 6-20 dagen per jaar
- 21-50 dagen per jaar
- >50 dagen per jaar
- Onbekend
- Weigering
-

4.8 Hoeveel uur per dag laste u wanneer u ging lassen?

- >4 uur per dag
- 1-3 uur per dag
- <1 uur per dag
- Onbekend
- Weigering

4.9 Had u tijdens het lassen over het algemeen de kabel over uw schouder hangen?

o Ja o Nee o Onbekend o Weigering

4.10 Heeft u ooit buiten uw werk om, bijvoorbeeld in uw eigen tuin of huis, chemische bestrijdingsmiddelen, ofwel pesticiden, toegepast om bijvoorbeeld insecten, onkruid, schimmel of knaagdieren te bestrijden?

o Ja o Nee → 4.21 o Onbekend → 4.21 o Weigering → 4.21

Als ja: Gebruikte u bestrijdingsmiddelen tegen:

4.11 Insecten?

o Ja o Nee o Onbekend o Weigering

4.12 Onkruid?

o Ja o Nee o Onbekend o Weigering

4.13 Schimmel?

o Ja o Nee o Onbekend o Weigering

4.14 Muizen of ratten?

o Ja o Nee o Onbekend o Weigering

4.15 Iets anders dan zojuist genoemd?

o Ja, namelijk…… o Nee o Onbekend o Weigering

4.16 **Per soort waarop ja geantwoord is (vraag 4.11-4.15):** Hoe oud was u ongeveer toen u bestrijdingsmiddelen tegen *(noem soort)* voor het eerst buiten uw werk om gebruikte?

Leeftijd:……of jaar……..

4.17 Wanneer ongeveer heeft u deze middelen voor het laatst buiten uw werk toegepast?

Leeftijd:……of jaar:……..

4.18 Hoeveel jaar ongeveer heeft u deze middelen in totaal buiten uw werk om gebruikt, als u de jaren aftrekt dat u dit middel niet gebruikt heeft.

Aantal jaar:…….

4.19 Hoeveel dagen in het jaar gebruikte u deze middelen gemiddeld?

- 1-5 dagen per jaar
- 6-20 dagen per jaar
- 21-50 dagen per jaar
- >50 dagen per jaar
- Onbekend
- Weigering

→ 4.16 (volgende soort) of 4.20

Vervolgens zou ik graag wat willen weten over hoe vaak u bepaalde elektrische apparaten gebruikt en gebruikte.

4.20 Hoeveel keer per week gemiddeld droogt u uw haar met een haardroger, föhn of gebruikt u een krul of stijltang?

- o Aantal keer per week:……
- o Nooit of minder dan 1 keer per week

4.21 **Als 65 jaar of ouder:** En toen u ongeveer 60 jaar oud was?

- o Aantal keer per week:……
- o Nooit of minder dan 1 keer per week

4.22 **Als 45 jaar of ouder:** En toen u ongeveer 40 jaar oud was?

- o Aantal keer per week:……
- o Nooit of minder dan 1 keer per week

4.23 **Als 25 jaar of ouder:** En toen u ongeveer 20 jaar oud was?

- o Aantal keer per week:……
- o Nooit of minder dan 1 keer per week

4.24 **Alleen vragen aan een man (als vrouw → 4.29):** Heeft u zichzelf ooit geschoren met een elektrisch scheerapparaat waarbij een stekker in het stopcontact zit?

o Ja o Nee → 4.29 o Onbekend → 4.29 o Weigering → 4.29

4.25 **Als ja:** Scheert u zich op dit moment met een scheerapparaat met stekker in het stopcontact

o Ja o Nee o Onbekend o Weigering

4.26 **Als 65 jaar oud ouder:** En toen u ongeveer 60 jaar oud was?

o Ja o Nee o Onbekend o Weigering

4.27 **Als 45 jaar of ouder:** En toen u ongeveer 40 jaar oud was?

o Ja o Nee o Onbekend o Weigering

4.28 **Als 25 jaar of ouder:** En toen u ongeveer 20 jaar oud was?

o Ja o Nee o Onbekend o Weigering

4.29 Slaapt u op dit moment meestal met een elektrische wekker of klok met een stekker in het stopcontact binnen een afstand van 1 meter van uw hoofd?

o Ja o Nee o Onbekend o Weigering

4.30 **Als 65 jaar of ouder:** En toen u ongeveer 60 jaar oud was?

o Ja o Nee o Onbekend o Weigering

4.31 **Als 45 jaar of ouder:** En toen u ongeveer 40 jaar oud was?

o Ja o Nee o Onbekend o Weigering

4.32 **Als 25 jaar of ouder:** En toen u ongeveer 20 jaar oud was?

o Ja o Nee o Onbekend o Weigering

4.33 Heeft u ooit geslapen met een elektrisch deken die een deel van de nacht of de hele nacht aanstond terwijl u sliep?

o Ja o Nee → 4.42 o Onbekend → 4.42 o Weigering → 4.42

4.34 **Als ja:** Slaapt u op dit moment in de winter meestal met een elektrisch deken die een deel van de nacht of de hele nacht aan staat terwijl u slaapt?

o Ja o Nee → 4.36 o Onbekend → 4.36 o Weigering → 4.36

4.35 **Als ja:** Staat dit deken dan de hele nacht aan?

o Ja o Nee o Onbekend o Weigering

4.36 **Als 65 jaar of ouder:** En toen u ongeveer 60 jaar oud was? Sliep u toen in de winter meestal met een elektrisch?

o Ja o Nee → 4.38 o Onbekend → 4.38 o Weigering → 4.38

4.37 **Als ja:** Stond dit deken dan de hele nacht aan?

o Ja o Nee o Onbekend o Weigering

4.38 **Als 45 jaar of ouder:** En toen u ongeveer 40 jaar oud was? Sliep u toen in de winter meestal met een elektrisch?

o Ja o Nee → 4.40 o Onbekend → 4.40 o Weigering → 4.40

4.39**Als ja:** Stond dit deken dan de hele nacht aan?

o Ja o Nee o Onbekend o Weigering

4.40 **Als 25 jaar of ouder:** En toen u ongeveer 20 jaar oud was? Sliep u toen in de winter meestal met een elektrisch deken?

o Ja o Nee → 4.43 o Onbekend → 4.43 o Weigering → 4.43

4.41 **Als ja:** Stond dit deken dan de hele nacht aan?

o Ja o Nee o Onbekend o Weigering

4.42 Heeft u ooit een waterbed gehad?

o Ja o Nee → 5.1 o Onbekend → 5.1 o Weigering → 5.1

4.43 **Zo ja:** Slaapt u op dit moment op een waterbed?

o Ja o Nee o Onbekend o Weigering

4.44 **Als 65 jaar of ouder:** Sliep u op een waterbed toen u ongeveer 60 jaar oud was?

o Ja o Nee o Onbekend o Weigering

4.45 **Als 45 jaar of ouder:** Sliep u op een waterbed toen u ongeveer 40 jaar oud was?

o Ja o Nee o Onbekend o Weigering

4.46 **Als 25 jaar of ouder** Sliep u op een waterbed toen u ongeveer 20 jaar oud was?

o Ja o Nee o Onbekend o Weigering

5. Rook en drinkgewoonten

Nu wil ik u een aantal vragen stellen over uw rook en drink gewoontes.

5.1 Heeft u gedurende uw leven in totaal meer dan 100 sigaretten gerookt? Dit zijn ongeveer 5 pakjes.

o Ja o Nee → 5.12 o Onbekend → 5.12 o Weigering → 5.12

5.2 **Als ja:** Op welke leeftijd bent u begonnen met roken?

Leeftijd…… of jaar……

5.3 Rookt u momenteel?

o Ja → 5.5 o Nee o Onbekend → 5.5 o Weigering → 5.5

5.4 **Als nee:** Op welke leeftijd bent u de laatste keer gestopt met het roken van sigaretten?

Leeftijd gestopt:……of jaar…….

5.5 Zijn er gedurende deze jaren dat u gerookt heeft, jaren geweest dat u niet gerookt heeft? En zo ja, hoeveel jaren waren dit ongeveer?

- o Ja, aantal jaren niet gerookt……
- o Nee

Kunt u bij de volgende leeftijdscategorieën aangeven hoeveel sigaretten u toen gemiddeld per dag rookte? Als u maar in een deel van die periode rookte noemt u het aantal sigaretten uit de periode dat u wel rookte:

5.6 **Als gerookt in die periode:** Hoeveel sigaretten rookte u per dag onder de 20 jaar oud?

Aantal sigaretten:……

5.7 **Als 20 of ouder en gerookt in die periode:** Hoeveel sigaretten rookte u per dag tussen de 20 en 29 jaar?

Aantal sigaretten:……

5.8 **Als 30 of ouder en gerookt in die periode:** Hoeveel sigaretten rookte u per dag tussen de 30 en 39 jaar?

Aantal sigaretten:……

5.9 **Als 40 of ouder en gerookt in die periode:** Hoeveel sigaretten rookte u per dag tussen de 40 en 49 jaar?

Aantal sigaretten:……

5.10 **Als 50 of ouder en gerookt in die periode:** Hoeveel sigaretten rookte u per dag tussen de 50 en 59 jaar?

Aantal sigaretten:……

5.11 **Als 60 of ouder en gerookt in die periode:** Hoeveel sigaretten rookte u per dag toen u 60 jaar of ouder was?

Aantal sigaretten:……

Ook vragen als deelnemer zelf geen sigaretten heeft gerookt:
5.12 Heeft u wel eens andere rookmiddelen gebruikt dan sigaretten zoals sigaren of pijp? Hier bedoelen we een gebruik van tenminste een jaar 1 of meer keer per week.

o Ja o Nee → 5.14 o Onbekend → 5.14 o Weigering → 5.14

5.13 **Als ja:** Welke andere rookmiddelen?

Soort:……

5.14 Heeft u voor u 20ste ooit een huisgenoot, partner of een collega gehad die binnenshuis of op de werkplek in uw nabijheid meer dan 1 sigaret, sigaar of pijp per dag rookte?

o Ja o Nee → 5.16 o Onbekend → 5.16 o Weigering → 5.16

5.15 **Als ja:** Hoeveel jaar **voor** uw 20ste heeft u dit gehad?

Aantal jaar:……

5.16 Heeft u na u 20ste ooit een partner, andere huisgenoot of collega gehad die binnenshuis of op de werkplek in uw nabijheid meer dan 1 sigaret, sigaar of pijp per dag rookte?

o Ja o Nee → 5.18 o Onbekend → 5.18 o Weigering → 5.18

5.17 **Als ja:** Hoeveel jaar **na** uw 20ste heeft u dit gehad?

Aantal jaar:……

5.18 Rookte uw moeder op het moment dat ze in verwachting van u was?

o Ja o Nee o Onbekend o Weigering

5.19 Heeft u ooit regelmatig alcoholhoudende dranken gedronken? We bedoelen hierbij minimaal 1 keer per maand.

o Ja o Nee → 5.28 o Onbekend → 5.28 o Weigering → 5.28

5.20 **Als ja:** Hoeveel glazen alcoholhoudende dranken drinkt u op dit moment gemiddeld per week? Een flesje bier is ongeveer 1.5 glas.

- o Geen of minder dan 1 glas per week
- o Aantal glazen……

5.21 **Als 1 of meer per week:** Drinkt u wel eens, 1 keer per maand of vaker, 5 of meer glazen alcoholhoudende dranken per gelegenheid?

o Ja o Nee o Onbekend o Weigering

5.22 **Als 65 jaar of ouder:** Toen u ongeveer 60 jaar oud was, hoeveel glazen alcoholhoudende dranken dronk u toen gemiddeld per week?

- o Geen of minder dan 1 glas per week → 5.24
- o Aantal glazen……

5.23 **Als 1 of meer per week:** Dronk u toen wel eens, 1 keer per maand of vaker, 5 of meer glazen alcoholhoudende dranken per gelegenheid?

o Ja o Nee o Onbekend o Weigering

5.24 **Als 45 jaar of ouder:** Toen u ongeveer 40 jaar oud was, hoeveel glazen alcoholhoudende dranken dronk u toen gemiddeld per week?

- o Geen of minder dan 1 glas per week → 5.26
- o Aantal glazen……

5.25 **Als 1 of meer per week:** Dronk u toen wel eens, 1 keer per maand of vaker, 5 of meer glazen alcoholhoudende dranken per gelegenheid?

o Ja o Nee o Onbekend o Weigering

5.26 **Als 25 jaar of ouder:** Toen u ongeveer 20 jaar oud was, hoeveel glazen alcoholhoudende dranken dronk u toen gemiddeld per week?

- o Geen of minder dan 1 per week → 5.28
- o Aantal glazen……

5.27 **Als 1 of meer per week:** Dronk u toen wel eens, 1 keer per maand of vaker, 5 of meer glazen alcoholhoudende dranken per gelegenheid?

o Ja o Nee o Onbekend o Weigering

5.28 Heeft u ooit regelmatig cafeïne houdende koffie gedronken? We bedoelen hierbij tenminste 1 keer per week.

o Ja o Nee → 5.33 o Onbekend → 5.33 o Weigering → 5.33

5.29 **Als ja:** Hoeveel kopjes cafeïne houdende koffie drinkt u op dit moment gemiddeld per week?

Aantal kopjes:……

5.30 **Als 65 jaar of ouder:** En toen u ongeveer 60 jaar oud was?

Aantal kopjes:……

5.31 **Als 45 jaar of ouder:** En toen u ongeveer 40 jaar oud was?

Aantal kopjes:……

5.32 **Als 25 jaar of ouder:** En toen u ongeveer 20 jaar oud was.

Aantal kopjes:……

5.33 Heeft u ooit regelmatig zwarte of groene thee gedronken? We bedoelen hierbij tenminste 1 keer per week.

o Ja o Nee → 5.38 o Onbekend → 5.38 o Weigering → 5.38

5.34 **Als ja:** Hoeveel kopjes zwarte of groene thee drinkt u op dit moment gemiddeld per week?

Aantal kopjes:……

5.35 **Als 65 jaar of ouder:** En toen u ongeveer 60 jaar oud was.

Aantal kopjes:……

5.36 **Als 45 jaar of ouder:** En toen u ongeveer 40 jaar oud was.

Aantal kopjes:……

5.37 **Als 25 jaar of ouder:** En toen u ongeveer 20 jaar oud was.

Aantal kopjes:……

Nu ga ik u vragen stellen over het gebruik van drugs. Nogmaals wil ik zeggen dat al uw antwoorden vertrouwelijk en anoniem behandeld zullen worden.

Heeft u ooit:

5.38 Marihuana, wiet, of hasj gebruikt? o Ja o Nee o Onbekend o Weigering

5.39 HXTC gebruikt? o Ja o Nee o Onbekend o Weigering

5.40 Amfetamines gebruikt? o Ja o Nee o Onbekend o Weigering

5.41 Heeft u ooit cocaïne gebruikt? o Ja o Nee o Onbekend o Weigering

5.42 Heeft u ooit LSD gebruikt? o Ja o Nee o Onbekend o Weigering

5.43 Heeft u ooit paddo's gebruikt? o Ja o Nee o Onbekend o Weigering

Als ja bij een van de soorten drugs (vraag 5.38-5.43):

5.44 Op welke leeftijd/in welk jaar gebruikte u voor het eerst *(noem drugssoort)*?

Leeftijd:…….Jaar:……

5.45 Op welke leeftijd/in welk jaar gebruikte u deze drugssoort voor het laatst?

Leeftijd:…….Jaar:……

5.46 Heeft u deze drugssoort vaker dan 10 keer gebruikt?

o Ja o Nee → 5.44/6.1 o Onbekend → 5.44/6.1 o Weigering → 5.44/6.1

5.47 **Als ja:** Hoeveel jaar ongeveer heeft u deze drugssoort in totaal gebruikt, als u de jaren aftrekt dat u dit middel niet gebruikt heeft.

Aantal jaar:……

5.48 Hoeveel dagen per maand gebruikte u deze drugssoort ongeveer?

Aantal dagen:…… →5.44 (volgende soort) of 6.1

6. Medische geschiedenis

Dank u wel. Nu ga ik u een aantal vragen stellen over het gebruik van een aantal medicijnen.

6.1 Heeft u ooit statines gebruikt? Deze medicijnen worden voornamelijk voorgeschreven om het cholesterol gehalte in het bloed te verlagen

o Ja o Nee → 6.6 o Onbekend → 6.6 o Weigering → 6.6

6.2 **Als ja:** Welke van de volgende statines heeft u gebruikt? (meerdere antwoorden mogelijk)

- o Atorvastatine, ook wel verkrijgbaar onder de merknaam lipitor
- o Fluvastatine, ook wel verkrijgbaar onder de merknaam lescol
- o Pravastatine, ook wel verkrijgbaar onder de merknaam selectine
- o Rosuvastatine, ook wel verkrijgbaar onder de merknaam crestor
- o Simvastatine, ook wel verkrijgbaar onder de merknaam zocor
- o Onbekend

6.3 In welk jaar of op welke leeftijd heeft u voor het eerst statines gebruikt?

Jaar:…… Leeftijd:……

6.4 In welk jaar of op welke leeftijd heeft u voor het laatst statines gebruikt?

- o Jaar:…… Leeftijd:……
- o Nog steeds gebruik

6.5 Zijn er gedurende deze jaren dat u dit medijn gebruik heeft, jaren geweest dat u het medicijn niet gebruik heeft? En zo ja, hoeveel jaren waren dit ongeveer?

Aantal jaren niet gebruikt:……

Voor vrouwen (als man → 6.17)
Nu komen er wat vragen die specifiek bedoeld zijn voor vrouwen.

6.6 Kunt u zich de leeftijd herinneren waarop u voor het eerst ongesteld werd?

Leeftijd……of jaar:……

6.7 Is uw menstruatie al voorgoed weggebleven?

o Ja o Nee → 6.10 o Onbekend → 6.10 o Weigering → 6.10

6.8 **Als ja:** op welke leeftijd bleef de menstruatie voorgoed weg?

Leeftijd wegblijven menstruatie:……of jaar:……

6.9 Was het wegblijven van de menstruatie het gevolg van het verwijderen van uw baarmoeder of eierstokken?

o Ja o Nee o Onbekend o Weigering

6.10 Heeft u ooit de anticonceptiepil gebruikt?

o Ja o Nee → 6.14 o Onbekend → 6.14 o Weigering → 6.14

6.11 Als **ja:** Hoe oud was u toen u begon met het gebruik van de pil?

Leeftijd begin……of jaartal:……..

6.12 Hoe oud was u toen u definitief bent gestopt met de pil? Of gebruikt u de pil nog steeds?

- Leeftijd gestopt:……
- Nog steeds gebruik

6.13 Hoeveel jaar heeft u de pil in totaal gebruikt, rekening houdend met de jaren dat u de pil niet gebruikt heeft?

- Minder dan een jaar
- 1-4 jaar
- 5-9 jaar
- 10-14 jaar
- 15-19 jaar
- 20 of meer jaar
- Onbekend
- Weigering

6.14 Gebruikt u of heeft u ooit oestrogenen, vrouwelijke hormonen, gebruikt wegens overgangsklachten?

o Ja o Nee → 6.17 o Onbekend → 6.17 o Weigering → 6.17

6.15 **Als ja:** Hoe oud was u toen u begon met het gebruik van deze hormonen in verband met de overgang?

Leeftijd start gebruik:……

6.16 Hoeveel jaar heeft u in totaal tot nu toe deze hormonen gebruikt?

- Minder dan een jaar
- Aantal jaar gebruik……
- Onbekend
- Weigering

6.17 Bent u een maand of langer te vroeg geboren?

o Ja o Nee o Onbekend o Weigering

6.18 Heeft u ooit een hersenschudding gehad, of bent u ooit buiten bewustzijn geraakt door een klap tegen het hoofd of door een val?

o Ja o Nee → 6.23 o Onbekend → 6.23 o Weigering → 6.23

6.19 **Als ja:** Hoe vaak heeft u ooit een hersenschudding gehad, of bent u ooit buiten bewustzijn geraakt door een klap tegen het hoofd of door een val?

Aantal keer:……

6.20 Op welke leeftijd voor het eerst?

Leeftijd:……..of jaartal……

6.21 Op welke leeftijd voor het laatst?

Leeftijd:……..of jaartal……

6.22 Wat is de langste periode dat u ooit, door een hersenschudding in verband met een klap tegen het hoofd of door een val, buiten bewustzijn bent geweest?

Tijd:……

6.23 Heeft u ooit een flinke elektrische schok gehad? Een kleine schok zoals van schrikdraad hoeft u niet mee te tellen, maar bijvoorbeeld een schok tijdens het klussen thuis wel.

o Ja o Nee → 6.27 o Onbekend → 6.27 o Weigering → 6.27

6.24 **Als ja:** Hoe vaak heeft u een dergelijke elektrische schok gehad?

Aantal keer:……

6.25 Op welke leeftijd het eerst?

Leeftijd:……..of jaartal……

6.26 Op welke leeftijd voor het laatst?

Leeftijd:…….. of jaartal……

6.27 Heeft een arts u ooit verteld dat u een beroerte, herseninfarct of een hersenbloeding gehad heeft?

o Ja o Nee o Onbekend o Weigering

6.28 Heeft een arts u ooit verteld dat u een hartinfarct gehad heeft?

o Ja o Nee o Onbekend o Weigering

6.29 Heeft een arts u ooit verteld dat u een verhoogd cholesterol gehalte in uw bloed gehad heeft?

o Ja o Nee o Onbekend o Weigering

6.30 Heeft een arts u ooit verteld dat u astma heeft?

o Ja o Nee o Onbekend o Weigering

6.31 Heeft een arts u ooit verteld dat u hooikoorts heeft?

o Ja o Nee o Onbekend o Weigering

6.32 Heeft een arts u ooit verteld dat u een andere allergie heeft?

o Ja o Nee → 7.1 o Onbekend → 7.1 o Weigering → 7.1

6.33 **Als ja:** waar bent voor allergisch voor?

Allergie……

7. De ziekte van Parkinson in de familie

Ik zou graag willen weten hoe vaak de ziekte van Parkinson voorkomt in uw familie. Het gaat hierbij om directe biologische familie. We zijn geïnteresseerd in uw ouders en uw broers en zussen. Telt u ook eventuele overleden familie mee.

7.1 Heeft een arts u ooit verteld dat u zelf de ziekte van Parkinson heeft?

o Ja o Nee o Onbekend o Weigering

7.2 Heeft uw vader de ziekte van Parkinson gehad?

o Ja o Nee → 7.4 o Onbekend → 7.4 o Weigering → 7.4

7.3 **Als ja:** Was er een diagnose gesteld door een arts, bij het vaststellen van de ziekte van Parkinson bij uw vader?

o Ja o Nee o Onbekend o Weigering

7.4 Heeft uw moeder de ziekte van Parkinson gehad?

o Ja o Nee → 7.6 o Onbekend → 7.6 o Weigering → 7.6

7.5 **Als ja:** Was er een diagnose gesteld door een arts, bij het vaststellen van de ziekte van Parkinson bij uw moeder?

o Ja o Nee o Onbekend o Weigering

7.6 Hoeveel broers heeft u? Aantal broers…….

7.7 **Als 1 of meerdere broers:** Heeft hij/een van hen de ziekte van Parkinson gehad?

o Ja o Nee → 7.10 o Onbekend → 7.10 o Weigering → 7.10

7.8 **Als ja en meer dan 1 broer:** Hoeveel broers hebben de ziekte van Parkinson gehad?

Aantal:……..

7.9 Was er een diagnose gesteld door een arts, bij het vaststellen van de ziekte van Parkinson bij uw broer(s)?

o Ja o Nee o Onbekend o Weigering

7.10 Hoeveel zussen heeft u? Aantal zussen…….

7.11 **Als 1 of meerdere:** Heeft zij/een van hen de ziekte van Parkinson gehad?

o Ja o Nee → einde o Onbekend → einde o Weigering → einde

7.12 **Als ja en meer dan 1 zus:** Hoeveel zussen hebben de ziekte van Parkinson gehad?

Aantal:……..

7.13 Was er een diagnose gesteld door een arts, bij het vaststellen van de ziekte van Parkinson bij uw zus(sen)?

o Ja→ einde o Nee→ einde o Onbekend→ einde o Weigering→ einde

Einde interview

Summary

Parkinson disease (PD) is characterized by movement problems that are the result of a loss of dopaminergic neurons in the substantia nigra, an area in the midbrain. However, the disease affects areas throughout the whole brain. For only a small proportion of patients PD is caused by specific gene mutations. For the majority of cases it is believed that the disease is caused by age related cellular changes that increase vulnerability to neurodegeneration combined with genetic susceptibility and environmental factors. Smoking is the only environmental factor for which a relation with PD is well-established. A strong inverse relation between smoking and PD risk has been observed in many studies including prospective cohort studies, suggesting that smoking protects against the development of PD. Also some occupational exposures have been associated with PD, whereby the strongest evidence is available for pesticide exposure. The main focus of the research described in this thesis was on studying the association between occupational exposures and PD risk.

The PAGES study is a case-control study conducted in the Netherlands. Cases and controls were selected from five hospitals. Detailed information about lifestyle and occupational risk factors were ascertained by a telephone interview. The goal was to include all patients diagnosed between January 2006 and December 2011 in the participating hospitals. Two age and sex matched controls per PD patient were selected from individuals who visited the hospital between 2006 and 2011 for a range of non-neurodegenerative neurological complaints. The participation rate was 45% for cases and 35% for controls. The analyses as reported in this thesis were performed on 444 PD cases and 876 controls.

We conducted analyses on smoking behavior and observed a strong inverse association of cigarette smoking with PD risk. This lends support to the internal validity of the case-control study. We observed a weak inverse association between PD risk and coffee consumption and no association with alcohol consumption. In addition, we applied a novel modeling approach to explore the potential effect modification by intensity, duration and time-since-cessation of smoking on the association between total smoking and PD risk. We observed effect modification by time-since-cessation with a diminishing protective effect of total smoking after cessation of smoking.

A systematic review and meta-analysis of the literature published on PD and use of pesticides was performed. A total of 39 case-control studies, 4 cohort studies and 3 cross-sectional studies were identified and a significantly increased summary risk ratio of 1.62 was found (ever vs. never use). Meta-analyses on studies that

investigated the use of functional sub-classes of pesticides indicated a positive association with herbicides and insecticides, but not with fungicides. We further investigated the observed heterogeneity in study outcomes via stratification and meta-regression. The results did not indicate that heterogeneity was related to type of study design, source of control population, adjustment of results for potential confounders, or geographical area of study. However, the results were suggestive for an effect of method of exposure assessment: studies using job-title based exposure assignment found higher risk estimates than studies using self-reported information on pesticide use.

For the present case-control study, we tried to improve upon methods for exposure assessment by combining self-reported information, a job-exposure matrix (JEM), an exposure algorithm and a crop-exposure matrix to assess occupational exposure to pesticides from both application and re-entry work. The results did not show statistically significant associations between pesticide exposure and PD. However, elevated odds ratios in the higher exposure categories for insecticides, herbicides and fungicides were suggestive for a positive association. The analyses on specific active ingredients for which we constructed a crop-exposure matrix revealed a significant association of PD risk with the fungicide benomyl. We also analyzed the association with occupational exposure to endotoxin. Endotoxins are the lipopolysaccharide components of gram-negative bacterial cell walls, and like pesticides, exposure is common during agricultural work. It has been suggested that exposure to endotoxin may increase PD risk, but the results of our study do not provide support for such an association.

Results of previous studies in the aggregate did not indicate an association between exposure to extremely low-frequency magnetic fields (ELF-MF) and PD. We performed the most comprehensive analyses to date on the association between electricity related exposures and PD. An improvement of our study compared to most previous studies is that we used PD patients as confirmed by neurologists instead of relying on information on death certificates on which PD is often not mentioned. We used JEMs that were linked to the occupational histories of participants to estimate lifetime occupational exposure to ELF-MF and electrical shocks. Furthermore, we analyzed ever having worked in so-called ‘electrical jobs’, self-reported number of electrical shocks and use of selected electric household appliances. No associations with PD were observed for any of these exposure indices. Given the results of the previous studies on ELF-MF and the absence of any postulated mechanism, this strongly suggests that no relation exists between PD and exposures related to electricity.

It is known that high exposure to some organic solvents and the metal manganese can induce neurological effects with Parkinsonian symptoms. However, the results of previous studies that investigated the potential associations between metals and organic solvents and PD risk were inconclusive. We estimated lifetime occupational exposure to metals and chlorinated and aromatic solvents using JEMs. Because welding fumes may contain low levels of manganese and other metals, we additionally analyzed self-reported (electric) welding. Although slightly elevated risk estimates for the highest exposure categories for aromatic solvents were observed, we did not observe statistically significant associations. As such, our results do not provide evidence for an increased chance on developing PD after exposure to metals or solvents. For frequent welding, we observed a reduced risk of PD. This is in line with previous research, but evidence for a plausible mechanism of a true protective effect is lacking.

Methodological issues that may have given some concern about generalizability of the results presented in this thesis are the low participation rate and a potential link between the neurological conditions of the controls and the occupational risk factors studied in this thesis. Similar results were obtained, however, when analyses were conducted with only the participants under age 70 for who participation rates were higher, and when the analyses where repeated with subgroups of neurological conditions removed from the control group, indicating that results were not unduly biased by the low participation or conditions in the control group. An important methodological factor that affects quality of an epidemiological study is which exposure assessment method has been applied. We used several JEMs for estimating lifetime exposure to occupational risk factors. A limitation of a JEM is that it does not account for variation in exposure between persons with similar jobs, most likely resulting in non-differential misclassification and attenuation of existing risks. For pesticides, we were able to use self-reported information about pesticide use to increase specificity of the exposure assessment. In analyses where the combined information was used we observed stronger associations. This suggests indeed that the combination of self-reported information and a JEM increased the validity of the exposure assessment. Another issue in exposure assessment is the choice of the appropriate exposure metric. This is illustrated in the analyses on smoking where we showed that not only cumulative exposure determines risk, but also time-since-cessation. Lastly, the analyzed categories of pesticides, organic solvents and metals constitute out of a large range of different agents. Possibly, existing effects of specific pesticides, solvents and metals were diluted in our analyses. This was exemplified by our finding of benomyl which was not observed in the broader analyses. New epidemiological studies should therefore focus on further improving methods to identify specific substances associated with

PD, possibly by using more detailed exposure matrices such as the crop-exposure matrix that we used, or by assessing specific exposures in large (occupational) cohort studies.

As summarized above, our study contributed to the literature on lifestyle and occupational exposures in relation to PD risk. The main findings were the observed diminishing protective effect of total smoking after cessation of smoking, the suggestive association with pesticide exposure in particular with the fungicide benomyl, and the absence of associations with exposures related to electricity.

Nederlandse samenvatting

De ziekte van Parkinson is een progressieve ziekte die wordt gekenmerkt door stoornissen in het bewegen. Deze worden veroorzaakt door een verlies aan dopamine producerende zenuwcellen in de substantia nigra, een gebied in de middenhersenen. De ziekte tast daarnaast ook andere delen van de hersenen aan, waardoor behalve problemen met bewegen ook allerlei andere klachten kunnen ontstaan zoals slaapproblemen of vergeetachtigheid. Een klein deel van de patiënten heeft een erfelijke variant van de ziekte, maar voor de meeste patiënten is de oorzaak onbekend. Het risico op het krijgen van de ziekte van Parkinson neemt toe met leeftijd. Omgevingsfactoren kunnen mogelijk dit risico beïnvloeden. Eerdere epidemiologische studies hebben laten zien dat de ziekte van Parkinson minder vaak voorkomt onder (ex-)rokers, wat suggereert dat roken een beschermend effect heeft. Daarentegen is een aantal beroepsmatige blootstellingen in verband gebracht met een hoger risico op het krijgen van de ziekte, waaronder blootstelling aan gewasbeschermingsmiddelen (pesticiden). Het onderzoek dat is beschreven in dit proefschrift richt zich voornamelijk op de relatie tussen beroepsmatige blootstellingen en het risico op het krijgen van de ziekte van Parkinson.

Om meer duidelijkheid te krijgen over de verschillende risicofactoren is een patiënt-controleonderzoek opgezet in Nederland. Voor dit onderzoek is een telefonisch interview afgenomen bij een groep van 444 patiënten met de ziekte van Parkinson en een groep van 876 controlepersonen. Het doel was om alle patiënten te includeren die tussen 2006 en 2011 waren gediagnosticeerd met de ziekte van Parkinson in één van de vijf deelnemende ziekenhuizen. Per deelnemende patiënt zijn vervolgens twee op leeftijd en geslacht gematchte personen geselecteerd als controles die hetzelfde ziekenhuis hebben bezocht in dezelfde periode met niet-neurodegeneratieve neurologische klachten. Van de uitgenodigde patiënten nam 45% deel en van de uitgenodigde controles 35%. In het telefonische interview zijn onder andere gedetailleerde vragen gesteld over uitgevoerde beroepen gedurende het hele leven. Deze beroepshistories hebben we gecodeerd aan de hand van een internationaal classificatiesysteem, zodat deze kunnen worden gekoppeld aan beroepsblootstellingsmatrices (“job-exposure matrices” - JEMs). JEMs geven per beroep een schatting weer van de mate van een specifieke blootstelling. Op deze manier kon voor alle deelnemers van het patiënt-controleonderzoek de mate van verschillende beroepsmatige blootstellingen worden geschat.

In overeenstemming met de literatuur vonden wij ook in onze studie een sterke relatie tussen roken en de ziekte van Parkinson. De resultaten gaven aan dat hoe meer sigaretten men had gerookt, hoe lager het risico was op de ziekte van

Parkinson. Ook hebben we een nieuwe manier van modelleren gebruikt om de invloed te onderzoeken van het gemiddeld aantal gerookte sigaretten per dag, het aantal jaren gerookt en het aantal jaar geleden dat is gestopt met roken. De resultaten gaven dat stoppen met roken het verband tussen het totaal aantal gerookte sigaretten en het risico op de ziekte van Parkinson beïnvloedt. Een minder sterk verband met het risico op de ziekte werd gevonden naarmate men meer jaren is gestopt met roken. Daarnaast hebben we ook gekeken naar de consumptie van koffie en alcohol. De resultaten hiervan lieten een mogelijk zwak beschermend effect van hoge koffieconsumptie zien, maar geen verband tussen alcoholconsumptie en de ziekte van Parkinson.

Alvorens de relatie tussen blootstelling aan pesticiden en de ziekte van Parkinson te hebben onderzocht met de data van het patiënt-controleonderzoek, hebben we een systematische review en meta-analyse uitgevoerd van eerder gepubliceerde onderzoeken. In totaal hebben we 39 patiënt-controleonderzoeken, 4 prospectieve cohortonderzoeken en 3 cross-sectionele onderzoeken gevonden. De meta-analyse liet een statistisch significant verband zien met het ooit hebben toegepast van pesticiden (gewogen gemiddeld relatief risico: 1.62). Dit betekent dat mensen die in het verleden pesticiden hebben toegepast, 1.62 keer zoveel kans op het krijgen van de ziekte van Parkinson hebben als mensen die dit niet hebben. Meta-analyses van eerdere onderzoeken die keken naar het toepassen van subgroepen van pesticiden lieten een verband zien met insecticiden en herbiciden, maar niet met fungiciden. De eerdere onderzoeken lieten wel grote verschillen zien in de uitkomsten (heterogeniteit). Dit hebben we verder onderzocht met behulp van gestratificeerde analyses en meta-regressie. We vonden geen aanwijzingen dat de heterogeniteit in uitkomsten is gerelateerd aan type onderzoek (patient-controleonderzoek, cohortonderzoek of cross-sectioneel onderzoek), bron van de controlegroep (ziekenhuis of algemene populatie), statistische correctie voor mogelijke confounders (andere gerelateerde factoren die het onderzochte verband kunnen verstoren zoals rookgedrag) of aan de locatie van het onderzoek (Noord-Amerika, Europa of elders). Wel suggereerden de uitkomsten van onze analyses een verband met de gebruikte methode om blootstelling te bepalen. Onderzoeken die blootstelling bepaalden aan de hand van uitgevoerde beroepen vonden sterkere verbanden dan onderzoeken die gebruik maakten van zelf-gerapporteerde informatie over het gebruik van pesticiden.

Voor ons eigen patiënt-controleonderzoek hebben we gebruik gemaakt van verbeterde methoden om beroepsmatige bloostelling aan pesticiden te schatten. Zelf-gerapporteerde informatie over gebruik van pesticiden is gecombineerd met het schatten van blootstelling aan de hand van de beroepshistories, waarbij we

onder meer gebruik hebben gemaakt van een JEM. Daarbij is zowel blootstelling tijdens het spuiten van pesticiden, als blootstelling die wordt opgelopen tijdens het werken met gewassen korte tijd nadat pesticiden gespoten zijn, meegenomen. De analyses lieten geen statistisch significante associaties zien, echter suggereerden de resultaten een licht verhoogd risico op de ziekte van Parkinson voor de hoogst blootgestelden aan insecticiden, herbiciden en fungiciden. Een gewasblootstellingsmatrix was ontwikkeld om blootstelling aan specifieke pesticiden te schatten voor deelnemers die in de land- of tuinbouw hebben gewerkt aan de hand van gerapporteerde gewassen en de tijdsperiode. We vonden hiermee een statistisch significante associatie met het fungicide benomyl. Daarnaast hebben we een JEM gebruikt om naar de invloed van blootstelling aan endotoxinen (afkomstig van bepaalde typen bacteriën) te kijken. Blootstelling aan endotoxinen in net als voor pesticiden in de agrarische sector veelvoorkomend. Het is eerder geopperd dat ook blootstelling aan endotoxinen het risico op de ziekte van Parkinson zou kunnen verhogen, maar wij vonden hier geen aanwijzingen voor.

Wij hebben de meest uitgebreide analyses op het gebied van blootstellingen gerelateerd aan elektriciteit uitgevoerd. Eerdere onderzoeken toonden geen verband aan tussen blootstelling aan extreem laagfrequente magnetische velden (ELF-MV) en de ziekte van Parkinson. Een verbetering van onze studie ten opzichte van de meeste eerdere onderzoeken is dat we de patiënten geselecteerd hebben in ziekenhuizen waarbij de diagnose is bevestigd door neurologen in plaats van te vertrouwen op informatie op overlijdensakten waarop de ziekte van Parkinson vaak niet wordt vermeld. Om beroepsmatige blootstelling te schatten aan ELF-MV en elektrische schokken hebben we gebruikt gemaakt van JEMs. Verder analyseerden we het ooit hebben uitgevoerd van een beroep op een lijst met ‘elektrische beroepen’, het zelf-gerapporteerde aantal elektrische schokken dat deelnemers gedurende het leven hebben ervaren en het gebruik van geselecteerde elektrische huishoudelijke apparaten. In geen van deze analyses vonden we een associatie met de ziekte van Parkinson. Gezien de resultaten van vorige onderzoeken naar ELF-MV en het ontbreken van een waarschijnlijk biologisch mechanisme, suggereert dit sterk dat er geen relatie bestaat tussen blootstellingen op het gebied van elektriciteit en de ziekte van Parkinson.

Het is bekend dat hoge blootstelling aan bepaalde organische oplosmiddelen en aan het metaal mangaan neurologische effecten kan veroorzaken met symptomen die lijken op de ziekte van Parkinson. Echter, de resultaten van eerdere onderzoeken die een relatie tussen blootstelling aan zware metalen of aan organische oplosmiddelen en de ziekte van Parkinson onderzochten waren niet eenduidig. Om beroepsmatige blootstelling aan metalen en chloorhoudende en aromatische

oplosmiddelen te schatten gebruikten we JEMs. Hoewel we een zwak verband waarnamen tussen de hoogste blootstellingscategorieën van aromatische oplosmiddelen en de ziekte van Parkinson, vonden we geen statistisch significante associaties. Als zodanig geven onze resultaten geen bewijs voor een verhoogde kans op het ontwikkelen van de ziekte van Parkinson na blootstelling aan metalen of oplosmiddelen. Omdat lasrook lage niveaus van mangaan en andere metalen kan bevatten, hebben we ook zelf-gerapporteerde informatie over (elektrisch) lassen geanalyseerd. Voor individuen die frequent hadden gelast, zagen we een verminderd risico op de ziekte van Parkinson. Alhoewel dit in lijn is met eerder onderzoek, ontbreekt bewijs voor een biologisch mechanisme voor een beschermend effect.

Methodologische factoren die enige bezorgdheid over de generaliseerbaarheid van de gepresenteerde resultaten in dit proefschrift zouden kunnen geven, zijn de lage participatie en een mogelijk verband tussen de neurologische klachten van de controles en de blootstellingen die we in dit proefschrift hebben onderzocht. Vergelijkbare resultaten werden echter verkregen in analyses met uitsluitend deelnemers jonger dan 70 waarbij het deelnamepercentage hoger was. Ook uit analyses waarbij subgroepen van controles (gebaseerd op neurologische aandoeningen) uit de controlegroep gehouden zijn kwamen vergelijkbare resultaten. Dit geeft aan dat de resultaten niet te veel werden beïnvloed door de lage participatie of de samenstelling van de controlegroep.

Ook de methode die is toegepast om blootstelling te bepalen, is een belangrijke methodologische factor die van invloed is op de kwaliteit van een epidemiologische studie. In ons onderzoek maakten we gebruik van verschillende JEMs voor het schatten van beroepsmatige blootstellingen. Een beperking van een JEM is dat geen rekening wordt gehouden met de variatie in blootstelling tussen personen met vergelijkbare beroepen waardoor de blootstelling niet altijd goed wordt ingeschat. Het meest waarschijnlijke gevolg hiervan is dat bestaande verbanden afgezwakt (of zelfs helemaal niet meer) waargenomen worden. Voor pesticiden was het mogelijk om zelf-gerapporteerde informatie over het gebruik van pesticiden te gebruiken om de specificiteit van de blootstellingsbepaling te verhogen. We zagen sterkere associaties wanneer we van deze informatie gebruik maakten, hetgeen suggereert dat de combinatie van zelf-gerapporteerde informatie en een JEM de beoordeling van de blootstelling verbetert. Een ander probleem is het bepalen van de meest relevante blootstelling. Dit werd duidelijk in de analyses van roken waar we zien dat niet alleen cumulatieve blootstelling het risico bepaalt, maar ook het aantal jaar dat is gestopt met roken. Ook beslaan de geanalyseerde categorieën van bestrijdingsmiddelen, organische oplosmiddelen en metalen een groot aantal

verschillende stoffen. Mogelijk zijn bestaande effecten van specifieke pesticiden, oplosmiddelen en metalen daardoor afgezwakt in onze analyses. Dit wordt geïllustreerd door het significant naar voren komen van de invloed van benomyl in de specifieke analyse, wat niet werd waargenomen in de bredere analyses. Nieuwe epidemiologische studies moeten zich daarom richten op de verbetering van de methoden om specifieke stoffen te identificeren die gerelateerd zijn aan de ziekte van Parkinson. Dit kan mogelijk door het gebruik van meer gedetailleerde blootstellingsmatrices, zoals de gewasblootstellingsmatrix die wij hebben gebruikt, of door het onderzoeken van blootstellingen in grote (industrie-specifieke) prospectieve cohortonderzoeken.

Onze studie heeft een bijdrage geleverd aan de bestaande literatuur over lifestyle-gerelateerde en beroepsmatige blootstellingen en het risico op de ziekte van Parkinson. De belangrijkste bevindingen omvatten: het waargenomen beschermend effect van roken dat afneemt met het aantal jaren dat is gestopt; de suggestieve associatie met blootstelling aan pesticiden, in het bijzonder met het fungicide benomyl; en de afwezigheid van associaties met blootstellingen gerelateerd aan elektriciteit.

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About the author

Marianne van der Mark werd geboren in Voorburg op 5 maart 1985. Na in 2003 haar VWO-diploma behaald te hebben aan College het Loo te Voorburg, verhuisde ze naar Wageningen om Voeding en Gezondheid te gaan studeren. Tijdens haar Master koos ze voor de specialisatie Moleculaire Voeding. Haar grote afstudeervak voerde ze uit bij het RIKILT, waar ze de effecten van polyfenolen op het energie metabolisme van vetcellen onderzocht. In 2008 verbleef ze 4 maanden in Stockholm voor een stage bij de afdeling Klinische Epidemiologie van het Karolinska Institutet, waar ze heeft meegewerkt aan verschillende projecten. Na haar afstuderen vertrok ze in 2009 naar Utrecht, waar ze aan het Institute for Risk Assessment Sciences (IRAS) aan het promotieonderzoek begon dat is beschreven in dit proefschrift. Sinds februari 2014 is ze werkzaam bij het Integraal Kankercentrum Nederland (IKNL).

Marianne van der Mark was born in Voorburg, the Netherlands, on March 5, 1985. After completing her secondary education (VWO) at College het Loo in Voorburg in 2003, she moved to Wageningen to study Nutrition and Health. During her Master's program she chose the specialization Molecular Nutrition. She conducted her major thesis at RIKILT, where she studied the effects of polyphenols on energy metabolism of adipocytes. In 2008 she spent 4 months in Stockholm for an internship, where she worked on several projects at the Unit of Clinical Epidemiology of Karolinska Institutet. After graduation she moved to Utrecht in 2009, where she started the PhD project presented in this thesis at the Institute for Risk Assessment Sciences (IRAS). In 2014 she started working at the Comprehensive Cancer Centre the Netherlands (IKNL).