2.1. Pesticide exposure and Parkinson’s disease (PD): epidemiological studies

In recent years the use of pesticides in agriculture has been increasing steadily all over the world. There are over 1,000 chemicals classified as the pesticides (Zeljezic and Garaj-Vrhovac, 2001). All populations have a degree of risk in relation to the pesticide exposure from agricultural and non-agricultural use and through the residues in the food. However, workers employed in the pesticide production / spraying and the farmers who use them have a higher risk of exposure and hence are more prone to the potential health effects of the pesticides.

International status

In humans, a neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) that specifically destroys substantia nigra cells, creates a condition which is practically indistinguishable from Parkinson’s disease (PD). Langston et al. (1984) reported that MPTP is rapidly metabolized after systemic administration in toxic amounts, and identified 1-methyl-4-phenylpyridinium ion (MPP+) as a possible major metabolite. This study provided evidence that MPTP, which is structurally similar to the herbicide paraquat, can cause degeneration of dopaminergic neurons and can cause Parkinsonism.

Barbeau et al. (1987) studied a homogeneous genetic and ethnic rural population of Quebec, Canada to find out the distribution of the prevalence of PD in the 9 rural hydrographic regions of the Province. Using three different ascertainment methods, the
authors demonstrated that the prevalence of PD was unevenly distributed within rural areas. By investigating the characteristics of the regions of high prevalence, they found that these regions are predominantly agricultural and areas of intensive market gardening were also the areas with the highest use of the pesticides.

To investigate possible risk factors for PD, Koller et al. (1990) performed a case-control study of 150 PD patients and 150 age- and sex-matched controls. The authors interviewed and examined all the subjects for the demographic details including lifetime histories of residence, drinking water source, and occupations for example farming. The subjects were given a detailed questionnaire regarding herbicide/pesticide exposure. Authors found that rural living and drinking well water were significantly increased in the PD patients. However, their study showed that epidemiological evidence linking PD with pesticides has been inconsistent.

Wong et al. (1991) conducted a case-control study of 19 families having two or more siblings with PD. Demographic data were collected, comprising drinking water sources, histories of residence, pesticides/herbicides exposure and occupations. The authors found that rural living and drinking well water were significantly increased in PD compared with control subjects but farming and herbicide exposure did not show association with PD. These data suggested that living in a rural environment and drinking well water are risk factors for PD and that the total life exposure to an environmental toxin may be more important than exposure in early life.

To determine whether a history of exposure to rural environmental factors leads to an increased risk of developing idiopathic PD, Semchuk et al. (1991) conducted a case-control study of 130 cases and 260 randomly selected community controls in the city of
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Calgary in Province of Alberta, Canada. Significant increase was not found in risk for PD associated with a history of rural / farm living, or drinking well water in early childhood or at any time during the first 45 years of life.

In an another study from Calgary, Semchuk et al. (1992) reported a population-based case-control investigation of 130 residents with neurologist-confirmed idiopathic PD and 260 randomly selected age- and sex-matched community controls to determine whether agricultural work or the occupational use of pesticide chemicals was associated with an increased risk for PD. The authors obtained the data by personal interviews regarding lifetime occupational histories, including chemical exposure data. A history of farming, herbicide or insecticide use resulted in a significantly increased crude estimate of the PD risk and based on this the authors suggested a dose response relation between the PD risk and the increasing lifetime exposure to farming. But in the multivariate statistical analysis, after controlling for confounding factors / interaction between the exposure variables, previous occupational herbicide exposure was consistently the only significant forecaster of PD risk. These results supported the hypothesis that the occupational exposure of herbicides is associated with an increased risk for PD.

Butterfield et al. (1993) conducted a study on young-onset Parkinson's disease (YOPD) to examine occupational and environmental factors associated with PD risk. This study included 63 YOPD patients and 68 controls. After controlling the variables of race (ethnicity), educational level, sex, age, age at diagnosis, and family history of the disease, PD was found to be positively associated with insecticide exposure, herbicide exposure and rural residency at the time of the diagnosis. These findings are consistent with the hypotheses linking PD to exposure to pesticides.
Permanent PD was observed in a subject with chronic exposure to the fungicide maneb (manganese ethylene-bis-dithiocarbamate) (Meco et al., 1994). Symptoms developed at 37 years of age, two years after exposure had ceased. According to the authors, this was the second report on PD associated with exposure to maneb. Manganese is a recognized PD toxin in humans and dithiocarbamates can also induce extrapyramidal syndromes. The authors suggested that the biochemical effects of manganese and dithiocarbamates and their plausible neurotoxic mechanisms may have played a role in this case.

Fleming et al. (1994) analysed organochlorine pesticides in postmortem brain samples of 20 PD, 7 Alzheimer's disease (AD), and 14 non-neurological control cases. These three groups were matched in age at death, sex, and demographic variables. Residual levels of an organochlorine pesticide dieldrin, a lipid-soluble, long-lasting mitochondrial poison, were found in the brain of one third of PD patients compared with the controls. Despite the relatively small number of brains analysed, the association between dieldrin and the diagnosis of PD was found to be highly significant. This finding reflects that unlike organophosphates and other pesticides, organochlorine insecticides persist in the tissues for many years after the exposure is ceased. Although these findings indicate that organochlorine pesticides reach the brain tissue, but this do not prove that they cause PD and also do not identify which pesticides may be responsible.

To explore environmental risk factors for PD in Taiwan, Liou et al. (1997) investigated 120 patients with PD and 240 age and sex matched control subjects. Based on a structured open-ended questionnaire, the authors carried out standardized interviews to obtain history of exposure to environmental factors, including residence, drinking
water source and environmental / occupational exposures to various agrochemicals. The history of living in a rural environment, farming, use of herbicide/pesticides, and use of paraquat were found to be associated with an increased PD risk in a dose-response relationship. The PD risk was greater among the subjects who had used paraquat than those who had used herbicide/pesticides other than paraquat. This study suggested that environmental factors, especially exposures to paraquat and herbicide/pesticides, may play important role in the development of PD in Taiwan.

Gorell et al. (1998) evaluated pesticides exposure, farming, well water drinking and rural living as risk factors for PD in a population based study consisting of men and women ≥ 50 years of age who had primary medical care at Henry Ford Health System in metropolitan Detroit, USA. The results suggested that PD is associated with occupational exposure to herbicides, insecticides and to farming.

The in vivo results presented by Yang and Sun (1998) provided evidence that neurotoxicity of paraquat may be a consequence of cellular lipid peroxidation, which leads to cell death and may have great implications in assessing the risk of exposure to paraquat in PD.

In a study of caudate nucleus, one of three basic structures that make up the basal ganglia, obtained from post mortem patients with PD and from controls, Corrigan et al. (1998) found significantly higher concentrations of the organochlorine insecticide dieldrin in the PD tissue. A higher amount of the insecticide in the PD tissue suggested the association between PD and rural living.
In an etiological study in the twins, Tanner et al. (1999) strongly suggested that environmental factors played an important role in typical, non-familial PD, beginning after 50 years of age. Epidemiological risk factor analyses of PD cases identified several neurotoxicants, including MPP(+), salsolinol, dieldrin, manganese and paraquat. The authors demonstrated that during dopaminergic cell death cascades, MPP(+), the neurotoxicants and an oxidant, H₂O₂ can equally induce the ROS dependent events.

Amr (1999) reported health profiles of 300 pesticide formulators and an equal number of the pesticide applicators and revealed peripheral neuritis (>40%), psychiatric manifestations (>40%), electroencephalographic (EEG) changes (>25%), and hepatorenal dysfunction (>80%) in the pesticide applicators. He found the pesticide residues such as DDT, HCH and dieldrin in cheese, milk, yoghurt, milk powder and butter samples.

Corrigan et al. (2000) compared the concentrations of organochlorine compounds in the substantia nigra in PD patients with the brains of cortical Lewy body dementia (CLBD), Alzheimer's disease (AD), and non-demented non-Parkinsonian controls. The levels of the gamma isomer of hexachlorocyclohexane (gamma HCH, lindane) were found to be significantly higher in PD brain tissues than in the other groups. Similarly, dieldrin, total Aroclor-matched polychlorinated biphenyls and 1,1'-(2,2-dichloroethenyl diene)-bis(4-chlorobenzene) (p,p-DDE) were found higher in PD substantia nigra than in AD or control brains.

Ritz and Yu (2000) employed a proportional odds mortality design to compare all cases of PD recorded as underlying (1984-1994) or associated causes (1984-1993) of death occurring in California, USA with all deaths from ischaemic heart disease during the same period. Based on pesticide use report data they classified California counties
into several pesticide use categories. Mortality from PD as the underlying cause of death was higher in agricultural pesticide using counties than in pesticide non using counties. A dose response was noted for insecticide use per county land treated using 1982 agricultural census data. However, no such observation was made for amounts of restricted pesticides used and length of residency in a country prior to death. The data showed an increased PD mortality in California counties using agricultural pesticides.

Engel et al. (2001) studied 310 subjects who were given a structured neurological examination and completed a self-administered questionnaire which elicited detailed information on the pesticide (insecticide, herbicide and fungicide) used throughout their working careers. A generalised linear model was used to estimate prevalence ratios for Parkinsonism relative to the history of farming and pesticide use. Parkinsonism may be associated with long term occupational exposure to the pesticides, although no associations with specific pesticides could be detected. This finding was consistent with most of the publications on this topic.

According to Jenner (2001) selective nigral degeneration with inclusion formation provoked by systemic administration of the herbicide rotenone brings up the issue of pesticide exposure and environmental factors in general, as a cause of PD. Toxin-induced complex I inhibition probably represents one of many potential causes of PD, but it alerts to the dangers of such substances in the environment and the need to identify genetically susceptible populations; when vulnerable individuals become known, they should stay out of the garden.

Uversky et al. (2002) stated that certain pesticides and metals induce a conformational change in α-synuclein and directly accelerate the rate of formation of α-
synuclein fibrils; the simultaneous presence of pesticide and metal led to synergistic effects on the rate of fibrillation. The authors proposed a model in which environmental factors in conjunction with genetic susceptibility may form the underlying molecular basis for idiopathic PD.

To determine whether working on a plantation in Hawaii and exposure to the pesticides are associated with an increased risk of PD decades later, Petrovitch et al. (2002) carried out a 30 year follow-up prospective cohort study in the Oahu island of Hawaii. A questionnaire at study enrolment in 1965 was used to assess years of plantation work. Self-reported information on the pesticide exposure was collected at a separate examination 6 years later. Incident of PD was determined by medical record review or by an examination conducted by a study neurologist at a later date. These longitudinal observations regarding plantation work in Hawaii support case-control studies suggesting that exposure to pesticides increased the risk of PD.

In a prospective cohort study of 1,507 French elderly (1992-1998), Baldi et al. (2003) investigated the hypothesis that exposure to the pesticides could be related to central nervous system disorders. Lower cognitive performance was observed in subjects who had been occupationally exposed to the pesticides. In men, the relative risks of developing PD and Alzheimer's disease for occupational exposure was assessed by a job exposure matrix. No association was found with environmental pesticide exposure or having a primary job in agriculture. These results suggested the presence of neurologic impairments in the elderly persons who were exposed occupationally to the pesticides.

Firestone et al. (2005) assessed self-reported pesticide exposures using a structured interview of 250 PD case patients and 388 age and sex matched healthy control subjects.
Odds ratios (ORs) and 95% confidence intervals (CIs) were determined using logistic regression models, controlling for age, sex, and smoking. The findings for occupational pesticide exposures were consistent with a growing body of information linking the pesticide exposures with PD.

**National status**

The pesticide production in India is a year-round activity. The pesticide industry workers work 8 hours per day, 6 days per week and are constantly exposed to different pesticides. However, studies on genetic damage in such workers exposed to pesticides in India are meagre (Padmavathi et al., 2000).

A case study on five patients in Mumbai demonstrated acute and reversible Parkinsonism due to organophosphate pesticide intoxication (Bhatt et al., 1999). The authors proposed genetic susceptibility to organophosphate pesticide-induced Parkinsonism for three family members developing this syndrome. They described the clinical syndrome affecting the patients who were presented with recent organophosphate exposure and showed symptoms of an acute akinetic-rigid syndrome. All patients developed Parkinsonism that resembled PD clinically. Three genetically related patients were exposed to the pesticides in a common environment before the onset of Parkinsonism while their other family members were not affected. The confounding factors of Parkinsonism were excluded. One patient was lost to follow up and remaining four patients recovered completely without any treatment. One patient experienced repeated occurrence of Parkinsonism with accidental re-exposure to a pesticide contaminated environment. The clinical course of these five patients suggested that their
syndrome represented an unreported toxic effect of organophosphate pesticides. These observations support the role of epidemiologic studies in implicating organophosphate pesticides in the aetiology of PD.

A series of meta-analysis of peer-reviewed studies was performed by Priyadarshi et al. (2000) using 19 studies published between 1989 and 1999. Significant heterogeneity among the studies was detected and the random effect model was used to calculate combined odds ratio (OR). Most of the studies reported consistent increase in the risk of PD with exposure to the pesticides. The risk for PD was found to be about 90% higher among individuals who reported exposure to pesticides than those who were not exposed.

The selective dopaminergic toxicity of rotenone and other pesticides has been demonstrated in experimental studies. Exposure to pesticides such as paraquat or manganese ethylene-bis-dithiocarbamate (maneb) during critical periods of development could permanently damage the nigrostriatal dopaminergic neurons that enhance its vulnerability to subsequent exposures to neurotoxicants (Thiruchelvam et al., 2000 a,b, 2002).

To investigate the possible impact of environmental risk factors for idiopathic PD, a case-control study was performed on 175 PD patients (140 men, 35 women) and 350 non-Parkinson age-sex matched controls in the Eastern India (Sanyal et al., 2010) during January 1st, 2006 to December 10th, 2009. The subjects were given a structured neurological examination and completed an administered questionnaire which elicited detailed information on demographic data, pesticides / herbicides usage, family history, occupation, dietary and smoking habits. The multivariate analysis revealed that smoking appeared to be a protective factor while family history of PD, history of depression,
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pesticide exposure, exposure to other toxins, and rural living were associated with increased risk of PD. Results of this study supported the hypothesis of multifactorial aetiology of PD with environmental factors acting on a genetically susceptible host.

2.2. Pesticide exposure and cytogenetic studies

Several bio-monitoring studies have evaluated cytogenetic effects in the pesticide exposed workers from different countries and some of them have found increase of cytogenetic damage in the exposed groups. Several studies in populations exposed to the pesticides have demonstrated that the analyses of chromosomal aberrations (CAs), sister chromatid exchanges (SCEs) and micronuclei (MN) are good methods of detecting increases of cytogenetic damage in the exposed individuals.

International status

Yoder et al. (1973) studied lymphocyte cultures from 42 pesticide applicators and 16 controls for chromosomal aberrations during the midwinter ebb in pesticide spraying operations, and again during the peak summer period of intense spraying activity in USA. Cultures prepared from the exposed individuals during the heavy spraying period showed a marked increase in the frequency of chromatid lesions. This trend was especially noticeable among workers exposed primarily to herbicides. A few chromatid exchanges were also noted among the exposed group.

Crossen and Morgan (1978) investigated herbicide and pesticide sprayers and controls for the incidence of sister chromatid exchanges (SCEs) and found no difference between them. Five sprayers had an SCE rate higher than the control group. According to
the authors, the possible factors contributing to this elevated SCE rate were the failure to use protective clothing and the length of spraying time.

Cytogenetic data on the workers exposed to the pesticides in Be´ke´s County, Hungary, were reported by Nehez et al. (1981). The aim of this investigation was to evaluate some pathognomonic phenomena in men by long-term effects of the pesticides. The chromosomes were evaluated and some of the data showed probable mutagenic effect.

Dulout et al. (1985) studied the SCE and chromosomal aberrations (CA) in a population of floriculturists occupationally exposed to carbamate, organochlorine and organophosphorus pesticides. The blood samples from 36 individuals from a community of 154 persons of the Asiatic origin were studied. In the total material sampled, 21 individuals exhibited at least one symptom of chronic intoxication. The SCE analysis was performed in 14 symptomatic and 13 asymptomatic persons. The case-control analysis of 9 pairs matched by sex and age also showed significant difference. In contrast, the frequencies of CA were not correlated with symptoms of intoxication, although a significant increase of exchange type aberrations in relation to a group of non-floriculturists was observed in the population studied.

Paldy et al. (1987) carried out cytogenetic analysis of 80 workers professionally exposed to a complex of pesticides and 24 control persons. There was a significant increase of chromosomal aberrations (CA) in relation to the duration of exposure. Internal examinations revealed a more frequent occurrence of acute as well as chronic diseases among the workers aged 21-40 years.
Carbonell et al. (1990) studied SCE in the lymphocytes of 27 agricultural workers occupationally exposed to several pesticides and 28 matched controls from el Maresme, an agricultural area near Barcelona, Spain. The t-test did not reveal significant differences between the studied groups and the findings suggested that possibly the exposure level was too low to increase SCE in the lymphocytes.

De Ferrari et al. (1991) studied CA and SCE in the lymphocytes of 32 healthy individuals working in the flower industry and exposed to pesticides, 32 individuals exposed as above and hospitalized for bladder cancer, and 31 controls. Compounds to which floriculturists were exposed included 4 hydrocarbon derivative herbicides, 5 inorganic insecticides and fungicides, 9 nitro-organic fungicides, 12 organothiophosphate and organophosphate insecticides and 18 nitro-organic fungicides and herbicides. A significant increase in the incidence of CA and SCE was observed in both exposed groups. Hyperdiploid and polyploid metaphases were also significantly increased in both the exposed groups compared to the controls.

Gomez-Arroyo et al. (1992) evaluated the cytogenetic damage by means of the analysis of the SCE in a rural population of Tlaxcala, Mexico occupationally in contact with the pesticides. A total of 170 men comprising 94 exposed and 76 not exposed were studied and in the exposed group no statistically significant symptoms provoked by these compounds were observed.

Bolognesi et al. (1993) carried out a study of the flower cultivators in Italy in the western part of the Region of Liguria. The extensive use of the pesticides professionally exposes the floriculturists operating in this area to a complex mixture of compounds. The frequency of MN in the peripheral lymphocytes was evaluated in 71 floriculturists and a
control group of 75 healthy blood donors living in the area. The results of this study showed a significant increase in micronucleated lymphocyte frequency in floriculturists occupationally exposed to the pesticides; the frequency was significantly higher in the females than in males in both the exposed and control groups.

Carbonell et al. (1993) studied SCE and CA in the lymphocytes of 70 male agricultural workers occupationally exposed to several pesticides and 69 matched controls, without indication of exposure to the pesticides, from 'El Maresme' (Barcelona, Spain). Comparison between the pesticide exposed and unexposed groups revealed that the former showed substantial clastogenic effects in their lymphocytes without indication of increases in the basal frequency of SCE. Moreover, these effects seemed to be compounding, i.e. increasing with the exposure duration in years. When two confounding factors such as age and smoking habits were considered, it was found that they significantly increased the expression of the SCE, although no effect was detected in the expression of the CA.

Lander and Ronne (1995) studied the frequency of the SCE in cultured lymphocytes and the number of blood erythrocytes, leucocytes, and thrombocytes in 134 greenhouse sprayers exposed to a complex mixture of almost 50 insecticides, fungicides, and growth regulators and in 157 referents/controls. There was a slight tendency towards an increased SCE frequency with decreasing degree of protection during the pesticide applications. The results suggested a genotoxic effect from combined sub-toxic occupational pesticide exposure. On the other hand, no hematogenic effects could be observed at the existing pesticide exposure level.
Kourakis et al. (1996) investigated the potential clastogenic effect of the pesticides in 56 agricultural workers (29 indoor and 27 outdoor) exposed to complex chemical mixtures. Exposed and referent/control subjects were selected from the same geographical area located in Ionia, province of Thessaloniki, Greece. The SCE and CA, were studied in the peripheral lymphocytes. Comparison between the workers and control group revealed that the individuals exposed to the pesticides showed substantial clastogenic effects in their lymphocytes without indication of increases in their basal frequency of SCE. Moreover, the condition of exposure was found to influence the occurrence of CA. It was found that the subjects working only in green houses (confined spaces) showed higher CA levels than the subjects working in the open fields. The present study included workers living in the close vicinity of a large industrial zone near Thessaloniki and the percentage of the CA in these indoor sprayers was higher compared to the previous study carried out in a non-industrial areas of Thessaloniki.

Scarpato et al. (1996a) investigated the induction of the SCE, structural CA and MN in the peripheral lymphocytes of a group of Italian floriculturists exposed to a mixture of the pesticides. The authors reported no statistically significant difference in the frequencies of cytogenetic damage between exposed and control subjects.

Joksic et al. (1997) examined the induction of CA, MN, and SCE in cultured lymphocytes of 27 vineyard growers exposed to the pesticides. The cytogenetic examinations were performed during the pre-spraying period, a month after spraying, and at the closing of the spraying season. Two control groups, the first consisting of of 15 individuals from a nearby town, and the second consisting of 20 volunteers living 200 km from the vine-growing area (reference control group) were monitored for the same
cytogenetic parameters. In comparison to the reference control group, a statistically significant difference in MN was observed in the first control group at the end of the spraying season. With respect to the incidence of MN in the control group in the vine-growing area, a poor but positive correlation with duration of the spraying season was found, which was attributed to pesticides carried by air in the area. The SCE frequencies of the workers' lymphocytes were found not significantly changed due to the exposure. The yield of CA as well as that of MN in exposed subjects correlated positively with the duration of exposure.

The frequencies of the CA and MN in the peripheral blood lymphocytes of 40 workers at a phosphate fertilizer factory in North China were studied by Meng and Zhang (1997). It was found that the chemicals used in the factory caused an increase in both the CA and MN in the workers. The difference in mean frequencies of major CA type (chromosome rings, translocations, and dicentrics) per 100 metaphases of the workers and the non-exposed controls were found to be statistically significant. Both CA frequency and MN frequency of the workers increased with the length of the chemical exposure period up to 10 years.

The aerial application of an organophosphate insecticide malathion has increased public concerns about its potential adverse health effects. Titenko-Holland et al. (1997) studied micronucleus formation in human lymphocytes as a biomarker of genotoxicity. The lymphocytes were cultured from whole blood or after Ficoll isolation and treated with malathion in doses. A significant increase in micronucleated cells was found in isolated lymphocytes at high dose levels, concurrent with cytotoxicity and a strong inhibition of proliferation.
Davies et al. (1998) evaluated MN in the peripheral blood lymphocytes from the British Columbia seasonal farm workers and controls using the cytokinesis-block technique. The farm workers harvested berry crops and were likely occupationally exposed to the pesticides. The subjects included 39 females of South Asian descent; 18 farmworkers employed during 1993 and 21 controls matched for age. No significant difference was found between the frequency of micronucleated binucleates in the farmworkers group, and the control group. However, among the farmworkers hired in 1993, a positive albeit statistically non-significant association was found between micronucleated cell frequency and duration worked. In those who had ever been employed as farm workers, there was an elevated frequency of micronucleated cells in the group with the longest history of employment as a farm worker compared to those with the shortest employment history.

To estimate the genetic risk associated with pesticide exposure in a defined population, Venegas et al. (1998) evaluated the frequency of MN in the peripheral blood lymphocytes in a group of 22 pesticide sprayers from Concepción, Chile, occupationally exposed to the pesticide mixtures. No significant increases were observed either for the total number of MN or for binucleated cells with MN compared with a control population.

Lebailly et al. (1998) used the comet assay to assess DNA damage in the farm workers and an increase in damage levels was detected after 1 day spraying with pesticide mixtures. Enrolment of the farmers was based on handling of heavily used pesticides at particular periods during one spraying season. Four groups of farmers were formed, according to exposure to (a) various fungicide-insecticide mixtures (including
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chlorothalonil), (b) the herbicide isoproturon, (c) fungicide triazoles, and (d) a fungicide (chlorothalonil)-insecticide mixture. An increase in DNA damage level was observed in those who were exposed to the pesticides. This increase was correlated with area sprayed and with the number of spraying tanks used over this 1-day period. Haematological parameters were also measured on the same samples. No individual characteristic significantly influenced the mean number of lymphocytes or eosinophils, and no correlation was observed between the pesticide exposure-related parameters and haematological parameters. The level of DNA damage was significantly higher in the pesticide exposed farmers than in the controls. DNA damage detected by the alkaline comet assay seemed to reflect ongoing exposure to genotoxic agents but not an accumulation of damage.

Amr (1999) observed cytogenetic changes in the pesticide formulators and applicators by measuring chromosomal aberrations (CAs) which were found to be more than twice than those in the controls.

Garaj-Vrhovac and Zeljezic (1999) studied the possible genotoxic effects of occupational exposure to complex pesticide mixture on workers employed in pesticide production. Two different cytogenetic assays on cultured peripheral blood lymphocytes were used including the analysis of micronucleus (MN) assay and structural chromosomal aberrations (CA). The statistically significant elevated number of chromosome breaks and chromatids as well as the presence of dicentric chromosomes and chromatid exchanges in exposed subjects was observed compared to the control subjects. There was also statistically significant difference in frequency and distribution of MN between the exposed and control subjects.
Antonucci and De Sylos (2000) evaluated the CA frequencies in temporary cultures of lymphocytes from the peripheral blood of 23 workers professionally exposed to the various pesticides. These workers were hired by the Agronomic Institute of Parana (Brazil) and used all of the prevention measures provided. A detailed history of the usage of pesticides, history of recent illnesses / medical treatments and smoking habits were collected through a standard questionnaire administered to each subject. The age, sex, and smoking habits matched non-exposed subjects were enrolled as the negative control. A significant increase in CA frequencies was observed in exposed individuals when compared to the controls. The characteristics including duration of pesticide exposure and smoking habits showed no association with CA. Therefore, the statistically significant results may be considered true effects of the pesticides on human somatic cells.

Gómez-Arroyo et al. (2000) evaluated the cytogenetic damage on the floriculturists of Morelos State, Mexico, exposed to pesticides by means of biological tests based on SCE in lymphocytes of the peripheral blood and MN in buccal mucosal cells. Apart from cytogenetic analysis, the effects of the pesticide exposure on the cell proliferation kinetics (CPK) by the replication index (RI) and the mitotic index (MI) to detect cytotoxic effects were also studied. Significant differences between exposed and non-exposed groups were found in SCE, CKP and MI. The MN frequency in the exposed group was found to be three times higher than in the non-exposed group.

In a cross-sectional and prospective study, Lander et al. (2000) studied CA frequencies in cultured lymphocytes in 116 green house workers exposed to a complex mixture of nearly 50 pesticides including insecticides and fungicides along with growth regulators and also for 29 pesticide non-exposed, non-smoking referents/controls. The
pre-season frequencies of CA were slightly but not statistically significantly elevated for the green house workers compared with the controls. After a pesticide spraying season in the green houses, the total frequencies of cells with CA were found to be significantly higher than in the pre-season samples and also higher than for the referents. According to the authors the results may reflect an additive genotoxic effect of the exposure to insecticides and growth regulators (but not fungicides). The highest elevation in the risk of chromatid gaps was observed for persons who did not use gloves during re-entry activities such as nipping, cutting, pricking, and potting. The present results suggested a genotoxic effect from a complex sub-toxic occupational pesticide exposure. In general, the findings indicated the importance of usage of safety measures while high exposure and re-entry activities.

Garaj-Vrhovac and Zeljezic (2001) described a longitudinal study of possible genetic damage in Croatian workers occupationally exposed to a complex mixture of pesticides. The methods used were CA analysis, SCE, MN and comet assay in workers and control subjects. To identify primary genotoxic effects in these workers, blood samples were collected after the workers spent 8 months in the production of the pesticides and 8 months after the workers were removed from the production. During the production all subjects were simultaneously exposed to a complex mixture of pesticides containing alachlor, atrazine, malathion, cyanazine and 2,4-dichlorophenoxyacetic acid. Regardless of the sampling time the exposed workers showed an increased number of CA, SCE frequency, MN frequency, and values of comet assay parameters. After 8 months of non-exposure workers showed a significantly decreased number of CA, MN frequency, and DNA migration compared to the results of the 8 months prior sampling.
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However, genotoxic damage was still significantly higher than in the control subjects. Furthermore, the SCE frequency in the exposed subjects did not drop after the 8 months of non-exposure, which indicated long-term effect of exposure to a mixture of pesticides.

In a study conducted by Zeljezic and Garaj-Vrhovac (2001), the CA analysis and the alkaline single cell gel electrophoresis (comet assay) were used to evaluate the extent of the DNA damage and DNA repair in the peripheral blood lymphocytes of the subjects employed in the manufacture of pesticides and controls. To study the primary genotoxic effects in these workers, the blood samples were collected after 8 month duration of pesticide exposure and after 8 month period of the pesticide non-exposure. Irrespective of the period of sampling (i.e. 8 months of exposure or non-exposure), in the pesticide exposed subjects a statistically significantly elevated breaks in chromatid and chromosome, acentric fragments, dicentric chromosomes and numbers of aberrant cells were found compared with the controls. After the workers had spent 8 months out of the pesticide exposure area the number of aberrant cells and all types of chromatid aberrations and CA decreased significantly compared with sampling after the high exposure. However, the DNA damage still remained significantly higher compared to the control subjects. After the high exposure period to the pesticide mixtures, statistically significant rise in the levels of DNA damage in the comet assay in terms of tail moment and tail length were observed. After the workers were away for 8 months from pesticide production work, both comet assay parameters decreased significantly compared with the first sampling, but they remained high compared to the control subjects.

In a bio monitoring study, Pastor et al. (2001a) investigated whether an occupational exposure to a complex mixture of chemical pesticides produce a significant
increase of MN in both peripheral blood lymphocytes and buccal cells. They enrolled 49 male workers exposed to pesticides from an agricultural area of Malopolska region in Southern Poland, along with 50 men from the same area without exposure to the pesticides served as controls. The statistically significant differences in the frequencies of cytogenetic damage were not detected between the exposed and control subjects, for either type of cells studied.

The potential cytogenetic damage associated with the pesticide use in Greek agricultural workers was evaluated by Pastor et al. (2001b) using MN as a biomarker in lymphocytes of peripheral blood and exfoliated cells of the buccal mucosa. In addition, the effects of the pesticide exposure and other variables on the cytokinesis block proliferation index (CBPI) in lymphocytes were also evaluated. The MN were analysed in 50 agricultural workers exposed to pesticides and in 66 non-exposed individuals that constituted the control group. The comparison between workers and controls did not reveal any statistically significant difference in the MN frequency for either lymphocytes or buccal cells. Regarding CBPI, the value found in the exposed group was statistically significantly lower than in the control group.

Shaham et al. (2001) measured the SCE in peripheral lymphocytes of 104 greenhouse farmers exposed to the pesticides and 44 unexposed workers. The adjusted means of both SCEs per chromosome and SCEs per metaphase were significantly higher among the farmers compared with the unexposed group. Elevation of the SCEs was found among those who did not use protective measures while handling the pesticides. The finding of a significant increase of SCEs frequency in the peripheral lymphocytes in greenhouse farmers indicated a potential cytogenetic hazard due to the pesticides exposure.
In a longitudinal study by Zeljezic and Garaj-Vrhovac, (2002), possible genetic damage in a population of workers occupationally exposed to a mixture of pesticides was evaluated by using SCE analysis. As an extra cytogenetic test, the lymphocyte proportion that undergo one, two or three cell divisions and proliferative rate index were determined. This study was performed on the exposed group of workers employed in production of pesticides, exposed to a pesticides mixtures such as alachlor, atrazine, malathion, cyanazine and 2,4-dichlorophenoxyacetic acid. The blood samples of the pesticide exposed subjects were collected in 3 different stages: before the initiation of the new pesticide production, after 8 months period of daily work in the production of pesticides, and 8 months after the abstinence of subjects from the pesticide production. In all the three sampling stages, the mean SCE value and cells with high frequency of SCE in the pesticide exposed group were found to be significantly higher compared to the control subjects. No differences in the proliferative rate index (PRI) was observed between the exposed and control groups, regardless of the sampling period. These results suggested that the increase in the number of SCE found in the exposed subjects was not the result of either cytotoxic or epigenetic action of the mixture of pesticides, but due to the chronic occupational exposure to pesticide mixtures.

Ündegër and Basaran (2002) studied DNA damage in the peripheral lymphocytes of 33 pesticide-exposed workers employed in the municipality of Ankara (Turkey) for at least 1 year by comet technique. The results were compared with those from 33 control subjects of age, sex and smoking habits matched that did not have occupational pesticide exposure. The DNA damage observed in the lymphocytes of the workers was significantly higher than that in the controls. The observed DNA damage was found to be
significantly lower in workers applying some of the necessary individual safety protections during their work. No significant association was found between the duration of occupational exposure to pesticides and the degree of DNA damage.

National status

As for the cytogenetic studies from India, relatively few workers have reported data on the pesticide exposed subjects. Rita et al. (1987) investigated the effect of the pesticides on workers employed in grape gardens of Andhra Pradesh. Their cytogenetic study revealed a significant increase in the chromatid breaks and gaps in chromosomes of the peripheral blood in the workers exposed to the pesticides. In addition, a high frequency of satellite associations was recorded in these workers.

Twenty five male workers occupationally exposed to DDT, BHC, malathion, dimethoate, parathion, gromor, fenitrothion and urea were selected by Rupa et al. (1988) as subjects for the analysis of the CA and SCE in the peripheral lymphocytes. The frequency of CA and SCE was found increased significantly, irrespective of the duration of exposure to pesticides when compared to the controls.

Rupa et al. (1989a) collected blood samples from 50 smokers who were exposed to the pesticide including DDT, BHC, malathion, endosulfan, monocrotophos, methyl parathion, quinolphos, phosphomidon, dimethoate, fenvelrate and cypermethrin. The samples were also collected from 20 non-smokers (control I) and 27 smokers (control II) who were unexposed to the pesticides. There was a significant increase in total CA in the smokers exposed to pesticides when compared to unexposed controls.
Rupa et al. (1989b) studied blood of 50 smokers who were exposed to pesticides for SCEs, cell kinetics (CK) and mitotic index (MI). As controls, blood samples were collected from 20 non-smokers (control I) and 27 smokers (control II) who were not exposed to pesticides. A significant increase in the SCEs was observed as the duration of the exposure increased. The frequency of M1 metaphases increased significantly whereas M2 and M3+ metaphases decreased in the exposed group. The mitotic index increased in the control II and in the exposed population while it showed a decrease at 11-25 years' exposure.

Fifty two pesticide sprayers in cotton fields were selected by Rupa et al. (1989c) for the analysis of the CA in the lymphocytes of peripheral blood. Twenty five blood samples were obtained from the healthy males who did not have any pesticide exposure. Statistical analysis showed that there was a statistically significant increase in the CA in the exposed population compared to the controls. Total CA increased irrespective of the duration of the exposure.

Rupa et al. (1991) studied the clastogenic effects in the peripheral lymphocytes of cotton-field workers who were exposed to different pesticides. The type of aberrations observed in the exposed group were breaks, gaps, rings, exchanges, dicentrics and polyploidy. The CA frequency was found to be increased significantly in the male pesticide applicators when compared to the controls. A significant decrease in the mitotic index was observed in the exposed group as compared to the control group. As many as 24 out of 26 individuals showed ill health effects such as severe giddiness and nervous disorders.
Thirty pesticide-exposed workers and 30 matched controls were monitored for expression of CA and SCE in their lymphocytes (Hoyos et al., 1996). It was assuring to note that the exposure conditions among these Indian farmers did not cause detectable increase of chromosome damage using standard assays, suggesting the lack of serious long-term health problems.

The analysis of SCEs by Padmavathi et al. (2000) showed clear evidence for the clastogenic effect of occupational exposure to the pesticides and chemicals in pesticide industry workers. However, as the workers were frequently exposed to a variety of organophosphorous pesticides and toxic chemicals, it was difficult for authors to attribute damage to any particular agent.

2.3. Pesticide exposure and Parkinson’s disease (PD): molecular studies

2.3.1. MDR1 gene polymorphisms

*International status*

P-glycoprotein is a membrane protein encoded by the *MDR1* gene which exhibits functional polymorphism. This protein is present in the blood-brain barrier endothelial cells, limiting its substrates’ build-up in the central nervous system. Numerous epidemiological studies demonstrated an association between pesticides (which are substrates for P-glycoprotein) and Parkinson’s disease (PD). It was hypothesized that the polymorphism of the *MDR1* gene could modulate inter individual susceptibility for the disease in the subjects exposed to the pesticides. Thus specific genotype of multidrug resistance (*MDR1*) gene, which codes for P-glycoprotein, ABC-transporter of the
MDR/TAP subfamily, leading to a decreased expression of P-glycoprotein may be a risk factor for PD (Furuno et al., 2002, Droźdžik et al., 2003).

To evaluate whether alterations in the *MDR1* gene correlate with intestinal *MDR1* expression and uptake of orally administered P-glycoprotein (PGP) substrates, Hoffmeyer et al. (2000) analyzed the *MDR1* sequence in 21 volunteers whose PGP expression and function in the duodenum had been determined by Western blots and quantitative immunohistology or by plasma concentrations after orally administered digoxin. The authors observed a significant correlation of a polymorphism in exon 26 (C3435T) of *MDR1* with expression levels and *MDR1* function. The subjects who are homozygous for this polymorphism reported to have significantly lower duodenal *MDR1* expression and the highest digoxin plasma levels. The polymorphism was expected to affect the absorption and tissue concentrations of numerous other substrates of MDR1.

In a pilot case-control study on 95 PD patients and 106 controls, Furuno et al. (2002) investigated three common polymorphisms of *MDR1* viz., 3435C > T in exon 26, 2677G > T,A in exon 21, and -129T > C in exon 1b. No statistically significant associations between any of these polymorphisms and PD was reported. However, the distribution pattern of the genotypes was consistent showing the frequency of the 3435T/T (which had been associated with decreased P-glycoprotein expression and function) being highest in the early-onset PD group, followed by the late-onset PD group and the control group. Furthermore, the authors found that the *MDR1* exon 21 and exon 26 polymorphisms were in significant linkage disequilibrium since [2677G, 3435C] and [2677T, 3435T] haplotypes were far more frequently observed than expected. According
to the authors, *MDRI* and other drug transporters represented plausible candidates as PD risk genes.

In a case control study in the Polish population involving 107 PD patients (30 early onset and 77 late onset; 59 exposed to the pesticides and 48 non-exposed) and 103 controls, C3435T polymorphism of the *MDRI* gene was studied by Droździk et al. (2003). No statistically significant association between *MDRI* gene polymorphism and PD was found. However, a significant association between PD patients exposed to the pesticides and C3435T polymorphism of the *MDRI* gene was found. Comparing the exposed and non-exposed PD patients, a statistically significant higher frequency of the heterozygous subjects was observed in the former, which was associated with almost three-fold increased risk of the disease. Similarly, a higher frequency of 3435TT subjects was revealed in the exposed PD patients compared to the non-exposed patients. In exposed versus non-exposed subjects, the patients carrying at least one 3435T allele (i.e. mutant homozygous and heterozygous) had a significant five-fold higher risk of PD. Thus, it appeared that mutation of the *MDRI* gene predisposes to damaging effects of the pesticides, and possibly other toxic xenobiotics transported by P-glycoprotein, leading to PD.

Seven SNPs, extending from the promoter to exon 28 of the *MDRI* gene in 158 PD patients and 139 healthy controls were studied by Tan et al. (2004). Specifically the authors examined the association of haplotypes containing SNPs 2677 G > T/A and 3435 C > T and risk of PD. The frequency of each individual SNPs did not differ between PD as well as control subjects. However, there was a significant correlation between risk of PD and 2677G-3435C haplotype. There was a weak protective effect of the haplotype in
the white population. However, authors concluded that the $MDR1$ haplotypes did not generally modulate the risk of PD.

Dermietzel et al. (2006) reported a five-fold increased risk for developing PD in exon 26 (C3435T) heterozygous ($CT$) and mutant homozygous ($TT$) patients exposed to the pesticides.

Zschiedrich et al. (2009) conducted a large case control study in Germany investigating the potential relationship between $MDR1$ polymorphisms and PD. The authors determined the frequency of three $MDR1$ polymorphisms in 599 European PD patients characterized by age at onset, ethnicity and exposure to pesticides along with controls. The distribution of c.3435C/T differed significantly between PD patients exposed to pesticides and those non-exposed, and the authors suggested that common $MDR1$ polymorphisms might influence the risk to develop PD in conjunction with exposure to the pesticides.

2.3.2. CYP2D6 gene polymorphisms

International status

SNPs of cytochrome P450 (CYP) genes including $CYP1A2$, $CYP2C9$, $CYP2C19$, $CYP2D6$, $CYP3A4$ and $CYP2E1$, among others have been extensively investigated in PD and a significant association of the disease with $CYP2D6$ was reported in several studies. PD may be caused by genetic susceptibility to neurotoxic substances. The $CYP2D6$ is reported as a candidate gene for PD since its role in regulating drug and toxin metabolism, however, the association studies shown to have inconsistent results.
Elbaz et al. (2004) performed a case control study of PD in a population characterized by a high prevalence of the pesticide exposure and investigated the joint effect of pesticide exposure and CYP2D6 polymorphism. Although the results are based on a small group of subjects (n = 190) with the joint exposure, the findings were consistent with a gene-environment interaction disease model according to which the pesticides had a modest effect in subjects who were not CYP2D6 poor metabolizers; the effect of pesticides was approximately twofold increased in poor metabolizers and they were not at an increased PD risk in the absence of the pesticide exposure.

Deng et al. (2004) examined the polymorphisms of three CYP2D6 alleles viz., CYP2D6*3, CYP2D6*4, and CYP2D6*5 in PD patients. The patients who were homozygous for these alleles and had been weekly exposed to the pesticides showed a significant increased risk for the disease (odds ratio 8.41) and heterozygotes with such an exposure had comparatively low but significant odds ratio (3.27). The authors reported interaction of CYP2D6 polymorphism with the pesticide exposure in development of PD.

National status

A case control study was carried out by Singh et al. (2010) to investigate the association, if any, of the polymorphism in CYP2D6 that is involved in the metabolism and detoxification of chemicals causing PD. The results demonstrated increased frequency of CYP2D6*2 (1749G/C and 2938C/T), CYP2D6*4 (1934G/A) and CYP2D6*10A (188C/T) polymorphisms in PD cases compared to the control subjects. The statistical analysis shown a statistically significant association of CYP2D6*4 (1934G/A) and CYP2D6*10A (188C/T) polymorphisms with PD.
2.3.3. GST gene polymorphisms

International status

Glutathione transferases (GSTs) metabolise xenobiotics, including the pesticides. Therefore, the role of GST polymorphisms in the pathogenesis of PD has been studied from time to time by different authors. Parkinson's disease (PD) is thought to be secondary to the presence of neurotoxins, and the pesticides have been implicated as possible causative agents.

Scarpato et al. (1996b) studied occupational exposure of the 23 Italian floriculturists and 22 matched controls using the cytogenetic parameters SCE, structural CA and MN in the peripheral lymphocytes. The molecular parameters including glutathione S-transferase GSTM1 and GSTT1 involved in metabolism of xenobiotic substances were also studied. The authors evaluated the possible role of these genes in individual genotoxic response to the pesticide exposure. Blood sampling was performed during and one month after the end of intensive pesticide exposure to cover a duration of high and low exposure, respectively. The only significant influence of phenotype composition on cytogenetic response was an increase in the SCE levels in the GSTT1 positive individuals compared with the GSTT1 nulls. This finding was, however, based on only four GSTT1 null donors.

Scarpato et al. (1997) studied the influence of polymorphic GSTM1 and GSTT1 on the rate of CA in the peripheral lymphocytes of 30 pesticide-exposed floriculturists and 32 control subjects. According to the authors the pesticide exposure was not found to be associated with the elevated frequencies of the CA. In addition, the few individuals null for both GSTM1 and GSTT1 showed significantly higher CA counts than GSTM1
positives and GSTT1 nulls. The findings may be explained by the reduced detoxification capacity of GSTM1 null and GSTT1 null individuals.

Menegon et al. (1998) genotyped four GST classes (GSTM1, GSTT1, GSTP1, and GSTZ1) by PCR polymorphisms in 95 PD patients and 95 controls. All the patients were asked for information about the pesticide exposure. The distribution of the GSTP1 genotypes differed significantly between patients and controls, who had been exposed to the pesticides. No association was found with any of the other GST polymorphisms. The pesticide exposure and a positive family history were risk factors for PD. The blood-brain barrier expressing GSTP1 gene may effect neurotoxin responses and demonstrates the Parkinsonism inducing effects of the pesticides in some people.

Falck et al. (1999) studied the frequency of MN in cultured peripheral lymphocytes used as a biomarker of genotoxic effects in 34 Italian pesticide-exposed green house workers and 33 age and smoking habits matched unexposed referents/controls. The plausible effect of the gene polymorphisms of xenobiotic metabolizing enzymes GSTM1 and GSTT1 were also evaluated. The results indicated that MN rates were increased in green house workers, especially in those involved in the pesticide spraying. The GSTM1 positive genotype appeared to be associated with elevated MN frequencies.

Lucero et al. (2000) evaluated whether occupational exposure to a complex mixture of the pesticides results in a significant increase of MN in both peripheral blood lymphocytes and buccal cells. Each donor was also assessed for the presence or absence of GSTM1 and GSTT1 genes, to look for relationships between the genotypes and the cytogenetic responses. Sixty four green house workers from Almería, Southeastern Spain, along with 50 pesticide non-exposed subjects inhabiting the same area served as controls.
were enrolled in this study. The results obtained indicated that there were no statistically significant differences in the MN frequency between the two groups. According to the results of *GSTT1* analysis, there was a difference between both groups only for the cytokinesis-block proliferation index (CBPI). Neither *GSTM1* nor smoking habit and age showed any effect in the overall analysis.

Gregorio d'Arce and Colus (2000) carried out cytogenetic monitoring and genotyping on a group of 20 male workers occupationally exposed to a mixture of the pesticides in the town of São Jerônimo da Serra, PR (Brazil). There was no significant difference between the CA frequencies between the exposed and control groups. The *GSTM1* gene polymorphism was found to be 33% null. When statistical tests were carried out to evaluate the relationship of the *GSTM1* genotypes with the mitotic indexes and CA, no statistically significant difference was observed. The authors noted that the results from these type of investigations are difficult to conclude because different pesticide combinations were used depending on the crops, areas, seasons, etc.

Taylor et al. (2001) tested 307 PD and 105 control subjects, of which 87 patients and 53 controls reported a history of regular pesticide exposure. In the pesticide exposed group there was a weak association between the nucleotide *GSTZ1* genotype and PD. Furthermore, in this group, the *GSTZ1*C genotype (G94G124C245) was less common in the patients with PD than in the controls.

Kelada et al. (2003) tested associations between genotypes of *GSTM1* (homozygous deletion vs. non-deleted), *GSTT1* (homozygous deletion vs. non-deleted), and *GSTP1* (Ile104Val and Ala113Val) and PD in a case-control study of 214 idiopathic PD cases of the Caucasian ethnicity and 330 age, sex and ethnicity matched unrelated controls. No
statistically significant correlation with any of the GST genotypes were detected. Nevertheless, there was a slightly significant difference in the distribution of GSTP1 Ile104Val genotypes between the cases and controls, with an excess of Ile104Val heterozygotes found among the cases. This difference in the genotype distribution was strongest between smokers and non-smokers, and between the males and females. The distribution of GSTP1 Ala113Val and GSTP1 Ile104Val haplotypes did not differ between the cases and control subjects. These results demonstrated a potentially negligible role of GSTP1 in PD, but did not give evidence for associations with either GSTM1 or GSTT1.

Vilar et al. (2007) studied the allelic distributions and genotype associations of three major brain-expressed drug metabolizing enzymes (DMEs), in sporadic PD cases and control subjects. No statistically significant correlation was found between PD and CYP2D6 genotype. However the subjects with extensive metabolizer (EM) CYP2D6 phenotype and GSTP1*B variant genotype were found at significantly higher PD risk than the poor / intermediate metabolizers. The statistically significant association was found between the risk of PD and GSTP1*B allele. This association was particularly strong in the elder patients group (≥69 years) who showed double PD risk for GSTP1*B heterozygous, whilst GSTP1*B/*B homozygous were exclusively found amongst the patients. An interaction between GSTM1 and GSTP1 was observed in the late onset PD group. These results suggested that native GSTP1 encoding the fully active transferase variant plays a relevant role in dopaminergic neuroprotection.

Kiyohara et al. (2010) investigated the relationship of seven GST polymorphisms including GSTM1 and GSTT1 deletions, GSTP1 (rs1695), GSTO1 (rs11191972), GSTO1
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(rs4925), GSTO2 (rs2297235) and GSTO2 (rs156697) polymorphisms and PD risk with special reference to the interaction with the pesticide use or cigarette smoking among 238 PD patients and 370 controls in a Japanese population. None of the GST polymorphisms were found to be associated with risk of PD. Two polymorphisms including GSTO1 (rs4925) and GSTO2 (rs2297235) were observed to be in strong linkage disequilibrium. There was no evidence of interaction between self-reported pesticide use and GST polymorphisms. These results suggested that the studied GST polymorphisms did not play an important role in PD susceptibility in the Japanese population and the authors attributed this observation to small sample size.

In a study by Longo et al. (2013), the PD patients were significantly more exposed to the pesticides compared with the control group, and the heterozygote genotype was found to be associated with exposure to the pesticides in patients. The authors concluded that the exposure to the pesticides is associated with PD, whose effect can be enhanced when combined with the heterozygote genotype of GSTP1-Alw26I.

Dai et al. (2014) conducted a meta-analyses from 17 studies between 3419 PD cases and 5686 controls for four polymorphisms of GSTM1, GSTT1, GSTP1 (rs1695) and GSTP1 (rs1799811). There was no significant association between the polymorphisms studied and PD. A further subgroup study by ethnicity observed a risky role of GSTM1 deletion polymorphism with PD in the Europeans, and a protective role of GSTM1 with PD in the Latin Americans. This meta-analysis suggested that GSTM1 deletion polymorphism increased the risk of PD in the Europeans, but reduced the risk of PD in the Latin Americans.
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To sum up, the present review demonstrated that as for epidemiological studies of the pesticide exposed PD patients, almost all of them found occupational exposure of the pesticides/herbicides, farming and rural living etc. associated with an increased risk for PD. However, no associations with specific pesticides could be detected because the sprayers / farmers use one or more pesticides containing different chemicals and chemical combinations on different crops. Numerous cytogenetic investigations using SCEs, CAs, MN assay and comet assay on the pesticide exposed subjects showed increased genotoxicity / DNA damage in them compared to controls. Several case-control molecular genetic investigations on the pesticide detoxification and metabolizing genes such as MDR1, CYP2D6 and GSTs suggested that certain genotypes of these genes increased the risk of PD in the pesticide sprayers.