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Association between environmental exposure to pesticides and neurodegenerative diseases

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ABSTRACT

Preliminary studies have shown associations between chronic pesticide exposure in occupational settings and neurological disorders. However, data on the effects of long-term non-occupational exposures are too sparse to allow any conclusions. This study examines the influence of environmental pesticide exposure on a number of neuropsychiatric conditions and discusses their underlying pathologic mechanisms. An ecological study was conducted using averaged prevalence rates of Alzheimer's disease, Parkinson's disease, multiple sclerosis, cerebral degeneration, polyneuropathies, affective psychosis and suicide attempts in selected Andalusian health districts categorized into areas of high and low environmental pesticide exposure based on the number of hectares devoted to intensive agriculture and pesticide sales per capita. A total of 17,429 cases were collected from computerized hospital records (minimum dataset) between 1998 and 2005. Prevalence rates and the risk of having Alzheimer's disease, Parkinson's disease, multiple sclerosis and suicide were significantly higher in districts with greater pesticide use as compared to those with lower pesticide use. The multivariate analyses showed that the population living in areas with high pesticide use had an increased risk for Alzheimer's disease and suicide attempts and that males living in these areas had increased risks for polyneuropathies, affective disorders and suicide attempts. In conclusion, this study supports and extends previous findings and provides an indication that environmental exposure to pesticides may affect the human health by increasing the incidence of certain neurological disorders at the level of the general population.

Introduction

Pesticides are extensively used throughout the world, particularly over the past 50 years. In Spain, pesticide sales for the year 2008 reached a total amount of 94,549 tons, of which 31,163 (33%) were used in Andalusia (South Spain) representing the highest amount of pesticides sold nationwide. However, a heterogeneous pattern of pesticide use is seen across this region (Fig. 1) because of the distinct agriculture practices, with areas of intensive farming within plastic greenhouses (particularly near the coastline) and other of traditional open-air agriculture (in inland areas). These amounts of pesticides create the potential for pervasive human exposures arising from environmental sources.

In humans pesticides are responsible for acute and long-term health effects. Although studies are not fully consistent, there is a growing body of evidence linking long-term low-dose pesticide exposure (along with the special agricultural working conditions) to a variety of clinical conditions including cancers, reproductive health issues and a range of neurological disorders, including neurobehavioral performance impairments in children.

Neurodegenerative disorders are characterized by disease selective profiles of adult-onset neuronal cell loss within areas of the cerebral cortex, basal ganglia, cerebellum, brain stem, and motor systems, leading to nervous system dysfunction (Brown et al., 2005). They are likely due to a combination of genetic and environmental factors. Evidence from epidemiological, animal and cell models suggests that gene–environment interactions produce selective neurodegeneration through mechanisms involving impairment of mitochondrial function, oxidative stress and excitotoxicity (Sherer et al., 2001; Tanner et al., 2011). Pesticide exposure at relatively low doses may affect brain cells producing a loss of neurons in particular regions of the brain that results in subsequent cognitive decline, impaired memory and attention, and motor function. These neurobehavioral disturbances may eventually lead to Alzheimer's disease (AD), Parkinson's disease (PD) and other forms of dementia in late life (Baldi et al., 2003; Hayden et al., 2010; Tanner et al., 2011).

Environmental stressors including pesticides contribute to the above mentioned neurological conditions through mechanisms involving

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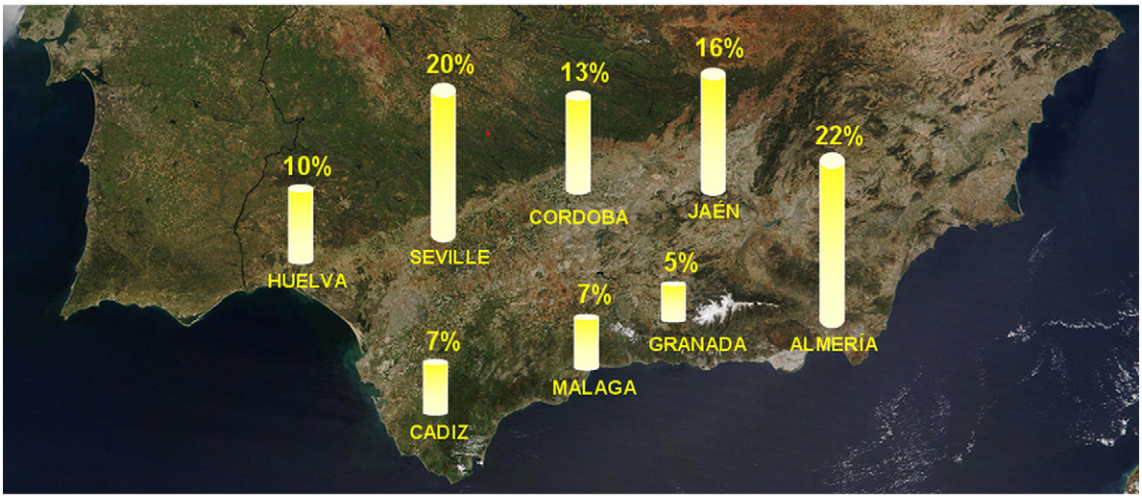

Fig. 1. Pesticides sales in the different provinces of Andalusia (South Spain) for the year 2008.

inflammation, oxidative stress and further apoptotic cell death (Franco et al., 2009). Stimulation of free radical production, induction of lipid peroxidation, and disturbance of the total antioxidant capability of the body are mechanisms of toxicity in most pesticides, including organophosphates (OPs), bipyridyl herbicides and organochlorines (Abdollahi et al., 2004). In addition to cholinergic overstimulation, anticholinesterase pesticides also induce activation of glutamatergic neurons. Glutamate release leads to activation of NMDA receptors and further generation of free radicals resulting in neuronal degeneration (Milatovic et al., 2010). Thus, oxidative stress and excitotoxicity may also contribute to the noncholinesterase mechanisms of OPs (Slotkin et al., 2010). However, despite the number of epidemiological and experimental studies conducted in the last decades, there is still controversy over the appearance of cognitive, sensorimotor, psychological and psychiatric effects occurring without actual or previous acetylcholinesterase inhibition (Moretto et al., 2010).

Exposure to pesticides at a high enough concentration to cause self reported poisoning symptoms has been associated with high depressive symptoms independently of other known risk factors for depression among farm residents (Stallones and Beseler, 2002) and farmers (Beseler and Stallones, 2008). Chronic exposure to OP pesticides has also been linked to psychiatric symptoms including affective disorders, especially depression, anxiety and unprovoked aggressive behavior (London et al., 2005). Thus, these compounds may contribute to suicide risk through biological pathways involving depressive central nervous system effects and/or impulsivity and lack of self control. OPs have been considered as risk factors for delayed polyneuropathy (Lotti and Moretto, 2005), a relative rare neurodegenerative disorder characterized by an axonal neuropathy with a sensory-motor dysfunction.

Most of the above mentioned nervous system disorders involve OP exposures, but recent evidence also suggests a role for the organochlorine DDT and fungicides. The relationship between pesticides and AD and PD appears strongest for long-term exposure to herbicides and insecticides (Brown et al., 2006; Doherty, 2006). The majority of studies have found associations primarily in the occupational setting and only very few studies have looked for chronic neurologic effects outside the occupational setting. Although occupational activity among farmers is the major way of exposure to pesticides, the presence of pesticide residues in water and foodstuff as well as in the environment near agriculture farms, implies that the general population has a significant background exposure to these compounds raising concerns as regards to their potential health impact. This study attempts to comprehend the potential association between environmental exposure to pesticides and the development of a number of neurodegenerative diseases, affective psychosis and suicide attempts by comparing disease prevalence and disease risk between geographical areas of Andalusia with different pesticide use. The possible mechanisms by which these environmental contaminants act and interplay with other risk factors are discussed.

Methods

Design. An ecological study was conducted using averaged prevalence rates of a number of nervous system conditions in selected areas from Andalusia (South Spain) with different environmental exposures to pesticides (Fig. 2) as a result of different pesticide use. Each study area corresponds to an administrative territorial division with a reference Hospital (referred to as health district).

Criteria for selecting the study areas. Andalusian health districts with high agriculture activity within plastic greenhouses were selected as target areas. Other health districts lacking intensive agriculture practices (but with extensive herbaceous crops) were also selected for comparison purposes. All these areas were classified into two groups based on the number of hectares devoted to intensive agriculture according to figures provided by the Andalusian Council of Agriculture. One thousand and two hundred hectares was used as a cut-off level for distinguishing areas of high and low use of pesticides. Agronomic criteria in the categorization of geographical areas (health districts) as regards to pesticide use are shown in Table 1. Pesticide sales for the year 2001 allowed to estimate the average pesticide exposure in the selected study areas, as this year represents the middle of the time period studied. Given that pesticide sales are available only at province level but not for health districts, the total amount of pesticides sold in each Andalusian province was divided by its total population and then multiplied by the population living in the selected health districts within each province (Table 1). Population data was obtained from the 2001 census. Finally, areas of high exposure included the following health districts: West Almeria (Poniente), Center of Almeria (Centro), South Granada and Huelva coastline. By contrast, low exposure areas were comprised by Axarquia (Malaga), Jerez coastline, East Almeria (Levante), Northeast Jaen, North Cordoba, and North Seville (Fig. 2).

Study population and target diseases. The study population consisted of 17,429 patients collected between 1998 and 2005 using the place of residence in Andalusia as inclusion criteria (see distribution in Table 2). The above population was diagnosed with any of the following neuropsychiatric conditions: Alzheimer disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), cerebral

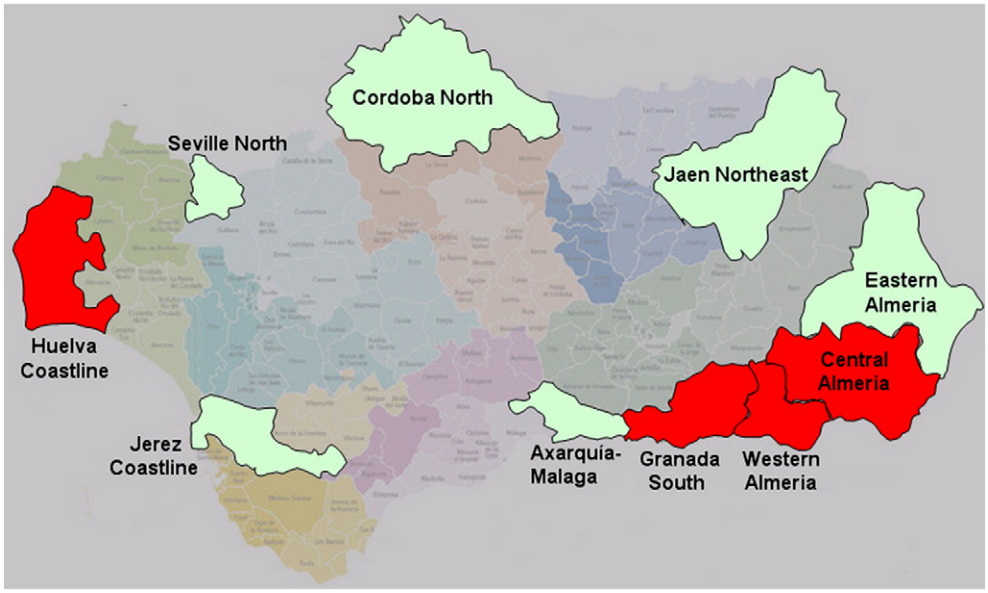

Fig. 2. Geographic distribution of the study areas (Andalusian health districts): high pesticide exposure (dark red) and low pesticide exposure (clear green).

degeneration (i.e., Pick disease, frontotemporal dementia, senile dementia, hydrocephalus-induced neural degeneration, Lewy bodies dementia), polyneuropathy (peripheral neuropathies of any kind), affective psychosis (manic, depressive, mixed type or bipolar disorders) and suicide attempts. Cases were collected from computerized records of the Andalusian Health Service (referred to as Minimum Dataset) for the 8-year study period. The Andalusian Minimum Dataset (AMD) collects public hospital discharge information, including coded clinical data for inpatients. AMD is recorded when a patient is discharged from a hospital after staying for at least one night or more. The main cause for admission (major diagnosis) and other secondary medical diagnoses is routinely recorded in AMD as are also age, gender, race and place of residence. The validity of the data gathered in AMD is determined by the quality of the discharge

Table 1
Amounts of pesticides used in health districts with high and low use of pesticides in Andalusia (South Spain).

	Western Almeria	Central Almeria	South Granada	Huelva Coastline	TOTAL (High use of pesticides)	Eastern Almeria	Jerez Coastline	North Córdoba	Northeast Jaen	Axarquia Malaga	Seville North	TOTAL (Low use of pesticides)
Population	170755	247406	129245	252611	800017	115007	300783	84819	171120	127.901	233.322	1032.952
Hectares of plastic greenhouses	18750	7514	2814	1213	30291	632	895	31	32	897	23	2.510
Total pesticides[a]	3085.43	4470.47	311.89	1015.96	8883.74	2078.10	735.91	556.77	1523.38	213.82	1037.87	6145.84
Insecticides[a]	1797.97	2605.06	87.79	286.68	4777.50	1210.97	355.46	95.99	162.96	46.36	339.15	2210.89
Fungicides[a]	764.47	1107.64	77.14	279.57	2228.81	514.89	142.24	193.86	454.44	106.76	254.09	1666.28
Herbicides[a]	73.66	106.73	92.24	327.16	599.80	49.61	187.70	165.53	573.95	65.41	337.81	1380.02
Plant growth regulators[a]	279.27	404.63	47.87	173.43	905.21	188.09	22.60	76.59	322.22	25.24	59.28	694.03
Other pesticides[a]	170.06	246.40	8.27	37.75	462.48	114.54	27.92	24.80	9.54	10.90	47.67	235.36
Total pesticides[b]	18.07	18.07	2.41	4.02	11.10	18.07	2.45	6.56	8.90	1.67	4.45	5.95
Insecticides[b]	10.53	10.53	0.68	1.13	5.97	10.53	1.18	1.13	0.95	0.36	1.45	2.14
Fungicides[b]	4.48	4.48	0.60	1.11	2.79	4.48	0.47	2.29	2.66	0.83	1.09	1.61
Herbicides[b]	0.43	0.43	0.71	1.30	0.75	0.43	0.62	1.95	3.35	0.51	1.45	1.34
Plant growth regulators[b]	1.64	1.64	0.37	0.69	1.13	1.64	0.08	0.90	1.88	0.20	0.25	0.67
Other pesticides[b]	1.00	1.00	0.06	0.15	0.58	1.00	0.09	0.29	0.06	0.09	0.20	0.23

Pesticides more commonly used in the study areas:
Insecticides: methylcarbamates (methomyl, oxamyl, methiocarb, pirimicarb, propoxur), organophosphates (malathion, parathion, parathion-methyl, methidathion, dimethoate, acephate, methamidophos, chlorpyrifos, diazinon, dichlorvos, pirimiphos-methyl, azinphos-methyl), pyrethroids (cypermethrin, deltamethrin, tralomethrin, acrynathrin, bifenthrin), neonicotinoids (imidachloprid), growth regulators insecticides (tebufenozide, diflubenzuron, teflubenzuron,, flufenoxuron, cyromazine, buprofezin), formamidine (amitraz, formetanate), organochlorine (endosulfan, dicofol), others (abamectin, thuringiensin).
Fungicides: (di)thiocarbamates (zineb, mancozeb, maneb, thiram), benzimidazole (benomyl, carbendazim, thiabendazole), phthalimide (folpet, captan), dicarboximide (procymidone, iprodione, vinclozolin), conazole (prochloraz, tebuconazole, triadimefon), others (copper, sulfur, propamocarb, nuarimol, cymoxanil, metalaxyl, oxadixyl, fosetyl, pyrazophos, kasugamycin).
Herbicides: bipyridyl (paraquat, diquat), organophosphonates (glyphosate, glufosinate), chlorotriazine (atrazine, simazine, terbuthylazine, cyanazine), phenylurea (chlorotoluron, isoproturon, linuron, diuron, monuron, fluometuron).
Plant growth regulators: auxins (4-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid), defoliants (cyanamide, metoxuron), cytokinins, gibberellins, growth inhibitors (abscisic acid, chlorpropham, glyphosine), growth retardants (chlormequat, uniconazol), ethylene.
Other pesticides: fumigants (methyl bromide, aluminum phosphide, 1,3-dichloropropene), molluscicides (metaldehyde, cooper salts, pentachlorophenol).
[a] Tons used in each health district.
[b] kg/person.

Table 2
Study population and distribution of the selected neuropsychiatric disorders stratified by the geographical areas studied (high and low pesticide exposure).

	Areas of high pesticide use	Areas of low pesticide use
Neuropsychiatric disorders* (n = 17,429 cases)	8561	8868
Alzheimer's disease	2185	1344
Parkinson's disease	2076	2059
Multiple Sclerosis	333	350
Cerebral degeneration	981	1790
Polyneuropathies	1012	1276
Affective psychosis	1177	1497
Suicides	797	552
Population** (Total)	800017	1032952
Male	400595	513185
Female	399422	519767
Employed population by economic activity***	$p = 0.340$	
Primary sector (agriculture and livestock, forestry and fishing)	77731 (25.26%)	63856 (19.61%)
Secondary sector (manufacturing industry and construction)	61955 (20.13%)	89841 (27.60%)
Tertiary sector (service industries, private and public activities)	168090 (54.61%)	171780 (52.79%)

* Cases collected from the Andalusian Minimum Dataset for the time period 1998–2005 in health districts with different pesticide use.
** Data obtained from the 2001 census, with this year being the middle of the time period studied.
*** Data obtained from the 2001 database from the Institute of Statistics of Andalusia.

Table 3
Prevalence (rate per 100,000 inhabitants), odds ratio and 95% confidence interval for a number of neuropsychiatric disorders in the population living in high exposure areas relative to that resided in low exposure areas.

	Prevalence rate		OR	95% CI	*P* value
	High exposure	Low exposure			
Total population					
Alzheimer's disease	273.12	130.11	2.10	1.96–2.25	<0.001
Parkinson's disease	259.49	199.33	1.30	1.22–1.39	<0.001
Multiple sclerosis	41.62	33.88	1.23	1.05–1.43	0.007
Cerebral degeneration	122.62	173.29	0.71	0.65–0.77	<0.001
Polyneuropathies	126.50	123.53	1.02	0.94–1.11	0.572
Affective psychosis	147.12	144.92	1.02	0.94–1.10	0.699
Suicide attempt	99.62	53.44	1.87	1.67–2.08	<0.001
Males					
Alzheimer's disease	198.45	98.79	2.01	1.80–2.25	<0.001
Parkinson's disease	243.39	201.29	1.21	1.11–1.32	<0.001
Multiple sclerosis	28.21	26.89	1.05	0.81–1.35	0.700
Cerebral degeneration	141.79	210.45	0.67	0.61–0.75	<0.001
Polyneuropathies	168.50	149.26	1.13	1.02–1.25	0.021
Affective psychosis	139.04	137.38	1.03	0.92–1.15	0.640
Suicide attempt	88.12	44.04	2.00	1.69–2.37	<0.001
Females					
Alzheimer's disease	348.00	161.03	2.17	1.99–2.36	<0.001
Parkinson's disease	275.65	224.52	1.40	1.28–1.52	<0.001
Multiple sclerosis	55.08	40.79	1.35	1.11–1.64	0.001
Cerebral degeneration	103.40	129.87	0.76	0.67–0.86	<0.001
Polyneuropathies	84.37	98.12	0.86	0.75–0.99	0.030
Affective psychosis	155.47	154.3	1.01	0.90–1.12	0.910
Suicide attempt	111.16	62.72	1.77	1.53–2.05	<0.001

report with respect to its inclusion of diagnoses and principal and secondary procedures and by exhaustiveness in the codification of hospital discharges.

Statistical analysis. Prevalence rates, odds ratios (OR) and their corresponding 95% confidence intervals for the selected neuropsychiatric conditions in areas of high versus low environmental pesticide exposure were calculated by using Chi square test. Student *t*-test was used to compare age differences between people living in the two study areas. Backwards stepwise multiple logistic regression analysis was used to estimate the effect of risk factors associated with the selected neuropsychiatric disorders. Models were adjusted for age, gender (1: males; 0: females), environmental pesticide exposure (1: areas of high pesticide use; 0: areas of low pesticide use), and an interaction term between pesticide use and gender (1: males residing in areas with high pesticide use areas; 0: remaining population). The comparative group used for each medical condition was comprised by the remaining cases included in the study with a distinct diagnosis. Data were analyzed using SPSS 18.0 and EPIINFO 3.5.1 statistical packages.

Results

Prevalence rates per 100,000 population for AD, PD, MS and suicide attempt were significantly greater in areas (health districts) with higher environmental pesticide exposure relative to those of low exposure (Table 3). In turn, the prevalence of cerebral degeneration was significantly reduced in areas of high exposure. When data are stratified by gender, similar differences were found for AD, PD, cerebral degeneration and suicide attempt. However, males failed to show significant differences for the prevalence of MS and their prevalence of polyneuropathies was greater in areas with high pesticide exposure. By contrast, females showed a significantly higher prevalence of MS and a lower prevalence of polyneuropathies in areas of high pesticide exposure.

Table 3 also shows the different risks for having neuropsychiatric disorders expressed as odds ratios for high exposure areas relative to those of low exposure. A significant increased risk was found for AD (OR 2.10), suicide attempt (OR 1.87), PD (OR 1.30) and MS (OR 1.23). No significant differences were found for polyneuropathies and affective psychosis between the two study areas. In turn, the population living in areas of high pesticide use had a lesser risk for presenting cerebral degeneration.

Stratification of data by gender is also shown in Table 3. Males living in high pesticide use areas had an almost two-fold higher risk of presenting AD and suicide attempts. They also showed a relatively small but significantly increased risk for PD and polyneuropathies (21 and 13%, respectively) as compared to males living in areas of low pesticide use. No statistically significant differences were found for MS and affective psychosis. Females showed similar results than males except for a significantly lower risk of polyneuropathies and an increased risk for MS in areas of high pesticide exposure. As occurred with males, no significant differences were found for affective psychosis between the two study areas.

Because some neurodegenerative disorders are associated with advanced age, the average age of our study population at the time of the first hospital discharge after diagnosis was compared between the two areas with different environmental exposure to pesticides (Table 4). The higher mean age observed for AD and PD only reflects that people with these disorders were discharged from Hospital at a slightly older age in the districts with high exposure to pesticides due to any cause of hospital admission, not necessarily their neuropsychiatric disorder. A further comparison of mean age between people living in districts of high exposure relative to those of low exposure failed to show significant differences in either males or females across 5-year age ranges for the age interval 35–84 years (data not shown).

Table 5 shows the multiple logistic regression analysis of the selected neuropsychiatric disorders adjusted for age, gender, environmental exposure to pesticides (coded as areas of high vs. low pesticide use) and an interaction term between exposure and gender. People living in areas with high pesticide use showed an increased

Table 4
Differences in mean age for the medical conditions studied at the time of the first hospital discharge after diagnosis (according to the Andalusian Minimum Dataset) between high exposure and low exposure areas in the study population and after stratifying by gender.

Medical condition	Exposure	Mean age (total population)	*P* value	Mean age (males)	*P* value	Mean age (females)	*P* value
Alzheimer's disease	High	82.45	<0.001	80.91	<0.001	83.32	<0.001
	Low	79.01		78.19		79.52	
Parkinson's disease	High	78.67	<0.001	77.97	<0.001	79.28	<0.001
	Low	76.05		75.30		76.83	
Multiple sclerosis	High	39.44	<0.05	41.33	0.10	38.47	<0.05
	Low	42.78		44.16		41.84	
Cerebral degeneration	High	61.70	<0.001	59.98	<0.001	64.08	<0.001
	Low	66.60		64.75		69.43	
Polineuropathies	High	62.31	0.5	60.89	0.99	65.16	0.79
	Low	62.72		60.90		65.46	
Affective psychosis	High	49.88	<0.001	48.77	<0.001	50.87	<0.05
	Low	46.66		44.70		48.45	
Suicide attempt	High	41.60	0.1	41.63	0.26	41.58	0.36
	Low	42.92		43.10		42.78	

risk for having AD and suicide attempts. Also, males living in these areas were at an increased risk for having polyneuropathies, affective psychosis and suicide attempts. However, a lower risk for cerebral degeneration was observed for the population residing in areas of high pesticide use.

Discussion

The present study was conducted to ascertain whether environmental pesticide exposure in areas with high level of pesticide use is associated with a number of neurodegenerative diseases and psychiatric disorders. To the best of our knowledge, this is the first study reporting seven different nervous system conditions among an elevated number of patients (n = 17429). The results provide further evidence to support an increased risk for AD, polyneuropathies, affective psychosis and suicide after environmental exposure to pesticides (Tables 3 and 5).

Table 5
Stepwise multiple logistic regression analysis of the selected neuropsychiatric disorders adjusted for exposure to pesticides, gender and age.

Medical condition	Risk factor	OR	95% CI	*P*-value
Alzheimer's disease	Age	1.10	1.09–1.10	<0.001
	Gender	0.64	0.59–0.70	<0.001
	Exposure	1.65	1.52–1.80	<0.001
Parkinson's disease	Age	1.06	1.05–1.06	<0.001
	Gender	1.18	1.10–1.28	<0.001
	Exposure	0.94	0.87–1.01	0.096
Multiple Sclerosis	Age	0.95	0.95–0.95	<0.001
	Gender	0.52	0.44–0.61	<0.001
Cerebral degeneration	Age	1.00	1.00–1.01	<0.001
	Gender	1.67	1.54–1.81	<0.001
	Exposure	0.46	0.42–0.50	<0.001
Polyneuropathies	Age	0.99	0.99–1.00	<0.001
	Gender	1.52	1.35–1.71	<0.001
	Exposure	0.68	0.58–0.79	<0.001
	Exposure*male	1.68	1.39–2.03	<0.001
Affective psychosis	Age	0.95	0.95–0.96	<0.001
	Gender	0.76	0.68–0.86	<0.001
	Exposure	0.71	0.63–0.80	<<0.001
	Exposure**male	1.18	0.99–1.41	0.068
Suicide attempts	Age	0.95	0.94–0.95	<0.001
	Gender	0.63	0.52–0.76	<0.001
	Exposure	1.76	1.49–2.08	<0.001
	Exposure*male	1.24	0.97–1.59	0.090

The following variables entered into the models: age, gender (0: female; 1: male), environmental pesticide exposure (1: areas of high pesticide use; 0: areas of low pesticide use) and the interaction term exposure*gender (1: males residing in areas of high pesticide use; 0: remaining population).

The favorable weather conditions in Andalusia make it possible to grow several crops a year within plastic greenhouses. Combinations of several classes of pesticides featuring additive or synergistic interactions are often used to prevent crops from the destructive effects of pests and to enhance crop productivity. Accordingly, besides agricultural workers the general population also presents background exposure to these compounds. A quantitative estimation for pesticide exposure clearly shows that the average pesticide use in high exposure areas is, on an individual basis, almost two-fold that estimated for low exposure areas with the exception of herbicides (Table 1). The predominance of extensive herbaceous crops (winter cereals, forages, olive tree) in the latter areas may account for the higher use of herbicides. Given that neither environmental nor biologic monitoring data were available to assess actual pesticide exposure of the target population for the time period studied, an ecologic design was used for the study.

The results of this study indicate that the prevalence and risk of AD, PD, MS and suicide are greater in populations living in areas of high pesticide use (indicating a higher potential exposure) relative to those of low pesticide use (Table 3). When data are stratified by gender, males living in high exposure districts showed a greater risk of polyneuropathies whereas females living in such areas presented a greater risk of MS and a reduced risk of polyneuropathies. The multiple logistic regression analyses shown in Table 5 confirm the raw results obtained in the bivariate analysis for AD, cerebral degeneration, polyneuropathies and suicide attempts. Besides, the multivariate analyses showed that males living in high pesticide use areas had an increased risk for affective psychosis. Generally, AD, PD and cerebral degeneration are age-related diseases, since increasing age represents the major risk factor for their development. In our study, no differences were found for age between the populations residing in districts of high relative to low exposure to pesticides, so age did not confound our results. As regards to gender, males had an increased risk for developing PD, cerebral degeneration and polyneuropathies, whereas females showed a higher risk for AD, MS, affective psychosis and suicide attempts. Our results are in line with previous studies reporting associations between pesticides and neurodegenerative disorders (Kamel and Hoppin, 2004).

Laske et al. (2004) reported an association between pesticide exposure and damage in certain brain areas related to PD and AD. Baldi et al. (2003) reported an association between PD and pesticides but only in men. In the Agricultural Health Study, long-term pesticide use was associated with incident PD but not with prevalent PD (Kamel et al., 2007). A 4.17-fold greater risk for PD was reported for people exposed to a combination of herbicides (maneb and paraquat) with respect to non-exposed populations (Costello et al., 2009). In our study, living in areas with high pesticide use, though a significant risk

factor for PD on bivariate analysis could not be proved significant in multivariate analysis. Certain environmental toxins, such as insecticides, herbicides, fungicides and, to a lesser extent, rural living and well water consumption have been considered as contributing factors for PD (Gill et al., 2010). Districts of low environmental exposure to pesticides had a greater use of herbicides (Table 1) and are located in inland areas (Fig. 2), where it is more likely to drink well water from groundwater aquifers. These two factors, along with methodological differences, may partially account for the contradictory results found for PD.

Several environmental factors have been considered as possible AD risk factors including metals, pesticides, solvents, electromagnetic fields, brain injuries, inflammation, educational levels, lifestyles and dietary factors (Migliore and Coppedè, 2009a). Few studies have examined the relationship of pesticide exposure and the risk of AD in later life. Baldi et al. (2003) reported a relative risk greater than 2-fold for AD in a prospective cohort study of French elderly persons who were exposed occupationally to pesticides. However, Gauthier et al. (2001) found no association between AD and environmental exposure to herbicides, insecticides and pesticides after controlling for genetic, occupational and sociodemographic factors. In our study, the population living in areas with high pesticide use had an increased risk for developing AD, independently of age and gender. Pesticides may affect the release of acetylcholine, a chemical that is important for memory and motor function, and AD is characterized by a disruption in memory, learning and other mental functions. It has been hypothesized that increased acetylcholine levels following acetylcholinesterase inhibition activate glutamatergic neurons causing the release of glutamate, which ultimately results in excitotoxicity and further neuronal death (Gill et al., 2010). However, other studies report that excitotoxicity is a non-cholinergic mechanism of OPs (Slotkin et al., 2010).

Although certain pesticides have been associated with MS (Blisard et al., 1986), other environmental toxins, such as heavy metal and solvents, show a stronger association. All these neurotoxins might trigger the immune system to damage brain cells and nerve tissue, leading to destruction of cells responsible for production of myelin. In our study, the population living in districts with a higher pesticide use, particularly females, showed a greater risk for having MS. Nevertheless, the multivariate analysis showed that younger age and female gender, but not pesticide exposure, were significantly associated with the disease.

Although uncommon, delayed polyneuropathy may occur in humans after massive OP poisoning. In turn, exposure to OPs at doses lower than those causing frank cholinergic toxicity may cause neuropathy only rarely. However, a pre-existing or underlying neuropathy (e.g., diabetics) may be amplified by exposure to certain pesticides (Lotti and Moretto, 2006). This lends support to our finding of a higher risk of polyneuropathies in males living in areas with high pesticide use. Though we are not aware of a differential distribution of the major causes of polyneuropathies (diabetes and alcohol consumption) between the two study areas, pesticide exposure appears to be a contributing factor in their development.

Acute high intensity pesticide exposure has been reported as a contributing factor for affective disorders such as depression (Beseler et al., 2008) whereas in the case of long-term, low-dose exposure such association is less accepted given the inconsistent epidemiological and experimental data (Moretto et al., 2010). However, in our study males living in areas with high pesticide use had an increased risk for affective disorders. Jaga and Dharmani (2007) described a potential risk of depression and suicide in agricultural workers exposed to OPs. London et al. (2005) also observed high rates of suicide in farming communities exposed to pesticides as well as a possible association between OPs and suicide. Although this effect may not be mediated through cholinesterase inhibition, much of evidence suggesting central nervous system effects after low level chronic exposure implies that other, more sensitive, brain proteins are likely targets (London et al., 2005). The average prevalence rate of suicide attempts for the year interval 1998–2005 was 99.62 per 100,000 in health districts with high pesticide use. This rate is 6-fold greater than the one reported for Western Almeria (a district of high pesticide exposure; 15.19 per 100,000) for the year interval 1976–1987 (Parrón et al., 1996), although the latter figure represented consummated suicides but not suicide attempts requiring hospital admission.

The etiology of neurodegenerative diseases is often multifactorial. There is a possibility that neurotoxic substances contribute to their occurrence in genetically vulnerable people by gene–environment interactions and thus may precipitate sporadic cases (Chang and Wu, 2009). Metals, pesticides and other environmental factors associated with neurodegeneration share the capability of producing reactive oxygen species (ROS) in nerve tissue. Different pesticide exposures, including OPs, have been reported to induce not only oxidative stress due to generation of free radicals, but also alteration in antioxidant defense mechanisms (López et al., 2007). Some tissues, especially the brain, are much more vulnerable to oxidative stress because of their elevated oxygen consumption that can produce large amounts of ROS. Moreover, oxidative damage is highly relevant in the brain because of its lower content in antioxidant enzymes, and the enriched composition of its membrane lipids in oxidizable polyunsaturated fatty acids (Slotkin and Seidler, 2010). The brain is also rich in metals, such as iron, which can act as a potent catalyst for ROS formation (Mariani et al., 2005; Migliore and Coppedè, 2009b). Accumulation of ROS leading to oxidative stress mediates a number of redox-dependent processes resulting in oxidative protein modifications, oxidative DNA damage and alterations in mitochondrial function (Franco et al., 2009; Chang and Wu, 2009). Cumulative oxidative modification in the mitochondrial DNA can result in erroneous transcription of genes encoding important subunits of the electron transport chain, leading to subsequent mitochondrial dysfunction, increased oxidative damage, and neuronal death (Fishel et al., 2007; Migliore and Coppedè, 2009b). In addition to the accumulation of oxidative damage in neurons, insufficient cellular repair mechanisms may contribute to premature aging and neuronal apoptosis. Because neurons are postmitotic cells, they are not self-renewed through cell proliferation and thus may lead to neurodegenerative disorders.

The risk of developing neurodegenerative diseases may be increased by interactions of environmental factors, such as pesticide exposure, with a number of genetic variants of degradative pathways (detoxification enzymes). Oxidative catabolism of pesticides, mediated by various cytochrome P450 enzymes, generates reactive free radicals and further oxidative damage. As regards to PD, differential expression or unfavorable genetic variants of CYP2D6, GSTP1 (the only GST family member expressed in substantia nigra) and the combined variant genotype MnSOD/NQO1 may increase the risk of the disease among farmers exposed to pesticides (Hubble et al., 1998; Menegon et al., 1998; Fong et al., 2007). Enzymes playing a role in toxicokinetic processes of xenobiotics may also contribute to the pathogenesis of PD with exposure to pesticides. This is the case of genetic variants of dopamine transporter (DAT) after paraquat and maneb exposure (Ritz et al., 2009). Inhibition of E1 ligase (an important degradative pathway) and inactivation of DJ1 (a mitochondrial protein involved in the protection against oxidative stress) have also been implicated in the etiology of PD after exposure to certain neurotoxins such as dithiocarbamate fungicides and paraquat, respectively (Chou et al., 2008), two pesticides that may act sinergistically.

This study has some limitations. The long latency period of the vast majority of neurodegenerative diseases makes it extremely difficult to track chemical exposures before the outcome in a longitudinal fashion and precludes to establish causal relationships. On the other hand, accurate exposure assessment may be difficult to perform in ecological studies because of the lack of exposure data at individual

level, which may result in misclassification (Brown et al., 2005). Besides, confounding from the grouping variable due to differential distribution of other risk factors (i.e., environmental or dietary exposure to other neurotoxins) over the health districts studied was partly solved by restricting the study to areas rather homogeneous with regard to farming practices (intensive agriculture within plastic greenhouses), which considerably diminished the within-district heterogeneity of pesticide exposures while large differences between districts remained. In addition, the potential bias from socioeconomic factors was ruled out because of the lack of significant differences in the distribution of the employed population across primary, secondary and tertiary sectors of the economic activity between areas with high and low pesticide use. Despite these limitations, the major strength of the present study is the collection of a large sample size of neuropsychiatric disorders that allows to achieve enough statistical power to counter the generally low magnitude of the association reported between pesticide exposure and nervous system health effects.

In conclusion, the results of this study indicate a higher prevalence and greater risk for certain neurodegenerative diseases and suicide attempts in populations, particularly males, living in areas with high use of pesticides. Given that a higher use is an indication of a higher potential exposure, this study provides indications that exposure to pesticides may be affecting the human health by increasing the incidence and risk or neurological disorders when evaluating at the level of the general population. Although ecological bias and other types of confounders preclude etiological interpretations, some of the observed excesses may be due to environmental exposures to pesticides. The results of this study support and extend previous associations of these chemicals with neurological disorders and may provide leads for further toxicological studies.

Conflict of interest statement

The authors declare that they do not have conflict of interest.

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