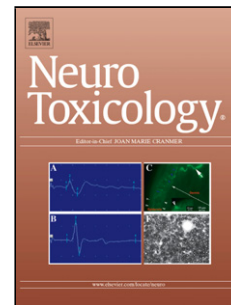# Accepted Manuscript

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Association between environmental exposure to pesticides and epilepsy

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Highlights

- The relationship between environmental exposure to pesticides and epilepsy is unknown
- 4007 subjects diagnosed with epilepsy over the years 1998 and 2010 were examined
- Areas of high vs. low pesticide used were defined based on agronomic data
- A high prevalence rate of epilepsy was found in areas of greater pesticide use
- Environmental exposure to pesticides might increase the risk of having epilepsy

Abstract

There is increasing evidence of an association between long-term environmental exposure to pesticides and neurodegenerative disorders; however, the relationship with epilepsy has not been addressed thus far. This study was aimed at determining the prevalence and risk of developing epilepsy among people from South-East Spain living in areas of high vs. low exposure to pesticides based on agronomic data. The study population consisted of 4007 subjects with a diagnosis of epilepsy and 580,077 control

subjects adjusted for age, sex and geographical area. Data were collected from hospital records of the Spanish health care system (basic minimum dataset) between the years 1998 and 2010. The prevalence of epilepsy was significantly higher in areas of greater pesticide use relative to areas of lesser use. Overall, an increased risk of epilepsy was observed in the population living in areas of high vs. low use of pesticides (OR:1.65; p <0.001). Although this study was exploratory in nature, the results suggest that environmental exposure to pesticides might increase the risk of having epilepsy.

Keywords: pesticides; environmental exposure; epilepsy.

1. INTRODUCTION

Epilepsy is the most common severe neurological disorder (Sander, 2003) affecting 0.5–1%, of the world population, with a lifetime incidence of 1–3% (Michael-Titus et al., 2010). It is estimated that 70 million patients around the world are diagnosed with epilepsy at any stage of life (OMS, 2015) as the disease can occur at any age, from birth to childhood and also among adults, including the older age groups (Rados, 2005). However, the occurrence, prevalence, and burden of epilepsy vary widely throughout the world (Banerjee et al., 2009), showing higher rates in developing countries than in developed countries (Perucca et al., 2001; Preux and Druet-Cabanac, 2005) as 90% of patients are thought to live in Africa, Asia, and Latin America (Houinato et al., 2013). In Spain, there are around 400,000 diagnosed epilepsy cases with 12,400 to 22,000 people showing symptoms for the first time every year. The majority of them are children, between 6 and 14 years old, with a rate of 3.7 cases per 1000 inhabitants (SEN, 2009).

Risk factors for epilepsy are multiple and include genetic background, trauma, brain tumor, metabolic disturbances, infections, autoimmune reactions, and chemical

exposures (Jovel et al., 2018; Pauschek et. al. 2016). Nevertheless, epilepsy likely result from interactions between the genetically-determined seizure threshold, underlying predisposing medical conditions or metabolic disorders, and acute triggers (Guberman and Bruni, 1999). Acquired epilepsy usually develops in three phases: injury (brain insult), epileptogenesis (latency) and chronic epilepsy (spontaneous recurrent seizure) (Raza et al., 2004).

A number of chemicals may cause seizures by a variety of molecular mechanisms and pathways. In addition, exposure to some chemicals during critical periods of development can disrupt neurodevelopment and potentiate the response to toxicants later in life (Ramsdell, 2010). In this regard, animals have shown to be more prone to generate seizures when stimulated in critical developmental periods (Stanojlović et al., 2013).

Seizurogenic chemicals are quite diverse and include toxic industrial chemicals, pesticides and natural toxins. While many of them are capable of producing seizures if exposure occurs at high enough doses, repeated administration of chemo-convulsants in subconvulsive doses may reduce the seizure threshold, a phenomenon referred to as chemical kindling (Mason and Cooper, 1972). A number of pesticides have been reported to induce kindling, such as lindane, endosulfan, chlordimeform, amitraz and chlorpyrifos (Wurpel et. al. 1993; Gilbert 1992; Gilbert and Mack 1989; Joy et. al. 1982). These pesticides elicit convulsant effects following repeated exposure at low doses (Gilbert 2001; Gilbert 1995).

The European Food Safety Authority (EFSA) grouped pesticide active substances according to their toxicological profiles in the so-called cumulative assessment groups (EFSA, 2014). To this end, regulatory studies were scrutinized to collect information on toxicological effects of authorized pesticides on the nervous

system. Convulsions were taken into account only if they were considered as a true specific effect, but not as a result of general toxicity following exposure to high doses. A number of pesticides belonging to different chemical classes were considered to produce convulsions as chronic effects (e.g., λ-cyhalothrin, fipronil, glufosinate, lufenuron and mepiquat). Interestingly, none of these pesticides were classified as producing convulsions as a result of acute exposure (the only pesticides eliciting this effect were zeta-cypermethrin, dimethoate, chlotianidin and pirimiphos-methyl) (EFSA, 2014). This experimental information supports the hypothesis that pesticides can produce epileptic seizures in humans as a result of long-term exposure to low doses and in the absence of acute poisoning.

In the last 50 years, pesticides have been widely and increasingly used throughout the world because of their benefits to agriculture and public health. According to the US-EPA, in 2011-2012, the amount of pesticides used around the world was approximately 2,7 million tons (Atwood and Paisley-Jones, 2017). However, the inadequate use of these substances may affect the health of applicators, farmers and consumers, as well as may impact the environment (Jabłońska-Trypuć et al., 2017; Pimentel et al., 1996). In fact, exposure to these substances has turned into an important issue of public health concern throughout the world.

The objective of this study was to assess whether environmental exposure to pesticides is associated with a greater prevalence rate and a higher risk of having epilepsy. To the best of our knowledge, this hypothesis has not been addressed so far.

2. MATERIAL AND METHODS

2.1. Design

A population-based case-control study was conducted wherein epilepsy cases and control subjects were selected from the province of Almeria (South-East Spain).

This province consists of three geographic areas (Centro [Center], Levante [East], and Poniente [West]), each of which corresponds to an administrative land division centered by a hospital of reference. Because the primary health care system is organized according to this organizational structure, the three areas mentioned above are also known as 'health care districts'.

2.2. Criteria for the selection of study areas

The three health care districts of Almeria province were categorized into two groups with different pesticide use according to agronomic criteria, which was considered as a surrogate for pesticide exposure (Table 1). The areas of high exposure included two health care districts: West Almeria (Poniente) and Center Almeria (Centro), whereas the low exposure area consisted of the health district of East Almeria (Levante). Up to 96% of the greenhouse surface of the province of Almería is located in the health districts of Poniente and Centro (Cartografía de Invernaderos de Cartografía de Almería, Granada y Málaga for 2017; http://www.juntadeandalucia.es/export/drupaljda/Cartografia%20_inv_AL_GR_MA_S EE.pdf). Accordingly, both health districts were categorized as areas of high pesticide use, since 79% of the total amount of pesticides sold in Almeria province was used in these districts. The remaining 21% was used in the health district of Levante, which contains only 4% of the total area of greenhouses in Almería province. Therefore, this district was categorized as of low pesticide use (see Supplementary material).

2.3. Study population

Cases were collected from computerized records of the Andalusian Public Health Service, referred to as Minimum Dataset, over a period of 13 years. 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 are routinely recorded in AMD as are also age, gender, race and place of residence. Subsequent hospital admissions of a same patient would not count as a new case because that patient has been previously identified. Accordingly, there is no possibility for duplicate cases. The validity of the data gathered in AMD is determined by the quality of the discharge report with respect to the collection of the principal and secondary diagnoses and procedures, and by exhaustiveness in the coding of hospital discharges.

A total of 4007 subjects having their place of residence in Almeria province and with a diagnosis of epilepsy were collected from the study areas between the years 1998 and 2010 (see distribution for adult and children epilepsy in the districts with high and low use of pesticides in Table 2). The diagnosis of epilepsy was defined according to the International Disease Classification, Ninth Revision, Clinical Modification (ICD-9CM) of the World Health Organization (code 345, epilepsy and recurrent seizures). Since the ICD-9CM was in force in Andalusia until January 2016, it was used for the data collection over the study period (1998-2010). The control group consisted of general population living in the same health care districts and over the same time period, but who were not diagnosed with epilepsy. The total number of participants included in the control group was 580,077, which was proportional to the number of epilepsy cases in each health care district of residence and matched by age and sex to be comparable (the distribution of the control population between areas of high and low pesticide use is shown in Table 2). Control subjects were taken from the 2004 census, which corresponds to the middle of study period.

2.4. Statistical analysis

Frequencies and percentages were calculated for categorical variables, and mean and standard deviation for quantitative variables. In addition, prevalence rates and risk of epilepsy were calculated in areas with high and low pesticide use (Odds Ratio –OR– and 95% confidence interval –95% CI– were calculated). The Mann Whitney U test was used to compare differences in age of the population between the two study areas as the prior use of Kolmogrov Smirnof's normality test indicated a non-normal distribution. The Chi-square test was used for qualitative variables. Multiple logistic regression analysis was conducted to assess the risk of epilepsy adjusted for age, gender and exposure to pesticides, as these were considered to have an influence on the statistical model. Models were also adjusted for the interaction term exposure X sex, and exposure X age. A mixed-model (multilevel) analysis was performed to examine the associations between epilepsy rates and pesticide exposure at the level of health district (districts of high vs. low use of pesticides). The level of statistical significance was set at p<0.05. The data was analyzed with the statistics packages SPSS 22.0 and EPIINFO 7.

3. RESULTS

The average age of the study subjects was 36.4 years with a standard deviation of 21.8 years. The group with higher exposure to pesticides had a mean age of 35.6 years and the lower exposure group 39.4 years, with the differences being statistically significant (p<0.001). Regarding gender, no statistically significant differences were observed between the two groups.

The total prevalence rate of epilepsy per 1000 inhabitants and for both sexes was significantly higher in areas with greater use of pesticides relative to those with lesser use (p<0.001). Regarding pediatric epilepsy, a higher number of cases was observed in areas with high use of pesticides as compared to those with low use; however, the differences were not statistically significant. Among adults, prevalence rates per 1000

inhabitants were significantly higher in areas of high *vs.* low pesticide use (p<0.001) (Table 3). Overall, a statistically significant higher risk of having epilepsy was observed in areas of greater use of pesticides (OR: 1.49, 95%CI: 1.36-1.62). When stratifying by sex, males showed a somewhat lower risk of epilepsy than females (OR: 1.43 and 1.56, respectively). However, when the adult population was considered, similar ORs were found for males and females and for both sexes together, with differences being statistically significant. Concerning pediatric epilepsy, girls had a significantly higher risk than boys (p<0.001) in areas of high *vs.* low pesticide use (OR: 1.69; 95%CI: 1.20-2.39).

Because of the hierarchical structure of the data (individuals were embedded in health districts to which a distinct level of pesticide use was assigned), a multilevel analysis was performed with epilepsy rates as the dependent variable and districts with high *vs.* low use of pesticides as the variable corresponding to the first level, treated as fixed effects. Since no statistically significant differences were found (p=0.12), we assumed that variables other than residing in districts with high vs. low use of pesticides did not influence the baseline model.

Table 4 shows the results of the multiple logistic regression models to assess the risk of epilepsy in relation to the different parameters included in the study. Hosmer and Lemeshow's goodness-of-fit test were found to be statistically significant (p<0.001). An increased risk of having epilepsy was observed both in the pediatric and adult populations living in areas of high vs. low exposure to pesticides (OR: 3.37 and 2.60, respectively). For the total population, a significantly increased risk of having epilepsy was observed in the population living in areas of high *vs.* low pesticide use (OR: 1.65).

4. DISCUSSION

The current study was conducted to assess whether living in areas with high pesticide use is associated with the occurrence of epilepsy. Results indicate that the prevalence and risk of having epilepsy is higher among populations living in areas of high pesticide use than in those of low pesticide use (Tables 3 and 4).

The increased use of pesticides for crop protection determines that, apart from applicators and agricultural workers, the general population is also exposed to these chemicals. In our study, although total greenhouses surface was over 40 times greater in the area of high pesticide use than in the one of low use, a relative estimate of pesticide use indicates that it is only 3.6 times greater (7835 vs. 2159 tons in the areas of high and low pesticide use, respectively) (Table 1). The reason behind this is the large number of woody crops (citrus, olive, almond and fruit trees) in the area of low pesticide use, as these crops require a lesser pesticide application.

This difference in the pesticide use might suggest potential differences in occupational and socioeconomic status among the three health district studied. Since a lower socioeconomic status has been reported as a risk factor for epilepsy in previous epidemiologic studies, we compared the distribution of the employed population across primary, secondary and tertiary sectors of the economic activity between areas with high and low pesticide use (Table 5). Although statistically significant differences were observed, these were driven by the fact that the primary sector predominated in the area of high pesticide use whereas the secondary sector was most prevalent in the area of low use of pesticides. The tertiary sector, which has a major socioeconomic impact, had a similar distribution between the two study areas (Table 5). However, no differences were observed between the disposable personal incomes between the areas of high and low pesticide use. Overall, socioeconomic factors can be considered as having no differential impact on the risk of epilepsy in our study.

Recognized risk factors for the development of epilepsy include birth injury, febrile seizures, traumatic brain injury, intracranial infection and family history of epilepsy (Jovel et al., 2018; Pauschek et. al. 2016). However, given the nature of this study it was not possible to account for all these potential factors as they were not included in the computerized hospital medical records (basic minimum data set). Despite this, and due to the relatively large size of the study population, the aforementioned factors are expected to be equally distributed in the population living in the study areas with distinct pesticide use.

To the best of our knowledge, there are no previous epidemiological studies examining pesticide exposure as a risk factor for epilepsy beyond cases of acute poisoning. However, various studies conducted in animals have shown *status epilepticus* (uninterrupted seizure activity for at least 30 minutes) after treatment with organophosphates (OPs) and carbamates (Todorovic et al., 2012; Shrot et al., 2014).

Exposure to OPs produces seizures that progress to *status epilepticus*, which can cause brain damage or death. Seizures may result from overstimulation of central muscarinic acetylcholine receptors (mAChRs) as a result of inhibition of acetylcholinesterase (Miller et al., 2017). This is followed by glutamatergic hyperactivity because of excessive release of glutamate from glutamatergic neurons, leading to intracellular calcium overload in postsynaptic neurons along with oxidative stress and increased neuroinflammatory responses (Chen, 2012). Such excessive calcium release causes excitotoxic lesion to the affected neurons which sustains and reinforces seizure activity as a result of neuronal excitotoxicity (Jett, 2007). This neural dysfunction could subsequently causes secondary neuronal damage and chronic neuropsychiatric consequences (Chen et al, 2012). There is evidence that elevated

intracellular calcium concentration and altered calcium homeostatic mechanisms may play a role in the development of acquired epilepsy (Raza et al., 2004).

Chronic low-level exposure to the OP dichlorvos in adult rats induced apoptotic neurodegeneration by raising mitochondrial calcium levels, impairing mitochondrial complexes I, III and IV, and increasing oxidative stress. In addition, low-level repeated exposure to other OPs (e.g., chlorpyrifos, acephate) has also been shown to induce inflammatory responses *in vitro* and upregulation of inflammatory cytokines *in vivo* (reviewed in Terry, 2012). Importantly, these OP chemicals were used as insecticides in our study areas (see Table 1), thus affording biological plausibility to this study.

Organochlorine and pyrethroid insecticides also exert neurotoxic effects causing a hyperexcitable state in brain leading to seizures and tremor (Stanojlović et al., 2013). All pyrethroids and DDT create a condition of hyperexcitability as a result of interacting with the sodium channel. Furthermore, most organochlorines and type II pyrethroids block the $GABA_A$ receptor and reduce the GABA-induced hyperpolarizing inward Cl^- flux, thus inducing a hyperexcitability syndrome in mammals accompanied by convulsions (Costa, 2015). In our study areas, type II pyrethroids (e.g., cypermethrin, tralomethrin, acrynathrin) and the organochlorine endosulfan were insectides commonly used (Table 1).

On the other hand, pesticides can induce oxidative stress as a result of an increased production of highly reactive molecules and/or a decrease in the antioxidant defenses against oxidative damage (Abdollahi et al., 2004). These included OPs, *N*-methylcarbamates, organochlorines, pyrethroids, triazines, neonicotinoids, paraquat and dithiocarbamates (Hernández et al., 2013), all of which were used in our study areas (Table 1), Since mitochondria are the primary site of production of highly reactive molecules, they are vulnerable to oxidative damage. Furthermore, mitochondria play a

key role in excitotoxicity and apoptosis (Waldbaum and Patel, 2010). Mitochondrial oxidative stress and dysfunction have been suggested to be contributing factors of epilepsy. *In vivo* studies have shown that mitochondrial oxidative stress can reduce the seizure threshold, so that oxidative stress cannot be considered only a consequence of seizures but also an active contributor to seizures and epileptogenesis (Waldbaum and Patel, 2010). Therefore, long-term exposure to low-environmental doses of toxic pesticides, in particular neurotoxic insecticides, may cause oxidative stress and hyperactivity in the nervous system and thus contribute to epileptogenesis. This pathomechanism might be facilitated by a particular genetic background that would make individuals more prone to undergo epileptic seizures. This hypothesis provides biological plausibility to the results of our study and, if confirmed with further epidemiological studies, would support long-term exposure to pesticides as a new environmental risk factor for epilepsy.

The Agricultural Health Study (AHS) looked at the risk of exhibiting a combination of neurological symptoms associated with exposure to pesticides and found that farmers with long-term moderate exposure to OP and organochlorine insecticides had a higher risk of neurological symptoms, such as dizziness, weakness, loss of balance, muscle spasms, tremors, difficulty speaking, loss of consciousness, etc. (Kamel et al., 2007). However, such study did not find significant differences between long-term and short-term exposure. A further study carried out in South-East Spain found that agricultural workers who applied pesticides had a higher likelihood of suffering from neurological symptoms lasting more than two days, such as cramps, tremors, muscle fatigue, loss of consciousness, and seizures (García-García et al., 2016). Park et al. (2018) evaluated the occurrence of seizures in acutely intoxicated patients and found the highest incidence in those who ingested ammonium glufosinate (31.5% of

patients), followed by those who ingested pyrethroids (5.9%) and glycine derivatives (5.4%). General grand mal seizures were the most frequent type (85.7% of cases). The majority of studies on the prevalence of epilepsy have found large differences, although most of them reported a prevalence rate of 5-10 per 1000 inhabitants, which can be extrapolated to a global level (Sander and Shorvon, 1996). In our study, we found a prevalence rate of 7.38 per 1000 inhabitants in areas of high pesticide use. This figure is somewhat higher than the rate reported by Sander and Shorvon (1996) for the general population and also higher than those reported for Southern European countries. A study conducted in the province of Malaga, near our study area, showed a prevalence rate of 4.79 per 1000 inhabitants (García-Martín et al., 2012). In the US, Helmers et al. (2015) found a prevalence rate of 8.5 per 1,000 individuals. Other studies reported differences between developed and developing countries, with a figure of 4-7 per 1000 inhabitants in developed countries and 5-74 per 1000 inhabitants in developing countries (Preux and Druet-Cabanc, 2005). However, there exist contradictory results as Pickrell et al. (2015) found a prevalence rate of 7.7 per 1000 inhabitants in developed countries and 5.6 per 1000 inhabitants in developing countries (Pickrell et al., 2015).

Another study found consistent prevalence rates in both rural and urban areas (12.8 and 12.2 per 1000 people, respectively), although differences were not statistically significant (Gaitatzis et al., 2004). However, Ngugi et al. (2010) reported a similar prevalence rate in rural areas (12.7 per 1000 inhabitants) but a lower prevalence for urban areas (5.9 per 1000 inhabitants). Regarding age, the prevalence of the disease typically increases with age, reaching a plateau at middle age and raising again later in life. This pattern is more frequently observed in developed countries, whereas in developing countries the prevalence does not tend to increase (or even decrease) after the age of 60 (Banerjee et al., 2009; Ngugi et al., 2010). In our study, we found a lower

prevalence rate of pediatric epilepsy cases in areas with a greater use of pesticides. However, in areas of low use of pesticides the rate of epilepsy in children was higher than that observed in adulthood (Table 3).

This study has a number of limitations. This is a mixed study where the outcome (epilepsy) and some individual confounders (age, sex) were assessed at individual level while pesticide exposure was assessed at ecological level, based on indirect proxies such as quantitative agronomical criteria. The study design used poses major problems of interpretation when making ecologic inferences and especially when making biologic inferences (i.e. due to ecologic bias). Ecologic bias was to some extent reduced because relatively small geographical units (health districts) were used, such that they were more homogeneous with respect to pesticide exposure. Additionally, a potentially greater migration between groups was not expected because no relevant socioeconomic differences were found between the districts of high and low pesticide use). A less precise estimation of disease rates across health districts can be ruled out because of the quality assessment of disease coding and registry aforementioned. Furthermore, the proportion of non-hospitalized cases of epilepsy was expected to be similar in the areas of high and low pesticide use. Likewise, it is possible that some of the other risk factors for epilepsy may vary by health district since social circumstances would be related to some of these; however, this potential bias would be non-differentially between the districts of high and low pesticide use. On the other hand, potential confounding factors (other than age and gender) could not be considered as the only available variables from the dataset examined were sex, age and place of residence. Therefore, the lack of adjustment for potential extraneous risk factors may not reduce the ecologic bias produced by these factors.

In our study there was no potential for differential health care seeking or recourse to hospitalization between health districts with distinct use of pesticides. The three districts studied (and their respective reference hospitals) had the same health resources and provided the same kind of medical assistance to their reference population. In addition, the patients' hospital medical record (and therefore the basic minimum dataset) was the same for the three hospitals as they used identical disease coding criteria.

5. CONCLUSION

While numerous studies have shown an association between acute pesticide exposure and further development of seizures, no study addressed the relationship between long-term low-dose exposure to pesticides and epilepsy. This study indicates that hospital-diagnosed epilepsy prevalence was higher in those health districts where a higher pesticide use was made in intensive agriculture under plastic greenhouses. However, the limitations inherent to the study design warrant further research to ascertain whether there exists a true causal association.

6. CONFLICT OF INTERSTS STATEMENT

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

7. ACKNOWLEDGEMENT

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Table 1: Agronomic criteria used to categorize health districts as areas with high or low use of pesticides in Almeria (South Spain).

Agronomic Criteria	High use of pesticides	Low use of pesticides
Hectares of plastic greenhouse [a]	26264	632
Total pesticides (tons used) [a]	7834.97	2158.61
Insecticides [b]	1125.05	309.96

	High use of pesticides	Low use of pesticides
Nematocides [c]	4083.4	1125.02
Fungicides [d]	1437.52	396.05
Herbicides [e]	133.94	36.9
Plant growth regulators [f]	873.39	240.63
Other pesticides [g]	181.67	50.05

[a]Source: Consejería de Agricultura, Ganadería y Pesca http://www.juntadeandalucia.es/organismos/agriculturapescaydesarrollorural.html. Data for the agricultural season 2006-2007.

[b,c]Organophosphates (malathion, dimethoate, acephate, chlorpyrifos, dichlorvos), *N*-methylcarbamates (methomyl, oxamyl, formetanate), pyrethroids (cypermethrin, tralomethrin, acrynathrin,), neonicotinoids (imidachloprid), growth regulators insecticides (tebufenozide, buprofezin), organochlorine (endosulfan), others (abamectin, thuringiensin).

[d](Di)thiocarbamates (zineb, mancozeb, maneb, thiram), benzimidazole (benomyl, carbendazim, thiabendazole), conazole (prochloraz, tebuconazole, triadimefon), others (copper, cymoxanil, oxadixyl, fosetyl).

[e]Bipyridyl (paraquat, diquat), organophosphonates (glyphosate, glufosinate), phenylurea (lufenuron).

[f]Auxins (4-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid), defoliants (cyanamide, metoxuron), plant growth inhibitors (chlorpropham), plant growth retardants (chlormequat, uniconazol),

[g]Fumigants (aluminium phosphide, 1,3-dichloropropene).

Data for individual pesticides most often used in greenhouses of the study areas are based on Hernández et al. (2003) and Parrón et al. (2011).

Table 1: Agronomic criteria used to categorize health districts as areas with high or low use of pesticides in Almeria (South Spain).

Agronomic Criteria	High use of pesticides	Low use of pesticides
Hectares of plastic greenhouse [a]	26264	632
Total pesticides (tons used) [a]	7,834.97	2,158.61
Insecticides [b]	1,125.05	309.96

Nematocides [c]	4,083.4	1,125.02
Fungicides [d]	1,437.52	396.05
Herbicides [e]	133.94	36.9
Plant growth regulators [f]	873.39	240.63
Other pesticides [g]	181.67	50.05

[a]   Source: Consejería   de   Agricultura,   Ganadería   y   Pesca http://www.juntadeandalucia.es/organismos/agriculturapescaydesarrollorural.html. Data for the agricultural season 2006-2007.

[b,c] Organophosphates (malathion, dimethoate, acephate, chlorpyrifos, dichlorvos), *N*-methylcarbamates   (methomyl,   oxamyl,   formetanate),   pyrethroids   (cypermethrin, tralomethrin, acrynathrin,), neonicotinoids (imidachloprid), growth regulators insecticides (tebufenozide, buprofezin), organochlorine (endosulfan), others (abamectin, thuringiensin).

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Data for individual pesticides most often used in greenhouses of the study areas are based on Hernández et al. (2003) and Parrón et al. (2011).

Table 2: Distribution of the study population as a whole and stratified by the geographical areas studied (high and low pesticide exposure).

Epilepsy	Total Population		Area of high pesticide use		Area of low pesticide use	
	Cases	*Controls*	*Cases*	*Controls*	*Cases*	*Controls*
Pediatric	731	102,907	608	82,913	123	19,994
Adults	3,276	477,170	2,768	370,793	508	106,377
Total	4,007	580,077	3,376	453,706	631	126,371

Table 3: Prevalence (rate per 1000 inhabitants), odds ratio (OR) and 95% confidence interval (95% CI) for epilepsy in the population living in areas of high pesticide exposure relative to areas of low exposure.

Epilepsy	High exposure			Low exposure			ORc			95% CI			*p* value*		
	Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total
Pediatric	8.15	6.35	7.28	8.71	3.88	6.34	0.97	1.69	1.19	0.76-1.23	1.20-2.39	0.98-1.44	0.80	0.002	0.07
Adults	7.96	6.84	7.40	5.01	4.41	4.72	1.57	1.54	1.56	1.38-1.79	1.34-1.77	1.42-1.71	<0.001	<0.001	<0.001
Total	7.99	6.75	7.38	5.58	4.33	4.96	1.43	1.56	1.49	1.28-1.60	1.37-1.78	1.36-1.62	<0.001	<0.001	<0.001

*Pearson's Chi Square test

Table 4: Stepwise multiple logistic regression analysis of pediatric, adult and total epilepsy adjusted for exposure to pesticides, gender and age.

Pathology	Risk factor	OR*	95% CI	p value
Pediatric Epilepsy	Age	1.15	1.09-1.21	<0.001
	Sex	2.12	1.44-3.13	<0.001
	Exposure	3.37	1.79-6.33	<0.001

	Age x Exposure	0.92	0.87-0.98	<0.001
	Exposure x Sex	0.59	0.39-0.90	<0.05
Adult Epilepsy	Age	1.04	1.03-1.04	<0.001
	Sex	1.30	1.22-1.40	<0.001
	Exposure	2.60	1.89-3.57	<0.001
	Age x Exposure	0.99	0.98-0.99	<0.05
Total Epilepsy	Exposure	1.65	1.51-1.79	<0.001
	Sex	1.28	1.20-1.36	<0.001
	Age	1.02	1.02-1.03	<0.001

* Models were adjusted for the following variables: age, gender (0: female; 1: male), environmental pesticide exposure (1: areas of high pesticide use; 0: areas of low pesticide use), and the interaction terms exposure x gender and exposure x age.

Table 5. Distribution of employed population by economic activity and average disposable

personal incomes

	High use of pesticides	Low use of pesticides	*p*
Economic sectors[*]			<0.001
Primary sector (agriculture and livestock, forestry and fishing)	55,411 (30.40%)	9,980 (22.43%)	
Secondary sector (manufacturing industry and construction)	31,396 (17.22%)	12,590 (28.30%)	
Tertiary sector (service industries, private and public activities)	95,464 (52.37%)	21,908 (49.25%)	
Disposable personal income[**] (Euros/person)	13,446 ± 1,895	14,340 ± 1,240	0,056

[*]Data obtained from the 2001 database from the Institute of Statistics of Andalusia

[**]Source: Spanish Tax Agency [Accessed on 26 may 2017] Available at:
http://www.agenciatributaria.es/AEAT/Contenidos_Comunes/La_Agencia_Tributaria/Estadisti
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