Neurotoxicology 96 (2023) 166–173

Contents lists available at ScienceDirect

Neurotoxicology

journal homepage: www.elsevier.com/locate/neuro

Occupational exposure to pesticides as a potential risk factor for epilepsy

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ARTICLE INFO

Edited by Dr. P Lein and Dr. R Westerink

Keywords:
Epilepsy
Pesticides
Occupational exposure
Farmers
Pesticide applicators
Personal protective equipment

ABSTRACT

Epilepsy is a chronic neurological disorder in which brain activity becomes abnormal, causing seizures. In a previous study we found that environmental exposure to pesticides was associated with a greater risk of epilepsy. The present study examined possible occupational risk factors that may contribute to the occurrence of epilepsy in farmers and pesticide applicators (sprayers). A case-referent study was conducted on 19,704 individuals over a 17-year study period (2000–2016). Epilepsy cases (n = 5091) were collected from Hospital records and referents (non-epilepsy cases, n = 14.613) from the Centre for Prevention of Occupational Risks, both from Almería (South-Eastern Spain). A significant increased risk of having epilepsy was found in farmers working in intensive agriculture (high-yield greenhouse crops) compared to extensive agriculture (open-air crops). The risk was greater for farmers residing in rural areas with high pesticide use (intensive farming crops in plastic greenhouses) and for those not wearing protective gloves. As for sprayers, the greatest risk of epilepsy was observed in those not wearing face mask, and in those living in areas with high pesticide use (greenhouse intensive agriculture). Overall, this study supports previous findings on the association between epilepsy and pesticide exposure in the general population, and extends the risk to farmers occupationally exposed to pesticides, mainly those engaged in intensive agriculture.

1. Introduction

Epilepsy is a chronic and serious neurological disorder that can cause unpredictable seizures. It is considered the fourth most common neurological disorder and affects people of all ages (Leonetti et al., 2020). According to the World Health Organisation (WHO), epilepsy is the second most common neurological disease regarding number of lost years of life or years lived with disability (GBD Global Burden of Diseases, 2015). In Europe, it affects about 6 million people with 400,000 new cases being diagnosed each year (Brodie et al., 1997; Baulac et al., 2015). In Spain, the age and sex-adjusted prevalence in people over 18 years of age is 14.87 per 1000 population, and the active prevalence, defined as epileptic seizures in the last five years, is 5.79 per 1000 population (Serrano Castro et al., 2015).

Multiple risk factors have been reported for epilepsy, including genetic background, trauma, brain tumour, metabolic disturbances, stroke, infections, autoimmune reactions and chemical exposures (Espinosa-Jovel et al., 2018; Pauschek et al., 2016). Some forms of epilepsy are considered as neurodevelopmental disorders (NDDs) since several developmental factors (including congenital brain malformations, altered neuronal signalling during embryonic life, and defects in postnatal maturation of neuronal networks) contribute to epileptogenesis (Bozzi et al., 2012). The developing brain is more vulnerable to chemicals than that of adults and numerous chemicals have been related with NDDs (Rice and Barone, 2000).

Certain chemicals, including pesticides, can cause convulsions by different mechanisms and molecular pathways. The most known include hyperstimulation of nicotinic and muscarinic acetylcholine receptors, blockage of voltage-gated sodium channels, altered function of GABAergic neurons, glutamatergic hyperactivity, neuronal

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https://doi.org/10.1016/j.neuro.2023.04.012
Received 2 March 2023; Received in revised form 26 April 2023; Accepted 27 April 2023
Available online 28 April 2023

excitotoxicity, intracellular calcium overload, oxidative stress and increased neuroinflammatory responses, among others (Becchetti et al., 2023; Chen, 2012; Miller et al., 2017; Jett, 2007).

The assessment performed by the European Food Safety Authority (EFSA) of in vivo studies performed according to OECD guidelines for the testing of chemicals, and submitted by applicants for the authorization of pesticides, identified pesticides from different chemical classes that produced convulsions. These were observed as part of acute or chronic toxicity tests. Bifenthrin, zeta-cypermethrin, dimethoate, pirimiphos-methyl, clothianidin, dieldrin and endosulfan caused convulsions in acute neurotoxicity studies, whereas beta-cypermethrin, lambda-cyhalothrin, phoxim, benfuracarb, fipronil, glufosinate, lufenuron, endrin, heptachlor, lindane and mepiquat induced convulsions in repeated-dose studies (90-days, 1- and 2-year studies) (EFSA European Food Safety Authority, 2014, 2019). This observation raised the hypothesis that pesticides might contribute to the occurrence of epileptic convulsions in humans as a result of repeated, low-dose exposure (Requena et al., 2018).

Pesticides are chemicals widely used to increase the global agricultural production. Though the main source of exposure of the general population is the ingestion of pesticide residues through the diet (mainly food commodities from vegetal origin), the highest exposure occurs in farmers, particularly those involved in intensive agriculture (e.g., plastic-covered greenhouses for growing vegetable crops) and to a lesser extent in extensive cultivation in open-field farming (Negatu et al., 2016a; and, 2016b; Machado and Martins, 2018). Total greenhouse area has increased worldwide, reaching in 2019 an estimate of near half a million hectares (https://www.cuestaroble.com/statistics.html). According to the World Vegetable Map 2018, Spain is the second country in the world after China in terms of surface of agricultural land dedicated to plastic-covered greenhouses for horticultural crop production. Of the 70,000 ha estimated in Spain, over 44,000 ha are located in Andalusia (Rabobank, 2018), most of them in the province of Almería, where this study was conducted (Supplementary Figure 1). This type of intensive agriculture requires a higher amount of pesticides to protect crops from harmful pests. Between the years 2000–2016 (study period), the pesticide use trend in Spain increased with respect to the European Union (EU), which remained roughly stable (Supplementary Figure 2).

Occupational pesticide handlers, including farmers and applicators, are often exposed to high levels of pesticides, mostly through the skin and inhalation. They are exposed at all stages of handling, from mixing/loading and spraying to cleaning the equipment used for pesticide application. Adverse health effects can occur when farmers and applicators do not wear personal protective equipment (PPE) and do not observe safety measures in handling pesticides. The Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) defined the reduction of health effects on workers exposed to pesticides, farmers and applicators as an objective within the framework of the International Code of Conduct on Pesticide Management (FAO/WHO, 2015). Indeed, wearing appropriate PPE and following safe pesticide handling practices can reduce the risks associated with pesticide exposure as these are considered the major modifying factors of exposure (Macfarlane et al., 2013; Yarpuz-Bozdogan, 2018).

In a previous study we found that environmental exposure to pesticides was associated with a greater prevalence rate and risk of having epilepsy (Requena et al., 2018). The present study, carried out in the same geographic area, sought to analyse possible occupational risk factors associated with agricultural practices which might contribute to the occurrence of epilepsies in farmers and pesticide applicators following long-term exposure to pesticides.

2. Materials and methods

2.1. Study design

A case-referent study was carried out in which all cases of epilepsy diagnosed in the province of Almería (South-East Spain) over 17 years were collected along with referent subjects from the same province lacking a clinical diagnosis of neurological disorders.

2.2. Study population

The study population consisted of 19,704 individuals who were selected during the study period 2000–2016. Epilepsy cases were collected from the Torrecardenas University Hospital, reference hospital where all epilepsy cases from Almeria province were referred to and followed up. For each epilepsy case, an average of 3 subjects without a diagnosis of epilepsy matched by age and from the same geographical area were selected. These were recruited from the Centre for the Prevention of Occupational Risks of the province of Almería, where they periodically attended the Health Monitoring Service for a routine medical surveillance examination. Both groups (cases and referents) were comprised by farmers and non-farmers (see below).

The case group consisted of 5091 subjects of both sexes and working age (18–65 years), residing in the province of Almería and diagnosed with epilepsy during the study period. The diagnosis was made according to the ICD9 (code 345) and the ICD10 (code G40) as both were in place over the study period. Of the total of epilepsy cases, 2761 subjects were farmers, of which 166 were certified and licenced as pesticide applicators (aka sprayers, i.e. farmers who as part of their farming work were in charge of mixing-loading and applying pesticides to crops).

The reference group consisted of 14,613 subjects of both sexes and working age (18–65 years), residents of the province of Almería and who had no previous medical diagnosis of neurological diseases. Of them, 6613 were farmers, of which 348 were also in charge of pesticide application (i.e., sprayers).

This study was approved by the Ethics and Research Committee of the Department of Nursing, Physiotherapy and Medicine of the University of Almeria (ID: EFM96/2021). All procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki.

2.3. Data collection

Information on sociodemographic variables (age, sex, level of education, smoking, profession, place of residence, years of residence) was collected from occupational and medical records. Additionally, farmers were interviewed to collect information on the agricultural tasks performed, years of work in agriculture, type of agriculture (intensive agriculture or open-air agriculture), application of pesticides and whether they regularly wore personal protective equipment (PPE, e.g., gloves, goggles, face mask, protective clothing) for skin, respiratory, and eyes. Pesticide applicators (sprayers) had PPE to apply pesticides, while the rest of farmers (who were not licensed to apply pesticides) also had the chance of using PPE to carry out agriculture tasks in crops wherein pesticides had previously been applied.

The place of residence for all study population (both farmers and non-farmers) was categorised into two zones (high pesticide use and low pesticide use), based on the percentage of surface area dedicated to intensive agriculture in plastic-covered greenhouses (Requena et al., 2018). For the crop season 2016–2017, the area with high pesticide use included 96 % of the greenhouse surface of the province of Almería (corresponding to Health Districts Centre and West), while the area of low pesticide use included the remaining 4 % of greenhouse surface (Health District East) (Consejeria de Agricultura, 2017).

Farmers carried out a combination of agricultural activities (40 h per week) such as pruning, weeding, thinning, and a subset of them (the so

Table 1
Pesticides most often used in intensive and extensive crops from the study area during the study period (2000–2016).

Active substance	Action	Chemical class	Crops treated	Year of banning
Intensive crops (plastic-covered greenhouses)				
Benomyl	Fungicide	Benzimidazole	Aubergine/ tomato/ pepper / zucchini	2004
Carbendazim	Fungicide	Benzimidazole	Cucumber	2014
Chlorpyrifos-methyl	Insecticide	Organophosphate	Tomato/ pepper/ cucumber/ melon/ watermelon	2020
Endosulfan	Insecticide	Organochlorinated (cyclodiene)	Tomato/ pepper	2007
Famoxadone	Fungicide	Oxazolidinedione	Tomato/ cucumber/ melon	2022
Flutriafol	Fungicide	Triazole	Tomato/ pepper	2021
Folpet	Fungicide	Dicarboximide	Tomato/ cucumber	2023[a]
Formetanate	Insecticide	*N*-methylcarbamate	Aubergine/ tomato/ pepper/ zucchini	2023[a]
Imidacloprid[b]	Insecticide	Neonicotinoid	Aubergine/ tomato/ zucchini	2020
Malathion	Insecticide	Organophosphate	Tomato	2023[a]
Mancozeb	Fungicide	Dithiocarbamate	Tomato/ pepper/ melon/ watermelon	2021
Methiocarb	Insecticide	*N*-methylcarbamate	Tomato/ pepper/ cucumber	2019
Methomyl	Insecticide	*N*-methylcarbamate	Aubergine/ tomato/ pepper/ melon/ watermelon	2019
Naled	Insecticide	Organophosphate	Zucchini	2012
Propineb	Fungicide	Dithiocarbamate	Tomato/ pepper/ cucumber	2019
Pymetrozine	Insecticide	Pyridine azomethine	Aubergine/ tomato/ pepper/ zucchini /cucumber	2015
Pyrimethanil	Fungicide	Pyrimidine	Aubergine/ tomato/ pepper/ cucumber	2023[a]
Thiophanate-methyl	Fungicide	Benzimidazole	Aubergine/ tomato/ melon/ watermelon	2020
Extensive crops (open-field agriculture)				
Acetamiprid	Insecticide	Neonicotinoid	Citrus	2033[a]
Chlorantraniliprole	Insecticide	Anthranilic diamide	Lettuce/ spinach/ broccoli	2024[a]
Chlorotoluron	Herbicide	Substituted phenylurea	Barley/ wheat/ oats	2023[a]
Glyphosate	Herbicide	Organophosphorus	Citrus	2023[a]
Imidacloprid	Insecticide	Neonicotinoid	Grapes/ citrus	2020
Indoxacarb	Insecticide	Oxadiazine	Lettuce/ spinach/ broccoli/ grapes	2021

[a] Expiration of approval period in the European Union (EU)
[b] Allowed in the EU only if used as insecticide in permanent greenhouses

Table 2
Pesticide application techniques for pest control in intensive and extensive crops during the study period.

Crop	Spraying equipment	Dose applied[a]	Number of treatments[b]	Application time[c]
Intensive crops (plastic-covered greenhouses)				
Aubergine	Spray gun	600	10	3
Tomato	Spray gun	1500	10	3
Pepper	Spray gun	1250	5	3
Cucumber	Spray gun	1000	15	3
Zucchini	Cannon spraying machine	600	10	30
Melon	Cannon spraying machine	700	5	30
Watermelon	Cannon spraying machine	600	5	30
Extensive crops (open-field agriculture)				
Lettuce/ spinach/ broccoli/	Tractor spraying	300–1000	2	0.5
	Tractor spraying	300–700[d]	6[d]	0.5[d]
Grape	Spray gun	200–1000[d]	3[d]	3[d]
Grape	Spray gun	1000–1500[e]	1[e]	3[e]
Citrus	Tractor spraying	500[f]	2[f]	0.5[f]
Citrus	Tractor spraying	100–400[g]	2[g]	0.5[g]
Citrus	Spray gun	1000–1500[e]	1[e]	3[e]
Barley/ wheat/ oats	Spray gun	200–400	2	3

Specific data for indoxacarb [d], imidacloprid [e], acetamiprid [f] and glyphosate [g].
[a] Litres per hectare of crop grown (except for citrus: 500 [f] grams per hectare).
[b] Number of treatments per crop season.
[c] Hours per hectare.

called 'sprayers' or 'pesticide applicators') were also in charge of mixing-loading and applying pesticides. The pesticides most used during the study period, their classification based on the target pest group, type of crops where they have been used, and the year when their use was banned are depicted in Table 1. In addition, the method of pesticide application according to the type of crop grown, dose of application and the number of times pesticides were applied per crop season, are shown in Table 2. This information was gained from the farmers' responses to interview/survey questions and from agronomists supervising the study area over the study period.

2.4. Data analysis

The information collected was captured and curated in a database created with the IBM SPSS statistical software package (SPSS 26.0 for Windows). The Kolmogorov-Smirnov test was used to assess whether the continuous variables (age, length of residence -years-, and years working in agriculture) were normally distributed. Sociodemographic data, farmer status and smoking habit were compared between the epilepsy and non-epilepsy groups using the Chi-square test for dichotomic and categorical variables, and the non-parametric Mann-Whitney U test for continuous variables as these were not normally distributed. In a further step, epilepsy in farmers and in the subset of pesticide applicators was compared by occupational features using the Chi-square test for dichotomic and categorical variables and the non-parametric Mann-Whitney U test for continuous variables. The risk of epilepsy was calculated for the entire study population and separately for farmers and pesticide applicators as odds ratios (OR) and 95 % confidence interval (CI) using Chi-square test.

Finally, multiple logistic regression analysis was used to assess the risk of epilepsy in the total study population, and in the subgroups of farmers and pesticide applicators. Models were adjusted for variables

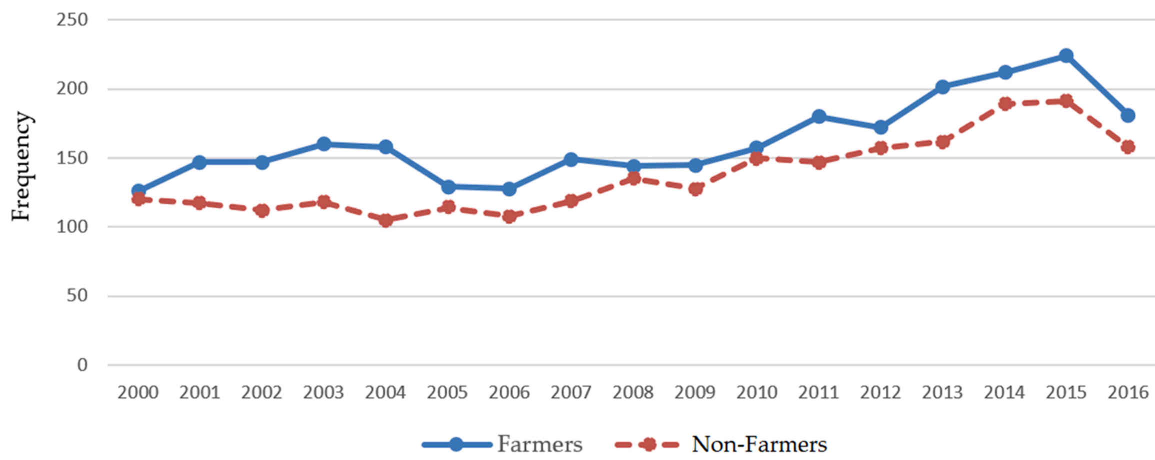

Fig. 1. Distribution of prevalent cases of epilepsy in farmers and non-farmers throughout the study period (2000–2016).

Table 3
Comparison of sociodemographic data, smoking habit and occupation between the two study groups, "epilepsy" and "non-epilepsy".

Characteristic		*Epilepsy (n = 5091)*	*Non-epilepsy (n = 14613)*	*p value*
Age (years)†		38.17 ± 7.62	38.19 ± 7.66	0.850*
Sex	Male	3537 (69.5 %)	10711 (73.3 %)	0.001**
	Female	1554 (30.5 %)	3902 (26.7 %)	
Agricultural Occupation	Yes (Farmer)	2761 (54.2 %)	6613 (45.3 %)	0.001**
	No (Non-farmer)	2330 (45.8 %)	8000 (54.7 %)	
Education level	No studies	1738 (35.7 %)	5073 (36.6 %)	0.020**
	Low	1637 (33.6 %)	4360 (31.4 %)	
	Medium	1335 (27.4 %)	3937 (28.4 %)	
	High	156 (3.2 %)	503 (3.6 %)	
Tobacco	Yes	2227 (45.8 %)	6639 (47.5 %)	0.040**
	No	2193 (45.1 %)	6021 (43.1 %)	
	Former smoker	440 (9.1 %)	1311 (9.4 %)	
Place of residence	Areas with high pesticide use	931 (18.3 %)	4049 (27.7 %)	0.001**
	Areas with low pesticide use	4160 (81.7 %)	10564 (72.3 %)	
Length of residence (years)†	Areas with high pesticide use	36.92 ± 8.34	37.21 ± 8.38	0.060*
	Areas with low pesticide use	36.71 ± 8.10	37.27 ± 7.88	0.070*

† Data expressed as mean ± standard deviation
The term “Farmer” also includes the subgroup of agriculture workers licensed to apply pesticides.
p value obtained using * Mann–Whitney *U* test for continuous variables or ** Chi-squared test for categorical variables.

that showed statistically significant differences in the bivariate analysis. These included age, sex, labour status (farmers vs. non-farmers), place of residence (areas of high vs. low use of pesticides) and several occupational features, such as years working in agriculture, type of agriculture (intensive greenhouse agriculture vs. extensive open-field agriculture), and use of gloves, goggles, face mask and protective clothing as personal protective equipment (PPE). The level of statistical significance was set at a p-value < 0.05.

3. Results

A total of 19,704 individuals participated in the study, 5091with a previous diagnosis of epilepsies and 14,613 without such a diagnosis. Fig. 1 shows the trend for epilepsies cases separately for farmers and non-farmers during the 17 years of study (2000–2016). The temporal pattern of epilepsies studied shows a similar trend for farmers and non-farmers, although farmers showed a significantly increased frequency over the entire study period (n = 2761, 54.2 % vs. n = 2330, 45.8 %; p = 0.001) as indicated in Table 3.

The main characteristics of the study population are shown in Table 3. No significant differences were observed for age, and length of residence (years) in the current farm. In contrast, statistically significant differences were observed for sex, occupation, education level, place of residence and smoking. Therefore, these variables were included in the multivariate analysis as potential confounders.

Table 4 shows the risk of epilepsy separately in farmers and sprayers, expressed as OR, when comparing for intensive farming (plastic-covered greenhouses) vs. extensive agriculture (open-air), areas of high vs. low pesticide use, and use of no vs. any PPE during their working day. A significantly increased risk of epilepsy was found for farmers working in intensive agriculture and for those not wearing goggles, masks and protective suits compared with their respective reference groups (see above). The greatest statistically significant risks were observed for farmers residing in areas of high pesticide use relative to those residing in areas of low pesticide use (OR: 1.86; $p < 0.001$), and for those who did not wear gloves while farming (OR: 1.85; $p < 0.001$).

A significantly increased risk of epilepsy was observed for farmers and for pesticide applicators when comparing by type of agriculture, i.e. intensive agriculture (greenhouses) versus extensive agriculture (open-air crops), yet the size of the effect was very similar for both subgroups. The highest risk of epilepsy was found for sprayers who did not wear face mask during the application of pesticides (OR of 3.11; $p < 0.001$) and for those residing in areas of high pesticide use (OR 2.40; $p < 0.001$). Farmers with a diagnosis of epilepsy had been working in agriculture for a slightly but significantly longer period of time (years) as those without epilepsy. Conversely, in the group of sprayers, those with epilepsy had been working in agriculture for less time (years) than those without epilepsy, with the differences being statistically significant (Table 4).

Table 5 shows the results of the multiple logistic regression analysis of possible factors that may be associated with the development of epilepsies in a) the entire study population, b) farmers, and c) the subgroup of pesticide applicators (farmers in charge of applying pesticides). For the total population, a significant greater risk of epilepsy was found for

Table 4
Risk of epilepsy by type of agriculture and use of PPE between farmers and sprayers.

Characteristic		*Farmers*				*Sprayers (pesticide applicators)*			
		Epilepsy (n = 2761)	*Non-epilepsy (n = 6613)*	*OR (95 % C. I.)*	*p value*	*Epilepsy (n = 166)*	*Non- epilepsy (n = 348)*	*OR (95 % C. I.)*	*p value*
Type of agriculture	Intensive (greenhouse)	2304 (83.4 %)	5142 (77.8 %)	1.44 (1.28–1.61)	0.001**	161 (96.7 %)	331 (95.1 %)	1.37 (1.24–1.50)	0.040**
	Extensive (open-air)	457 (16.6 %)	1471 (22.2 %)			5 (3.3 %)	17 (4.9 %)		
Place of residence	Areas of high pesticide use	2291 (83 %)	4782 (72.3 %)	1.86 (1.66–2.08)	0.001**	145 (87.3 %)	258 (74.1 %)	2.40 (1.43–4.03)	0.001**
	Areas of low pesticide use	470 (17 %)	1831 (27.7 %)			21 (12.7 %)	90 (25.9 %)		
PPE (use of gloves)	No	1715 (62.1 %)	3102 (46.9 %)	1.85 (1.69–2.04)	0.001**	115 (69.3 %)	227 (65.2 %)	1.20 (0.80–1.78)	0.360**
	Yes	1046 (37.9 %)	3511 (53.1 %)			51 (30.7 %)	121 (34.8 %)		
PPE (use of glasses)	No	1973 (71.5 %)	4577 (69.2 %)	1.11 (1.02–1.22)	0.030**	118 (71.1 %)	259 (74.4 %)	0.42 (0.55–1.22)	0.840**
	Yes	788 (28.5 %)	2036 (30.8 %)			48 (28.9 %)	89 (25.6 %)		
PPE (use of mask)	No	1909 (69.1 %)	4091 (61.9 %)	1.38 (1.25–1.51)	0.001**	127 (76.5 %)	178 (51.1 %)	3.11 (2.05–4.71)	0.001**
	Yes	852 (30.9 %)	2522 (38.1 %)			39 (23.5 %)	170 (48.9 %)		
PPE (use of protective clothing)	No	2527(91.5 %)	5898 (86.2 %)	1.32 (1.12–1.52)	0.001**	-	-		
	Yes	234 (8.5 %)	715 (10.8 %)			166 (100 %)	348 (100 %)		
Years working in agriculture		13.98 ± 6.05	13.60 ± 5.81	-	0.005*	11.30 ± 6.47	13.80 ± 6.94	-	0.001*
Devices used for pesticide application	Spray gun			-		83 (50 %)	150 (43.1 %)	1.32	0.140**
	Cannon spraying machine			-		83 (50 %)	198 (56.9 %)		

p value obtained using * Mann–Whitney *U* test for continuous variables or ** Chi-squared test for categorical variables.

farmers and for those living in areas with high pesticide use (OR 1.58 and 1.69, respectively, $p < 0.001$). For farmers, the regression model was adjusted for age, sex, years worked in agriculture, place of residence, and wearing gloves, goggles, face mask and protective clothing, as independent variables (predictors). The highest risk of epilepsy was observed for male farmers (OR=1.76; p = 0.001), those who resided in areas of high pesticide exposure (OR=2.01; p = 0.001) and those who did not wear gloves during working days (OR=2.00; p = 0.001).

For pesticide applicators, the model was adjusted for age, type of agriculture and wear face mask as these variables showed a significant association in the bivariate analyses. Table 5 shows that the greatest risk of epilepsy was found for sprayers who applied pesticides in greenhouses (intensive agriculture) relative to those who applied pesticides in open-air crops (extensive agriculture) (OR: 2.57; p = 0.03), for those who resided in areas with high pesticide use (OR: 2.64; p = 0.001) and those who did not wear face mask while spraying (OR: 3.34; p = 0.001).

4. Discussion

Few studies have examined pesticide exposure as a possible risk factor for epilepsy, beyond cases of acute poisoning. In a previous study conducted in the same study areas, we found that environmental exposure to pesticides as a result of a high pesticide use in intensive greenhouse horticulture increased the prevalence and risk of epilepsy (Requena et al., 2018). Another study, also conducted in South-Eastern Spain, reported that workers who applied pesticides were more likely to have neurological symptoms lasting more than two days, such as cramps, tremors, muscle fatigue, loss of consciousness and convulsions (García-García et al., 2016). Based on the associations found, the present study sought to analyse possible occupational risk factors for epilepsy in farmers and pesticide applicators exposed to pesticides. An increased risk of having a diagnosis of epilepsy was observed for farmers and pesticide applicators, particularly those with poor use of PPE (Table 5).

It should be noted that, in our study, a subset of farmers was qualified to apply pesticides by obtaining a pesticide applicator license, which enabled them to spray pesticides inside greenhouses (referred to as sprayers or pesticide applicators). Farmers not licensed to use pesticides could enter greenhouses where pesticides had been recently applied, hence they could be exposed to pesticide residues while working inside greenhouses. Thereby, they had the possibility of using PPE for skin, respiratory, and eye protection.

Factors contributing to adverse health effects following occupational exposure to pesticides include mixing and spraying pesticides, wind/agricultural pesticide drift, poor compliance with PPE, knowledge, perceptions, wearing contaminated clothes, warm weather, eating, drinking, smoking during or after pesticide handling and personal hygiene behaviour (washing hands or taking a shower) (Suratman et al., 2015). As a matter of fact, farmers complying with recommendations for pesticide handling and use of PPE have a significantly lower risk of having high urinary pesticide metabolite levels (Barrón Cuenca et al. (2020). PPE are indeed important modifiers of occupational pesticide exposure and are critical for reducing the risk of developing pesticide-related symptoms and diseases (Ye et al., 2013).

Our study found that farmers who did not wear gloves during their farming work had twice the risk of having epilepsy compared to those not wearing gloves (Table 5). In the Agricultural Health Study (AHS), the algorithm used for estimating pesticide exposure intensity predicted that farmers would reduce exposure by up to 90 % when chemical-resistant gloves are worn together with coverall (Hines et al., 2011; Thomas et al., 2010). In our study, sprayers failing to use face mask while handling pesticides had a more than three times higher risk of epilepsy than those who did; however, no statistically significant differences were found when compared by the use of gloves or goggles (Table 5). The comparison of ORs for having epilepsy between farmers and pesticide applicators using the same PPE (face mask, gloves, goggles) showed that pesticide applicators had a lower risk than farmers (Table 4). These findings underscore the higher compliance of pesticide applicators with respect to farmers regarding the use of PPE and its effectiveness. Similarly, farmers re-entering sprayed fields may have even greater pesticide absorption than applicators, likely because of less

Table 5
Stepwise multiple binary logistic regression analysis of the risk of developing epilepsy in the total population, and separately in farmers and in the farmers' subset of pesticide applicators, adjusted for potential risk factors.

	Parameters	*OR*	*95 % C.I.*	*p-Value*
Total population[a]	Sex (Male)	1.42	1.31–1.53	0.001
	Farmer	1.58	1.48–1.69	0.001
	Place of residence (area with high pesticide use)	1.69	1.56–1.84	0.001
Farmers[b]	Age (in years)	1.02	1.01–1.03	0.001
	Sex (male)	1.76	1.53–2.03	0.001
	Years working in agriculture	0.97	0.96–0.98	0.001
	Place of residence (area with high pesticide use)	2.01	1.77–2.28	0.001
	Use of gloves (no)	2.00	1.49–8.91	0.001
	Use of goggles (no)	1.30	1.17–1.45	0.001
	Use of face mask (no)	1.33	1.20–1.48	0.001
	Use of protective clothing (no)	1.47	1.24–1.74	0.001
Sprayers (pesticide applicators)[c]	Place of residence (area with high pesticide use)	2.64	1.46–4.77	0.001
	Type of agriculture (greenhouse)	2.57	1.67–6.18	0.030
	Use the face mask (no)	3.34	2.19–5.11	0.001

[a] The regression model was adjusted for age, sex (0: female; 1: male), labour status (0: non-agriculture worker; 1: agriculture worker), place of residence (0: area with low use of pesticides; 1: area with high use of pesticides).
[b] The regression model was adjusted for age, sex (0: female; 1: male), years working in agriculture, place of residence (0: area with low use of pesticides; 1: area with high use of pesticides), wear gloves (0: yes; 1: no), wear goggles (0: yes; 1: no), wear face mask (0: yes, 1: no), wear protective clothing (0: yes; 1: no).
[c] The regression model was adjusted for type of agriculture (0: extensive crops; 1: greenhouse crops), place of residence (0: area with low use of pesticides; 1: area with high use of pesticides), wear face mask (0: yes, 1: no).

safety training and use of PPE, and a longer duration of exposure than pesticide applicators (Macfarlane et al., 2013).

Despite all farmers and the subgroup of pesticide applicators had been exposed to pesticides for more than 10 years (Table 4), the time working in agriculture was not significantly associated with an increased risk of epilepsy (Table 5). In contrast, Pratama, Setiani (2021) reported that farmers with a working period of more than 10 years were more likely to complain of neurological symptoms. The length of time worked as a farmer can be an important factor for pesticide exposure as the longer the duration of exposure, the higher the farmers' risk of adverse health effects. However, the years of past experience in farming are not always associated with pesticide use practices (Sumudumali et al., 2021). In line with this, Barrón Cuenca et al. (2020) reported a non-linear association between urinary concentrations of 3,5,6-trichloro-2-pyridinol (a metabolite of chlorpyrifos) and years working as a farmer, as significantly higher concentrations were found in farmers working 1–3 years compared to those working more than 8 years (Barrón Cuenca et al., 2020).

Regulatory toxicology studies have shown that pesticides from different chemical classes can cause convulsions after chronic exposure, while none of them elicited convulsions following acute exposures (EFSA, 2014, 2019). These findings suggest that pesticides might contribute to the occurrence of epileptic seizures upon long-term exposure to low doses and not only as a consequence of acute poisoning. The potential underlying mechanisms may therefore differ depending on the dose and duration of exposure.

Seizures may result from different mechanisms at molecular or subcellular level. These include overstimulation of central muscarinic acetylcholine receptors following acetylcholinesterase inhibition by organophosphate insecticides, which can raise intracellular calcium levels ultimately leading to postsynaptic neuronal excitotoxicity (Lee et al., 2016). Other insecticides cause hyperexcitability following blockage of voltage-gated sodium channels (e.g., pyrethroids) or the $GABA_A$ receptor (fipronil, most organochlorines and type II pyrethroids) (Requena et al., 2018). Furthermore, many pesticides such as OPs, *N*-methylcarbamates, organochlorines, pyrethroids, triazines, neonicotinoids, paraquat and dithiocarbamates have been shown to induce oxidative stress by an imbalance between the production of highly reactive molecular species and their removal by a decrease in the cellular antioxidant defences (Abdollahi et al., 2004; Hernández et al., 2013; Sule et al., 2022). Mitochondrial oxidative stress and dysfunction of mitochondrial complexes can reduce the seizure threshold in vivo and has been considered as a contributing factor of epilepsy (Aguiar et al., 2012; Waldbaum and Patel, 2010).

Our previous study carried out in the same geographical area found a modest increased risk of epilepsy in children living in areas with greater use of pesticides (Requena et al., 2018). Some forms of epilepsy have been considered as NDDs (Bozzi et al., 2012; Heyne et al., 2019) and the high co-occurrence (comorbidity) between epilepsy and NDDs suggests a shared, underlying biological mechanism (Chow et al., 2019). Some pesticides are known or suspected to pose a neurodevelopmental hazard (e.g., organophosphates -with chlorpyrifos being the one most studied-, neonicotinoids, pyrethroids, dithiocarbamates…), which has important implications for pregnant farmers and children of farmers/applicators occupationally exposed to pesticides.

This study has some limitations that should be considered for the adequate interpretation of the findings. Specific data on frequency and duration of exposure to individual pesticides were not available and the pattern of pesticides used in crop production changed over the study period (17 years) because of innovations and restrictions in the European pesticide market implemented since the first decade of the 2000s. These consisted of a more stringent legal framework for the approval of pesticides and the gradual implementation of integrated pest management and biological control in the study area. On the other hand, lack of adjustment of multivariate models for potential confounders other than those used in this study (e.g., including the known risk factors for the development of epilepsy mentioned in the Introduction) and the potential contribution of co-exposure to other chemicals and environmental stressors might have also impacted on the occurrence of epilepsy. However, the large size of the study population, which amounted to over five thousand cases, may contribute to minimize the impact of residual confounding on the results. Furthermore, it is expected that the known risk factors for epilepsy are not unevenly distributed among the study groups because of the large sample size. This study also provides an approach to a real-world exposure scenario to pesticides in a large agriculture area over 17 years; therefore, it can be linked to the novel approaches proposed for simulating real-life exposures (Hernández et al., 2020; Tsatsakis et al., 2019), thus contributing to a better understanding of the real-life risk associated with long-term exposure to multiple pesticides.

In conclusion, this study supports previous findings suggesting a higher risk of epilepsy in the general population associated to pesticide exposure and extends the presumed increased risk to farmers occupationally exposed to pesticides, particularly those with lack of or improper use of PPE.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Raquel Alarcón: Formal analysis, Data curation, Writing, Belén Giménez: Formal Analysis, Data curation, Antonio F. Hernández: Formal analysis, Writing, Antonia López-Villén: Formal Analysis, Methodology, Tesifón Parrón: Conceptualization, Methodology, Formal analysis, Jessica García-González: Formal Analysis, Methodology, Mar Requena: Conceptualization, Supervision, Writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neuro.2023.04.012.

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