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Brief Report

Relationship between Butyrylcholinesterase Activity and Cognitive Ability in Workers Exposed to Chlorpyrifos

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Abstract: Background. The use of Chlorpyrifos leads to a public, environmental, and occupational health problem associated with adverse effects in the exposed population, generating alterations mainly in the central nervous system, such as cognitive function. This study aimed to estimate the association between butyrylcholinesterase activity (BChE) and cognitive ability in workers exposed to chlorpyrifos. Methods. We designed a cross-sectional study, where we measured BChE in serum samples as an indicator of exposure to chlorpyrifos. The cognitive ability was assessed by the mean score of the Peruvian version of the Mini-Mental State Examination (MMSE). We also used a questionary to collect demographic and occupational information. Results. We evaluated 120 farmers with a predominance of males (92%) and a mean age of 32.1 ± 9.0 years. We found most of the workers in fumigation activities (84%). The mean BChE was 6144.7 ± 2355.0 U/L, and 46% presented inhibition enzyme (<5500 U/L). The median MMSE score was 28 (interquartile range: 26.5–31.5; 6% showed an alteration in cognitive ability (score < 24)). The MMSE test found a significant association between BChE inhibition and MMSE score (β: −0.071, 95%CI: −0.108 to −0.025). Conclusion. In this study, 45.8% of workers exposed to chlorpyriphos presented BChE inhibition. The BChE inhibition is significantly associated with the MMSE score in workers exposed to chlorpyrifos.

Keywords: cognitive ability; butyrylcholinesterase; chlorpyrifos; organophosphorus compounds

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1. Introduction

Pesticides are essential compounds in pest control and guarantee the production of various agricultural foods; however, when they are not applied safely, they generate environmental pollution and adverse effects on human health, which are sometimes irreversible [1,2]. Chlorpyrifos is an organophosphorus pesticide widely used in the fumigation of agricultural fields [3]. Occupational exposure to chlorpyrifos is usually high, and the lack of personal protective equipment (waterproof overalls, caps, safety glasses, gloves, boots, and respirators with filters) and limited training for the safe handling of pesticides are risk factors for the occurrence of occasional problems such as poisoning [4].

In Peru, the use of pesticides is increasing owing to agro-export activities, and chlorpyrifos is one of the most used [5], despite that, in 2020, the European Commission prohibited its use in agro-export products [6], which should reduce the use of this pesticide in the medium and long term. Reports from 2016–2018 have shown 5976 accumulated cases of pesticide poisoning and 94 deaths [7], so pesticide exposure is considered a public health problem in Peru. Continuous and long-term exposure to chlorpyrifos generates adverse effects on the central nervous system, deteriorating different areas, with progressive involvement in the cognitive aspect [8]. Cognitive ability involves reasoning, problem-solving, planning, abstract thinking, understanding complex ideas, and learning from experience [9]. This ability is affected by pesticide exposure [10], which also increases the risk of long-term neurodegenerative diseases by 50% [11]. Therefore, it is reasonable to think that continuous exposure to pesticides progressively impairs cognitive ability, and this could serve as a potential indicator of risk for neurodegenerative diseases [12].

Occupational exposure to pesticides is evaluated with biomarkers, among which the enzymatic activity of cholinesterase stands out [13]. Cholinesterases are enzymes that belong to the group of serine hydrolases. They catalyze the neurotransmitter acetylcholine and terminate its action. There are two types of cholinesterase: butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) [14]. AChE plays a key role in the termination of cholinergic neurotransmission and its activity is on neurons and erythrocytes, while BChE, synthesized in liver, does not have a known physiological function, but is used as a biomarker for organophosphorus and carbamate pesticides exposure [15]. BChE has major activity on plasma and its inhibition has better sensitivity than AChE in identifying long-term exposure at low doses of organophosphate pesticides [16]. Previous studies in Peru have shown inhibition of BChE in farmers exposed to pesticides [17,18]; however, its attribution to a specific type of pesticide is not reported.

There are few studies regarding cognitive ability and its relationship with BChE activity in farmers exposed to pesticides [19,20]. Studies focus on impaired cognitive function in newborns and children with environmental exposure to pesticides [21–24]. Exposure to chlorpyrifos generates BChE inhibition and cognitive and motor alterations that are observed even several months after intoxication [19,25,26]. In the case of Peru, there is no clear evidence of this association, especially in regions that export grapes, asparagus, lima beans, cotton, and avocados, among other vegetables that require pesticide mixtures to guarantee their cultivation. Some companies export different products and use various combinations of pesticides. The limited information on the relationship of interest limits the comprehensive health approach that all workers exposed to pesticides should receive during occupational health surveillance. Hence, this study aimed to estimate the relationship between BChE and the cognitive ability of workers exposed to chlorpyrifos. The study findings strengthen primary prevention activities, focused on the timely identification of alterations in the worker's mental health and improving work performance [27].

2. Materials and Methods

2.1. Study Design

We designed a cross-sectional study to evaluate workers at risk of exposure to chlorpyrifos. We carried out the study between January and March 2018. It was complementary to the annual occupational medical examinations, which did not include measuring butyrylcholinesterase activity and evaluating cognitive ability. It should be noted that the use of chlorpyrifos was exclusive in the fumigation of the asparagus crop.

2.2. Population and Sample Study

We evaluated workers in an asparagus agro-export industry in the department of Ica, located 310 km from Lima, the capital of Peru. Workers performed activities that generated direct exposure to chlorpyrifos at different levels, such as warehouse workers, collecting agricultural products, and spraying. We excluded workers older than 65 with chronic liver and psychiatric diseases, psychotropic treatment, and alcohol or cocaine addiction. We evaluated 120 workers and performed a post-estimation of power, whose value was 83.8%. We compared means in two independent groups, considering a difference of 0.9 points on the MMSE scale (test for cognitive ability) with a deviation of 1.65 points, as evidenced by Corral et al. [19], at a 95% confidence level. We calculated power with Epidat program version 4.2 (Dirección Xeral de Saúde Pública, Xunta de Galicia, Spain).

2.3. Techniques and Procedures

Data collection. Through an interview, we obtained sociodemographic information such as age, sex, and degree of academic instruction. Regarding labor characteristics, information on the number of years working in the agro-export company, the type of work activity carried out, and the system used for spraying was collected.

Biological sampling. We obtained blood samples by venous puncture in 5 mL tubes without anticoagulant and centrifuged at 3500 rpm for 5 min. Moreover, serum samples were obtained and kept at 2–8 °C to continue the analysis of BChE. The workers fasted for 12 h before the obtention of these samples.

Butyrylcholinesterase activity. We measured the activity of this enzyme because it is more sensitive than AChE in identifying enzyme inhibition in populations with chronic exposure to pesticides [13]. For the measurement, an auto-analyzer Wiener Lab model CM250 (Wiener Lab, Buenos Aires, Argentina), with a kinetic kit (Cholinesterase AA liquid, Wiener Lab, Buenos Aires, Argentina), with absorbance readings for 90 s and at a wavelength of 405 nm, was used. According to the manufacturer, we considered BChE inhibition when an activity less than 5500 IU/L was identified under reaction condition at 37 °C, applicable for children, men, and women adults. To ensure the reliability of the measurements, an internal quality control (Standatrol, Wiener lab, Buenos Aires, Argentina), whose coefficients of variation were 4.2% and 6.3% for normal and pathological level control, respectively, was performed. The laboratory conducting these measurements has ISO/IEC 15,189 accreditation and external quality assessment programs with Oneworld Accuracy Company (One World Accuracy, Vancouver, Canada).

Cognitive ability. The Mini-Mental State Examination (MMSE), developed and validated by Folstein et al. [28], was used to measure cognitive ability. The test consisted of items that addressed the dimensions of orientation, fixation, concentration and calculation, memory, language, and construction, with a maximum score of 35 points. It was administered in 5–10 min. Two occupational psychologists administered the test. A score below 24 points was considered a sign of cognitive impairment, according to what was proposed by Lobo et al. [29], who revalidated and normalized the original version to Spanish and reported a sensitivity and specificity of 89.8% and 75.1%. Custodio et al. [30] adapted the Peruvian version. They found an AUC of 0.97 in identifying dementia in the adult population, and it has been used in several studies in the Peruvian population [31].

2.4. Statistical Analysis

We presented numerical variables with their average and standard deviation and categorical variables in absolute and relative frequencies. We calculated the median and interquartile range (IQR) for MMSE score according to BChE activity (inhibition or not) and the other study variables. We evaluated the association between BChE activity and cognitive ability (MMSE score) by a generalized linear model (GLM) crude and adjusted by confounders selected by epidemiological criteria based on a directed acyclic graph. Among the confounders are age, sex, time, type of work, and academic level. According to scientific evidence, these variables are associated with cholinesterase activity and cognitive ability [14,32]. We estimated the coefficient and its 95% confidence interval. We considered a p-value less than 0.05 as a significant association. We carried out analyses in the Stata corporation software version 17.0 (Stata Corporation, College Station, TX, USA).

2.5. Ethical Considerations

All participants gave informed consent after explaining the investigation's objectives, benefits, and risks. Universidad Alas Peruanas of Peru approved the study by The Research Committee (RD Nº 018-2022-DA-GT-D-FMHyCS-UAP) in January 2017. Moreover, we obtained the administrative permission of the agro-export company to conduct the study.

3. Results

The study population consisted of 120 workers whose mean age was 32.0 ± 8.8 years. The workers had a median working time of 3 years. Most workers were engaged in fumigation (84%); three of each four participants had completed secondary education and 13.33% had technical or university education. The median of the butyrylcholinesterase activity was 5802 IU/L (IQR: 4351–8355). Regarding BChE inhibition, 45.8% of the farm workers presented BChE inhibition. On the other hand, the MMSE test score was 28.9 ± 3.1. We show additional descriptive information about sociodemographic characteristics in Table 1.

Table 1. Descriptive characteristics of the study participants (n = 120).

Characteristics	*n*	%
Age, years [a]	31.9 ± 8.5 (21–59)	
Working time, years [b]	3.0 (1–15)	
Sex		
Male	110	91.67
Female	10	8.33
Type of job		
Harvest	1	0.83
Warehouse	19	15.83
Fumigation	100	83.33
Academic level		
Elementary School	6	5.00
Incomplete High School	5	4.17
Completed High School	93	77.50
Non University Education	10	8.33
University	6	5.00
System used for spraying		
Manual back pump	12	10.00
Pump with back motor	60	50.00
Tractor with nebulizer	28	23.33
No fumigation job	20	16.67
BChE activity, IU/L [b]	5802 (4351–8355)	
BChE inhibition (<5500 IU/L)		
No	65	54.17
Yes	55	45.83
MMSE score, points [b]	28 (26.5–31.5)	

[a] Mean ± standard deviation (minimum–maximum), [b] median (IQR). BChE: butyrylcholinesterase; MMSE: Mini-Mental State Examination.

The median and IQR for MMSE score according to study variables are presented in Table 2. We found differences in MMSE score by working time (p = 0.008) and BChE inhibition (p < 0.001). Additionally, we found that the medians of BChE between people with and without cognitive impairment were 5320 (IQR: 2832–8475) and 5805 (IQR: 4351–8235), but without a significant difference (p = 0.767, Mann–Whitney test).

Table 2. Mini-Mental State Examination (MMSE) score by independent variables in bivariate analysis.

Characteristics	MMSE Score		*p*-Value
	Median	IQR	
Sex			0.239 [a]
Male	30	29–33	
Female	28	26–31	
Age group (years)			0.901 [a]
18–29	28	27–32	
30–59	29	26–31	
Working time			0.008 [a]
<3 years	30	27–33	
≥3 years	28	26–29	
Type of job			0.641 [b]
Harvest	29	29–29	
Warehouse	30	26–33	

Table 2. *Cont.*

Characteristics	MMSE Score		*p*-Value
	Median	IQR	
Fumigation	28	26.5–31	
Academic level			0.348 [b]
Elementary School	27	27–29	
Incomplete High School	30	27–30	
Completed High School	28	26–32	
Non University Education	29	27–30	
University	33	29–33	
BChE inhibition			<0.001 [a]
No	30	27–33	
Yes	27	26–30	

[a] Mann–Whitney test, [b] Kruskal–Wallis test.

Regarding BChE activity and MMSE test scores, the crude model found a significant association (coefficient: −0.059; 95%CI: −0.097 to −0.022). This association was also found in the adjusted model, showing a significant association between BChE inhibition and a decrease in the MMSE score (coefficient: −0.07; 95%CI: −0.108 to −0.025) after adjusting by age, sex, working time, type of job, and academic level (Table 3).

Table 3. Association between BChE inhibition and MMSE score in multivariate analysis.

MMSE Score	Crude Model		Adjusted Model [a]		*p*-Value
	Coef.	CI95	Coef.	CI95	
No BChE inhibition	1.00		1.00		
BChE inhibition	−0.059	−0.097 to −0.022	−0.071	−0.108 to −0.025	0.002

[a] GLM adjusted for age, sex, working time, type of job, and academic level. BChE: butyrylcholinesterase; MMSE: Mini-Mental State Examination.

4. Discussion

This study evaluated the association between butyryl cholinesterase (BChE) activity and cognitive ability (MMSE score). Nearly 50% of the farm workers presented BChE inhibition, and BChE activity was found to be associated with MMSE scores. The literature describes substantial evidence that agricultural workers are affected in the long term by the development of neurodegeneration events [33]. The inhalation and dermal routes are the main routes of entry for pesticides. The exposure is exacerbated by a lack of adequate personal protective equipment and training in risk prevention and control [34].

The most commonly used biological indicator of pesticide exposure is butyrylcholinesterase activity (BChE), whose inhibition allows identifying risk groups [35]. In the sample, the proportion of people with inhibited activity was close to 50%. This proportion is worrisome, considering that adverse health conditions could be manifested at some point if the working conditions and exposure levels remain the same. The identification of an association between BCHE inhibition in farm workers and a decrease in cognitive abilities is relevant to promote strategies to prevent or at least reduce risk exposure. Moreover, BChE inhibition is oriented to take preventive measures based on the worker's relocation to a new job position to reduce exposure to pesticides, as recommended by various authors who have addressed the health problems generated by chlorpyrifos [36].

The health problems related to exposure to chlorpyrifos are diverse and evidenced when there is acute exposure, with manifestations such as seizures, sphincter relaxation, loss of consciousness, vomiting, cardiac arrest, and death; in these cases, laboratory tests are dispensed [37]. However, with chronic exposure to low doses of chlorpyrifos, clinical manifestations in the short term are not obvious, especially events such as neurodegeneration, cancer, and other chronic diseases [38–40]. The evaluation of cognitive ability is relevant in people exposed to chlorpyrifos, considering its neurotoxic potential [19]. This study assessed cognitive ability by a screening test applied under specific requirements defined by the same trial [41]. The study findings indicate that BChE inhibition is related to a reduction in the MMSE score. This test has been widely used in the geriatric population to identify diseases such as Alzheimer's or senile dementia; however, some studies have shown its usefulness within labor populations [42].

We found a significant association between BChE inhibition and the MMSE score. The population with the inhibited enzyme presented a reduced MMSE score. A previous study in the Chilean population evidenced that people with direct and indirect exposure to pesticides show higher cognitive deficit rates than those without pesticide exposure [19]. A scooping review regarding the health effects of pesticide exposure in Latin American and Caribbean Populations also reported some evidence that exposure to pesticides may adversely impact the health of people, including a reduction in the MMSE score (however, this review indicates important limitations in the studies that undermine the strength of the results) [43]. Another study in Greece also found this association among greenhouse workers [44]. However, the information generated in recent years is inconclusive, especially in cases of chronic exposure, This is because of the limitation of evaluating the occurrence of adverse clinical events in follow-up studies. There is no consensus on the association between specific cognitive abilities and exposure to pesticides [25]. Perhaps, the most relevant evidence is that of Blanc et al., who reported the association between organophosphate exposure and cognitive performance in a prospective cohort of grapevine workers in France, whose results support the hypothesis that cognitive disorders may be associated with exposure to specific organophosphates [26]. In addition, Baldi et al. showed the long-term cognitive effects of low-level exposure to pesticides under occupational conditions [45]. The association between BChE inhibition and MMSE scores found in this study provides a baseline to evaluate the effect of the exposition to chlorpyrifos (and BChE inhibition) and cognitive ability in the Peruvian population. The influence of exposure to chlorpyrifos on cognitive function has been studied in animal models with alteration of cholinergic synapses [46]. Recently, it has been shown that continuous and simulated occupational exposure generates neurobehavioral deficits and possibly depression in adult rats [47]. More longitudinal epidemiological studies and systematic reviews with meta-analysis are needed to improve the consistency of the evaluated association.

The study's limitations derive from the cross-sectional design, which lacks temporality. Hence, we conducted a multivariate analysis to control the effect of confounders. Likewise, cholinesterase activity has a high rate of genetic variability reflected in its dispersed enzyme activity; however, we use a normal range obtained from previous studies in the Peruvian population. On the other hand, the sample size was small; therefore, the relationship between BChE activity and the total MMSE score was evaluated, and not by dimensions, to avoid loss of power. Likewise, we used non-parametric statistics and estimated the relationship in a generalized linear model. Although we found a significant association between BChE activity and MMSE score, this finding cannot be generalized to other populations whose occupational exposure may differ because of the type of pesticide used and the working conditions. Likewise, BChE activity can vary according to liver status and overestimate inhibition. Hence, we included only workers with no history of liver disease and transaminase values in the normal range. Despite the limitations, it is important to consider that the workers evaluated were at risk of exposure only to chlorpyrifos, so the confounding effect due to other possible pesticides is ruled out. Finally, it is important to consider that exposure to chlorpyrifos can be assessed by measuring specific metabolites such as 3,5,6-trichloro-2-pyridinol (TCPy), which is already used in occupational surveillance programs [47–49], and even in national monitoring programs like the National Report on Human Exposure to Environmental Chemicals [50]. Therefore, its implementation and use in the evaluation of workers exposed to pesticides should be considered.

5. Conclusions

We found BChE inhibition as a biomarker of chorpyrifos exposure in 45.8% of workers in the study. Likewise, a significant relationship between BChE inhibition and cognitive ability became apparent. It is important to improve strategies to prevent and control the risk of exposure to chlorpyrifos. In addition, the use of biomarkers such as cholinesterases in human biomonitoring and neuropsychological test batteries may be powerful tools to timely detect and hopefully prevent cognitive impairment and other functions in workers' central nervous system.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author. The data are not publicly available due to privacy considerations.

Conflicts of Interest: The authors declare no conflict of interest in the preparation or publication of this article.

References

1. Nicolopoulou-Stamati, P.; Maipas, S.; Kotampasi, C.; Stamatis, P.; Hens, L. Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. *Front. Public Health* **2016**, *4*, 148. [CrossRef] [PubMed]
2. Hu, R.; Huang, X.; Huang, J.; Li, Y.; Zhang, C.; Yin, Y.; Chen, Z.; Jin, Y.; Cai, J.; Cui, F. Long- and short-term health effects of pesticide exposure: A cohort study from China. *PLoS ONE* **2015**, *10*, e0128766. [CrossRef] [PubMed]
3. Saunders, M.; Magnanti, B.L.; Correia Carreira, S.; Yang, A.; Alamo-Hernández, U.; Riojas-Rodriguez, H.; Calamandrei, G.; Koppe, J.G.; Krayer von Krauss, M.; Keune, H.; et al. Chlorpyrifos and neurodevelopmental effects: A literature review and expert elicitation on research and policy. *Environ. Health* **2012**, *11*, S5. [CrossRef] [PubMed]
4. Baker. *, E.L.R.M.B. Organophosphate Toxicity*; StatPearls Publishing: Treasure Island, FL, USA, 2019.
5. Delgado-Zegarra, J.; Alvarez-Risco, A.; Yáñez, J.A. Indiscriminate use of pesticides and lack of sanitary control in the domestic market in PeruUso indiscriminado de pesticidas e falta de controle sanitário do mercado interno no Peru. *Rev. Panam. De Salud Publica Pan Am. J. Public Health* **2018**, *42*, e3. [CrossRef]
6. European Comission. Chlorpyrifos & Chlorpyrifos-Methyl. Available online: https://food.ec.europa.eu/plants/pesticides/approval-active-substances/renewal-approval/chlorpyrifos-chlorpyrifos-methyl_en (accessed on 29 October 2022).
7. CDC. *Intoxicaciones Agudas Por Plaguicidas Por Semanas Epidemiológicas. Perú 2016-2018*; Centro Nacional de Epidemiología, Prevención y Control de Enfermedades: Lima, Peru, 2019.
8. Lee, Y.S.; Lewis, J.A.; Ippolito, D.L.; Hussainzada, N.; Lein, P.J.; Jackson, D.A.; Stallings, J.D. Repeated exposure to neurotoxic levels of chlorpyrifos alters hippocampal expression of neurotrophins and neuropeptides. *Toxicology* **2016**, *340*, 53–62. [CrossRef]
9. Ispas, D.; Borman, W.C. Personnel Selection, Psychology of. In *International Encyclopedia of the Social & Behavioral Sciences*, 2nd ed.; Wright, J.D., Ed.; Elsevier: Oxford, UK, 2015; pp. 936–940. [CrossRef]
10. Aloizou, A.M.; Siokas, V.; Vogiatzi, C.; Peristeri, E.; Docea, A.O.; Petrakis, D.; Provatas, A.; Folia, V.; Chalkia, C.; Vinceti, M.; et al. Pesticides, cognitive functions and dementia: A review. *Toxicol. Lett.* **2020**, *326*, 31–51. [CrossRef]
11. Gunnarsson, L.G.; Bodin, L. Occupational Exposures and Neurodegenerative Diseases-A Systematic Literature Review and Meta-Analyses. *Int. J. Environ. Res. Public Health* **2019**, *16*, 337. [CrossRef]
12. Gonzales, M.M.; Garbarino, V.R.; Pollet, E.; Palavicini, J.P.; Kellogg, D.L., Jr.; Kraig, E.; Orr, M.E. Biological aging processes underlying cognitive decline and neurodegenerative disease. *J. Clin. investigation* **2022**, *132*, e158413. [CrossRef]
13. Dalmolin, S.P.; Dreon, D.B.; Thiesen, F.V.; Dallegrave, E. Biomarkers of occupational exposure to pesticides: Systematic review of insecticides. *Environ. Toxicol. Pharmacol.* **2020**, *75*, 103304. [CrossRef]
14. Jokanović, M.; Maksimović, M. Abnormal cholinesterase activity: Understanding and interpretation. *Eur. J. Clin. Chem. Clin. Biochem. J. Forum Eur. Clin. Chem. Soc.* **1997**, *35*, 11–16.
15. Lockridge, O. Review of human butyrylcholinesterase structure, function, genetic variants, history of use in the clinic, and potential therapeutic uses. *Pharmacol. Ther.* **2015**, *148*, 34–46. [CrossRef]

16. Strelitz, J.; Engel, L.S.; Keifer, M.C. Blood acetylcholinesterase and butyrylcholinesterase as biomarkers of cholinesterase depression among pesticide handlers. *Occup. Environ. Med.* **2014**, *71*, 842–847. [CrossRef]
17. Rosales, J. Uso de marcadores genotoxicológicos para la evaluación de agricultores expuestos a plaguicldas organofosforados. *An. De La Fac. De Med.* **2015**, *76*, 247–252. [CrossRef]
18. Cataño, H.C.; Carranza, E.; Huamaní, C.; Hernández, A.F. Plasma cholinesterase levels and health symptoms in peruvian farm workers exposed to organophosphate pesticides. *Arch. Environ. Contam. Toxicol.* **2008**, *55*, 153–159. [CrossRef]
19. Corral, S.A.; de Angel, V.; Salas, N.; Zúñiga-Venegas, L.; Gaspar, P.A.; Pancetti, F. Cognitive impairment in agricultural workers and nearby residents exposed to pesticides in the Coquimbo Region of Chile. *Neurotoxicology Teratol.* **2017**, *62*, 13–19. [CrossRef]
20. Ramírez-Santana, M.; Zúñiga-Venegas, L.; Corral, S.; Roeleveld, N.; Groenewoud, H.; Van der Velden, K.; Scheepers, P.T.J.; Pancetti, F. Reduced neurobehavioral functioning in agricultural workers and rural inhabitants exposed to pesticides in northern Chile and its association with blood biomarkers inhibition. *Environ. Health* **2020**, *19*, 84. [CrossRef]
21. Dobbins, D.L.; Chen, H.; Cepeda, M.J.; Berenson, L.; Talton, J.W.; Anderson, K.A.; Burdette, J.H.; Quandt, S.A.; Arcury, T.A.; Laurienti, P.J. Comparing impact of pesticide exposure on cognitive abilities of Latinx children from rural farmworker and urban non-farmworker families in North Carolina. *Neurotoxicology Teratol.* **2022**, *92*, 107106. [CrossRef]
22. Benavides-Piracón, J.A.; Hernández-Bonilla, D.; Menezes-Filho, J.A.; van Wendel de Joode, B.; Lozada, Y.A.V.; Bahia, T.C.; Cortes, M.A.Q.; Achury, N.J.M.; Muñoz, I.A.M.; Pardo, M.A.H. Prenatal and postnatal exposure to pesticides and school-age children's cognitive ability in rural Bogotá, Colombia. *Neurotoxicology* **2022**, *90*, 112–120. [CrossRef]
23. Kalloo, G.; Wellenius, G.A.; McCandless, L.; Calafat, A.M.; Sjodin, A.; Sullivan, A.J.; Romano, M.E.; Karagas, M.R.; Chen, A.; Yolton, K.; et al. Chemical mixture exposures during pregnancy and cognitive abilities in school-aged children. *Environ. Res.* **2021**, *197*, 111027. [CrossRef]
24. van Wendel de Joode, B.; Mora, A.M.; Lindh, C.H.; Hernández-Bonilla, D.; Córdoba, L.; Wesseling, C.; Hoppin, J.A.; Mergler, D. Pesticide exposure and neurodevelopment in children aged 6-9 years from Talamanca, Costa Rica. *Cortex A J. Devoted Study Nerv. Syst. Behav.* **2016**, *85*, 137–150. [CrossRef]
25. Munoz-Quezada, M.T.; Lucero, B.A.; Iglesias, V.P.; Munoz, M.P.; Cornejo, C.A.; Achu, E.; Baumert, B.; Hanchey, A.; Concha, C.; Brito, A.M.; et al. Chronic exposure to organophosphate (OP) pesticides and neuropsychological functioning in farm workers: A review. *Int. J. Occup. Environ. Health* **2016**, *22*, 68–79. [CrossRef] [PubMed]
26. Blanc-Lapierre, A.; Bouvier, G.; Gruber, A.; Leffondre, K.; Lebailly, P.; Fabrigoule, C.; Baldi, I. Cognitive disorders and occupational exposure to organophosphates: Results from the PHYTONER study. *Am. J. Epidemiol.* **2013**, *177*, 1086–1096. [CrossRef] [PubMed]
27. Kell, H.J.; Lang, J.W.B. Specific Abilities in the Workplace: More Important Than g? *J. Intell.* **2017**, *5*, 13. [CrossRef] [PubMed]
28. Folstein, M.F.; Folstein, S.E.; McHugh, P.R. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* **1975**, *12*, 189–198. [CrossRef] [PubMed]
29. Lobo, A.; Saz, P.; Marcos, G.; Día, J.L.; de la Cámara, C.; Ventura, T.; Morales Asín, F.; Fernando Pascual, L.; Montañés, J.A.; Aznar, S. Revalidation and standardization of the cognition mini-exam (first Spanish version of the Mini-Mental Status Examination) in the general geriatric population. *Med. Clin.* **1999**, *112*, 767–774.
30. Custodio, n.; García, A.; Montesinos, R.; Lira, D.; Bendezú, L. Validación de la prueba de dibujo del reloj—versión de Manos—como prueba de cribado para detectar demencia en una población adulta mayor de Lima, Perú. *Rev. Peru. De Med. Exp. Y Salud Publica* **2011**, *28*, 29–34. [CrossRef]
31. Custodio, n.; Lira, D. Adaptación peruana del Minimental State Examination (MMSE). *An. De La Fac. De Med.* **2014**, *75*, 69. [CrossRef]
32. Plassman, B.L.; Williams, J.W., Jr.; Burke, J.R.; Holsinger, T.; Benjamin, S. Systematic review: Factors associated with risk for and possible prevention of cognitive decline in later life. *Ann. Intern. Med.* **2010**, *153*, 182–193. [CrossRef]
33. Ziem, G. Pesticide spraying and health effects. *Environ. Health Perspect.* **2005**, *113*, A150–A151. [CrossRef]
34. Baharuddin, M.R.; Sahid, I.B.; Noor, M.A.; Sulaiman, N.; Othman, F. Pesticide risk assessment: A study on inhalation and dermal exposure to 2,4-D and paraquat among Malaysian paddy farmers. *J. Environ. Sci. Health. B Pestic. Food Contam. Agric. Wastes* **2011**, *46*, 600–607. [CrossRef]
35. Rastogi, S.K.; Singh, V.K.; Kesavachandran, C.; Jyoti; Siddiqui, M.K.J.; Mathur, N.; Bharti, R.S. Monitoring of plasma butyrylcholinesterase activity and hematological parameters in pesticide sprayers. *Indian J. Occup. Environ. Med.* **2008**, *12*, 29–32. [CrossRef]
36. Krenz, J.E.; Hofmann, J.N.; Smith, T.R.; Cunningham, R.N.; Fenske, R.A.; Simpson, C.D.; Keifer, M. Determinants of butyrylcholinesterase inhibition among agricultural pesticide handlers in Washington State: An update. *Ann. Occup. Hyg.* **2015**, *59*, 25–40. [CrossRef]
37. Peter, J.V.; Sudarsan, T.I.; Moran, J.L. Clinical features of organophosphate poisoning: A review of different classification systems and approaches. *Indian J. Crit. Care Med. Peer Rev. Off. Publ. Indian Soc. Crit. Care Med.* **2014**, *18*, 735–745. [CrossRef]
38. Franco, R.; Li, S.; Rodriguez-Rocha, H.; Burns, M.; Panayiotidis, M.I. Molecular mechanisms of pesticide-induced neurotoxicity: Relevance to Parkinson's disease. *Chem. Biol. Interact.* **2010**, *188*, 289–300. [CrossRef]
39. Barrett, J.R. More Concerns for Farmers: Neurologic Effects of Chronic Pesticide Exposure. *Environ. Health Perspect.* **2005**, *113*, A472. [CrossRef]
40. Takahashi, n.; Hashizume, M. A systematic review of the influence of occupational organophosphate pesticides exposure on neurological impairment. *BMJ Open* **2014**, *4*, e004798. [CrossRef]

41. Xu, X.; Chong, E.; Hilal, S.; Ikram, M.K.; Venketasubramanian, N.; Chen, C. Beyond Screening: Can the Mini-Mental State Examination be Used as an Exclusion Tool in a Memory Clinic? *Diagnostics* **2015**, *5*, 475–486. [CrossRef]
42. Bertolucci, P.H.; Brucki, S.M.; Campacci, S.R.; Juliano, Y. The Mini-Mental State Examination in a general population: Impact of educational status. *Arq. De Neuro-Psiquiatr.* **1994**, *52*, 1–7. [CrossRef]
43. Zúñiga-Venegas, L.A.; Hyland, C.; Muñoz-Quezada, M.T.; Quirós-Alcalá, L.; Butinof, M.; Buralli, R.; Cardenas, A.; Fernandez, R.A.; Foerster, C.; Gouveia, N. Health Effects of Pesticide Exposure in Latin American and the Caribbean Populations: A Scoping Review. *Environ. Health Perspect.* **2022**, *130*, 096002. [CrossRef]
44. Akhoundzardeini, M.; Zare Sakhvidi, M.J.; Teimouri, F.; Mokhtari, M. Association between Exposure to Pesticides and Cognitive Function in Greenhouse Workers (Case Study: Ahmadabad Village of Yazd Province). *J. Environ. Health Sustain. Dev.* **2021**, *6*, 1388–1398. [CrossRef]
45. Fiedler, n.; Rohitrattana, J.; Siriwong, W.; Suttiwan, P.; Ohman Strickland, P.; Ryan, P.B.; Rohlman, D.S.; Panuwet, P.; Barr, D.B.; Robson, M.G. Neurobehavioral effects of exposure to organophosphates and pyrethroid pesticides among Thai children. *Neurotoxicology* **2015**, *48*, 90–99. [CrossRef] [PubMed]
46. Jett, D.A.; Navoa, R.V.; Beckles, R.A.; McLemore, G.L. Cognitive Function and Cholinergic Neurochemistry in Weanling Rats Exposed to Chlorpyrifos. *Toxicol. Appl. Pharmacol.* **2001**, *174*, 89–98. [CrossRef] [PubMed]
47. Ribeiro, A.C.R.; Hawkins, E.; Jahr, F.M.; McClay, J.L.; Deshpande, L.S. Repeated exposure to chlorpyrifos is associated with a dose-dependent chronic neurobehavioral deficit in adult rats. *Neurotoxicology* **2022**, *90*, 172–183. [CrossRef] [PubMed]
48. Mora, A.M.; Baker, J.M.; Hyland, C.; Rodríguez-Zamora, M.G.; Rojas-Valverde, D.; Winkler, M.S.; Staudacher, P.; Palzes, V.A.; Gutiérrez-Vargas, R.; Lindh, C.; et al. Pesticide exposure and cortical brain activation among farmworkers in Costa Rica. *Neurotoxicology* **2022**, *93*, 200–210. [CrossRef]
49. Saraji, M.; Talebi, K.; Balali-Mood, M.; Imani, S. Urinary metabolites of diazinon and chlorpyrifos in sprayer operators and farm workers of a potato farm. *J. Environ. Sci. Health B.* **2022**, *57*, 745–755. [CrossRef]
50. CDC. Chemicals in CDC's National Report on Human Exposure to Environmental Chemicals. Center for Disease, Control and Prevention of United States. 2022. Available online: https://www.cdc.gov/exposurereport/pdf/Report_Chemical_List-508.pdf (accessed on 30 October 2022).