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Cognitive impairment in agricultural workers and nearby residents exposed to pesticides in the Coquimbo Region of Chile

Sebastián A. Corral[a,b,d,f], Valeria de Angel[e,f,g], Natalia Salas[c], Liliana Zúñiga-Venegas[a], Pablo A. Gaspar[e,f,g], Floria Pancetti[a,*]

[a] *Laboratory of Environmental Neurotoxicology, Department of Biomedical Sciences, Faculty of Medicine, Universidad Católica del Norte, Coquimbo, Chile*
[b] *School of Psychology, Faculty of Social Sciences, Universidad Central de Chile, Santiago, Chile*
[c] *Facultad de Educación, Universidad Diego Portales, Santiago, Chile*
[d] *Department of Psychology, Faculty of Social Sciences, Universidad de Chile, Santiago, Chile*
[e] *Department of Psychiatry, Clinical Hospital, Universidad de Chile, Santiago, Chile*
[f] *Translational Psychiatry Laboratory, Physiology and Biophysics Department, Faculty of Medicine, Universidad de Chile, Santiago, Chile*
[g] *Biomedical Neuroscience Institute, Santiago, Chile*

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ABSTRACT

Chronic exposure to organophosphate pesticides is a worldwide public health concern associated with several psychiatric disorders and dementia. Most existing studies on the effects of pesticides only evaluate agricultural workers. Therefore, this study sought to establish if individuals indirectly exposed to pesticides, such as residents in agricultural areas, also suffer cognitive impairments. Neuropsychological evaluations were carried out on three groups ($n = 102$): agricultural workers directly exposed to pesticides ($n = 32$), individuals living in agricultural areas indirectly (i.e. environmentally) exposed to pesticides ($n = 32$), and an unexposed control group ($n = 38$). The assessed cognitive processes included memory, executive functions, attention, language praxis, and visuoconstruction. The direct exposure group performed significantly lower in executive function, verbal fluency, and visual and auditory memory tests than the indirect exposure group, which, in turn, performed worse than the unexposed group. Even after adjusting for age, gender, and educational level, both exposure groups showed higher rates of cognitive deficit than control individuals. In conclusion, both direct and indirect chronic exposure to pesticides affects cognitive functioning in adults and, consequently, actions should be taken to protect the health of not only agricultural workers, but also of residents in agricultural areas.

1. Introduction

Worldwide pesticide use in the agricultural industry and for domestic purposes is associated with serious occupational health problems and deleterious environmental impacts (Suratman et al., 2015). Growth of the Chilean agricultural sector in recent decades has necessitated the expansion of areas used for crop production. Consequently, pesticide use has also increased, leading to acute intoxication outbreaks in agricultural areas, mainly during the spraying season (Pancetti et al., 2011; Zúñiga-Venegas et al., 2015).

In the Coquimbo Region, most of the applied agricultural pesticides belong to the organophosphate chemical family, which is used for insect management (Moretto, 1998). Acute organophosphate poisoning produces cholinergic symptoms resulting from the molecular inhibition of acetylcholinesterase, a key enzyme in central and peripheral synapses (Marrs, 1993). On the other hand, chronic exposure often goes unnoticed, with long-term consequences only becoming evident with the occurrence of neuropsychiatric and carcinogenic disorders, congenital malformations (Mostafalou and Abdollahi, 2013), and neurodegenerative diseases (Wang et al., 2014).

Abundant evidence supports that prolonged exposure to organophosphate and carbamate pesticides produces cognitive impairment that can be detected in exposed individuals through neuropsychological performance evaluations (Colosio et al., 2009; Rohlman et al., 2007). Indeed, exposure has been linked with impairments in intellectual functioning, academic skills, abstraction abilities, reasoning, and motor and social skills, among others. Furthermore, some studies specifically highlight psychomotricity, short-term memory, working memory, and attention as the most affected cognitive functions (Baldi et al., 2003; Kamel and Hoppin, 2004), and major impairments in planning abilities

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Abbreviations: FAB, Frontal Assessment Battery; MANOVA, multivariate analysis of variance; MMSE, Mini-Mental State Examination; ROCF, Rey-Osterrieth Complex Figure

[*] Corresponding author at: Laboratory of Environmental Neurotoxicology, Faculty of Medicine, Universidad Católica del Norte, Larrondo 1281, 178-1421 Coquimbo, Chile.
E-mail address: pancetti@ucn.cl (F. Pancetti).

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and mood have also been described (Mackenzie-Ross et al., 2010).

Despite the notable impacts to human health, most studies focus assessments only on directly and chronically exposed individuals, such as agricultural workers. Indeed, little research has considered populations of environmentally, or indirectly, exposed individuals, and most of these studies center on environmentally-exposed children (Guillette et al., 1998; Rauh et al., 2011; Rohlman et al., 2005). One such study reported that children had a higher risk of cognitive impairment when the gestation period occurred while mothers lived in proximity to agricultural fields treated with pesticides (Shelton et al., 2014). Similarly, lower-than-expected IQ scores, as measured by the Wechsler Intelligence Scale (3rd Ed.), have been reported in Chilean children living near an important center for agricultural activity (Muñoz-Quezada et al., 2011).

Although frequent cases of acute intoxications occur when spraying crops with pesticides, there are very few studies in Chile that assess the impact of exposure on cognitive performance. This information gap continues to persist even despite evidence linking low cognitive performance with severe, lifelong consequences, including dementia (Meyer-Baron et al., 2015). Therefore, the aim of this study was to determine the cognitive impacts of pesticide exposure in directly (i.e. agricultural workers) and indirectly (i.e. agricultural area residents) exposed individuals. For this, cognitive assessments of memory, attention, executive function, and language and visuoconstruction praxis were performed in three groups with different degrees of pesticide exposure: direct, indirect, and unexposed.

2. Methods

2.1. Study design

This was an exploratory cross-sectional study that simultaneously ascertained information regarding exposure and outcome. Due to the pilot nature of the study, non-probability convenience sampling was carried out. The following three groups, with different degrees of pesticide exposure, were assessed: direct exposure, corresponding to agricultural workers with occupational and daily exposure to organophosphate pesticides; indirect exposure, corresponding to residents living in proximity to agricultural activities and that would therefore be environmentally exposed to pesticides; and non-exposure (controls), corresponding to individuals living far from agricultural activities. Exposure levels were estimated from a questionnaire completed by the volunteers (see supplementary Fig. S1). Informed written consent was obtained from voluntary participants prior to recruitment.

2.2. Study groups

The direct-exposure group included 32 individuals living in proximity to and directly working with agricultural pesticides. This group included pesticide mixers and sprayers, as well as crop harvesters, that performed agricultural work within the study area, which included the Elqui Valley (Montegrande, Pisco Elqui, and Pan de Azúcar villages) and Limarí Valley (Chañaral Alto village), Chile. The indirect-exposure group included 32 residents of the above-cited villages. Finally, the unexposed group consisted of 38 individuals recruited from coastal areas lacking agricultural activities. The studied geographical locations are depicted in Fig. 1. Group recruitments and evaluations took place between November 2009 and January 2011.

2.3. Inclusion and exclusion criteria

Men and women between the ages of 18 and 55 were considered. Inclusion criteria of the direct-exposure group were at least three years of daily contact with and direct manipulation of pesticides within a context of work. Inclusion criteria of the indirect-exposure group were at least three years of living in proximity to agricultural areas that applied pesticides, but without direct manipulation. For the direct and indirect exposed groups, individuals that reported an episode of acute intoxication during the study period were also included. Inclusion criteria of the unexposed group were not living in proximity to, coming into contact with, or directly manipulating pesticides.

Exclusion criteria included alcoholism, drug addiction, diagnosis of a psychiatric disease, and neurodegenerative/neurological disorders. Individuals under treatment with drugs affecting the central nervous system were also excluded since these drugs can alter neuropsychological outcomes, therefore affecting study results. Finally, left-handed individuals were also excluded to discard the bias produced by the laterality of neuropsychological functions.

2.4. Volunteer recruitment

Study locations were chosen based on the expected levels of pesticide exposure based on the agricultural activity carried out in that locations (mainly grapes and citrics farming). Direct-exposure individuals were recruited from three different locations. In Pisco Elqui, agricultural workers were interviewed and evaluated at a village office. In Pan de Azúcar, initial contact was through the Rural Health Service and Residents' Association, while subject evaluations took place at the local health center. Finally, in Chañaral Alto, volunteers were evaluated in the outbuildings of a local farm company.

Indirect-exposure individuals were recruited from four locations. In Montegrande, initial contact and volunteer evaluations took place at the Gabriela Mistral School. In Pisco Elqui, participants were contacted through home visits and were evaluated in their own homes. In Pan de Azúcar, volunteers were evaluated at the Rural Health Center. Finally, in Chañaral Alto, subjects were assessed at the administrative buildings of a farm company.

Unexposed subjects were recruited from the towns La Serena and Coquimbo. In an attempt to homogenize the groups and standardize educational levels, subjects in the control group were employed in manual labor jobs, mostly in construction or service areas such as cleaning, maintenance, and security. It is important to mention that the educational curriculum at every grade level is regulated by the Ministry of Education; therefore a lack of homogeneity among schools does not constitute a bias in this study.

2.5. Neuropsychological evaluations

Subjects who met the inclusion criteria were evaluated individually through seven neuropsychological tests that assessed general mental state, memory, attention, visuoconstruction praxis, and executive functions. All tests were administered by the same trained psychologist. Following is a brief summary of the applied neuropsychological tests and of the cognitive areas evaluated by each:

- Mini-Mental State Examination (MMSE) (Folstein et al., 1975): Brief evaluation of spatial and temporal orientation, short-term memory, attention, calculation, language, and praxis (Peña-Casanova et al., 2005).
- Digit span test, applying the Wechsler Adult Intelligence Scale in its revised version (WAIS-R) (Hermosilla, 1982). For further information about this version see also Rosas et al. (2014). Briefly, digit span test consists of forward and backward digits and is used to evaluate memory and attention, respectively. This test has been used in neuropsychological batteries to assess people exposed to pesticides (Rohlman et al., 2007; Roldán-Tapia et al., 2005).
- Rey-Osterrieth Complex Figure Test (ROCF) (Rey, 2003): Determines visuoconstruction skills and visual memory. The ROCF test also helps determine cognitive performance in different areas, such as planning, organizing, problem solving strategies, and motor functions (Lezak et al., 2012; Peña-Casanova et al., 2005). This test has also been found sensitive to cognitive impairment induced by

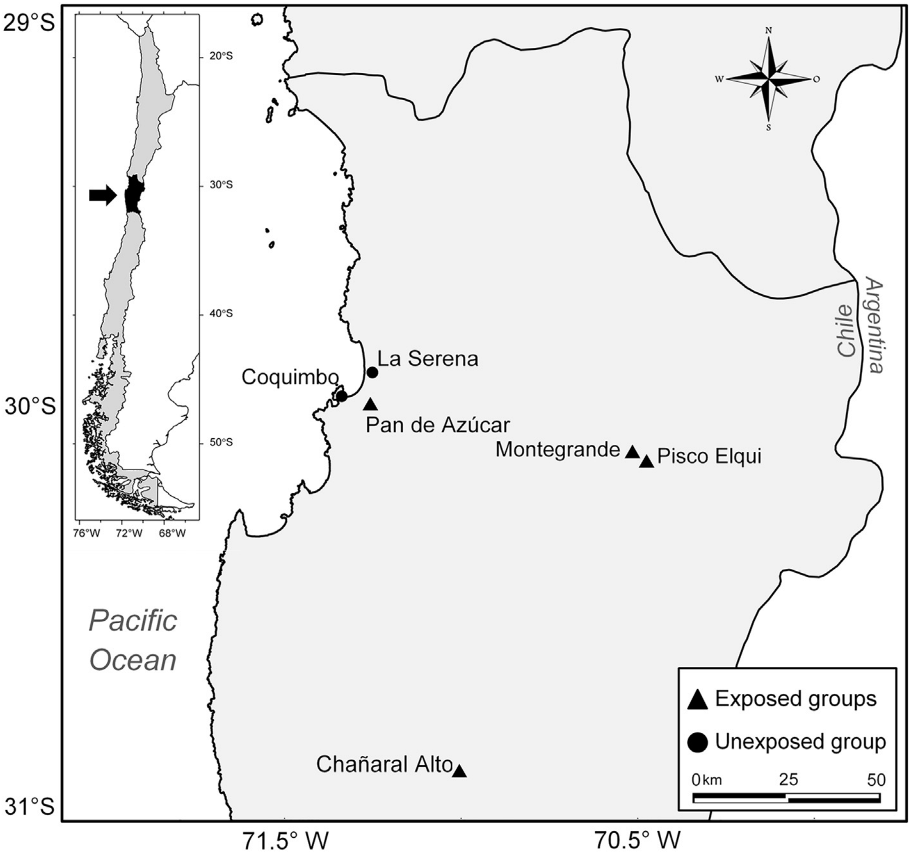

Fig. 1. Map showing the towns at which volunteers belonging to each group were recruited.

pesticide exposure (Roldán-Tapia et al., 2006).

- Stroop Test: Evaluates the ability for divided attention and resistance to interference. This test has been used in batteries evaluating cognitive impairment due to pesticide exposure (Roldán-Tapia et al., 2005). In the present study, only scores of divided attention were considered.
- d2 Test of Attention, Spanish version (Brickenkamp, 2004): Adapted from the Aufmerksamkeits-Belastungs-Test and used to evaluate selective attention and concentration skills.
- Frontal Assessment Battery (FAB) (Dubois et al., 2000): Rapidly evaluates executive functions. The FAB is composed of six semantic sub-tests, including verbal fluency, motor programming, sensitivity to interference, inhibitory control (go/no-go), and environmental autonomy through prehension behavior.
- Semantic Verbal Fluency, Barcelona Sub-test for Animals and Letter P (Peña-Casanova, 2003): Evaluates the ability to access semantic and lexicon storages (Peña-Casanova et al., 2005).

Table 2 summarizes the applied text by cognitive function and the respective cut-off scores of normality. It is important to mention that at the time of this study, only the MMSE and digit span test from WAIS-R were validated in Chile. Consequently, this study used an unexposed group as a control, with performance comparisons against the exposed groups.

2.6. Statistical analysis

Descriptive statistics were first obtained from all collected data. Information on age, gender, years of exposure, and educational level was tabulated and analyzed to detect differences among groups. To minimize the bias of potential confounders, the variables should be equally distributed among the three groups.

Inter-group differences between the seven cognitive tests were determined using one-way multivariate analysis of variance (MANOVA) followed by a post-hoc comparison test with Bonferroni correction. Significant differences between groups were established at $p < 0.005$.

To determine if direct- and indirect-exposure individuals showed higher rates of cognitive deficit, as measured by the proportion of individuals scoring below the cut-off values established for each test, logistic regression analyses were used. For this, the direct- and indirect-exposure groups were used as predictors; the unexposed group was used as the reference category, and the dichotomized (i.e. present or absent) variable of cognitive deficit for each test was used as the outcome variable. The obtained results were adjusted for age, gender, and educational level.

All analyses were conducted with the SPPS 22.0 software (IBM, 2013).

3. Results

The following variables were considered potential confounders when analyzing scores from the cognitive tests: gender, educational level, age, and years of exposure (Table 1). Furthermore, although most tests were completed by all participants, FAB test data were only obtained for 77 individuals (21 direct-exposure; 26 indirect-exposure; 30 unexposed).

3.1. Confounders

3.1.1. Gender

There were twice as many men (66.7%) as women (33.3%). A chi-squared test of independence showed a significant relationship between gender and exposure group ($X^2 = 8.61$, $n = 102$, $p < 0.05$). Women were more likely to be in the indirect-exposure group than men, while males were more likely to be in the unexposed group than women. Despite these differences, *t*-tests and Mann-Whitney-*U* tests were used

Table 1
Study group demographics.

	Direct exposure (n = 32)		Indirect exposure (n = 32)		Controls (n = 38)		Total (n = 102)	
	n	%	n	%	n	%	n	%
Gender[*]								
Male	23	71.9	15	46.9	30	66.7	68	66.7
Female	9	28.1	17	53.1	8	33.3	34	33.3
Educational level								
Primary	15	46.9	8	25	10	32.4	33	32.4
Secondary	11	34.4	13	40.6	19	42.2	43	42.2
Technical	6	18.8	11	34.4	9	25.5	26	25.5

	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Age	39.1 ± 9.5	39.8 ± 9.7	39.3 ± 9.8	39.4 ± 9.6
Years of exposure	14.06 ± 8.1	16.2 ± 13.9	–	15.2 ± 11.4

[*] $p < 0.05$.

to compare all cognitive scores between men and women, but no differences were found. Furthermore, chi-squared tests investigated if there were gender-based differences in terms of abnormal cognitive results. Both genders were found equally likely to have abnormal results in all cognitive tests. These analyses support that gender was not a study confounder.

3.1.2. Educational level

A large proportion (67.7%) of participants had completed secondary education or technical schooling, but almost one-third of participants had only completed basic education. To reduce the confounding effects of educational level, control volunteers were recruited at the end of experimental sampling to homogenize the years of education between groups. Furthermore, a chi-squared test was conducted and showed no significant differences among groups ($X^2 = 5.70$, $n = 102$, $p > 0.05$).

3.1.3. Age

For each of the cognitive tests, the general population below the age of 55 should show no cognitive declines. Despite this, the sample group as a whole was below this threshold. Therefore, a linear regression analyses was used to determine if a higher age predicted low scores in any of the cognitive tests, with results showing that age was a significant predictor of scores for the copy subtest of the ROCF ($F_{(1, 100)} = 7.15$, $p < 0.01$, adjusted $R^2 = 0.06$, $R = 0.26$) and recall subtest of the ROCF ($F_{(1, 100)} = 9.93$, $p < 0.01$, adjusted $R^2 = 0.08$, $R = 0.30$). Nevertheless, the obtained R-values indicated that correlations between age and the two variables were weak. Additionally, the R^2 value indicates that age can only explain 6 and 8%, respectively, of the variation in each test. The remaining tests did not show significant differences. Considering these results, age was not a confounder of the study.

3.1.4. Years of exposure

The mean (± SD) years of exposure in the direct-exposure group (14.1 ± 8.1) did not significantly differ from the indirect-exposure group (16.2 ± 13.9). Linear regression analyses were performed in the exposure groups to assess if longer exposure times predicted lower scores in any of the cognitive tests. Regression analysis results showed that exposure time did not significantly predict cognitive scores.

3.2. Group differences in cognitive scores

A one-way MANOVA was conducted to determine differences between the exposure groups in regard to each test of cognitive ability. The cognitive tests eligible for MANOVA were MMSE, the Semantic

Table 2
Mean cognitive test scores with comparisons between the different exposure groups.

Cognitive function	Test	Normal cut-off score	Direct exposure (Mean ± SD)	Indirect exposure (Mean ± SD)	Un-exposed (controls) (Mean ± SD)	Statistic	p-Value	Post-hoc comparisons
General mental status	MMSE	> 24	26.9 ± 2.4	28.1 ± 2.1	29.0 ± 1.2	F(2.74) = 11.95	< 0.001	DE < IE, C
Memory	Digits span forward	≥5	4.5 ± 1.0	4.6 ± 1.0	5.3 ± 1.0	H = 11.30	0.004	DE, IE < C
	ROCF memory recall	> 25 Pc	28.5 ± 22.9	36.9 ± 31.7	57.9 ± 33.6	H = 12.60	0.002	DE, IE < C
Attention	Digits span backward	≥4	3.1 ± 0.8	3.3 ± 1.0	3.9 ± 0.9	H = 15.53	< 0.001	DE, IE < C
	d2 test	> 25 Pc	19.0 ± 18.6	20.1 ± 19.5	25.4 ± 18.6	H = 3.74	0.154	–
	Stroop word-colour	> 40	45.5 ± 7.9	45.0 ± 8.1	48.0 ± 6.6	F(2.99) = 1.71	0.189	–
Visuoconstruction praxis	ROCF copy	> 25 Pc	71.1 ± 30.3	82.3 ± 27.9	87.1 ± 18.5	H = 7.64	0.022	–
Executive functions	Verbal fluency:							
	Animals	> 16	18.4 ± 6.0	20.3 ± 7.3	22.0 ± 5.4	F(2.74) = 6.33	< 0.01	DE < IE, C
	Words (letter P)	> 24	21.4 ± 10.6	26.2 ± 10.9	32.7 ± 8.6	F(2.74) = 17.25	< 0.001	DE < IE < C
	Frontal Assessment Battery	> 16	12.8 ± 2.0	14.9 ± 1.7	16.3 ± 1.5	F(2.74) = 23.69	< 0.001	DE < IE < C

Pc: percentile/DE: direct exposure, IE: indirect exposure, C: controls.

Verbal Fluency test, and FAB. Parametric statistical tests could not be performed for the remaining cognitive assessments, with the exception of the Stroop Test, which had less than a mild correlation with the other variables, thus violating MANOVA collinearity assumptions. MANOVA analyses detected statistically significant differences in the cognitive ability tests by exposure group ($F_{(8,\ 142)} = 6.33$, $p < 0.001$, Wilk's $\Lambda = 0.54$), where higher pesticide exposure was related to lower cognitive scores. Subsequent ANOVA analyses found significant main effects for all tests entered in the MANOVA (Table 2).

An independent ANOVA was carried out on the Stroop Test, with no significant differences in test results across groups. An independent samples Kruskal-Wallis test was carried out for the remaining cognitive tests. The ROCF recall ($H = 12.60$, $p < 0.005$); the forward digit span ($H = 11.30$, $p < 0.005$); and backward digit span tests ($H = 15.53$, $p < 0.005$) were all significant. The ROCF copy test ($H = 7.64$, $p = 0.022$) was not significant after applying Bonferroni correction, and the d2 test was also non-significant.

Post-hoc pairwise comparisons showed that the direct-exposure group had significantly lower cognitive scores than the indirect-exposure group in the MMSE, the FAB, and the Semantic Verbal Fluency test for both animals and the letter P. In all cases, the direct-exposure group performed significantly worse than the unexposed control group. The indirect-exposure group was comparable to control individuals in the MMSE and Semantic Verbal Fluency test for animals, but this group did significantly worse than the control group in the remaining five tests.

3.3. Cognitive deficit

Excepting the ROCF recall and Stroop Tests, a higher percentage of direct-exposure individuals scored below the cut-off point for each cognitive test as compared to indirect-exposure individuals (Fig. 2). Similarly, more participants scored poorly in the indirect-exposure group than unexposed participants for every test.

Logistic regression analyses revealed that the odds of scoring below the cut-off point if directly exposed to pesticides were no different than for indirect exposure. However, a second set of logistic regression analyses for all tests using the control group as the reference category did result in significant differences. Age, gender, and educational level were included in these analyses to adjust for potential confounding effects. In the MMSE, ROCF (copy and recall), d2, and Stroop Tests, the odds of being in one of the exposure groups and of showing signs of cognitive deficit were comparable to the control group. In contrast, the FAB, Digital Span (forward and backward), and verbal fluency (animals and letter P) tests demonstrated at least a three-fold increase in the risk of cognitive deficit compared to controls (Table 3). Importantly, these odds ratios should be interpreted with care as the small sample size means that the confidence intervals are very large.

Table 3
Table of odd ratios (OR) and 95% confidence intervals (CI) of scoring below the cut-off for each test[a] compared to controls.

Test	Direct exposure		Indirect exposure	
	OR	(95% CI)	OR	(95% CI)
FAB	44.9	(5.6–359.7)	7.3	(1.7–32.4)
Digit span (forward)	4.9	(1.6–14.9)	4.5	(1.4–13.8)
Digit span (backward)	7.4	(2.4–22.4)	2.9	(1.02–8.3)
Verbal fluency (animals)	5.7	(1.3–25.6)	4.5	(1.04–19.4)
Verbal fluency (letter P)	16.7	(4.3–64.6)	8.1	(2.1–31.3)

[a] The shown ORs pertain only to the five cognitive tests that produced significant results. Not included are the MMSE, ROCF copy and recall, d2, and Stroop Tests.

4. Discussion

Acute and chronic (> 10 years) exposure to organophosphate and carbamate pesticides, both of which act by inhibiting cholinesterases, is widely associated with cognitive and motor impairments that can be observed even several months post-intoxication (Kamel et al., 2003; Roldán-Tapia et al., 2006; Rohlman et al., 2007). To date, only one report exists about the impact of pesticide exposure on neurocognitive performance in a Chilean population. This is despite having a large agricultural sector and a clear record of acute intoxication outbreaks provided through *Surveillance System of Acute Intoxications Reports* from the Chilean Ministry of Health (Pancetti et al., 2011). This prior study reported impairments in verbal comprehension and processing speed, as well as low intellectual coefficients, in agricultural workers from the Maule Region of Chile that were exposed for at least five years to organophosphate pesticides (Muñoz-Quezada et al., 2016a).

While performed in 2011, the present study supports Muñoz-Quezada et al. (2016a), finding that a group of direct, or occupationally, exposed workers had worse cognitive performance than an unexposed control group. Specifically, the direct-exposure group showed lower scores in tests of executive functions, verbal fluency, and visual and auditory memory than individuals not exposed to high pesticide levels (Table 2 and Fig. 2). Worth noting, the present study also recruited an indirect, or environmentally, exposed group. Interestingly, individuals from this group also showed decreased performance in the same cognitive areas as occupationally-exposed subjects. This indicates that individuals who do not daily or directly manipulate pesticides still

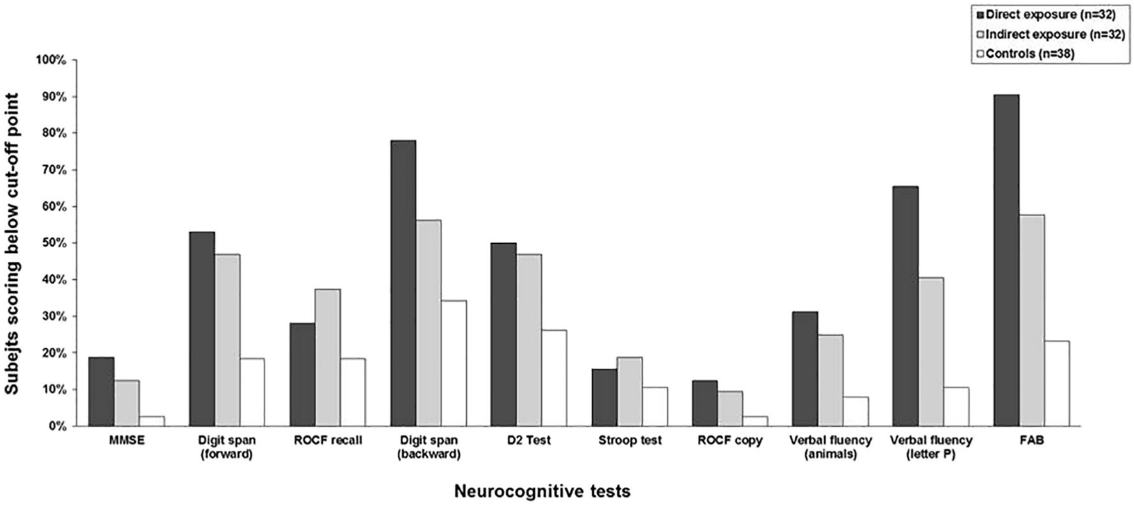

Fig. 2. Percentage of volunteers scoring below the cut-off point in each cognitive test.

suffer negative consequences simple as a result of living near agricultural areas that make a widespread use of pesticides.

Cognitive differences between exposure groups were mostly seen in tests related to attention and verbal fluency. Despite a clear tendency of poorer performance in groups with higher pesticide exposure, no statistically significant differences were obtained for visuoconstruction alterations (ROCF copy test, Table 2). There were also no differences in the Stroop and d2 tests, which measure specific cognitive abilities related to sustained and selective attention. These results are in line with existing literature that suggests pesticide damage is diffuse and not focal (Kamel and Hoppin, 2004). Nevertheless, other researchers have found these areas affected by pesticides (e.g. Mackenzie-Ross et al., 2010). A study performed in France, which used a methodological design very similar to that currently reported, classified vineyard workers as indirectly and directly exposed to pesticides. Impaired performance was observed in relation to exposure level in tests containing components of selective attention and cognitive inhibition (Baldi et al., 2011). Considering these contrasting results, it would be interesting to evaluate these effects on a larger sample to assess if an increase in power would in fact yield significant differences between exposure groups. Indeed, this point was discussed in the review of Kamel and Hoppin (2004), who indicated that many studies employing fewer than 100 exposed subjects lack statistical power.

The present study not only found that lower exposure groups performed better, but also that the risk of showing signs of cognitive deficit varied across the exposure groups. When looking at the proportion of scores below the cut-off points for each test, the exposure groups once again exhibited a higher risk for showing signs of cognitive decline than unexposed individuals (Table 3). This tendency was observed for tests of executive functions and memory. Although risks were higher for individuals that were directly exposed to pesticides, those living in proximity to areas with high pesticide use were also at risk for cognitive deficits, particularly in regards to memory and executive functions (Table 3).

In the assessed groups, the years of pesticide exposure did not predict cognitive deficits (Table 1). Subjects in this study had mean and median exposure times of over 10 years, and were therefore “chronically” exposed. In other words, the data indicate that after a 10-year threshold of exposure, cognitive damage has already taken place, and each additional year of pesticide exposure would not significantly increase the risk of poorer cognitive performance. This 10-year threshold has been previously reported, even in cases where pesticide exposure occurs at very low doses (Roldán-Tapia et al., 2005). Nevertheless, since study subjects were not followed over time, it cannot be ruled out that additional years of exposure might produce worse cognitive performance. Indeed, a follow-up of a vineyard farmer cohort from the study conducted by Baldi et al. (2011) demonstrated that these groups had a risk of 1.97 to obtain a two-point lower score on the MMSE over a span of 4–5 years, meaning worse performance over time while exposure conditions were maintained.

The present study has several limitations. First, a short version of the battery was used to evaluate cognitive functioning. A full battery including psychomotricity and psychiatric symptoms might have been more apt considering the high prevalence of neurological and psychiatric disorders observed in pesticide-exposed populations (Colosio et al., 2009). Similarly, a larger sample size and longitudinal follow-up could prospectively detect problems in other cognitive domains and other mental health disorders. Another limitation is that this study did not determine the exact types of substances to which participants were exposed. Although this was not a study objective, it is possible to infer that individuals were probably exposed to chlorpyrifos, the most widely sold organophosphate in the studied region and one of the most commonly used insecticides in grapes and other fruits (Zúñiga-Venegas et al., 2015). In fact, a recent study by Muñoz-Quezada et al. (2017) found that almost 50% of the interviewed pesticide applicators from the Maule Region of Chile reported using chlorpyrifos. Finally, no biomarkers of effect (e.g. inhibition of cholinesterases in blood) or exposure (e.g. metabolites of organophosphates in urine) were quantified. However, it is worth mentioning that the present study was planned as a pilot for a larger research project conducted in the same area between 2011 and 2014. This latter study did assess blood biomarkers, thus providing a more complete idea about exposure type, chemical agent usage, and internalized pesticide doses (Ramírez-Santana et al., 2015). Briefly, the inhibition of plasma cholinesterase (or butyrylcholinesterase) activity, instead of erythrocyte acetylcholinesterase activity, appears to be directly correlated with the level of pesticide exposure (unpublished results).

5. Conclusion

This study reports that the cognitive deficits frequently observed in agricultural workers that directly manipulate pesticides represent only part of the population that suffers cognitive impairments. Indeed, indirectly, i.e. environmentally, exposed individuals also presented cognitive deficits compared to unexposed individuals. These results were independent of age, gender, educational level, and other confounding factors. Moreover, the present data were consistent with previous reports in which individuals chronically exposed to organophosphate pesticides showed severe cognitive impairments (for a recent review see Muñoz-Quezada et al., 2016b). Considering these results, preventive mental health programs should include cognitive evaluations for agricultural workers and residents living near agricultural areas. Health programs should also consider other at-risk populations, such as the children of mothers who were indirectly or direct exposed to pesticides.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ntt.2017.05.003.

Declaration of interest

The authors declare that there are no conflicts of interest.

Transparency document

The http://dx.doi.org/10.1016/j.ntt.2017.05.003 associated with this article can be found, in the online version.

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References

Baldi, I., Lebailly, P., Mohammed-Brahim, B., Letenneur, L., Dartigues, J.F., Brochard, P., 2003. Neurodegenerative diseases and exposure to pesticides in the elderly. Am. J. Epidemiol. 157 (5), 409–414.

Baldi, I., Gruber, A., Rondeau, V., Lebailly, P., Brochard, P., Fabrigoule, C., 2011. Neurobehavioral effects of long-term exposure to pesticides: results from the 4-year follow-up of the PHYTONER study. Occup. Environ. Med. 68 (2), 108–115.

Brickenkamp, R., 2004. d2 test de atención. TEA, Madrid.

Colosio, C., Tiramani, M., Brambilla, G., Colombi, A., Moretto, A., 2009. Neurobehavioural effects of pesticides with special focus on organophosphorus compounds: which is the real size of the problem? Neurotoxicology 30 (6), 1155–1161.

Dubois, B., Slachevsky, A., Litvan, I., Pillon, B., 2000. The FAB: a frontal assessment battery at bedside. Neurology 55 (11), 1621–1626.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. “Mini-mental state”. A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12 (3), 189–198.

Guillette, E.A., Meza, M.M., Aquilar, M.G., Soto, A.D., Garcia, I.E., 1998. An anthropological approach to the evaluation of preschool children exposed to pesticides in Mexico. Environ. Health Perspect. 106 (6), 347–353.

Hermosilla, M. La Escala de Inteligencia de Wechsler para adultos. Unpublished manuscript. School of Psychology, Pontificia Universidad Católica de Chile, Santiago, Chile, 1982.

IBM Corp, 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, IBM Corp (Released 2013).

Kamel, F., Hoppin, J.A., 2004. Association of pesticide exposure with neurologic dysfunction and disease. Environ. Health Perspect. 112 (9), 950–958.

Kamel, F., Rowland, A.S., Park, L.P., Anger, W.K., Baird, D.D., Gladen, B.C., Moreno, T., Stallone, L., Sandler, D.P., 2003. Neurobehavioral performance and work experience in Florida farmworkers. Environ. Health Perspect. 111 (14), 1765–1772.

Lezak, M., Howieson, D., Bigler, E., Tranel, D., 2012. Neuropsychological Assessment, 5th ed. Oxford University Press.

Mackenzie-Ross, S.J., Brewin, C.R., Curran, H.V., Furlong, C.E., Abraham-Smith, K.M., Harrison, V., 2010. Neuropsychological and psychiatric functioning in sheep farmers exposed to low levels of organophosphate pesticides. Neurotoxicol. Teratol. 32 (4), 452–459.

Marrs, T.C., 1993. Organophosphate poisoning. Pharmacol. Ther. 58 (1), 51–66.

Meyer-Baron, M., Knapp, G., Schäper, M., van Thriel, C., 2015. Meta-analysis on occupational exposure to pesticides-neurobehavioral impact and dose-response relationships. Environ. Res. 136, 234–245.

Moretto, A., 1998. Experimental and clinical toxicology of anticholinesterase agents. Toxicol. Lett. 102–103, 509–513.

Mostafalou, S., Abdollahi, M., 2013. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicol. Appl. Pharmacol. 268 (2), 157–177.

Muñoz-Quezada, M.T., Iglesias, A.V.P., Lucero, M.B.A., 2011. Exposure to organophosphate and cognitive performance in Chilean rural school children: an exploratory study. Rev. Fac. Nac. Salud Pública 29 (3), 256–263.

Muñoz-Quezada, M.T., Lucero, B., Iglesias, V., Muñoz, M.P., Achú, E., Cornejo, C., Concha, C., Grillo, A., Brito, A.M., 2016a. Organophosphate pesticides and neuropsychological and motor effects in the Maule Region, Chile. Gac. Sanit. 30 (3), 227–231.

Muñoz-Quezada, M.T., Lucero, B.A., Iglesias, V.P., Muñoz, M.P., Cornejo, C.A., Achu, E., Baumert, B., Hanchey, A., Concha, C., Brito, A.M., Villalobos, M., 2016b. Chronic exposure to organophosphate (OP) pesticides and neuropsychological functioning in farmworkers: a review. Int. J. Occup. Environ. Health 22 (1), 68–79.

Muñoz-Quezada, M.T., Lucero, B., Iglesias, V., Levy, K., Muñoz, M.P., Achú, E., Cornejo, C., Concha, C., Brito, A.M., Villalobos, M., 2017. Exposure to organophosphate (OP) pesticides and health conditions in agricultural and non-agricultural workers from Maule, Chile. Int. J. Environ. Health Res. 27 (1), 82–93.

Pancetti, F., Ramírez, M., Castillo, C., 2011. Epidemiological studies of anticholinestarase pesticides poisoning in Chile. In: Satoh, T., Gupta, R.C. (Eds.), Anticholinesterase Pesticides: Metabolism, Neurotoxicity, and Epidemiology. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 357–364.

Peña-Casanova, J., 2003. Programa integrado de exploración neuropsicologica revisado - Barcelona. Masson, Barcelona.

Peña-Casanova, J., Gramunt, N., Gich, J., 2005. Test neuropsicologicos. Masson, Barcelona.

Ramírez-Santana, M., Zúñiga-Venegas, L., Corral, S., Sandoval, R., Scheepers, P.T., Van der Velden, K., Roeleveld, N., Pancetti, F., 2015. Assessing biomarkers and neuropsychological outcomes in rural populations exposed to organophosphate pesticides in Chile—study design and protocol. BMC Public Health 15, 116–124.

Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D., et al., 2011. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environ. Health Perspect. 119 (8), 1196–1201.

Rey, A., 2003. Rey, test de copia y reproduccion de memoria de figuras complejas geométricas. TEA, Madrid.

Rohlman, D., Arcury, T., Quandt, S., Lasarev, M., Rothlein, J., Travers, R., et al., 2005. Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. Neurotoxicology 26 (4), 589–598.

Rohlman, D.S., Lasarev, M., Anger, W.K., Scherer, J., Stupfel, J., McCauley, L., 2007. Neurobehavioral performance of adult and adolescent agricultural workers. Neurotoxicology 28 (2), 374–380.

Roldán-Tapia, L., Leyva, A., Laynez, F., Santed, F.S., 2005. Chronic neuropsychological sequelae of cholinesterase inhibitors in the absence of structural brain damage: two cases of acute poisoning. Environ. Health Perspect. 113 (6), 762–766.

Roldán-Tapia, L., Nieto-Escamez, F.A., del Aguila, E.M., Laynez, F., Parron, T., Sanchez-Santed, F., 2006. Neuropsychological sequelae from acute poisoning and long-term exposure to carbamate and organophosphate pesticides. Neurotoxicol. Teratol. 28 (6), 694–703.

Rosas, R., Tenorio, M., Pizarro, M., Cumsille, P., Bosch, A., 2014. Standardization of the Wechsler Intelligence Scale for adults – fourth edition in Chile. Psykhe 23 (1), 1–18.

Shelton, J.F., Geraghty, E.M., Tancredi, D.J., Delwiche, L.D., Schmidt, R.J., Ritz, B., Hansen, R.L., Hertz-Picciotto, I., 2014. Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: the CHARGE study. Environ. Health Perspect. 122 (10), 1103–1109.

Suratman, S., Edwards, J.W., Babina, K., 2015. Organophosphate pesticides exposure among farmworkers: pathways and risk of adverse health effects. Rev. Environ. Health 30 (1), 65–79.

Wang, A., Cockburn, M., Ly, T.T., Bronstein, J.M., Ritz, B., 2014. The association between ambient exposure to organophosphates and Parkinson's disease risk. Occup. Environ. Med. 71 (4), 275–281.

Zúñiga-Venegas, L., Aquea, G., Taborda, M., Bernal, G., Pancetti, F., 2015. Determination of the genotype and phenotype of serum paraoxonase 1 (PON1) status in a group of agricultural and nonagricultural workers in the Coquimbo Region, Chile. J. Toxic. Environ. Health A 78 (6), 357–368.