Neurological Deficits After Long-term Pyrethroid Exposure

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ABSTRACT: Pyrethroid pesticides have been suggested to be a cause of Parkinson disease and other neurodegenerative diseases. To investigate this, a cross-sectional study was conducted among 120 Bolivian public health vector program spray men, primarily exposed to pyrethroids. Pesticide exposure and central nervous system (CNS) symptoms were determined by a structured interview, whereas neuromotor and neurocognitive performance was assessed using the computerized Behavioral Assessment and Research System and CATSYS system. Individuals exposed to higher levels reported significantly more CNS symptoms (adjusted odds ratio per quintile of cumulative exposure = 2.01 [1.22-3.31]). There was no association seen between pyrethroid exposure and neuromotor performance. Higher spraying intensity was associated with significantly worse neurocognitive performance in structural equation models (adjusted β per quintile = −0.405 [−0.660 to −0.150]), and workers only exposed to pyrethroids performed worse than workers also exposed to other pesticides (adjusted β = −1.344 [−2.224 to −0.464]). Chronic pyrethroid exposure may cause deterioration in neurocognitive performance, and exposure control is recommended.

KEYWORDS: Pyrethrins, pyrethroids, pesticides, insecticides, Parkinson disease, neurocognitive disorders

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Introduction

Pesticides are a heterogeneous group of compounds used to kill organisms considered unwanted by humans. Studies have shown that long-term exposure to pesticides may be associated with neurodegenerative diseases such as Parkinson disease (PD),[1–3] Alzheimer dementia,[4] and amyotrophic lateral sclerosis (ALS).[5,6] However, study results are somewhat heterogeneous, and most studies consider exposure to pesticides in general and not specific compounds or classes of compounds.

Pyrethroids are a class of insecticides whose primary toxicodynamic mode of action is delayed closure of voltage-gated sodium channels in the central nervous system (CNS) of exposed organisms.[7] Due to differences in the structure of sodium channels (and, to a lesser extent, toxicokinetic factors), the acute toxicity of pyrethroids to mammals is relatively low compared with insects,[8] and pyrethroids do not have the acute human toxicity of carbamate and organophosphate insecticides.[9] However, the potential for chronic neurological damage due to long-term pyrethroid exposure has been debated, especially in Germany.[10] But few epidemiologic studies specifically addressing pyrethroids have been conducted.

In Bolivia (and many other tropical countries), vector-borne diseases, such as malaria, dengue fever, and Chagas disease, continue to pose serious public health problems, and indoor residual spraying of insecticides is an integral part of the fight against them.[11] The spraying workers of the Bolivian vector control programs have a very well-defined exposure to pesticides. In the 1950s and 1960s, they used organochlorine insecticides; in the 1970s, they changed to organophosphates and carbamates; and since 1980s, they have almost exclusively used pyrethroids (cypermethrin, alpha-cypermethrin, and deltamethrin), with only very limited use of the carbamate insecticide bendiocarb.[12] The spraying workers are also relatively easy to recruit because they are employed by the authorities, and due to spraying practices (indoor spraying using hand-held equipment and poor use of personal protective equipment), workers have high (peak) exposure to pyrethroids. These factors combined make them an ideal population for a study on adverse effects of pyrethroids.

As part of a larger study on health effects of pyrethroid exposure,[13] the objective of this part of the study was to investigate the possible association between pyrethroid exposure and neuromotor performance, neurocognitive performance, and CNS symptoms among vector control spray men in Bolivia in 2012.

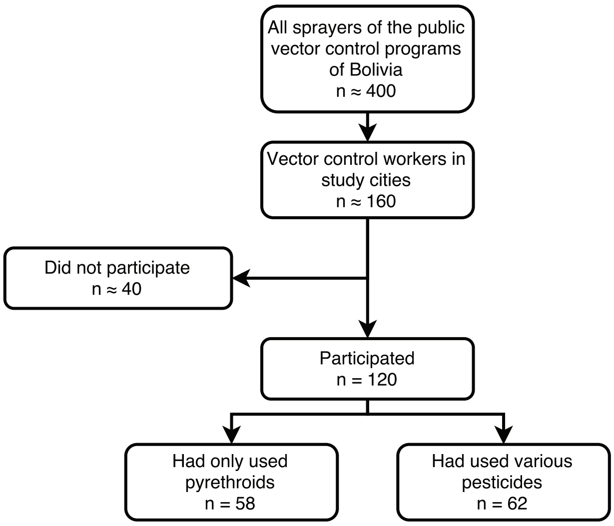

Figure 1. Participant recruitment.

Methods

Recruitment

The public vector control units in the Bolivian cities La Paz, Santa Cruz, and Cochabamba employed roughly 160 vector control workers (or former vector control workers, now working in administration), and 120 of these workers (~75%) chose to participate in the study from June to July 2012 (see Figure 1). All participants signed an informed consent form before inclusion.

Before statistical analysis, results from Behavioral Assessment and Research System (BARS) and/or CATSYS tests (see below) from 7 subjects were (partly) deleted due to poor eyesight, cough, or illiteracy (not shown in Figure 1).

Interview

A specialist in occupational medicine conducted a structured interview, including questions about exposure to pesticides, use of personal protective equipment, demographic information, consumption of alcohol/tobacco/coca leaves, and history of hospitalizations due to pesticide intoxication. The interview questions were based on questionnaires from similar investigations of pesticide health effects in developing countries,[14] adapted to the special setting of vector control.

Based on the interview information, we derived the following 3 exposure metrics: spraying duration (number of years employed as a pesticide sprayer), intensity (number of hours of spraying per week in the weeks with actual spraying), and cumulative spraying (total number of hours of pesticide exposure). To reduce the influence of outliers and to allow nonlinear statistical modeling, each of the 3 exposure metrics was divided into quintiles.

Measurement of height and weight

A standard bathroom scale was used to weigh participants, and 1.5 kg was subtracted to account for clothes. Height without shoes was measured using a medical scale in La Paz and a measuring tape fixed to a wall in Santa Cruz and Cochabamba.

Neuromotor performance

CATSYS (Danish Product Development Ltd., www.catsys.dk) is a computer-based system for quantifying human neuromotor performance, used to detect effects of environmental compounds on the CNS.[15,16] All examinations were conducted in a standardized manner[17] by the same medical doctor trained in the use of CATSYS.

Hand tremor. Hand tremor was measured for both left and right hands (recoded to dominant/nondominant hand) using an accelerometer the size and approximate shape of a ballpoint pen. Tremor intensity was defined as the root mean square of the accelerations of the tip of the accelerometer in the frequency band of 0.9 to 15.0 Hz.

Postural balance. Postural balance was assessed using a platform that recorded the position of the center of gravity of the person standing on top of it for 60 seconds. Subjects wore socks, but not shoes. Measurements were repeated 4 times—with eyes open/closed and with/without a 2-cm-thick piece of polystyrene foam under the feet. Performance was assessed by the sway area, defined as the area of the smallest polygon that completely covers the trajectory of the center of gravity during the test.

Neurocognitive performance

Boston Naming Test. The Boston Naming Test (BNT) is a test of vocabulary consisting of a number of simple drawings that have to be named by the participant.[18] We administered a shortened 12-item version of the BNT designed for and validated in a Spanish-speaking population.[19] The outcome measure was the number of correct answers.

Reaction time tests. Simple reaction time tests (RTT) determined the average time to respond to an audiovisual signal by pressing a button on a handle. The test was conducted for both left and right hands and was a part of the CATSYS system, described above.

Behavioral Assessment and Research System. The Behavioral Assessment and Research System (BARS, Northwest Education, Training and Assessment, www.nweta.com/bars) is an automated computer-based system for assessing cognition. It was developed for use in populations with limited education.[20] A Spanish version was used for this study. The tests took place in a standardized manner.[21]

Continuous performance test. A complex reaction time test, the continuous performance test (CPT) measured sustained visual attention. A total of 100 geometric shapes were displayed in succession, and a button had to be pressed only when a circle was shown. The outcome measure was average reaction time for correct key presses and standard deviation of the former.

Symbol-digit test. The average latency to correctly match numbers to abstract symbols was determined in the symbol-digit test (SDT) as a measure of complex cognitive functions. The decoding matrix was shown on-screen at the same time as each symbol. Only correct responses were used when calculating average latency.

Digit span test forward. This test measured attention and memory, expressed as the longest number span that could correctly be reproduced after being briefly displayed on-screen.

Digit span test backward. Digit span test backward (DST backward) was similar to digit span test forward (DST forward), but subjects had to type the digits in reverse of the displayed order, meaning the task was more complex.

Serial digit learning. The serial digit learning test (SDL) was an overflow version of the DST forward of attention and memory. A 9-digit number was displayed briefly and had to be reproduced. All participants were shown the same number twice. The number of correct digits from both rounds was recorded. In single-level regression models (see below), a value of 1 was added to each result to allow for logistic transformation as a few persons did not have any correct digits.

Matching to sample. A pseudo-random matrix of black and white blocks was displayed for a few seconds. Then, 3 similar matrices were shown, and as a test of visual memory, participants had to select the matrix that was identical to the original. In total, 40 rounds of matching to sample (MTS) were completed.

Subjective CNS symptoms

Subjects were asked specifically whether they had experienced 62 subjective symptoms during the last year. The list was based on symptoms previously reported to be associated with pyrethroid exposure.[7,22] In this article, only the 15 symptoms primarily related to the CNS were included—blurred vision, headache, hyperactivity/restlessness/irritability, dizziness, confusion, lethargy, slurred speech, difficulty concentrating, difficulty remembering, disturbed sleep, unconsciousness, lack of coordination, trembling hands, trembling body, and cramps. A total score was calculated equal to the number of CNS symptoms reported.

Statistical analysis

Data from interviews and BNT were double entered using EpiData 3.1 (www.epidata.dk, Denmark). Data clean-up was performed with Stata 12.1 (StataCorp, College Station, TX, USA). Single-level regression analyses were conducted using Stata and multilevel regression analyses using *Mplus* 7.4 (Muthén & Muthén, Los Angeles, CA, USA).

Correlations between exposure metrics

The correlation between the 3 exposure metrics was weak to moderate with $R^2 = 0.06$ ($P = .0054$) for duration vs intensity, $R^2 = 0.44$ ($P < .0001$) for duration vs cumulative spraying, and $R^2 = 0.28$ ($P < .0001$) for intensity vs cumulative spraying.

Neuromotor performance

Hand tremor and postural balance data were analyzed using both crude and adjusted single-level regression models for each of the exposure metrics (duration, intensity, and cumulative spraying). Analyses were conducted with quintiles of the exposure metric as a categorical variable and a continuous variable. A priori–selected confounders included in the adjusted analyses were age, educational level, and location.

Initial attempts to analyze data using linear regression were unsuccessful as residuals (defined as the difference between observed and predicted values) were not normally distributed, and this issue could not be solved by transformation. Data were therefore dichotomized using the median as the cutting point and analyzed using logistic regression.

We excluded participants who at some point had been hospitalized due to acute pesticide poisoning (n = 4). Participants with known neurologic illness or use of psychotropic drugs were also excluded (n = 9). Analyses were conducted for the remaining 107 persons and in the subgroup of 52 participants who had only been exposed to pyrethroids and not to other types of pesticides.

A number of sensitivity analyses were performed. First, the 13 workers who had otherwise been excluded were reentered. Second, analysis was restricted to the subgroup of persons who had not sprayed for the last month. Third, we included an extended number of confounders—average alcohol consumption per week for the past 5 years, number of months working with other potentially neurotoxic substances, use of stimulants on the day of testing, and ratio between body weight and height (for postural balance data only).

Neurocognitive performance

Neurocognitive performance was analyzed in multilevel regression models. The first level consisted of a confirmatory factor analysis, in which an estimate of a latent variable for general mental ability (Spearman *g*) was calculated from the individual neurocognitive test results. The tests covered a broad variety of cognitive domains—speed and stability in simple reactions and choice reactions, processing speed of graphical and numerical stimuli, capacity in working memory and learning, performance in visuospatial perception and memory, verbal understanding, and general knowledge.[23–34] Estimation was done by the full information maximum likelihood method, making the analysis less sensitive to sporadic missing values. The statistical fit of the model was good, and the latent variable (*g*) explained 25% of

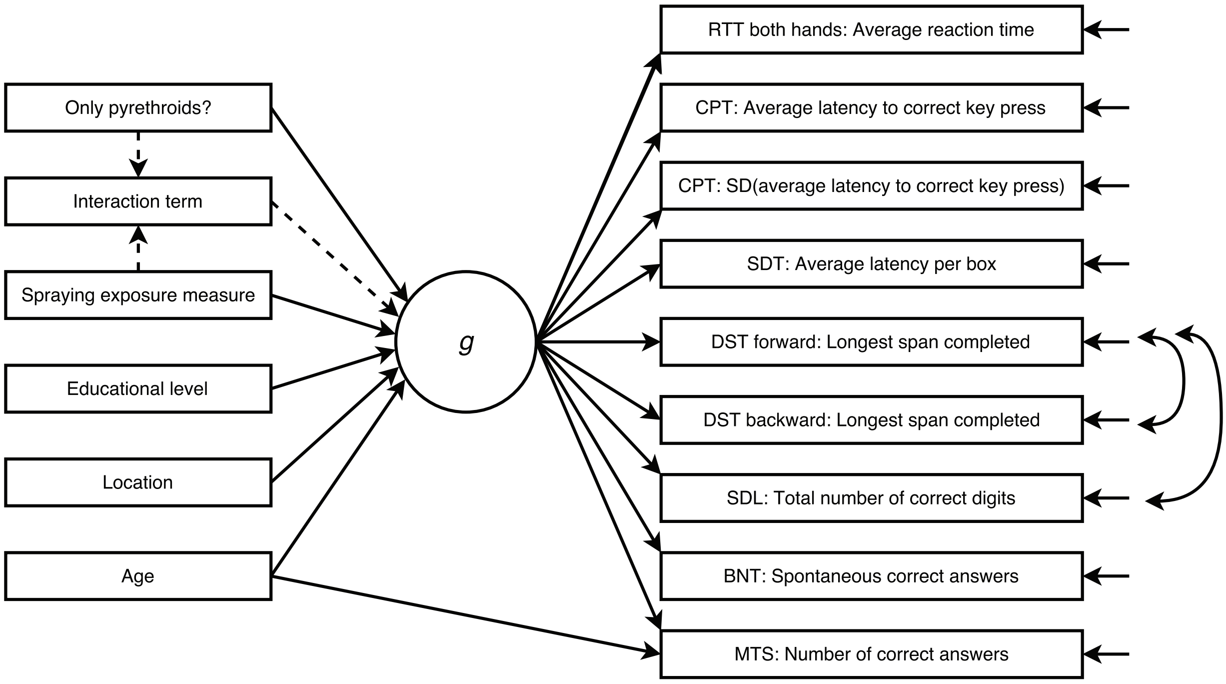

Figure 2. Adjusted structural equation model of neurocognitive performance. BNT indicates Boston Naming Test; CPT, continuous performance test; DST backward, digit span test backward; DST forward, digit span test forward; MTS, matching to sample; RTT, reaction time tests; SDL, serial digit learning; SDT, symbol-digit test.
Age was allowed to influence not only *g* but also MTS as this improved the model fit. All covariates were intercorrelated, but for ease of viewing, this is not shown in the figure.

the total variance (see Figure S1, Online Appendix). The second level of the analyses consisted of structural equation models (SEMs) in which predictor variables and sets of covariates influenced the latent variable (*g*) instead of results from each single neurocognitive test results. Both crude and adjusted analyses were conducted with each of the exposure metrics (duration, intensity, and cumulative spraying) as the relevant exposure measure. As with neuromotor analyses, confounders included in the adjusted models were age, educational level, and location.

We did not have power to reliably build the SEMs if any of the 120 participants were excluded. Therefore, we took other pesticides than pyrethroids into account by including a dichotomous variable (0 for ever-users of other pesticides than pyrethroids and 1 for never-users). A graphical representation of the adjusted SEMs can be seen in Figure 2.

Sensitivity analyses were conducted with an extended number of confounders—the same as for neuromotor sensitivity analyses, plus a single dichotomous variable used to adjust for having been hospitalized, having known neurological illness, or using psychotropic drugs.

Furthermore, all neurocognitive data were analyzed in single-level models, using either linear regression (if normality of residuals could be confirmed) or dichotomization followed by logistic regression.

Subjective CNS symptoms

Analyzing every subjective CNS symptom on its own would have led to a high risk of mass significance (ie, finding a few P values $\leqslant .05$ simply because a large number of analyzes were conducted, even if no real associations existed). Therefore, only the symptom score (ie, the total number of symptoms reported by each participant) was analyzed statistically. Due to non-normally distributed residuals in linear regression models, the symptom score was dichotomized (using the median as the cutting point) to allow logistic regression. The dichotomous variable was analyzed in the same manner as the neuromotor data.

Results

Demographics

Demographic information on the participants is shown in Table 1. Subjects who had only used pyrethroids were younger, with higher level of instruction, and had sprayed less than subjects who had also used other classes of pesticides.

Spraying practices and knowledge about pesticides

Even though all participants were employed in public health vector control programs, their safety practices during spraying were suboptimal (see Table S1, Online Appendix). Although most participants always or sometimes used a form of mask when handling pesticides, only 14% used rubber gloves and 80% used leather gloves. Around 44% of participants always or sometimes ate or drank during spraying, and 48% blew on the nozzle of the pesticide pump (using their mouths) if it got obstructed. Even though most participants washed their hands and their bodies and changed clothes after spraying, a substantial proportion only did so sometimes.

Table 1. Demographic information.

	ONLY PYRETHROIDS	VARIOUS PESTICIDES	*P*
n	58	62	—
Age, y[a]	43.9 [34.8–52.2]	54.5 [47.7–58.3]	<.0001
Height, cm[a]	168 [164–171]	168 [163–171]	.78
Weight, kg[a]	76.5 [68.5–81.5]	77.0 [70.0–86.5]	.27
Spraying duration, y[a]	6.0 [3.0–10.0]	18.6 [10.0–27.0]	<.0001
Spraying intensity, h[a]	35.0 [21.0–42.0]	42.0 [28.0–56.0]	.01
Cumulative spraying (10 000 of hours)[a]	0.3 [0.1–0.8]	1.5 [0.5–2.7]	<.0001
Location, No. (%)[b]			.38
La Paz	16 (27.6)	17 (27.4)	
Santa Cruz	24 (41.4)	32 (51.6)	
Cochabamba	18 (31.0)	13 (21.0)	
Education, No. (%)[b]			.11
Less than primary school	2 (3.5)	4 (6.5)	
Primary school	20 (34.5)	33 (53.2)	
Secondary school or technical education	34 (58.6)	23 (37.1)	
University	4 (3.5)	2 (3.2)	

[a]Numbers are median [interquartile range]. *P* values derived from the Wilcoxon rank sum test.
[b]*P* values derived from Fischer exact test.

Participants were not optimally trained on the handling of pesticides. Only 18% of participants had taken a course within the last year, 56% had taken a course longer ago, and 26% had never done so.

Neuromotor performance

Main results with quintiles of exposure entered as a continuous variable are presented in Table 2. Results from analyses using quintiles of exposure as a categorical variable and results from sensitivity analyses can be seen in Tables S2, S7 to S9 (Online Appendix), respectively.

No consistent associations were found between pesticide exposure and postural balance, whereas a tendency was noted for higher exposed individuals to have less hand tremor. The few statistically significant results may be due to mass significance (5% of all analyses are expected to be statistically significant, even in the absence of any real associations).

Neurocognitive performance

Multilevel regression models. Table 3 contains main results from the SEM analyses of neurocognitive performance where quintiles of exposure were entered as continuous variables. Remaining results are shown in Table S3 (Online Appendix).

A clear pattern appeared in the SEMs. Spraying intensity was consistently and, in many cases, statistically significantly associated with poor neurocognitive performance (expressed by lower *g*). A few analyses also showed a negative association with cumulative exposure, but much less consistently.

Interestingly, only having used pyrethroids was associated with lower *g* in almost all adjusted analyses (shown by $\beta < 0$), but not in analyses where only having used pyrethroids was the only independent variable (labeled "without covariates" in Table 3).

Single-level regression models. Results from the main single-level regression analyses of neurocognitive performance can be seen in Tables S4 and S5 (Online Appendix), whereas results from sensitivity analyses are found in Tables S7 to S9.

Symbol-digit test results showed a slight tendency toward poor performance (longer average response time) among higher exposed individuals, but effect sizes were small and generally nonsignificant. The only significant trend was for spraying duration among sprayers who had only used pyrethroids.

All analyses showed trends toward poor performance (only being able to recall a short number span) on DST forward test with increasing exposure levels. Among all sprayers, high spraying intensity was significantly associated with poor performance (adjusted odds ratio (OR): 1.87 (1.22-2.88) per quintile. Results were similar, but not statistically significant, among

Table 2. Results from analyses of neuromotor performance.

NEUROMOTOR TEST			TYPES OF PESTICIDES USED	SPRAYING DURATION		SPRAYING INTENSITY		CUMULATIVE EXPOSURE	
				RAW ANALYSES	ADJUSTED ANALYSES	RAW ANALYSES	ADJUSTED ANALYSES	RAW ANALYSES	ADJUSTED ANALYSES
				OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Hand tremor	Dominant		Any	1.00 (0.76–1.31)	0.87 (0.61–1.25)	0.91 (0.66–1.25)	0.89 (0.62–1.28)	0.97 (0.74–1.27)	0.91 (0.65–1.28)
			Only pyrethroids	0.69 (0.42–1.13)	0.66 (0.35–1.27)	0.95 (0.60–1.49)	1.21 (0.68–2.14)	0.59 (0.34–1.02)	0.64 (0.32–1.29)
	Nondominant		Any	0.97 (0.73–1.29)	1.01 (0.70–1.46)	0.87 (0.63–1.22)	0.86 (0.60–1.23)	0.91 (0.69–1.21)	0.92 (0.65–1.29)
			Only pyrethroids	0.61 (0.35–1.07)	0.62 (0.28–1.35)	0.74 (0.45–1.21)	0.84 (0.45–1.57)	0.44 (0.23–0.86)	0.45 (0.18–1.13)
Postural balance	No foam	Open eyes	Any	1.10 (0.82–1.46)	0.94 (0.63–1.40)	0.87 (0.63–1.22)	0.86 (0.57–1.29)	1.17 (0.88–1.55)	1.24 (0.86–1.79)
			Only pyrethroids	1.03 (0.63–1.69)	1.11 (0.56–2.18)	0.78 (0.47–1.28)	0.96 (0.49–1.89)	1.08 (0.63–1.83)	2.20 (0.96–5.01)
		Closed eyes	Any	1.32 (0.98–1.78)	1.03 (0.69–1.54)	1.03 (0.74–1.44)	0.94 (0.62–1.42)	1.21 (0.90–1.63)	1.01 (0.69–1.49)
			Only pyrethroids	1.21 (0.73–2.00)	1.01 (0.50–2.04)	0.97 (0.59–1.60)	1.04 (0.52–2.10)	1.10 (0.64–1.89)	1.48 (0.67–3.27)
	Foam	Open eyes	Any	1.01 (0.75–1.35)	0.72 (0.45–1.15)	1.00 (0.71–1.41)	0.92 (0.59–1.44)	0.95 (0.71–1.28)	0.71 (0.45–1.11)
			Only pyrethroids	0.70 (0.41–1.19)	0.53 (0.23–1.23)	1.21 (0.73–2.00)	3.83 (1.08–13.57)	0.69 (0.39–1.21)	0.92 (0.40–2.12)
		Closed eyes	Any	1.16 (0.86–1.55)	1.14 (0.76–1.71)	1.00 (0.71–1.41)	0.83 (0.55–1.25)	1.29 (0.95–1.75)	1.23 (0.84–1.80)
			Only pyrethroids	1.28 (0.77–2.13)	1.25 (0.58–2.67)	0.99 (0.60–1.63)	0.52 (0.24–1.14)	1.49 (0.85–2.61)	1.17 (0.56–2.45)

Abbreviations: CI, confidence interval; OR, odds ratio.
Quintiles of pesticide exposure are seen as continuous variables.
OR = ratio of performing worse than the median per 1 increase in exposure quintile. Grayed out results are statistically significant.
Analyses labeled "Any" include participants who had only used pyrethroids, as well as participants who had used various pesticides.

Table 3. Results from structural equation model analyses of neurocognitive performance.

				β (95% CI)
Crude analyses	No interaction	Spraying duration	Quintiles	–0.120 (–0.340 to 0.100)
		Spraying intensity	Quintiles	–0.242 (–0.448 to –0.036)
		Cumulative exposure	Quintiles	–0.124 (–0.338 to 0.090)
		Only pyrethroids?	Only pyrethroids?	–0.262 (–0.683 to 0.159)
	With interaction	Spraying duration	Quintiles	–0.241 (–0.559 to 0.077)
			Only pyrethroids?	–0.507 (–1.585 to 0.571)
			Interaction	0.016 (–0.454 to 0.486)
		Spraying intensity	Interaction	0.214 (–0.117 to 0.545)
			Only pyrethroids?	–0.955 (–1.935 to 0.025)
			Quintiles	–0.404 (–0.682 to –0.126)
		Cumulative exposure	Quintiles	–0.333 (–0.639 to –0.027)
			Only pyrethroids?	–0.878 (–1.944 to 0.188)
			Interaction	0.121 (–0.228 to 0.470)
Adjusted analyses	No interaction	Spraying duration	Quintiles	0.008 (–0.227 to 0.243)
		Spraying intensity	Quintiles	–0.204 (–0.400 to –0.008)
		Cumulative exposure	Quintiles	–0.033 (–0.253 to 0.187)
		Only pyrethroids?	Only pyrethroids?	–0.578 (–0.960 to –0.196)
	With interaction	Spraying duration	Quintiles	–0.229 (–0.529 to 0.071)
			Only pyrethroids?	–1.189 (–2.187 to –0.191)
			Interaction	0.184 (–0.135 to 0.503)
		Spraying intensity	Quintiles	–0.405 (–0.660 to –0.150)
			Only pyrethroids?	–1.344 (–2.224 to –0.464)
			Interaction	0.255 (–0.051 to 0.561)
		Cumulative exposure	Quintiles	–0.348 (–0.634 to –0.062)
			Only pyrethroids?	–1.372 (–2.319 to –0.425)
			Interaction	0.211 (–0.107 to 0.529)

Abbreviations: CI, confidence interval; OR, odds ratio.
Quintiles of pesticide exposure are seen as continuous variables.
"Quintiles" are quintiles of the pesticide exposure measure. "Only pyrethroids" is a dichotomous variable with a value 1 for workers who had only used pyrethroids and 0 for workers who had also used various other pesticides. "Interaction" means the interaction of only having used pyrethroids on the association between pesticide exposure and *g*. Adjusted analyses only include the variables shown in Figure 2. Grayed out results are statistically significant.

sprayers who had only used pyrethroids. Similar (but nonsignificant) trends toward poor performance with higher exposure were also noted in the related DST backward test.

All pesticide exposure metrics were associated with lower performance (only being able to recall a short number span) on SDL, and similar to DST, the clearest association was seen for spraying intensity among all sprayers—relative number of correct digits per quintile: 0.86 (0.77-0.97).

Continuous performance test results showed nonsignificantly lower performance among higher exposed individuals for latency to correct key press, whereas no clear trends were seen for standard deviation (latency to key press) (Table S4).

No consistent associations were found between pesticide exposure and performance in RTT, BNT, and matching to sample (MTS).

Subjective CNS symptoms

Table 4 shows that the most common symptoms reported (in order of decreasing prevalence) were headache, dizziness,

Table 4. Symptom prevalence.

	ONLY PYRETHROIDS	VARIOUS PESTICIDES
n	55 (100)	52 (100)
Blurred vision	12 (22)	9 (17)
Headache	31 (56)	26 (50)
Hyperactivity/restlessness/irritability	8 (15)	12 (23)
Dizziness	22 (40)	17 (33)
Confusion	2 (4)	1 (2)
Lethargy	0 (0)	1 (2)
Slurred speech	2 (4)	6 (12)
Difficulty concentrating	5 (9)	8 (15)
Difficulty remembering	9 (16)	10 (19)
Disturbed sleep	2 (4)	4 (8)
Unconsciousness	0 (0)	0 (0)
Lack of coordination	0 (0)	0 (0)
Trembling hands	11 (20)	12 (23)
Trembling body	6 (11)	7 (13)
Cramps	6 (11)	14 (27)
Total number of CNS symptoms[a]	2 [1–3]	1.5 [0–4]

Abbreviation: CNS, central nervous system.
[a]Values are median [interquartile range]. All other values are counts (%).

Table 5. Result from analyses of subjective CNS symptoms.

OUTCOME	TYPES OF PESTICIDES USED	RAW ANALYSES	ADJUSTED ANALYSES
		OR (95% CI)	OR (95% CI)
Spraying duration	Any	1.12 (0.85–1.48)	1.59 (0.97–2.60)
	Only pyrethroids	1.66 (0.98–2.81)	1.98 (0.91–4.35)
Spraying intensity	Any	1.35 (0.97–1.88)	1.92 (1.13–3.25)
	Only pyrethroids	1.51 (0.91–2.51)	1.47 (0.74–2.91)
Cumulative exposure	Any	1.28 (0.96–1.71)	2.01 (1.22–3.31)
	Only pyrethroids	2.16 (1.18–3.94)	2.24 (0.99–5.06)

Abbreviations: CI, confidence interval; CNS, central nervous system; OR, odds ratio.
Quintiles of pesticide exposure are seen as continuous variables.
OR = odds ratio of reporting more symptoms than the median per 1 increase in exposure quintile. Grayed out results are significant.
Analyses labeled "Any" include participants who had only used pyrethroids, as well as participants who had used various pesticides.

trembling hands, blurred vision, hyperactivity/restlessness/irritability, cramps, and difficulty remembering.

Consistent relationships were found between all pesticide exposure metrics and OR of reporting a higher than median number of CNS symptoms, in both the raw and adjusted analyses (Table 5 and Table S6 [Online Appendix]). For example, in the adjusted analyses including all sprayers, the OR per quintile was 1.59 (0.97-2.60) for spraying duration, 1.92 (1.13-3.25) for spraying intensity, and 2.01 (1.22-3.31) for cumulative exposure. Estimates were similar in analyses limited to sprayers who had only used pyrethroids (Table 5 and Table S6 [Online Appendix]) but did not reach statistical significance. The results in the sensitivity analyses were similar to the results in the main analyses (Table S7-S9, Online Supplement).

Discussion

In our analyses, high-level pyrethroid exposure compared with low-level pyrethroid exposure was associated with reduced neurocognitive performance and with reporting more CNS symptoms, whereas no association was seen for neuromotor performance.

Our findings on neurocognitive performance are supported by a recent review that concluded pesticide exposure, in general, may be linked to poor neurocognitive performance and dementia.[4] However, we have not been able to locate any previous studies specifically about neurocognitive dysfunction after pyrethroid exposure. Investigators have instead used broad categories such as exposure to pesticides, exposure to insecticides, proxies such as farm work and rural residence, and questions about other classes of pesticides. Caution is therefore warranted when comparing our results with previous studies.

The lack of an effect on neuromotor performance in this study is inconsistent with previous findings. Recent meta-analyses have shown that pesticide exposure statistically significantly increases the risk of PD[1–3] and ALS,[5,6] and a few studies have looked specifically at pyrethroids. An American multicenter case-control study showed a significant association between exposure to pesticides and PD (adjusted OR: 1.9 [1.1-3.2]), whereas the association was nonsignificant for exposure specifically to the pyrethroid permethrin (adjusted OR: 3.2 [0.7-15.8]).[35] The Agricultural Health Study (AHS) is a population-based study among American professional pesticide applicators and their spouses. In the AHS, a significant positive exposure-response relationship was seen between incident (but not prevalent) PD and cumulative days of pesticide exposure.[36] The main AHS results did not show any association between PD and permethrin,[36] but in a nested case-control study based on the AHS, permethrin exposure was nonsignificantly associated with PD (adjusted OR: 1.5 [0.8-3.0]), and an exposure-response relationship was suggested by the fact that glove use significantly modified the association (adjusted OR: 4.7 [1.5-14.6] for ever-users of permethrin who used gloves $\leqslant$50% of the time, adjusted OR: 0.8 [0.3-2.3] for ever-users who used gloves >50% of the time, P for interaction = .02).[37] In the AHS, exposure to pyrethroids was also nonsignificantly associated with ALS (adjusted OR: 1.4 [0.6-3.4]).[6] A population-based case-control study from France showed a statistically significant exposure-response relationship between occupational pesticide exposure and OR for PD. It also showed a nonsignificant association between occupational exposure specifically to pyrethroids among men (adjusted OR: 1.8 [0.6-5.1] for men >65 years), but without an exposure-response relationship.[38] It should be noted that the population in the latter study had much lower exposure levels to pyrethroids than our population (median cumulative number of hours of professional pyrethroid exposure was 72 for cases and 60 for controls).[38]

In light of previous results, one should not take the effect of pyrethroid exposure on postural stability and hand tremor in this study as evidence that pyrethroids are associated with reduced neurocognitive performance only and not with impaired neuromotor performance. In this cross-sectional study, we would expect a healthy worker effect, where susceptible workers have left the spraying work, leaving the more resistant (and therefore, healthier) workers as our study population. But because of the manual character of spraying, minor intellectual impairment is probably less likely to lead to a sprayer quitting his job than movement disorder symptoms are. Furthermore, we may not have had sufficient statistical power due to a relatively small study population.

Only about 75% of the sprayers employed at the time of data collection chose to participate in the study, possibly leading to selection bias. If nonparticipants were both healthier and more heavily exposed than participants, it would lead to bias away from the null hypothesis, possibly explaining the effects demonstrated on CNS symptoms and neurocognitive performance. However, although healthy and highly exposed workers could theoretically have declined participation because of a lack of personal interest in the study, participants were financially compensated for their time, and we believe this was a sufficient incentive for participation in itself. On the contrary, we know of at least 2 workers who declined participation because they had health problems associated with pesticide exposure, and they feared losing their jobs if performing poorly on the study tests. It is therefore also possible that nonparticipants were highly exposed and in poor health condition, but that would lead to bias toward the null hypothesis and cannot explain the associations seen.

Results from sensitivity analyses limited to persons who had not sprayed within the last month (only conducted for the single-level regression models) were similar to results for the study population as a whole, suggesting that the effect of pyrethroids and other pesticides on CNS function and symptoms is chronic rather than acute.

Because we are studying long-term neuromotor/neurocognitive performance, we would expect poor outcomes to have stronger correlations with cumulative spraying than with spraying intensity or duration. But in the SEMs, the strongest correlations were seen for spraying intensity, and in the single-level regression models, there was no clear pattern. One could speculate that this was because the exposure metrics (duration, intensity, and cumulative spraying) were highly correlated, but our analyses showed that correlations between them were actually weak to moderate.

In the single-level regression models, the same trends were seen for sprayers who had only used pyrethroids as for sprayers who had also been exposed to other classes of pesticides. Moreover, in the SEMs, only having used pyrethroids had a significant negative influence on g in almost all analyses—except in the analyses where only having used pyrethroids was the only independent variable. The latter finding may be due to lower overall exposure in the group of sprayers who had only used pyrethroids (see Table 1). In short, our analyses suggest that on the long term, pyrethroids may not be safer than other classes of pesticides.

Although pyrethroid pesticides are used all over the world, this project was conducted in Bolivia because of 2 important characteristics of the population of sprayers employed in the public vector control programs: In recent years, they have almost exclusively been exposed to pyrethroids and not other pesticides.[12] Furthermore, they have high peak exposure levels due to spraying taking place indoors and poor use of personal protective equipment. Associations demonstrated in this high-exposure population may not be clinically relevant in low-exposed populations (e.g., passengers seated in aircraft during "disinfecting" with pyrethroids), and results cannot immediately be extrapolated to these groups.

Vector-borne diseases, such as malaria and dengue fever, affect millions of people each year,[39,40] and spraying with pyrethroids is considered a cornerstone in the fight against them.[11] Because the acute toxicity of other classes of pesticides is typically much higher than that of pyrethroids,[9] it is not a viable strategy to substitute pyrethroids with other pesticides out of fear of long-term toxicity, and exposure must be minimized by other means. First, only the necessary amount of pesticides should be used. Second, workers should be educated on the proper handling of pesticides—blowing or sucking the nozzle of the spraying equipment when obstructed has been shown to severely increase the risk of acute pesticide intoxication (OR: 4.0 [1.7-9.5]),[14] yet almost half of our population did this. Finally, proper personal protective equipment should be used—as previously mentioned, a case-control study among American pesticide applicators showed that the use of gloves significantly weakened the association between exposure to the pyrethroid permethrin and PD.[37]

There is a clear need for more robust research on possible chronic health effects of pyrethroid exposure. If our findings can be replicated in a follow-up study, it would underline the importance of exposure minimization. Rejection would be equally important, as irrational fears of chronic health damage should not hinder the use of an important weapon against vector-borne diseases.

Conclusions

Long-term exposure to pyrethroids may adversely affect the CNS, manifested by a number of subjective symptoms and deterioration of neurocognitive performance. No evidence was found of an association between pyrethroid exposure and poor neuromotor performance. Our results demonstrate a need for pyrethroid exposure control by efficient spraying and adequate use of personal protective equipment.

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Author Contributions

MRHH, EJ, FL, FD, and VS conceived and designed the experiments and jointly developed the structure and arguments for the paper. MRHH and FD analyzed the data and contributed to the writing of the manuscript. MRHH wrote the first draft of the manuscript. MRHH, EJ, FL, GC, FD, NTB, and VS agree with manuscript results and conclusions and made critical revisions and approved the final version. All authors reviewed and approved the final manuscript.

Data Sharing

Study data are available for any researcher from the Danish Data Archive, archival number DDA-26874 (http://dda.dk/catalogue/26874).

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