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High pesticide exposure events and central nervous system function among pesticide applicators in the Agricultural Health Study

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Abstract

Purpose—While acute pesticide poisoning can be associated with persistent adverse central nervous system (CNS) effects, little is known about the effect of episodic and unusually high pesticide exposure events (HPEEs) that typically do not result in acute poisoning. The objective of this investigation was to examine the association between HPEEs and CNS function among licensed pesticide applicators enrolled in the Agricultural Health Study (AHS).

Methods—In 2006–2008, 693 male participants, with no history of a physician-diagnosed pesticide poisoning, completed nine neurobehavioral tests to assess memory, motor speed, sustained attention, verbal learning, and visual scanning and processing. Information on HPEEs and pesticide poisonings was obtained from previous AHS interviews. Associations between HPEEs and neurobehavioral outcomes were estimated with linear regression controlling for age and outcome-specific covariates.

Results—A history of at least one HPEE was reported by 156 (23%) participants. Adverse associations were observed between HPEEs and two of the nine neurobehavioral tests. On a test of visual scanning and processing (*Digit-Symbol*), participants with HPEEs were 4.2 seconds slower (95% CI: −7.27, −1.11) than those without HPEEs, equivalent to the effect of 3.9 years of age in this population. On a test of visual scanning and motor speed (*Sequences A*), participants with HPEEs were 2.5 seconds slower (95% CI: −4.53, −0.41) than those without HPEEs, equivalent to the effect of 3.9 years of age. No significant associations were observed between HPEEs and the other neurobehavioral tests.

Conclusions—HPEEs may contribute to adverse CNS outcomes independent of diagnosed pesticide poisoning.

Keywords

agricultural workers; epidemiology; neurobehavioral testing; pesticide exposure

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Conflict of interest The authors declare that they have no conflict of interest.

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Introduction

More than 18,000 pesticide products are licensed for use in the United States (EPA 2003). Internationally, approximately 5.2 billion pounds of active pesticide ingredient were used in 2007 of which approximately 1.1 billion pounds were used in the United States (EPA 2011a). Insecticidal pesticides accounted for 17% of all active ingredient applied worldwide and 8% in the United States (EPA 2011a). Internationally, the number of persons with regular exposure to pesticides is not readily available, although it is likely in the millions. In the United States, approximately 538,000 private certified pesticide applicators and 399,000 commercial certified pesticide applicators were registered in 2007 (EPA 2011a).

Pesticides are designed to selectively prevent, destroy, repel, or mitigate target organisms (EPA 2011a). However, many pesticides are poorly selective and toxic to non-target species, including humans. Furthermore, prior to application, active pesticides are often mixed with "inert" ingredients that may also have human toxicity (e.g., organic solvents). Consequently, pesticide applicators are ultimately exposed to a complex mixture of substances with a range of toxicity.

The human toxicological potency of pesticides varies widely. With sufficient dose, many have been reported to result in acute neurological toxicity (Kamel and Hoppin, 2004). Specifically, acute neurotoxicity has been described following exposure to organophosphate (OP), carbamate, and organochlorine insecticides, as well as following exposure to some herbicides (e.g., 2,4-D and paraquat), fungicides (e.g., methylmercury), and fumigants (e.g., methyl bromide) (Costa et al. 2008; Keifer and Firestone 2007).

The neurotoxicity of OP insecticides have been studied in greater detail than that of other pesticides and the acute neurological effects of OP exposure sufficient to cause overt clinical toxicity (*i.e.*, acute OP pesticide poisoning) are especially well described (Marrs 1993). Mild acute effects include headache, dizziness, nausea, vomiting and diarrhea whereas more severe acute toxicity includes cardiac rhythm disturbances, seizures, respiratory failure and coma (Bardin et al. 1994). Acute OP pesticide poisoning has been associated with long-term neurological sequelae, including an increase in neurological symptoms and deficits in neurobehavioral test performance (Steenland et al. 1994; London et al. 1998; Rosenstock et al. 1991; Stallones and Beseler 2002; Wesseling et al. 2002). While some consensus exists regarding the short- and long-term effects of overt OP pesticide poisoning, associations between persistent neurological impairment and OP pesticides exposure at levels insufficient to cause clinical toxicity are inconsistent (Costa 2006; Ray and Richards 2001). The long-term neurotoxicity of exposure to non-OP pesticides is even less well characterized, regardless of exposure dose or timing.

Exposure to pesticides can be minimized with use of appropriate application equipment, work practices, and personal protective equipment. However, equipment malfunction or poor work practices during mixing, loading or applying pesticides or during the repair or maintenance of application equipment can result in short-term but high-level exposure either with or without clinical evidence of acute toxicity. Such events are common. For example, at the time of enrollment in the Agricultural Health Study (AHS), a large prospective study of pesticide applicators, 14% of pesticide applicators reported having at least one *high pesticide exposure event* (HPEE) (defined as "an incident or experience while using any pesticide which caused an unusually high personal exposure") during their working lifetime (Alavanja et al. 1999). The majority of HPEEs did not result in a pesticide-associated health care visit nor the reporting of neurological symptoms (Bell et al. 2006).

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Because they represent a transient, but potentially substantial, increase in exposure, HPEEs may be toxicologically important. However, little is known about the neurotoxicity of HPEEs that do not result in clinically overt pesticide poisoning. To address this question, we examined associations between HPEEs and measures of central nervous system (CNS) function among private pesticide applicators enrolled in the AHS.

Methods

We studied the association between neurobehavioral function and HPEEs among participants enrolled in the AHS, a large prospective study of licensed pesticide applicators from Iowa and North Carolina (Alavanja et al. 1996). Between 1993 and 1997, 52,394 private applicators enrolled in the AHS by completing a self-administered enrollment questionnaire at the time of pesticide licensing and recertification. A self-administered "take-home" questionnaire was completed within one month of enrollment. These two questionnaires comprised Phase 1 data collection. Two, five-year follow-up phone interviews were administered to AHS participants (Phases 2 and 3 data collection). The Phase 3 interview was administered no more than one year prior to participation in the present study. The three phases collected information on demographic characteristics, pesticide exposure, pesticide application methods, use of personal protective equipment, occupational exposure to other toxicants, and other activities that may influence exposure or disease risk as well as demographic and lifestyle information. Copies of AHS questionnaires are available online (AHS 2010).

Study participants

Private pesticide applicators were invited to participate in the present study if they had completed all AHS interviews (Phases 1–3), resided in Iowa or North Carolina, and lived within approximately 150 miles of the testing facilities. AHS participants were not invited if they had previously reported stroke, amyotrophic lateral sclerosis, multiple sclerosis, Parkinson's disease, retinal or macular degeneration, hypothyroidism or diabetes, as these outcomes may influence neurobehavioral testing results. In addition, participants who, during the Phase 3 interview, reported drinking more than 41 alcoholic beverages per week were also excluded. To study a population who were using pesticides agriculturally, the sample was limited to participants who were farming at the time of AHS enrollment. We also excluded women because they represented fewer than 1% of licensed pesticide applicators in the AHS cohort. A total of 1,807 AHS participants were initially eligible to participate in the present study after eligibility criteria were applied.

Because this sample was recruited for a study of associations between multiple pesticide use metrics and neurological outcomes, we enriched the sample for applicators with higher lifetime use of OP pesticides based on reported OP use in Phase 1. Specifically, a stratified random sample was selected from among eligible AHS participants with equal sampling from the upper quartile versus the lower 75% of the distribution of lifetime OP pesticide use. Thus the sampling frame allowed us to recruit a sample enriched for OP use, but was not used as an analytical variable and therefore did not bias the observed associations. In Iowa, testing was conducted in Iowa City and Dubuque between November 2006 and March 2007. In North Carolina, testing was conducted in Greenville and Wilmington between January and March 2008.

Among 1,807 eligible participants, 759 (42%) agreed to participate and were scheduled for neurobehavioral testing. Of these, 58 participants either cancelled or failed to attend their scheduled appointment. In total, neurobehavioral testing was administered to 701 participants for an overall response rate of 39 percent. Of the 701 participants, analyses were

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restricted to the 693 who did not report a past physician diagnosed pesticide poisoning event.

Participants were reimbursed for time and travel expenses to and from the testing facility. The study was approved by all relevant Institutional Review Boards and all participants provided written informed consent for the present study.

High pesticide exposure assessment

Information on HPEEs was obtained from AHS questionnaires and interviews; each of the three data collections asked a slightly different question:

> Phase 1. Have you ever had an incident or experience while using any type of pesticide which caused you unusually higher personal exposure?
>
> Phase 2. Since (year of enrollment) did you have any incidents with fertilizers, herbicides, or other pesticides that caused you an unusually high personal exposure?
>
> Phase 3. Since (date of last interview) have you had any incidents or spills that resulted in an unusually high exposure to pesticides from contact with your skin, from breathing fumes or dust, or from accidental ingestion?

Because we were interested in the effect of having at least one HPEE in an applicator's lifetime, participants who reported "yes" to at least one of these questions during an AHS interview were classified as having a HPEE. Information on HPEEs was obtained prior to enrollment in the present study. Individuals who reported an HPEE were asked to provide information regarding the pesticide or chemical involved in the most recent HPEE at each interview.

Neurobehavioral testing

Neurobehavioral testing was administered in private rooms by trained technicians unaware of participants' exposure status. Eight computerized tests from the Neurobehavioral Evaluation System, Version 3 (NES3), were administered and are described in detail in the NES3 User's Manual (Letz et al. 2000; Letz et al. 1996; Letz 2000). In addition, the manually-administered Grooved Pegboard test was used. These tests have been used extensively in investigations of neurotoxicants and are considered to be sensitive indicators of a wide range of CNS functions; the tests are described below.

Continuous Performance Test—The Continuous Performance Test was used to assess sustained attention. The participant was instructed to press the space bar on a computer keyboard (Dell, Model SK-8135, Round Rock, TX, USA) as quickly as possible when the letter "S" appeared on screen, but not when any other letter appeared. A new letter appeared every second for 300 seconds. The summary measure was the mean reaction time in milliseconds for responding to the letter "S". A higher score indicated poorer test performance.

Digit-Symbol Test—The Digit-Symbol test is a modification of a commonly used test from the Wechsler Adult Intelligence Scale (Wechsler 1981). It measured visual scanning and information-processing speed. The test consisted of nine digit-symbol pairs displayed across the top of a touch-screen computer monitor (Elo Touchsystems, Menlo Park, CA, USA) and a row of nine symbols displayed at the bottom of the screen. A random integer (1 to 9) appeared in the middle of the screen. The participant's task was to touch the symbol at the bottom of the screen that was paired with the number as quickly as possible. The summary measure was the latency in seconds to complete responses to 36 items. A higher score indicated poorer test performance.

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Finger Tapping—The Finger Tapping test was used to measure manual motor speed and dexterity. Using the index finger of the dominant hand, the participant was instructed to press the space-bar on a computer keyboard as many times as possible until instructed to stop. A practice trial was administered followed by four, 10-second trials. The summary measure was the total number of finger taps for the four trials. The test was repeated using the non-dominant hand. A lower score indicated poorer test performance.

Grooved Pegboard—The manually-administered Grooved Pegboard test was used to assess dexterity and fine motor coordination (Klove 1963). The Grooved Pegboard test consisted of a metal board with 25 holes with randomly positioned slots and 25 notched pegs (Lafayette Instruments, Lafayette, IN, USA). Using the dominant hand, the participant's task was to insert the pegs into the slots in sequence, as quickly as possible. The test was completed when all pegs were placed or after three minutes. The summary measure was the time required in seconds to place all of the pegs. The test was repeated using the non-dominant hand. A higher score indicated poorer test performance.

Auditory Verbal Learning Test (AVLT) Total Recall—The NES3 AVLT Total Recall was used to assess verbal learning and memory (Letz et al. 2003a). At the beginning of the test, the participant was instructed to listen to a recorded list of 12 words. The participant was instructed to repeat verbally as many of the words as he could remember. The number correct was recorded by the examiner. Three trials were administered using an identical word list. The summary measure was the total number of correct responses for the three trials. Possible scores ranged from 0 to 36 with a lower score indicating poorer test performance.

AVLT Delayed Recall—AVLT Delayed Recall assessed memory and was administered approximately 20 minutes following the AVLT Total Recall trials. The participant was instructed to recall as many words as possible from the original 12-word list. The summary measure was the total number of correct responses and possible scores ranged from 0 to 12. A lower score indicated poorer test performance.

AVLT Recognition—AVLT Recognition was used to assess memory and was administered following the AVLT Delayed Recall test. This test consisted of a list of 24 words that included the original 12 words and 12 "distractor" words in random order. The participant was instructed to listen to the list and to correctly identify words that were included on the original list. The summary measure was the number of true positives minus the number of false positives. Possible scores ranged from -12 to 12 with a lower score indicating poorer test performance.

Sequences A—Sequences A is a test of motor speed and tracking similar to the Trail-making Test. Circles containing the letters "A" through "U" were displayed on a touch-screen computer monitor without special order. The participant was instructed to touch the circles in alphabetic order as quickly as possible. The summary measure was the number of seconds to complete the sequence correctly. A higher score indicated poorer test performance.

Sequences B—Sequences B is also a test of motor speed and tracking and was administered following the Sequences A test. Circles containing the numbers "1" through "11" and the letters "A" through "J" were displayed on the touch-screen computer monitor without special order. This test required that the participant alternate between number and letter sequences. The participant was instructed to touch the circles in alternating numerical and alphabetical order (i.e. 1, A, 2, B, 3, C, etc.) as quickly as possible. The summary

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measure was the number of seconds to complete the sequence correctly. A higher score indicated poorer test performance.

Assessment of potential confounders

Potential confounding variables were identified *a priori* and were selected from the AHS data and the health history questionnaire administered on the day of neurobehavioral testing. Information was obtained on age, height, education, state of residence, smoking status, alcohol and caffeine consumption, head injury, total lifetime days of any pesticide use, use of personal protective equipment and exposure to other potential neurotoxicants such as solvents and welding fumes. NES3 Adult Reading Test (ART) scores were measured at the time of neurobehavioral testing to estimate intellectual functioning (Letz et al. 2003b). Positive and negative affectivity were measured using the NES3-administered version of the Positive and Negative Affect Schedule (PANAS) (Watson et al. 1988). Visual acuity with corrective lenses (eyeglasses or contact lenses) was measured using a standard testing instrument, the Optec 1000 (Stereo Optical Co, Chicago, IL, USA). Possible visual acuity scores ranged from 20/20 to 20/200. Scores of 20/50 to 20/200 were considered indicators of poorer visual acuity.

Statistical methods

A small number of participants were excluded from analysis of individual neurobehavioral outcomes after standard linear regression diagnostics were performed. Regression diagnostics included studentized residual plots and checks for leverage and influence (Kleinbaum 1998). Two subjects were excluded from Digit-Symbol, one from Sequences A and one from Sequences B models. These observations were found to be extreme outliers from the overall sample and each had a studentized residual value that exceeded the absolute value of 4.0.

We used a backward elimination procedure to create separate base models for each neurobehavioral outcome measure with outcome-specific covariates. First, we examined the unadjusted association between each covariate and each outcome with linear regression. Covariates associated with a neurobehavioral outcome with a p-value <0.20 were then selected for inclusion in an initial full multiple linear regression base model for that outcome. Covariates with p-values ≥ 0.20 were removed sequentially from the initial full base model. The final base model for each neurobehavioral outcome included only those covariates with p-values <0.20.

Adjusted associations between each neurobehavioral outcome and the HPEE variable were estimated with linear regression models in which the outcome was regressed on the HPEE variable while controlling for the base model covariates. We changed the sign of the parameter estimates for the timed tests (Continuous Performance, Digit-Symbol, Grooved Pegboard, Sequences A and B) so that lower scores indicated poorer test performance for all neurobehavioral outcomes.

To compare HPEE age-equivalent effect sizes across the neurobehavioral outcome measures, each adjusted HPEE parameter estimate was converted into an age-equivalent value by dividing it by the base model parameter estimate for age for that outcome.

To evaluate whether associations between HPEEs and adverse neurobehavioral outcomes were attributable to medication use or medical conditions, we excluded from the analyses participants who reported a) use of benzodiazepines (n=18), opiates (n=12), anticonvulsants (n=3), barbiturates (n=2), antipsychotics (n=3), and donepezil (n=1) and b) history of alcoholism (n=6), brain tumor (n=5), alcohol use on day of testing (n=3), struck by lightning

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(n=1), renal failure (n=1), macular degeneration (n=1), and severe dementia (n=1) and compared the parameter estimates to estimates from models that included these individuals.

We used the P1RE1071201, P2RE1071202 and 07222008 releases of the AHS dataset. All analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of the study participants

Of the 693 participants included in the analyses, 156 (23%) reported one or more HPEEs in their lifetime. A total of 186 HPEEs were reported; some participants reported an HPEE on more than one interview. Of the 186 HPEEs, 73 (39%) resulted from exposure to insecticides, 68 (37%) from exposure to herbicides, 7 (4%) from exposure to fungicides, 6 (3%) from exposure to nematicides, and 32 (17%) were unknown. Given the complexity of pesticide application tasks, over a working lifetime, some participants experienced more than one HPEE involving more than one pesticide; additionally some HPEEs involved multiple chemicals.

Among the participants with HPEEs, 54% were from Iowa and 46% were from North Carolina (Table 1). Participants with HPEE had higher education, lower caffeine use and higher cumulative days of pesticide use than those without HPEE. Use of personal protective equipment when applying pesticides was similar between the two groups (~85%) as was the use of solvent additives when mixing or applying pesticides (~10%). The proportion of participants who personally mixed, loaded, handled or applied pesticides at the time of the most recent AHS interview was greater among those reporting HPEEs than among those who did not report HPEEs (85% vs. 75%).

Descriptive summary statistics for the neurobehavioral test results are presented in Table 2. The Finger Tapping and Grooved Pegboard results were similar for both hands, therefore only the results of the dominant hand are presented. The total number of participants completing each test varied because some study participants were unable to complete the tests in the allowed time or after two attempts, or because of computer problems or test administrator error.

Linear regression base model covariates

Base model regression coefficients for each neurobehavioral outcome are presented in Table 3. For all outcome measures, age and Adult Reading Test (ART) scores were statistically significant covariates. State was included in the base models for all outcome measures with the exception of the Continuous Performance Test and AVLT Total Recall test. Visual acuity score was strongly associated with two tests which required visualization of small stimuli (Digit-Symbol and Grooved Pegboard). Log_{10} lifetime days of use of any pesticide was a statistically significant covariate in AVLT Delayed Recall models. The total variance accounted for by the regression models was the highest for Digit-Symbol ($R^2 = 0.48$) and lowest for Finger Tapping ($R^2 = 0.16$).

Associations between HPEEs and neurobehavioral outcomes

HPEEs were significantly adversely associated with two of the nine neurobehavioral tests (Table 4). Participants with HPEEs were, on average, 4.2 seconds slower on the Digit-Symbol test than those without HPEEs. This effect size is equivalent to 3.9 years of age in this sample. Participants with HPEEs were, on average, 2.5 seconds slower on the Sequences A test; an effect size equivalent to 3.9 years of age in this sample. No significant

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associations were observed for the seven other neurobehavioral outcomes. The associations between HPEEs and test performance did not significantly differ by state.

Sensitivity analyses

Analyses were repeated after excluding 39 participants taking CNS active medications and 18 participants reporting medical conditions that may affect the CNS. No meaningful changes in estimates of association were observed.

Discussion

HPEEs are a relatively common event among pesticide applicators enrolled in the AHS. We observed modest but meaningful associations between HPEEs and adverse performance on the Digit-Symbol test (a measure of visual scanning and processing) and Sequences A (a measure of visual scanning and motor speed). The Digit-Symbol test is widely used in neurotoxicology research and is among the most responsive to neurotoxicant exposure (Anger 2003). Our results suggest that high-level pesticide exposures that do not result in physician-diagnosed pesticide poisoning may contribute to persistent adverse neurological effects. The overlap in neurobehavioral domains between the Digit-Symbol test and Sequences A suggests that the observed results were not chance observations.

In the present study, we examined associations between neurological outcomes and all HPEEs, rather than stratifying them by specific pesticide agent or class. We did so because pesticide applicators work in complex chemical environments that are not easily deconstructed into homogeneous exposure categories. For example, pesticide formulations used in agricultural settings often include substantial quantities of non-pesticide “inert” ingredients (*e.g.*, organic solvents) that may contribute substantially to the neurological toxicity of the formulation (Cox and Surgan 2006; Weinhold 2010). These non-pesticide ingredients may have contributed to the observed associations between HPEEs and measures of neurobehavioral function independently of the specific active pesticide ingredient. Furthermore, although recent studies of neurological outcomes among pesticide-exposed workers have focused on organophosphate pesticide exposure, many other pesticides have some neurotoxic potency as well (Reuber 1983; Singer et al. 1982; Keim and Alavanja 2001; Kamel and Hoppin, 2004; Costa et al. 2008). In the present study, pesticide-specific information was available for only the most recently reported HPEE at the time of each AHS interview and little or no additional information was available about the formulation of the involved pesticide. Hence, participants with more than one HPEE may have experienced especially heterogeneous exposure. Stratifying HPEEs by specific agents would over-simplify the complexity of HPEEs as actually experienced by pesticide applicators. Consequently, in our analyses, HPEEs are best considered an undesirable occupational event experienced by pesticide applicators rather an indicator of exposure to any particular pesticide.

Although the literature is mostly consistent in demonstrating that acute OP pesticide poisoning is associated with long-term central nervous system impairment, the effect on the nervous system of prolonged OP and other pesticide exposure without evidence of previous poisoning is still controversial. Several studies of OP pesticide exposed (but not poisoned) workers have reported neurobehavioral deficits in measures of memory, motor speed, simple reaction time, sustained attention and visual scanning and processing (Kamel et al. 2003; Rohlman et al. 2007; Roldan-Tapia et al. 2005; Bazylewicz-Walczak et al. 1999; Fiedler et al. 1997), while others have reported little or no evidence of long-term neurobehavioral deficits (London et al. 1998; Ames et al. 1995; Daniell et al. 1992; Steenland et al. 2000). To our knowledge, this is the first study to examine the association between unusually high pesticide exposures (among persons without a diagnosis of pesticide poisoning) and

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neurobehavioral function. Consequently, the results of this study fill a gap in knowledge about the long-term neurobehavioral effects of transient increases in exposure at levels insufficient to result in overt pesticide poisoning.

The participation rate in this study was low (39%), raising concern that our study sample may not represent all eligible pesticide applicators enrolled in the AHS. However, on several important characteristics, including age and total lifetime days of pesticide use, participants were similar to eligible non-participants, suggesting comparability (data not shown). Thus, there is little reason to believe that the exposure-effect association among participants is meaningfully different from that among those who were eligible but did not participate.

One limitation resulting from the cross-sectional design of the study is ambiguity of temporal association. One possible interpretation of the observed associations is that those with subclinical neurobehavioral impairment were more likely to have HPEEs rather than the interpretation that those with HPEE's were more likely to demonstrate neurobehavioral impairment. Given the toxicological literature showing elevated risk of neurobehavioral impairment among persons with overt pesticide poisoning, we believe that the observed results are consistent with HPEEs leading to neurological impairment, rather than the reverse.

A major strength of the study is that it was based on a relatively large sample of pesticide applicators randomly selected from the AHS, a population whose lifetime pesticide use has been well characterized. The sample included pesticide applicators from two distinct geographic regions, Iowa and North Carolina, with varying crops and farming practices. As such, the results of the present study are relevant to a large segment of the farming population.

It is unlikely that the observed adverse associations were the result of an acute cholinergic response to recent pesticide exposure. Neurobehavioral testing was conducted during the winter months when pesticide application is minimal. Furthermore, we excluded participants with a history of physician-diagnosed pesticide poisoning, suggesting that our findings are not due to any long-term sequelae of a previous acute pesticide poisoning event. While it is possible that some pesticide poisonings were not reported, the signs and symptoms of clinically overt pesticide poisoning are easily recognizable, making it unlikely that past pesticide poisoning was unrecognized.

In this sample of licensed pesticide applicators, a history of high pesticide exposure events was associated with adverse results on two neurobehavioral tests. These findings add to the increasing evidence that pesticide exposure at levels that do not produce acute pesticide poisoning may be associated with long-term adverse neurological function. If these events do contribute to adverse neurological outcomes, then efforts aimed at preventing high pesticide exposures should be a public health priority.

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Table 1

Demographic characteristics, personal health information, and chemical and pesticide exposures by high pesticide exposure events (HPEEs) among 693 pesticide applicators

Characteristic	No HPEEs (n=537)				HPEEs (n=156)			
	Mean	SD	No.	%	Mean	SD	No.	%
Age (yrs)	62.2	11.5	--	--	57.8	11.5	--	--
Adult Reading Test (0–60)	29.0	10.0	--	--	33.2	10.3	--	--
Positive affect (1–5)	3.5	0.7	--	--	3.5	0.6	--	--
Negative affect (1–5)	1.4	0.4	--	--	1.5	0.5	--	--
Lifetime pesticide use (days)	1,451	1,510	--	--	2,065	1,865	--	--
Testing location								
Iowa	--	--	267	50	--	--	85	54
North Carolina	--	--	270	50	--	--	71	46
Education								
≤ High school	--	--	291	54	--	--	61	39
> High school	--	--	246	46	--	--	95	61
Alcohol (drinks/wk)								
0 drinks	--	--	308	57	--	--	87	56
1–7 drinks	--	--	180	34	--	--	50	32
>7 drinks	--	--	49	9	--	--	19	12
Visual acuity								
20/20 – 20/40	--	--	447	83	--	--	139	89
20/50 – 20/200	--	--	90	17	--	--	17	11
Head injury								
No injury	--	--	422	79	--	--	110	71
Injury, no loss of consciousness	--	--	53	10	--	--	17	11
Injury, w/loss of consciousness	--	--	62	12	--	--	29	19
Caffeine use (drink regularly)	--	--	401	75	--	--	39	25
Solvents exposure (ever)	--	--	225	42	--	--	59	38
Personal protective equipment use			463	86			133	85
Ever used solvent additives *			57	11			15	10

Characteristic	No HPEEs (n=537)				HPEEs (n=156)			
	Mean	SD	No.	%	Mean	SD	No.	%
Mixed/applied pesticides in past year			401	75			133	85

*Ever used solvent additives when mixing or applying pesticides

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Table 2

Frequencies and means of neurobehavioral outcome measures by high pesticide exposure events (HPEEs) (n=693)

Outcome	No HPEEs (n=537)				HPEEs (n=156)			
	N	Mean	SD	Range	N	Mean	SD	Range
Continuous Performance (ms)	533	430.1	46.0	3186 – 612.3	152	420.5	40.5	338.7 – 595.4
Digit-Symbol (s)	532	118.2	23.3	75.3 – 210.4	152	114.4	21.7	73.6 – 213.6
Finger Tapping, dominant hand (# of taps)	532	53.4	9.8	9 – 86	155	54.6	8.7	22 – 73
Grooved Pegboard, dominant hand (s)	537	93.3	24.8	51 – 180	156	87.4	21.3	57 – 159
AVLT Total Recall (# correct)	532	19.5	5.0	5 – 31	156	21.1	5.1	10 – 34
AVLT Delayed Recall (# correct)	532	6.5	2.8	0 – 12	155	7.2	2.7	0 – 12
AVLT Recognition (tp-fp)	532	8.3	2.6	−3 – 12	154	8.6	2.3	2 – 12
Sequences A latency (s)	522	43.4	14.7	20.0 – 92.6	150	40.8	13.2	14.8 – 91.3
Sequences B latency (s)	515	65.8	21.8	29.7 – 144.4	149	59.7	18.0	22.8 –s 114.7

AVLT Auditory Verbal Learning Test, *tp* true positives, *fp* false positives

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Table 3

Base model regression coefficients for neurobehavioral outcome measures (n=693)

Outcome	Age (yrs)	ART score	Lifetime pesticide days [c]	NA score	PA score	Caffeine	Education	State	Visual acuity score	Base model R^2
CPT* (ms)	−1.60[b]	0.63[b]	--	--	4.32	−8.07[a]	--	--	--	0.23
Digit-Symbol* (s)	−1.07[b]	0.42[b]	--	--	4.85[b]	--	3.26[a]	−4.53[b]	−5.06[b]	0.48
Finger Tapping, dominant (# of taps)	−0.24[b]	0.13[b]	--	--	0.94	--	--	−2.29[b]	--	0.16
Grooved Pegboard, dominant* (s)	−1.09[b]	0.17[a]	--	--	--	-3.67[a]	--	−3.14[a]	−6.83[b]	0.35
AVLT Total Recall (# correct)	−0.18[b]	0.10[b]	--	−1.14[b]	0.63[a]	--	0.91[a]	--	--	0.28
AVLT Delayed Recall (# correct)	−0.09[b]	0.04[b]	0.52[b]	−0.60[a]	0.35 [a]	--	0.68[b]	−0.30	--	0.27
AVLT Recognition (tp-fp)	−0.06[b]	0.04[b]	--	--	0.31[a]	--	0.73[b]	−0.76[b]	--	0.20
Sequences A* (s)	−0.64[b]	0.40[b]	--	--	2.04[b]	--	--	−2.83[b]	--	0.42
Sequences B* (s)	−0.95[b]	0.53[b]	--	--	4.10[b]	--	--	−4.88[b]	--	0.42

Age, ART, Lifetime pesticide days ($\log_{10}$), NA and PA are continuous variables. Caffeine, education, state of residence, and visual acuity are categorical variables. The reference groups for the categorical variables are: caffeine = drink regularly, education = ≤ high school education, state = Iowa, visual acuity = 20/20 – 20/40 vision

ART Adult reading test, *NA* negative affectivity, *PA* positive affectivity, *CPT* Continuous Performance Test, *AVLT* Auditory Verbal Learning Test, *tp* true positives, *fp* false positives

* Signs of scores have been changed so that lower scores indicate poorer performance

[a] $p<0.05$

[b] $p<0.01$

[c] Log_{10} lifetime days of any pesticide use

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Table 4

Adjusted linear regression models of associations between high pesticide exposure events (HPEEs) and neurobehavioral outcome measures (n=693)

Outcome	N	β	95% CI	Age equivalence in years[a]
CPT* (ms)	684	0.50	−7.85, 6.85	−0.3
Digit-Symbol* (s)	683	**−4.19**	**−7.27, −1.11**	3.9
Finger Tapping, dominant (# of taps)	687	−0.48	−2.10, 1.15	2.0
Grooved Pegboard, dominant* (s)	693	0.02	−3.59, 3.55	−0.02
AVLT Total Recall (# correct)	688	0.52	−0.29, 1.28	−2.9
AVLT Delayed Recall (# correct)	687	0.01	−0.45, 0.46	−0.1
AVLT Recognition (tp-fp)	686	−0.26	−0.69, 0.16	4.3
Sequences A* (s)	671	**−2.47**	**−4.53, −0.41**	3.9
Sequences B* (s)	663	−0.48	−3.52, 2.55	0.5

Models are adjusted for the base model covariates presented in Table 3.

CPT Continuous Performance Test, *AVLT* Auditory Verbal Learning Test, *tp* true positives, *fp* false positives

* Signs of scores have been changed so that lower scores indicate poorer performance

[a] Calculated as β for NB outcome divided by β for age in Table 3