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Neurobehavioral function and organophosphate insecticide use among pesticide applicators in the Agricultural Health Study

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ABSTRACT

Although persistent decrements in cognitive function have been observed among persons who have recovered from clinically overt organophosphate (OP) pesticide poisoning, little is known about the cognitive effects of chronic OP exposures that do not result in acute poisoning. To examine associations between long-term pesticide use and neurobehavioral (NB) function, NB tests were administered to licensed pesticide applicators enrolled in the Agricultural Health Study (AHS) in Iowa and North Carolina. Between 2006 and 2008, 701 male participants completed nine NB tests to assess memory, motor speed and coordination, sustained attention, verbal learning and visual scanning and processing. Data on ever-use and lifetime days of use of 16 OP pesticides were obtained from AHS interviews conducted before testing between 1993 and 2007 and during the NB visit. The mean age of participants was 61 years (SD = 12). Associations between pesticide use and NB test performance were estimated with linear regression controlling for age and outcome-specific covariates. NB test performance was associated with lifetime days of use of some pesticides. Ethoprop was significantly associated with reduced performance on a test of motor speed and visual scanning. Malathion was significantly associated with poor performance on a test of visual scanning and processing. Conversely, we observed significantly better test performance for five OP pesticides. Specifically, chlorpyrifos, coumaphos, parathion, phorate, and tetrachlorvinphos were associated with better verbal learning and memory; coumaphos was associated with better performance on a test of motor speed and visual scanning; and parathion was associated with better performance on a test of sustained attention. Several associations varied by state. Overall, we found no consistent evidence of an association between OP pesticide use and adverse NB test performance among this older sample of pesticide applicators. Potential reasons for these mostly null results include a true absence of effect as well as possible selective participation by healthier applicators.

1. Introduction

Organophosphate (OP) pesticides are widely used in the United States and internationally to protect crops from insect damage. OP pesticides accounted for approximately 35% of all insecticide pesticides used in the U.S. in 2007 with over 33 million pounds used annually (EPA, 2011). Exposure is common among agricultural workers as well as the general population (Centers for Disease Control and Prevention, 2009).

The acute toxicity of OP pesticides is well described and results from inhibition of the enzyme acetylcholinesterase (AChE) (Bardin et al., 1994). Long-term exposure to low or moderate levels of OP pesticides does not cause clinically overt cholinergic toxicity (Lotti, 2002; Romana et al., 2001). However, evidence for an association between long-term low or moderate exposure to OPs and impaired neurobehavioral (NB) function or other neurological effects is inconsistent (Bazylewicz-Walczak et al., 1999; Farahat et al., 2003; Kamel and Hoppin, 2004; Kamel et al., 2003; Pilkington et al., 2001; Rohlman et al., 2007; Rohlman et al., 2011; Roldan-Tapia et al., 2005; Rothlein et al., 2006) and no clear consensus on the association between low- or moderate-level exposure and NB function has emerged among authors of recent reviews of this topic (comparison of reviews by

Abbreviations: AHS, Agricultural Health Study; ART, Adult Reading Test; AVLT, Auditory Verbal Learning Test; BMI, body mass index; CPT, Continuous Performance Test; CNS, central nervous system; NA, negative affect; NB, neurobehavioral; OP, organophosphate; PA, positive affect.

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Colosio et al. (2009) and Rohlman et al. (2011) illustrates the divergence of opinion). The heterogeneity of findings reported in the literature may be due to a number of methodological limitations including small sample size (Bazylewicz-Walczak et al., 1999; Fiedler et al., 1997), use of poor or inaccurate exposure estimates (Farahat et al., 2003; Kamel et al., 2003; London et al., 1998; Rohlman et al., 2007; Roldan-Tapia et al., 2005), referent groups that may have differed from the exposure group on characteristics other than exposure (e.g., sheep dippers versus ceramic workers) (Bazylewicz-Walczak et al., 1999; Farahat et al., 2003; Fiedler et al., 1997; Pilkington et al., 2001; Stephens and Sreenivasan, 2004), and inadequate control for potential confounding variables, such as previous pesticide poisoning (Baldi et al., 2001; Bazylewicz-Walczak et al., 1999; Farahat et al., 2003; Pilkington et al., 2001).

The purpose of this investigation was to examine associations between OP pesticide use and measures of neurobehavioral function in a large cohort of pesticide applicators with well characterized pesticide use histories. The primary hypothesis we tested was whether long-term OP pesticide use was associated with adverse NB outcomes.

2. Materials and methods

2.1. Study participants

This study was conducted among private pesticide applicators enrolled in the Agricultural Health Study (AHS). The AHS is a large prospective study of private pesticide applicators, their spouses, and commercial pesticide applicators from Iowa and North Carolina. Details have been described elsewhere (Alavanja et al., 1996; Waggoner et al., 2011). Briefly, between 1993 and 1997, participants completed the AHS Enrollment questionnaire at the time of pesticide licensing and recertification. Forty-percent of applicators also completed a "take-home" questionnaire within 1 month of enrollment. Detailed information on some OPs was collected on this second questionnaire, thus, only individuals who completed both questionnaires at enrollment were eligible. These two questionnaires comprised Phase 1 data collection. After enrollment, a five-year and a ten-year follow-up telephone interview were administered (Phases 2 and 3 data collection).

Male participants who completed all AHS interviews, resided in Iowa or North Carolina, and lived within approximately 150 miles of the testing facilities were invited to participate in the present study. Participants were not eligible if, either at the time of the most recent AHS interview (Phase 3) or during a brief telephone screening interview prior to enrollment, they had medical conditions that could influence central or peripheral nervous system testing results (i.e., amyotrophic lateral sclerosis, multiple sclerosis, Parkinson's disease, retinal or macular degeneration, stroke, hypothyroidism and treatment for diabetes), reported drinking $\geq$42 alcoholic beverages per week, or reported a history of pesticide poisoning at the most recent interview. Initially, 1807 male AHS participants were eligible to participate in the present study after these criteria were applied.

To enrich the sample for applicators with higher lifetime use of OP pesticides, we oversampled the high end of the OP lifetime use distribution based on the lifetime days of use 10 OPs assessed in detail in Phase 1. Among eligible participants, a stratified sample was selected based on equal sampling from the upper and lower portion of the OP lifetime day's distribution. In Iowa, a cutpoint of ~75% was used to separate individuals; in NC the cutpoint was lower (66%) because the NC cohort is more geographically dispersed and has fewer members. Individuals were randomly selected from within the high and low groups. While the cutpoint was shifted for selection, all analyses were done based on lifetime use of pesticides which included data from all AHS phases as well as the NB appointment. Thus the sampling frame allowed us to have an enriched sample for OP use, but was not used as an analytical variable. 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. All participants were fluent in English. Participants were reimbursed for time and travel expenses. Appropriate Institutional Review Boards approved the study protocol, and all participants provided written informed consent for the present study.

2.2. Exposure assessment

Information on pesticide exposure history, farm practices, medical history, and demographic factors was obtained from the questionnaires used during all three AHS phases as well as a NB study questionnaire administered at the time of testing. Copies of AHS questionnaires are available online (AHS, 2010).

Use of specific pesticides was quantified for each participant using information from AHS questionnaires and the NB study questionnaire. In each phase of the AHS, pesticide use was assessed in slightly different ways, and we integrated these data from all phases to create variables on ever use and lifetime days of use. In Phase 1 questionnaires, participants were queried in detail about 50 specific pesticides and asked to provide information on ever use, frequency of use and years of use. Additionally, on the "take-home" questionnaire, participants were asked to complete a checklist indicating ever use of specific chemicals, but were not asked to provide information on frequency or duration of use. In Phases 2 and 3, participants provided open-ended responses regarding pesticide use since last interview, and this information was used to create lifetime use for those time periods. The NB study questionnaire collected pesticide use information for the past 12 months including ever mix or apply and duration of use (days).

We evaluated the 16 OP pesticides used by at least 50 NB participants. Nine of these were reported in detail on the AHS enrollment questionnaire (chlorpyrifos, coumaphos, diazinon, dichlorvos, fonofos, malathion, parathion, phorate, and terbufos); six were initially queried on the take-home questionnaire checklist (acephate, dimethoate, disulfoton, ethoprop, phosmet, and tetrachlorvinphos); and a new chemical introduced in 1995 (tebupirimfos) was reported initially on the Phase 2 questionnaire. Using information from all AHS interviews and the NB study questionnaire, we created two lifetime exposure metrics: 1) ever use — based on any positive report at any interview and 2) lifetime days of use based on the sum of lifetime days of use reported at each interview. For the Phase 1 lifetime days summary measure, we multiplied the number of days used per year by the numbers of years used. As chemicals on the "take-home" checklist did not have this information, we assumed i) that the days of use per year was equal to the median number of days of insecticide use per year for that individual and ii) that the number of years used was equal to the median number of years that individual had applied insecticides using categories comparable to the Phase 1 questionnaire. For Phases 2 and 3 as well as the NB interview information, we multiplied the number of days used per year by the number of years since last interview to create the lifetime days accrued during that period. We then summed the lifetime days for Phases 1, 2, 3 and the NB interview to create the cumulative lifetime days of use of each pesticide. All pesticide use occurred prior to NB testing. An overall summary measure of all OP pesticide use (cumulative lifetime days of all OP pesticides) was also created by summing the lifetime days reported for each individual OP pesticide. In addition to variables on OP use, we also created similar variables for four carbamate pesticides (aldicarb, benomyl, carbaryl and carbofuran). An overall measure of cumulative lifetime pesticide use was estimated for the 50 pesticides assessed in detail during Phase 1. This variable integrated data from all AHS interviews.

2.3. Neurobehavioral testing

NB tests were administered by trained technicians unaware of the participants' exposure status (Starks et al., in press). Eight computerized tests from the English language version of the Neurobehavioral Evaluation System, Version 3 (NES3), (Letz, 2000; Letz et al., 2003; Letz et al., 2000) and the manual Grooved Pegboard test (Lafayette Instruments, Lafayette, IN) (Klove, 1963) were administered. These tests have been used extensively in investigations of neurotoxicants and were selected to be sensitive indicators of a wide range of neurobehavioral functions. A description of the nine NB tests is presented in Table 1.

2.4. Assessment of potential confounders

To construct outcome-specific models, we evaluated a common set of covariates potentially associated with neurobehavioral outcomes. We selected the following questionnaire covariates as potential confounders of the NB outcomes: age, height, education, state, smoking status, alcohol consumption, head injury, current antidepressant use, caffeine consumption and exposure to other potentially neurotoxic substances such as organic solvents, soldering and welding fumes. Additionally, we administered tests of reading ability, affect, and visual acuity to control for these potential confounders as well. NES3 includes an Adult Reading Test (ART) to estimate intellectual functioning and the Positive and Negative Affect Schedule (PANAS) to measure positive and negative affect (Watson et al., 1988). We measured best corrected visual acuity using the Optec 1000 (Stereo Optical Co, Chicago, IL).

2.5. Statistical methods

2.5.1. Linear regression analyses

We used a backward elimination procedure to create separate base models for each NB 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 NB outcome in unadjusted linear regression models with a p-value <0.20 were selected for inclusion in an initial full multiple linear regression base model for that outcome. Covariates with p-values >0.20 were then removed sequentially from the initial full base model. The final multivariate base model for each NB outcome included only those covariates with p-values <0.20. All final multivariate base models included age (years) and ART score. In addition, the Continuous Performance Test final multivariate base model included positive affect (PA) score and caffeine consumption; the Digit-Symbol final multivariate base model included PA score, education, state, and visual acuity score; the Finger Tapping final multivariate base model included PA score and state; the Grooved Pegboard final multivariate base model included caffeine consumption, state and visual acuity score; the AVLT Total Recall final multivariate base model included negative affect (NA) score, PA score, and education; the AVLT Delayed Recall final multivariate base model included NA score, PA score, education and state; the AVLT Recognition final multivariate base model included PA score, education and state; and the Sequences A and B final multivariate base model included PA score and state.

For each NB outcome, we excluded subjects who had studentized residual values that exceeded the absolute value of 4.0. For Digit-Symbol, two subjects were excluded; for Sequences A one subject was excluded; and for Sequences B one subject was excluded (Belsley et al., 2004).

Each pesticide was examined both as a continuous variable (cumulative lifetime days of use) and as a dichotomized variable (ever/never use). The lifetime days of pesticide use variables were $\log_{10}$-transformed to normalize the distribution of residuals. Adjusted associations between NB outcomes and pesticide exposures were estimated with linear regression models in which the NB outcome was regressed on the pesticide exposure variable while controlling for the covariates included in the base model. Parameter estimates for the timed tests (Continuous Performance Test, Digit-Symbol, Grooved Pegboard, Sequences A and B) were multiplied by -1 so that lower scores indicated poorer test performance for all NB outcomes. In addition, pesticide use by state was examined with the inclusion of a state by pesticide interaction term.

2.5.2. Confounding by related pesticide exposures

Pesticide applicators typically use more than one pesticide. Potential confounding of the association between NB outcomes and each pesticide by other pesticides was examined. Specifically, Spearman correlations were calculated for pesticides associated with NB outcomes with a p-value <0.10. Moderately correlated pesticide pairs ($r \geq 0.30$) were added simultaneously to final base models and the pesticide variable parameter estimates were compared to models with only one pesticide.

2.5.3. Sensitivity analyses

For our primary analysis, we analyzed data from all participants. To assess the robustness of our results, we conducted two analyses where we excluded: 1) individuals with medical conditions or use of medication that may affect NB outcomes (ascertained at the time of neurobehavioral testing and not during earlier AHS interviews or the screening telephone call), and 2) individuals with a history of physician-diagnosed pesticide poisoning (reported at the time of neurobehavioral testing and not during earlier AHS interviews). For the first sensitivity analysis, we excluded participants who reported use of specific medications (i.e., benzodiazepines ($n=18$), opiates ($n=12$), anticonvulsants ($n=3$), barbiturates ($n=2$), antipsychotics ($n=3$), and donepezil ($n=1$)) or medical conditions not reported on the Phase 3 interview (i.e., a history of alcoholism ($n=6$), brain tumor ($n=5$), alcohol use on day of testing ($n=3$), struck by lightning ($n=1$), renal failure ($n=1$), macular degeneration ($n=1$), and severe dementia ($n=1$)). Parameter estimates from models excluding these individuals were then compared to estimates from models that included these individuals. A similar strategy was employed to assess whether excluding individuals with a history of diagnosed pesticide poisoning at enrollment ($n=8$) influenced our results.

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).

Table 1
Neurobehavioral tests of central nervous system (CNS) function administered to pesticide applicators.

Test	Function assessed	Units
Continuous performance[a]	Sustained attention	m/s
Digit-symbol[a]	Visual scanning and processing	Seconds
Finger tapping, dominant hand	Motor speed	Avg # of taps (4, 10-second trials)
Grooved pegboard, dominant hand[a]	Fine motor coordination	Seconds
Auditory verbal learning total recall	Verbal learning and memory	# Correct (0–36)
Auditory verbal learning delayed recall	Memory	# Correct (0–12)
Auditory verbal learning recognition	Memory	True positives–false positives (−12–12)
Sequences A latency[a]	Motor speed and scanning	Seconds
Sequences B latency[a]	Motor speed and scanning	Seconds

[a] Higher scores indicate poorer performance.

3. Results

3.1. Participation

NB testing was administered to 701 participants from the 1807 eligible AHS participants. The overall participation rate was 39%. Participants were similar to non-participants with respect to age and pesticide use history (data not shown).

3.2. Characteristics of the study participants

3.2.1. Demographics

Descriptive statistics for potential confounding demographic characteristics, personal health information and chemical exposures are presented in Table 2. Among the 701 participants included in the analyses, 51% were from Iowa and 49% were from North Carolina. The mean age of the participants was 61 years (SD = 12) and approximately half reported completing more than a high school education. Of those reporting up to 12 years of education, nearly all (83%) reported completing high school on the most recent AHS questionnaire (data not shown). At the time of the most recent AHS interview, 78% of the participants (N = 542) were still applying pesticides and 69% were still farming (N = 484) (data not shown). Over 20% of the participants reported a past head injury with or without loss of consciousness and eight (1%) reported a previous physician-diagnosed pesticide poisoning.

3.2.2. Pesticide exposures

Frequencies of use of specific pesticides and means of cumulative lifetime days for the 16 OPs, four carbamates and two pesticide summary variables are presented in Table 3. Ever use of specific OPs ranged from 77% for malathion to ≤10% for dimethoate, tebupirimfos and tetrachlorvinphos. Carbaryl (63%) was the most commonly used carbamate pesticide. Most participants reported ever using any OP (97%) and all but one participant reported using at least one pesticide in their lifetimes. Lifetime days of all OP pesticides and lifetime days of all pesticides were similar between Iowa and North Carolina participants (data not shown). Carbamate pesticide use, however, was more prevalent in North Carolina (93%) than in Iowa (67%).

Table 2
Demographic characteristics, personal health information and Chemical exposure for the 701 Agricultural Health Study male pesticide applicators.

Characteristic	Mean	SD	No.	%
Age (years)	61	12	–	–
Height (cm)	179	6	–	–
Adult reading test (0–60)	30	10	–	–
Positive affect (1–5)	3.5	0.7	–	–
Negative affect (1–5)	1.4	0.4	–	–
Testing location				
Iowa	–	–	356	51
North Carolina	–	–	345	49
Education				
≤High school	–	–	355	51
>High school	–	–	346	49
Smoking status				
Never smoked	–	–	403	57
Current smoker	–	–	47	7
Past smoker	–	–	251	36
Alcohol consumption (drinks/week)				
0 drinks	–	–	401	57
1–7 drinks	–	–	231	33
>7 drinks	–	–	69	10
Visual acuity				
20/20–20/40	–	–	592	84
20/50–20/200	–	–	109	16
Head injury				
No injury	–	–	536	76
Injury, no loss of consciousness	–	–	71	10
Injury, w/loss of consciousness	–	–	94	13
Antidepressants (current use)	–	–	51	7
Caffeine use (drink regularly)	–	–	525	75
Solvent exposure (ever)	–	–	288	41
Soldering exposure (ever)	–	–	36	5
Pesticide poisoning (ever)	–	–	8	1

Table 3
Frequencies and means of cumulative lifetime days of pesticide use for 701 male pesticide applicators.

Pesticides	N[a]	%	Mean	SD	Min.	Median	Max.
Organophosphates							
Acephate	166	24	85	90	3	56	501
Chlorpyrifos	418	60	75	103	2	39	767
Coumaphos	94	13	74	245	1	12	1683
Diazinon	302	43	55	93	1	20	846
Dichlorvos	128	18	443	1064	1	58	8680
Dimethoate	66	9	46	68	2	25	457
Disulfoton	110	16	43	42	2	25	236
Ethoprop	121	17	45	50	3	25	316
Fonofos	201	29	64	84	2	39	457
Malathion	541	77	99	201	2	37	2625
Parathion	147	21	103	272	1	20	1668
Phorate	230	33	70	130	1	25	1628
Phosmet	101	14	61	83	3	26	600
Tebupirimfos	69	10	51	46	4	40	250
Terbufos	356	51	101	116	2	56	752
Tetrachlorvinphos	69	10	65	99	3	25	582
Carbamates							
Aldicarb	131	19	86	118	2	29	742
Benomyl	116	17	62	122	1	13	767
Carbaryl	440	63	103	153	1	47	1388
Carbofuran	290	41	55	92	1	25	752
Summary variables							
All organophosphates	682	97	420	663	2	241	8763
All pesticides	700	100	1604	1617	10	1045	11,677

[a] Number of participants who reported ever use.

3.3. Associations between OP pesticide use and neurobehavioral outcomes

Descriptive statistics of the NB test results and comparisons to results presented in other studies using similar or identical test administration methods are presented in Appendix A. Because a small number of study participants were unable to complete individual tests in the allowed time or after two attempts, the total number of participants completing each test varied slightly. Specific pesticides were associated with some NB tests. Three of the nine NB outcomes we examined had at least one significant adverse association with ever-use (Table 4) or lifetime days of pesticide use (Table 5). Lifetime days of all OP use was not significantly associated in either direction with any NB test. Ever-use of ethoprop and lifetime days of malathion use were both significantly associated with poorer performance on the Digit-Symbol test. Ever-use of disulfoton, ethoprop and terbufos were significantly associated with poorer performance on the Sequence A test. Conversely, six of the nine outcomes had significantly better test performance with ever-use or lifetime days of use. Better test performance among was observed more frequently for the three Auditory Verbal Learning tests (Total Recall, Delayed Recall and Recognition). For several NB outcomes, we observed a significant state by pesticide interaction, suggesting differential effects for chlorpyrifos, coumaphos, and malathion in North Carolina and Iowa (Table 6).

3.4. Associations between carbamate use and neurobehavioral outcomes

No statistically significant adverse associations were observed between carbamate pesticide use and adverse NB test results. Rather, each of the four carbamate pesticides was significantly associated

Table 4
Regression coefficients from linear regression models for neurobehavioral outcomes and ever used pesticides for the 701 male pesticide applicators.

Pesticide	CPT[a] (n = 692)		Digit-Symbol[a] (n = 691)		Finger Tapping, dominant (n = 695)		Grooved Pegboard, dominant[a] (n = 699)		AVLT Total Recall (n = 696)		AVLT Delayed Recall (n = 695)		AVLT Recognition (n = 694)		Sequences A[a] (n = 679)		Sequences B[a] (n = 671)	
	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE
Organophosphates																		
Acephate	1.68	3.55	−2.12	1.83	0.22	0.97	−0.04	2.12	−0.04	0.39	0.22	0.27	−0.02	0.25	−2.07	1.25	−1.36	1.83
Chlorpyrifos	−1.57	3.12	int	–	−0.64	0.70	**3.47***	1.53	0.54	0.34	0.37	0.19	0.17	0.18	−1.60	0.89	−0.55	1.30
Coumaphos	0.92	4.39	2.30	1.87	1.24	1.00	1.82	2.19	**1.25***	0.48	0.50	0.27	0.40	0.26	0.75	1.26	2.93	1.82
Diazinon	−1.61	3.07	0.37	1.32	0.71	0.70	0.60	1.54	0.16	0.33	0.19	0.19	−0.03	0.18	−0.74	0.89	0.78	1.30
Dichlorvos	−0.46	3.90	−0.47	1.76	0.30	0.94	0.97	2.05	0.51	0.43	0.18	0.26	0.17	0.24	1.53	1.18	1.34	1.72
Dimethoate	2.00	5.13	−1.58	2.17	−1.06	1.15	−0.71	2.53	0.84	0.56	0.21	0.32	0.55	0.30	1.98	1.45	1.42	2.11
Disulfoton	3.73	4.18	−1.97	1.93	−1.20	1.03	−2.08	2.24	−0.18	0.46	0.37	0.28	−0.30	0.27	**−2.55***	1.31	0.72	1.93
Ethoprop	1.51	3.99	**−3.66***	1.84	0.33	0.99	−1.33	2.15	**−0.86***	0.44	−0.23	0.27	−0.43	0.25	**−3.27****	1.25	−1.46	1.85
Fonofos	1.70	3.33	0.90	1.56	0.24	0.83	0.57	1.82	0.36	0.36	0.34	0.23	0.03	0.22	−0.73	1.05	−0.29	1.53
Malathion	−6.13	3.62	−2.68	1.53	0.30	0.81	int	–	0.58	0.39	0.24	0.22	−0.19	0.21	−0.91	1.04	0.20	1.51
Parathion	3.47	3.73	0.40	1.60	−0.33	0.85	0.61	1.86	−0.26	0.40	0.41	0.23	0.06	0.22	−0.65	1.08	0.62	1.58
Phorate	−1.54	3.20	0.24	1.40	−0.21	0.75	−0.99	1.64	0.22	0.35	0.25	0.21	0.33	0.19	−0.52	0.95	0.03	1.38
Phosmet	−2.83	4.35	−2.00	1.92	0.19	1.02	0.89	2.25	0.29	0.47	−0.15	0.28	−0.21	0.27	−0.15	1.29	0.00	1.86
Tebupirimfos	−0.04	5.04	2.35	2.23	0.23	1.19	−1.29	2.62	0.36	0.55	0.19	0.33	−0.03	0.31	−0.38	1.51	1.60	2.18
Terbufos	−0.62	3.02	0.78	1.33	1.21	0.70	−1.22	1.55	0.06	0.33	0.17	0.19	0.04	0.18	**−1.74***	0.90	−0.66	1.31
Tetrachlorvinphos	3.28	5.03	0.78	2.17	−1.28	1.16	−0.72	2.54	**1.24***	0.55	0.35	0.32	0.53	0.30	1.45	1.46	1.69	2.10
Carbamates																		
Aldicarb	−0.69	3.89	1.00	1.84	−0.51	0.97	2.00	2.12	0.25	0.47	0.30	0.27	**0.51***	0.25	−0.66	1.24	−0.01	1.81
Benomyl	3.56	4.06	2.64	1.83	−0.44	0.97	−1.29	2.13	0.56	0.47	0.44	0.27	0.28	0.25	2.27	1.24	−1.78	1.81
Carbaryl	int	–	0.48	1.48	0.49	0.79	−1.76	1.74	**0.83***	0.38	**0.56****	0.22	0.05	0.21	−0.31	1.01	1.00	1.46
Carbofuran	4.75	3.08	1.81	1.30	1.20	0.69	2.08	1.52	0.21	0.34	0.19	0.19	−0.07	0.18	−0.75	0.88	int	–

CPT = Continuous Performance Test, AVLT = Auditory Verbal Learning Test, int = significant interaction term for state by pesticide exposure; All models were adjusted for age (years) and ART score. In addition, CPT models were adjusted for positive affect score (PA) and caffeine consumption; Digit-Symbol models were adjusted for PA, education, state, and visual acuity score; Finger Tapping models were adjusted for PA and state; Grooved Pegboard models were adjusted for caffeine consumption, state and visual acuity score; AVLT Total Recall models were adjusted for negative affect score (NA), PA score, and education; AVLT Delayed Recall models were adjusted for NA, PA, education and state; AVLT Recognition models were adjusted for PA, education and state; Sequences A and B were adjusted for PA and state.

[a] Regression coefficients have been multiplied by −1 so that lower scores indicate poorer performance.

* $p<0.05$.

** $p<0.01$.

with better performance on one or more NB tests (Tables 4–5). A significant interaction between state and ever-use of carbaryl was observed for the Continuous Performance Test (Table 6). Among North Carolina participants, significantly decreased test performance was observed with ever-use of carbaryl, whereas better, but non-statistically significant test performance was observed among Iowa participants. We also observed a significant interaction between state and ever-use of carbofuran for the Sequence B test.

3.5. Confounding by related pesticide exposures

The simultaneous inclusion of correlated pesticides in the models did not attenuate any statistically significant associations between the pesticide exposures and the NB outcome measures.

3.6. Sensitivity analyses

When we excluded the 57 participants with medical conditions or medications which may influence NB performance, we saw no difference in the results. However, when the eight individuals with a history of diagnosed pesticide poisoning were removed from the analyses, the parameter estimate of the association between ever ethoprop use and Digit-Symbol test performance was reduced from −3.66 s ($p=0.05$) to −3.19 s ($p=0.09$). No other meaningful changes in estimates of association were observed.

4. Discussion

This is one of the largest studies of NB function among OP pesticide-exposed workers published to date. The study included good characterization of specific pesticide use patterns and quantitative measures of NB function. Overall, among this sample of pesticide applicators, we found no consistent evidence of an adverse association with OP pesticide use. Specifically, ever-use or lifetime days of use of at least one OP pesticide was associated with significantly poorer performance on three of nine NB tests and with significantly better performance on six of nine tests. Given the large number of statistical tests performed and the limited number of significant associations, it is possible that some of our results were due to chance.

The three tests for which adverse associations were observed assessed visual scanning and processing (Digit-Symbol), verbal learning and memory (AVLT Total Recall), and motor speed and scanning (Sequences A). Our findings are somewhat comparable to the results of several previous studies of agricultural workers without previous pesticide poisoning. Rohlman et al. (2007) administered a battery of 10 NB tests to 119 Hispanic adults and adolescents working in agriculture and 56 Hispanic adult and adolescent referents. Statistically significantly poorer test performance was observed on four NB measures, including the Continuous Performance Test, among those with any experience mixing or applying pesticides. Farahat et al. (2003) studied 52 male workers occupationally exposed to OP pesticides and 50 unexposed male controls with similar demographic characteristics. After adjustment for age and education, workers occupationally exposed to OP pesticides performed significantly worse than unexposed workers on six of 12 NB tests, including Digit-Symbol and Trailmaking parts A and B (similar to Sequences A and B in the current study). Kamel et al. (2003) conducted a cross-sectional study of NB test performance among 288 farm workers with at least 1 month of farm work exposure and 51 controls without farm work exposure. Ever having done farm work was associated with poorer performance on four of eight NB tests including tests of verbal memory, motor speed and motor coordination.

Table 5
Regression coefficients from linear regression models for neurobehavioral outcome measures and cumulative lifetime days of pesticide use (log_{10} transformed) for 701 male pesticide applicators.

Pesticide	CPT[a] (n = 692)		Digit-Symbol[a] (n = 691)		Finger Tapping, dominant (n = 695)		Grooved Pegboard, dominant[a] (n = 699)		AVLT Total Recall (n = 696)		AVLT Delayed Recall (n = 695)		AVLT Recognition (n = 694)		Sequences A[a] (n = 679)		Sequences B[a] (n = 671)	
	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE
Organophosphates																		
Acephate	0.95	1.98	−1.13	1.00	−0.08	0.53	0.02	1.16	−0.07	0.22	0.12	0.15	−0.05	0.14	−0.86	0.68	−0.33	1.00
Chlorpyrifos	−0.29	1.74	−0.99	0.73	−0.22	0.39	1.77	0.85	0.26	0.19	**0.22***	0.11	0.06	0.10	−0.85	0.50	−0.49	0.72
Coumaphos	1.42	3.09	2.24	1.31	1.03	0.70	1.94	1.54	**0.82***	0.34	0.30	0.19	int	–	0.50	0.88	**3.05***	1.28
Diazinon	−0.67	1.96	−0.46	0.86	0.23	0.45	0.03	1.00	0.01	0.21	0.03	0.13	−0.02	0.12	−0.22	0.58	0.70	0.85
Dichlorvos	−0.63	1.82	0.13	0.82	−0.05	0.44	−0.28	0.96	0.30	0.20	0.16	0.12	0.13	0.11	0.55	0.55	0.60	0.80
Dimethoate	1.65	3.45	−0.79	1.46	−0.14	0.78	−0.51	1.71	0.39	0.37	0.07	0.21	0.37	0.20	1.37	0.98	−1.16	1.42
Disulfoton	3.08	2.72	−1.46	1.25	−0.76	0.66	−1.25	1.44	−0.23	0.30	0.19	0.18	−0.24	0.17	−1.52	0.85	0.28	1.25
Ethoprop	2.17	2.58	−1.81	1.19	0.05	0.63	−0.51	1.39	−0.60	0.28	−0.18	0.17	−0.27	0.16	**−1.65***	0.81	−0.97	1.18
Fonofos	0.78	2.02	0.41	0.94	0.08	0.50	0.85	1.09	−0.27	0.22	0.26	0.14	−0.05	0.13	−0.38	0.63	−0.53	0.91
Malathion	−0.90	1.80	**−1.75***	0.75	0.11	0.40	int	–	0.14	0.20	0.10	0.11	−0.10	0.10	−0.52	0.52	0.16	0.75
Parathion	**4.65***	2.32	0.35	0.99	−0.60	0.53	−0.11	1.16	0.10	0.25	**0.35***	0.15	0.20	0.14	0.24	0.67	0.03	0.97
Phorate	−0.25	1.93	0.82	0.83	−0.40	0.44	−0.50	0.97	0.18	0.21	0.21	0.12	**0.25***	0.12	−0.02	0.56	0.28	0.82
Phosmet	−1.47	2.68	−1.15	1.18	0.21	0.63	0.45	1.38	0.12	0.29	−0.16	0.17	−0.12	0.16	−0.11	0.79	−0.01	1.14
Tebupirimfos	−0.44	3.12	1.51	1.38	0.08	0.73	−1.13	1.61	0.34	0.34	0.20	0.20	0.00	0.19	−0.21	0.93	1.01	1.34
Terbufos	−0.22	1.60	0.48	0.70	0.65	0.37	−0.98	0.82	0.04	0.17	0.06	0.10	0.04	0.10	−0.65	0.48	−0.47	0.69
Tetrachlorvinphos	2.34	3.11	0.45	1.34	−0.77	0.72	−0.43	1.57	**0.76***	0.34	0.18	0.20	0.36	0.19	1.19	0.90	0.89	1.30
Carbamates																		
Aldicarb	1.05	2.27	1.12	1.06	−0.26	0.56	1.88	1.23	0.30	0.27	0.27	0.15	**0.30***	0.15	−0.04	0.72	0.72	1.06
Benomyl	2.59	2.72	2.13	1.22	−0.03	0.65	−0.01	1.42	0.60	0.31	**0.41***	0.18	0.27	0.17	**2.25****	0.82	1.34	1.20
Carbaryl	0.60	1.64	−0.04	0.84	−0.12	0.45	−0.79	0.98	0.21	0.22	**0.25***	0.12	0.04	0.12	−0.02	0.57	0.36	0.83
Carbofuran	1.56	1.97	0.68	0.83	0.63	0.44	1.50	0.96	0.20	0.21	0.21	0.12	0.05	0.11	−0.45	0.56	−0.52	0.81
Summary variables																		
ALL OPs	−0.95	2.17	−1.22	0.93	−0.16	0.49	−0.93	1.08	0.10	0.23	0.16	0.14	−0.05	0.13	−0.68	0.63	0.18	0.91
ALL pesticides	2.38	3.13	−1.45	1.33	0.42	0.70	−0.12	1.54	0.43	0.34	**0.54***	0.19	0.10	0.18	−1.26	0.90	−0.77	1.30

CPT = Continuous Performance Test, AVLT = Auditory Verbal Learning Test, int = significant interaction term for state by pesticide exposure; All models were adjusted for age (years) and ART score. In addition, CPT models were adjusted for positive affect score (PA) and caffeine consumption; Digit-Symbol models were adjusted for PA, education, state, and visual acuity score; Finger Tapping models were adjusted for PA and state; Grooved Pegboard models were adjusted for caffeine consumption, state and visual acuity score; AVLT Total Recall models were adjusted for negative affect score (NA), PA score, and education; AVLT Delayed Recall models were adjusted for NA, PA, education and state; AVLT Recognition models were adjusted for PA, education and state; Sequences A and B were adjusted for PA and state.

[a] Regression coefficients have been multiplied by −1 so that lower scores indicate poorer performance.

* $p<0.05$.

** $p<0.01$

Although a number of studies have reported significant associations between pesticide exposure and NB test performance (e.g., Farahat et al., 2003; Kamel et al., 2003; Mackenzie Ross et al., 2010; Rohlman et al., 2007; Rothlein et al., 2006; Stephens et al., 1995), others have been essentially null. For example, no consistent associations between pesticide exposure and neurobehavioral outcomes were observed among 163 fruit farm spray operators when compared to 84 non-spraying laborers in South Africa (London et al., 1997). Similarly, a recent study of 191 current and former chlorypyrifos-exposed termiticide applicators and 189 unexposed referents found little difference in neurobehavioral measures across the exposure groups (Steenland et al., 2000). Additional examples of essentially

Table 6
Regression coefficients from linear regression models for neurobehavioral outcome measures and pesticide exposures with an interaction term for state by pesticide exposure for the 701 male pesticide applicators.

	CPT[a] (n = 692)		Digit-Symbol[a] (n = 691)		Grooved Pegboard[a] (n = 699)		AVLT Recognition (n = 695)		Sequences B[a] (n = 671)	
	Iowa	N.C.	Iowa	N.C.	Iowa	N.C.	Iowa	N.C.	Iowa	N.C.
Ever used										
Carbaryl	6.60	**−14.52***	–	–	–	–	–	–	–	–
Carbofuran	–	–	–	–	–	–	–	–	2.99	**−3.78***
Chlorpyrifos	–	–	**−4.67****	1.06	–	–	–	–	–	–
Malathion	–	–	–	–	**5.85***	−3.98	–	–	–	–
Lifetime days (log_{10} transformed)										
Coumaphos	–	–	–	–	–	–	0.03	**1.09****	–	–
Malathion	–	–	–	–	**2.46***	−1.11	–	–	–	–

CPT = Continuous Performance Test, AVLT = Auditory Verbal Learning Test. Results are presented for models with a significant interaction term ($p<0.05$); Models are adjusted for the base model covariates and an interaction term for state by pesticide exposure.

[a] Regression coefficients have been multiplied by −1 so that lower scores indicate poorer performance.

* $p<0.05$.

** $p<0.01$.

negative studies are provided in recent reviews (Colosio et al., 2009; Rohlman et al., 2011). Of course, in addition to numerous methodological differences, effect size variability across studies may be due to differences in total delivered pesticide dose. In the absence of a readily available biological indicator of lifetime pesticide dose delivered to the nervous system, differences in dose may be an important, but currently unknown, source of variability across studies.

The predominantly null results of our study may be due to the possibility that the sample was highly selected. Although we randomly sampled from the AHS cohort, we required individuals to have completed all AHS questionnaires, and we excluded individuals with a number of health conditions. It is possible that individuals who left farming or who were unable to complete the AHS questionnaires may have been more affected by pesticides than those who were eligible and participated in the current study. Furthermore, the average age of our population was older compared with most previous study populations. An older cohort is more likely to manifest selective survival than a younger cohort. This potential selection bias may have attenuated the observed associations between long-term pesticide use and NB outcomes. Because healthy older individuals are also likely to have used pesticides for longer, it may also have created the appearance of an association between pesticide use and improved NB test performance. Additionally our study did not capture the full range of OP exposures in the AHS cohort, as our sample sites were limited and thus, we did not include all geographic regions. For example, orchard growers are generally limited to the western part of North Carolina which was outside our sampling area. There are unique OPs and application methods used in orchards and we were limited in our ability to assess these. In addition, the overall response rate of the study was slightly less than 40%, further suggesting that our study sample may not have been representative of all pesticide applicators enrolled in the AHS. However, participants were similar to non-participants on several important characteristics including age and total lifetime days of pesticide use suggesting that the groups were comparable.

Several studies have reported a positive association between long-term OP pesticide exposure and neurological symptom prevalence among agricultural workers (Bazylewicz-Walczak et al., 1999; Kamel et al., 2007; London et al., 1998; Ohayo-Mitoko et al., 2000; Smit et al., 2003). Kamel et al. examined associations between long-term pesticide use and self-reported neurological symptoms among 18,782 licensed pesticide applicators enrolled in the AHS (Kamel et al., 2007). They reported associations between neurological symptoms and cumulative exposure to OP pesticides among pesticide applicators with no history of previous pesticide poisoning. While seemingly inconsistent with our findings, individuals may experience subtle differences in neurological function and report symptoms before dysfunction can be identified with objective measures (Kamel and Hoppin, 2004).

We also observed several statistically significant interactions between state and specific chemical use. The explanation for these interactions is unclear. However, the difference in findings observed between Iowa and North Carolina participants may be due to uncaptured differences in pesticide use and application methods. For example, chlorpyrifos is more often used in a granular form in Iowa and more often used in a liquid form in North Carolina (Thomas et al., 2010); thus, state differences may be indicative of different patterns of exposure.

One of the strengths of the AHS is the detailed pesticide exposure assessment, which allowed us to create relatively precise exposure estimates. Whereas most studies in the literature have used dichotomized exposure variables, we estimated cumulative lifetime days of use to specific OP pesticides, as well as to this class as a whole, for each study participant. In addition to cumulative lifetime days of use, we also created a metric of ever use of specific OP pesticides. Because it is unlikely that a farmer would apply a specific pesticide only one day in his lifetime, a farmer's ever use of a specific pesticide likely represents at least one season of use.

Although misclassification of pesticide use is possible, methodological studies have shown that AHS participants provide accurate and reliable pesticide use and duration of exposure information (Blair et al., 2002; Hoppin et al., 2002). Using pesticide registration information, Hoppin et al. showed that AHS participants provide plausible data regarding lifetime duration of use, with fewer than 5% reporting implausible values for specific chemicals (Hoppin et al., 2002). Related findings were reported by Blair et al. (2002) who found that for repeated interviews of AHS participants the percentage agreement for specific pesticide use and application practices were high, ranging from 70% to more than 90%.

A major strength of the study is that it was based on a large sample of pesticide applicators selected from the AHS. The sample included pesticide applicators from two distinct geographical locations with different crops and farming practices. Therefore, the results of the present study are likely relevant to a large segment of the farming population. Unlike many prior studies, we had sufficient power to examine the associations of individual pesticides with NB outcomes while controlling for important covariates. However, we were not well powered for examination of interactions, so for those associations that differed between states or for those pesticides used in only one state, we had limited power.

In conclusion, organophosphate pesticide use was not consistently associated with adverse NB test performance among this older sample of pesticide applicators. While specific associations may have been due to chance, some of the findings are consistent with previous studies. Potential reasons for these mostly null results include a true absence of effect as well as possible selective participation by healthier applicators.

Conflict of interest statement

We declare that we do not have any competing interest.

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Appendix A

Neurobehavioral outcome measures

We administered the Finger Tapping and Grooved Pegboard tests separately for the dominant and non-dominant hands. However, because the overall results were similar for both hands, only the results for the dominant hand are presented. Some study participants were unable to complete individual tests in the allowed time or after two attempts, therefore, the total number of participants completing each test varied. Descriptive summary statistics for the NB test results are presented in Table 1, Appendix A. Comparison of values obtained in the current study to those of studies using NES II or NES III tests are presented in Table 2, Appendix A.

Table 1
Descriptive summary statistics of neurobehavioral outcome measures of 701 male pesticide applicators in the Agricultural Health Study.

Outcome	N	Mean	SD	Min.	Median	Max.
Continuous performance (ms)	693	427.9	44.9	318.6	421.2	612.3
Digit-Symbol (s)	692	117.6	23.1	73.6	112.1	213.6
Finger tapping, dominant hand (# of taps)	695	53.6	9.6	9.0	55.0	86.0
Grooved pegboard, dominant hand (s)	700	92.0	24.0	51.0	86.0	180.0
Auditory verbal learning total recall (# correct)	696	19.9	5.1	5.0	20.0	34.0
Auditory verbal learning delayed recall (# correct)	695	6.6	2.8	0	7.0	12.0
Auditory verbal learning recognition (tp-fp)	694	8.3	2.6	−3.0	9.0	12.0
Sequences A latency (s)	680	42.9	14.6	14.8	40.3	93.8
Sequences B latency (s)	672	64.6	21.2	22.8	59.5	144.4

ms = milliseconds, s = seconds, # = number, tp = true positives, fp = false positives.

Table 2
Comparison of neurobehavioral measures of pesticide applicators in the Agricultural Health Study to results of other published studies using identical measures.

Test	Reference	Study population; mean age	N	Mean	SD
Continuous Performance Test (ms)	Present study	Male pesticide applicators; 61 years	693	427.9	44.9
	Letz et al., 1996	Male, U.S. Army Veterans; 40 years	757	352.2	35.7
Digit-Symbol (s)	Present study	Male pesticide applicators; 61 years	692	117.6	23.1
	White et al., 2003	Non-cognitively impaired; 46 years	66	91.5	20.9
	Letz et al., 2003	Neurology clinic patients; 44 years	299	137.8	40.4
	Gerr, 2011 (unpublished)	Rural community residents; 57 years	686	115.0	34.9
Finger tapping, dominant (# of taps)	Present study	Male pesticide applicators; 61 years	695	53.6	9.6
	White et al., 2003	Non-cognitively impaired; 46 years	66	71.9	10.2
	Frumkin et al., 2001	Male industrial workers; 49 years	95	*51.2	8.2
Grooved pegboard, dominant (s)	Present study	Male pesticide applicators; 61 years	700	92.0	24.0
	Letz et al., 2000	Industrial plant workers; 71 years	82	93.5	25.2
	Letz et al., 1996	Male, U.S. Army Veterans; 40 years	738	71.6	11.0
Verbal learn total recall (#correct)	Present study	Male pesticide applicators; 61 years	696	19.9	5.1
	Letz et al., 2003	Neurology clinic patients; 44 years	311	20.9	5.9
	Gerr, 2011 (unpublished)	Rural community residents; 57 years	687	23.2	6.4
Verbal learn delayed recall (#correct)	Present study	Male pesticide applicators; 61 years	695	6.6	2.8
	Letz et al., 2003	Neurology clinic patients; 44 years	311	6.9	3.3
Sequences A (s)	Present study	Male pesticide applicators; 61 years	680	42.9	14.6
	Letz et al., 2003	Neurology clinic patients; 44 years	300	26.6	11.8
	Letz et al., 2000	Industrial plant workers; 71 years	83	41.8	16.3
	Gerr, 2011 (unpublished)	Rural community residents; 57 years	694	38.4	15.4
Sequences B (s)	Present study	Male pesticide applicators; 61 years	672	64.6	21.2
	Letz et al., 2003	Neurology clinic patients; 44 years	297	47.8	24.2
	Letz et al., 2000	Industrial plant workers; 71 years	83	90.5	32.0
	White et al., 2003	Non-cognitively impaired; 46 years	66	42.1	19.2
	Gerr, 2011 (unpublished)	Rural community residents; 57 years	694	53.9	23.5

*Calculated for 10 second trial used in present study.

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