

## Original Investigation

# Association of Environmental Toxins With Amyotrophic Lateral Sclerosis

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**IMPORTANCE** Persistent environmental pollutants may represent a modifiable risk factor involved in the gene-time-environment hypothesis in amyotrophic lateral sclerosis (ALS).

**OBJECTIVE** To evaluate the association of occupational exposures and environmental toxins on the odds of developing ALS in Michigan.

**DESIGN, SETTING, AND PARTICIPANTS** Case-control study conducted between 2011 and 2014 at a tertiary referral center for ALS. Cases were patients diagnosed as having definitive, probable, probable with laboratory support, or possible ALS by revised El Escorial criteria; controls were excluded if they were diagnosed as having ALS or another neurodegenerative condition or if they had a family history of ALS in a first- or second-degree blood relative. Participants completed a survey assessing occupational and residential exposures. Blood concentrations of 122 persistent environmental pollutants, including organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and brominated flame retardants (BFRs), were measured using gas chromatography-mass spectrometry. Multivariable models with self-reported occupational exposures in various exposure time windows and environmental toxin blood concentrations were separately fit by logistic regression models. Concordance between the survey data and pollutant measurements was assessed using the nonparametric Kendall  $\tau$  correlation coefficient.

**MAIN OUTCOMES AND MEASURES** Occupational and residential exposures to environmental toxins, and blood concentrations of 122 persistent environmental pollutants, including OCPs, PCBs, and BFRs.

**RESULTS** Participants included 156 cases (mean [SD] age, 60.5 [11.1] years; 61.5% male) and 128 controls (mean [SD] age, 60.4 [9.4] years; 57.8% male); among them, 101 cases and 110 controls had complete demographic and pollutant data. Survey data revealed that reported pesticide exposure in the cumulative exposure windows was significantly associated with ALS (odds ratio [OR] = 5.09; 95% CI, 1.85-13.99;  $P = .002$ ). Military service was also associated with ALS in 2 time windows (exposure ever happened in entire occupational history: OR = 2.31; 95% CI, 1.02-5.25;  $P = .046$ ; exposure ever happened 10-30 years ago: OR = 2.18; 95% CI, 1.01-4.73;  $P = .049$ ). A multivariable model of measured persistent environmental pollutants in the blood, representing cumulative occupational and residential exposure, showed increased odds of ALS for 2 OCPs (pentachlorobenzene: OR = 2.57; 95% CI, 1.31-5.02;  $P = .006$ ; and *cis*-chlordane: OR = 6.51; 95% CI, 2.05-20.73;  $P = .002$ ) and 1 PCB (PCB 151: OR = 1.66; 95% CI, 1.03-2.67;  $P = .04$ ). There was modest concordance between survey data and the measurements of persistent environmental pollutants in blood; significant Kendall  $\tau$  correlation coefficients ranged from  $-0.18$  (Dacthal and "use pesticides to treat home or yard") to  $0.24$  (*trans*-nonachlor and "store lawn care products in garage").

**CONCLUSIONS AND RELEVANCE** In this study, persistent environmental pollutants measured in blood were significantly associated with ALS and may represent modifiable ALS disease risk factors.

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**A**myotrophic lateral sclerosis (ALS) is a progressive, fatal neurodegenerative disease.<sup>1</sup> Toxic exposures, combined with genetic susceptibility, may trigger motor neuron degeneration explained via the gene-time-environment hypothesis.<sup>2</sup>

In Michigan, geographic variations in ALS death rates may correspond to locations of environmental toxins identified by the US Environmental Protection Agency's Toxics Release Inventory<sup>3</sup> and National Priorities List.<sup>4</sup> We hypothesized that toxic exposures are more likely to be identified in patients with ALS compared with healthy controls. Organochlorine pesticides (OCPs),<sup>5</sup> polychlorinated biphenyls (PCBs; electrical insulation),<sup>6</sup> and polybrominated diphenyl ethers (PBDEs; brominated flame retardant [BFRs])<sup>7,8</sup> are of particular interest as potential ALS risk factors owing to known neurotoxic properties and high persistence in the environment and body.

In 2011, we initiated a case-control study to evaluate ALS environmental risk factors with the strength of combining assessments of environmental pollutants in blood with detailed exposure reporting. Importantly, the ability to validate self-reported exposures with biospecimens is necessary for large-scale efforts identifying ALS risk factors. We previously showed that reported pesticide exposure is associated with ALS.<sup>9</sup> This article extends and advances our previous work by incorporating blood measurements of persistent organic pollutants in conjunction with reported exposures.

## Methods

### Study Populations and Data Collection

This case-control study was conducted between 2011 and 2014. Cases and controls were older than 18 years and able to communicate in English. All patients with ALS meeting inclusion criteria with definitive, probable, probable with laboratory support, or possible ALS by revised El Escorial criteria<sup>10</sup> were asked to participate during visits at the University of Michigan (UM) ALS Clinic; recruitment announcements were also sent via the National ALS Registry. Controls, recruited through postings and a UM clinical research volunteer database (<https://umclinicalstudies.org/>), with a neurodegenerative condition, ALS, or family history of ALS in a first- or second-degree blood relative were excluded. The study received approval from the UM institutional review board. All participants provided written informed consent. Controls received compensation.

Participants completed a self-administered written survey, derived from Agency for Toxic Substances and Disease Registry instruments,<sup>11</sup> assessing residential history, occupational history, military service, smoking history, and demographic characteristics. Questionnaires were distributed in person or via mail. Telephone follow-up clarified responses as needed; next of kin was contacted for patients with communication impairments. Double data entry was performed for quality assurance in a random sample of surveys. Blood samples were collected for measurements of pollutants.

### Data Types

Demographic characteristics included age, sex, ethnicity, education, marital status, tobacco history, and military service his-

## Key Points

**Question** What is the role of environmental toxins on the risk of developing amyotrophic lateral sclerosis (ALS)?

**Findings** In this case-control study measuring concentrations of persistent environmental pollutants in blood involving 156 patients with ALS and 128 controls, pesticides were found to be an ALS risk factor based on both self-reported and measured exposures.

**Meaning** Pesticides and other persistent environmental pollutants may represent modifiable ALS disease risk factors.

tory. Job titles, workplace, dates of employment, and 22 exposure-related questions assessed occupational risk factors in each job (2 most recent and 2 longest-held jobs; eAppendix in the [Supplement](#)). This article focuses on occupational exposures, from which 98 candidate covariates were derived from exposure-related questions, including job titles and workplace settings for each job. Specifically, 58 identified exposure risk factors, 20 occupational groups, and 20 industrial groups were queried for each job. Residential exposures were assessed using 54 questions for the current house and 18 for the house where the participant was born and the 2 houses where the participant lived the longest (eAppendix in the [Supplement](#)). Surveys with more than 70% missing responses were excluded from further analyses.

Blood samples measured 122 persistent neurotoxic organic pollutants (OCPs, PCBs, BFRs) using National Health and Nutrition Examination Survey and National Human Exposure Assessment Survey methods (eAppendix in the [Supplement](#)).<sup>12</sup> These compounds had wide use and existing quality-assured measurement methods.<sup>5-7</sup> Owing to a protocol change, some participants provided whole blood, whereas others provided plasma samples. Partition coefficients between plasma and whole blood for all measured compounds (eTable 1 in the [Supplement](#)) were determined in a small study involving 21 volunteers (data not shown) and used to convert whole-blood concentrations to plasma concentrations (eAppendix in the [Supplement](#)). Compounds analyzed had a detection frequency greater than 30% (eTable 1 in the [Supplement](#)).

### Exposure Time Windows

Four exposure time windows categorized past occupational exposures: window 1 indicates exposure occurring in at least 1 period during the entire job history; windows 2, 3, and 4 indicate exposures in the last 10 years, 10 to 30 years ago, and more than 30 years ago, respectively. Reference dates for cases and controls were symptom onset and survey consent date, respectively. Occupational exposure variables in each job were assigned to the appropriate time windows. An individual could be exposed in multiple time windows depending on job history.

### Job-Exposure Matrix for Occupational Pesticide Exposures

Lifetime cumulative occupational exposures to pesticides were derived from a job-exposure matrix developed using the participants' reported job titles and workplace. The probability ( $0 \leq$

$P \leq 1$ ) and intensity (scored from 0 [lowest] to 4 [highest]) of exposure were estimated for each occupation and industry group based on previous studies<sup>13,14</sup> and judgments of 2 experienced exposure scientists (F.-C.S. and S.B.). Exposure scores equaled the product of probability and intensity and were mapped to 4 levels (scores = 0, 1, 2, and 3 for probability-intensity products of 0, 1-2, 3-6, and  $\geq 7$ , respectively; eTable 2 in the [Supplement](#)). The lifetime cumulative exposure was estimated by weighting exposure scores by job duration.<sup>15</sup>

### Statistical Analysis

Descriptive analyses, including central tendency and dispersion, examined demographic characteristics, smoking status, and exposure to pollutants by case type. We used *t* tests for continuous variables and  $\chi^2$  tests for categorical variables to examine differences between cases and controls. The normality of pollutant data was evaluated using Kolmogorov-Smirnov tests and stratified by case type. Spearman rank-order correlations assessed correlations between pollutants within chemical groups.

Multivariable logistic regression models tested associations between ALS and reported occupational exposures. Variable selection used a 3-step method. First, the 98 candidate covariates in window 1 were fitted into a logistic regression model using the stepwise method to select potential risk factors. Next, identified factors, including smoking status and military service,<sup>16,17</sup> were incorporated into a model with adjustments for age, sex, and education levels. Finally, the stepwise-selected risk factors with the largest *P* values were sequentially excluded from the model until all remaining stepwise-selected risk factors were significant or marginally significant ( $P \approx .05$ ). The same covariates were used in each exposure time window. Hosmer-Lemeshow tests examined goodness of fit, with the null hypothesis representing that observed and predicted values of the dependent variable were not different.

Associations between ALS and pollutants were tested using 2 approaches. First, single-pollutant logistic regression models provided an initial screen of the pollutant's association. The Bonferroni correction was applied to adjust the critical value (ie,  $\alpha/\text{number of tests} = .05/28$ ), which tends to increase the chance of false-negative results. Tests of individual chemicals increase the likelihood of significant results by chance alone because the correction is conservative as a result of multiple comparisons<sup>18</sup> and does not reflect effects of multiple exposures; therefore, the second approach used a multiple-pollutant logistic regression model. This stepwise variable selection method accounts for multicollinearity occurring due to high correlations between pollutants. Both models adjusted for age, sex, and educational level and used standardized pollutant levels (obtained by subtracting the mean and dividing by the standard deviation).

The nonparametric Kendall  $\tau$  correlation coefficient, which measures agreement between rankings of 2 ordinal variables, examined concordance between self-reported risks and occupational pollutant concentrations. The OCP concentrations were first partitioned into 5 groups, from the lowest to the highest levels, and were displayed in a matrix plot of the correlation coefficients. Because pollutant measurements integrate exposures

that may occur in both occupational and residential settings, the survey questions regarding potential residential exposures that might account for OCP exposure were also tested.

Missing responses and exposure measurements underwent multiple imputation, provided the missing rate was less than 30%. Ten imputations for incomplete values were obtained using the Markov chain Monte Carlo method.<sup>19</sup> Both observed and imputed data sets were tested. Comparisons between the observed and imputed data sets are found in eTable 3, eTable 4, eTable 5, eTable 6, and eTable 7 in the [Supplement](#).

Logistic and linear regression analyses and multiple imputations were performed in SAS version 9.2 statistical software (SAS Institute, Inc). The correlation plots used the package "corrplot" in R version 3.2.1 statistical software (R Foundation for Statistical Computing). A flowchart for the data analysis is shown in the eFigure in the [Supplement](#).

## Results

### Descriptive Analyses

One hundred fifty-six patients with ALS (mean [SD] age, 60.5 [11.1] years; 61.5% male) and 128 controls (mean [SD] age, 60.4 [9.4] years; 57.8% male) were recruited (66 cases and controls were described in our previous survey-only study<sup>9</sup>). Age, sex, educational level, smoking status, and occupational risk factor information was complete for 126 cases and 118 controls. Pollutant measurements were performed on participants who provided blood (129 cases and 119 controls). Together, 101 cases and 110 controls had complete demographic and pollutant data.

Demographic characteristics are summarized in [Table 1](#). Twenty-eight of the 122 persistent organic pollutants had detection frequencies higher than 30% (eTable 1 in the [Supplement](#)).

### Occupational ALS Risk Factors by Exposure Time Window

Of the 98 candidate occupational risk factor variables in each time window, a stepwise logistic regression model identified 14 risk factor variables meeting significance levels for entering and removing effects of  $P = .30$  and  $P = .35$ , respectively. The question "Did you get material on your skin or clothing?" was excluded owing to a missing rate higher than 70%. When including smoking status and military service into the model and adjusting for age, sex, and educational levels for each window, the following 6 occupational risk factors with  $P < .05$  composed the final model: (1) lead exposure; (2) pesticide exposure; (3) working in the US Armed Forces; (4) working in health care or social assistance; (5) working in accommodation (ie, lodging) or food services; and (6) working in public administration ([Table 2](#)). Goodness-of-fit tests for the 4 models did not indicate differences between observed and predicted values ( $P > .05$ ). Survey data revealed that reported pesticide exposure in the cumulative exposure windows was significantly associated with ALS (odds ratio [OR] = 5.09; 95% CI, 1.85-13.99;  $P = .002$ ). Military service was also associated with ALS in 2 time windows (exposure ever happened in entire occupational history: OR = 2.31; 95% CI, 1.02-5.25;  $P = .046$ ; exposure ever happened 10-30 years ago: OR = 2.18; 95% CI, 1.01-4.73;  $P = .049$ ).

**Table 1. Demographic Characteristics and Smoking Status for Cases With Amyotrophic Lateral Sclerosis and Controls Using Observed Data**

Variable	Cases	Controls	P Value <sup>a</sup>
Age, y	(n = 156)	(n = 128)	
Mean (SD)	60.5 (11.1)	60.4 (9.4)	.95
Median (range)	61 (28-86)	60 (40-81)	
Sex, No. (%)	(n = 156)	(n = 128)	
Female	60 (38.5)	54 (42.2)	.52
Male	96 (61.5)	74 (57.8)	
Distance from UM, No. (%)	(n = 156)	(n = 128)	
<20 km	45 (28.9)	90 (70.3)	<.001
≥20 km	111 (71.2)	38 (29.7)	
Education, No. (%)	(n = 128)	(n = 119)	
<Bachelor's degree	81 (63.3)	47 (39.5)	<.001
≥Bachelor's degree	47 (36.7)	72 (60.5)	
Smoking status, No. (%)	(n = 129)	(n = 120)	
Nonsmoker	60 (46.5)	53 (44.2)	.72
Former smoker	56 (43.4)	51 (42.5)	
Current smoker	13 (10.1)	16 (13.3)	

Abbreviation: UM, University of Michigan.

<sup>a</sup> Calculated by *t* test for continuous variables and  $\chi^2$  test for categorical variables.

### Chemical Concentrations in Blood and ALS

Fitted models using single pollutants are shown in **Figure 1** (imputed data) and eTable 3 in the **Supplement** (observed data). Figure 1 depicts ORs for 28 chemicals. Using the stepwise selection with imputed data, 10 compounds were selected into the multiple-compound model, including 4 OCPs, 3 PCBs, and 3 BFRs (Figure 1 and **Table 3**); 6 were significantly associated with ALS (3 OCPs, 2 PCBs, and 1 BFR). Increased odds of ALS were found with 2 OCPs (pentachlorobenzene: OR = 2.57; 95% CI, 1.31-5.02; *P* = .006; and *cis*-chlordane: OR = 6.51; 95% CI, 2.05-20.73; *P* = .002) and 1 PCB (PCB151: OR = 1.66; 95% CI, 1.03-2.67; *P* = .04). Odds of ALS were decreased with 1 OCP (Dacthal: OR = 0.34; 95% CI, 0.15-0.79; *P* = .01), 1 PCB (PCB 202: OR = 0.47; 95% CI, 0.25-0.87; *P* = .02) and 1 BFR (PBDE 66: OR = 0.57; 95% CI, 0.33-0.99; *P* = .047). Results of single- and multiple-compound models were similar, which may be explained by low to moderate correlation between most pairs of compounds (Spearman correlation coefficients were mostly below 0.5 among the PCB, BDE, pesticide, and other compounds). A number of compound pairs within the PCB or the BDE groups had higher correlation, eg, for compounds selected in the final models that were statistically significant, PCB 202 and PCB 175 had the highest correlation (Spearman correlation coefficient of 0.65).

### Concordance Between Survey Data and Pollutant Exposures

As both the survey and exposure measurements showed significant associations between pesticide exposures and ALS, we considered the concordance of these independent data sets. Kendall  $\tau$  correlation coefficients (**Figure 2**) showed weak agreement between OCP concentrations and survey-based cumulative occupational exposure to pesticides. The use of occupational and residential variables provided modest correlation with measurements of environmental pollutants, suggesting that pesticide exposure in the study population occurred in both occupational and residential/environmental settings and that survey data may help indicate the exposure source. Sig-

nificant Kendall  $\tau$  correlation coefficients ranged from  $-0.18$  (Dacthal and “use pesticides to treat home or yard”) to  $0.24$  (*trans*-nonachlor and “store lawn care products in garage”).

### Sensitivity Analysis in a Smaller Geographic Region

Given the geographic variation between cases and controls, a subgroup analysis on participants who lived less than 70 km from UM was performed. Fifty-three cases and 90 controls were selected to balance sample size and geographic matching. Single-pollutant logistic regression models for all participants and this subgroup showed high agreement for OCPs, PCBs, and BFRs (5 OCPs, 4 PCBs, and 1 BFR showed significant associations with ALS before Bonferroni correction; eTable 3 in the **Supplement**). Therefore, geographic variation between the cases and controls was a limited confounder.

## Discussion

The gene-time-environment hypothesis supports the contention that toxic exposures combined with genetic susceptibility may trigger motor neuron degeneration and ALS.<sup>2</sup> However, most studies assessing ALS environmental and occupational risks use surveys and are limited by recall bias and exposure misclassification.<sup>20</sup> Our study used both surveys and measurements of pollutants in blood. Notably, the survey used assesses exposure windows to identify periods of susceptibility for developing ALS to help clarify exposure-time interaction effects. Exposure to critical risk factors during such periods may have a greater effect than during other periods,<sup>21</sup> but other than in our previous study,<sup>9</sup> windows of ALS susceptibility have not been investigated to our knowledge.

We report that service in the US military is statistically or marginally significant in multiple exposure windows, agreeing with reports that military personnel are high-risk populations for ALS.<sup>17,22,23</sup> While triggers remain unknown,<sup>17</sup> sus-

**Table 2. Identified Occupational Risk Factors of Amyotrophic Lateral Sclerosis for 4 Exposure Time Windows**

Variable	OR (95% CI) <sup>a</sup>	
	Imputed	Observed
<b>Exposure Ever Happened in Entire Occupational History</b>		
Age, y	1.00 (0.97-1.03)	1.00 (0.97-1.04)
Male	0.76 (0.37-1.56)	0.80 (0.37-1.72)
Educational level ≥college	0.30 (0.17-0.54) <sup>b</sup>	0.28 (0.15-0.51) <sup>b</sup>
Smoker		
Current	1.06 (0.33-3.43)	0.72 (0.25-2.07)
Former	0.79 (0.35-1.78)	0.85 (0.46-1.57)
Ever worked in US Armed Forces	2.31 (1.02-5.25) <sup>c</sup>	2.20 (0.95-5.10)
Occupational exposure		
Lead	0.38 (0.15-0.94) <sup>c</sup>	0.32 (0.13-0.81) <sup>c</sup>
Pesticides	5.09 (1.85-13.99) <sup>b</sup>	5.46 (2.00-14.88) <sup>b</sup>
Industry		
Health care or social assistance	0.35 (0.17-0.74) <sup>b</sup>	0.38 (0.18-0.81) <sup>c</sup>
Accommodation or food services	0.23 (0.07-0.77) <sup>c</sup>	0.19 (0.06-0.60) <sup>b</sup>
Public administration	0.38 (0.14-1.04)	0.33 (0.12-0.88) <sup>c</sup>
<b>Exposure Ever Happened in Last 10 y</b>		
Age, y	1.00 (0.97-1.03)	1.00 (0.97-1.04)
Male	1.00 (0.52-1.94)	1.13 (0.55-2.32)
Educational level ≥college	0.32 (0.18-0.57) <sup>b</sup>	0.31 (0.17-0.56) <sup>b</sup>
Smoker		
Current	0.92 (0.30-2.82)	0.65 (0.23-1.79)
Former	0.83 (0.37-1.84)	0.83 (0.45-1.53)
Ever worked in US Armed Forces	2.00 (0.93-4.31)	1.76 (0.79-3.89)
Occupational exposure		
Lead	1.42 (0.45-4.53)	1.31 (0.41-4.14)
Pesticides	5.50 (1.40-21.52) <sup>c</sup>	6.18 (1.64-23.35) <sup>b</sup>
Industry		
Health care or social assistance	0.54 (0.25-1.18)	0.58 (0.26-1.28)
Accommodation or food services	0.29 (0.06-1.28)	0.30 (0.06-1.45)
Public administration	1.41 (0.21-9.39)	1.74 (0.26-11.79)
<b>Exposure Ever Happened 10-30 y Ago</b>		
Age, y	1.00 (0.97-1.03)	1.00 (0.97-1.03)
Male	0.94 (0.48-1.85)	0.99 (0.48-2.05)
Educational level ≥college	0.33 (0.18-0.58) <sup>b</sup>	0.33 (0.19-0.60) <sup>b</sup>
Smoker		
Current	0.91 (0.29-2.89)	0.60 (0.22-1.67)
Former	0.85 (0.39-1.85)	0.88 (0.48-1.61)
Ever worked in US Armed Forces	2.18 (1.01-4.73) <sup>c</sup>	2.34 (1.04-5.28) <sup>c</sup>
Occupational exposure		
Lead	0.62 (0.24-1.61)	0.59 (0.22-1.59)
Pesticides	4.85 (1.56-15.03) <sup>b</sup>	4.53 (1.44-14.23) <sup>c</sup>
Industry		
Health care or social assistance	0.63 (0.31-1.32)	0.64 (0.29-1.41)
Accommodation or food services	0.23 (0.05-0.96) <sup>c</sup>	0.24 (0.06-1.01)
Public administration	0.31 (0.09-1.12)	0.27 (0.08-0.95) <sup>c</sup>

(continued)

**Table 2. Identified Occupational Risk Factors of Amyotrophic Lateral Sclerosis for 4 Exposure Time Windows (continued)**

Variable	OR (95% CI) <sup>a</sup>	
	Imputed	Observed
<b>Exposure Ever Happened &gt;30 y Ago</b>		
Age, y	1.01 (0.98-1.04)	1.01 (0.98-1.05)
Male	1.11 (0.57-2.17)	1.20 (0.57-2.54)
Educational level ≥college	0.33 (0.18-0.58) <sup>b</sup>	0.28 (0.15-0.53) <sup>b</sup>
Smoker		
Current	0.73 (0.24-2.23)	0.33 (0.11-1.02)
Former	0.88 (0.41-1.89)	0.71 (0.38-1.32)
Ever worked in US Armed Forces	2.05 (0.93-4.51)	2.08 (0.89-4.83)
Occupational exposure		
Lead	0.51 (0.18-1.44)	0.46 (0.16-1.36)
Pesticides	3.30 (1.08-10.02) <sup>c</sup>	2.91 (0.91-9.31)
Industry		
Health care or social assistance	0.93 (0.36-2.40)	0.85 (0.33-2.21)
Accommodation or food services	0.11 (0.01-0.94) <sup>c</sup>	0.11 (0.01-0.92) <sup>c</sup>
Public administration	0.46 (0.15-1.41)	0.43 (0.14-1.34)

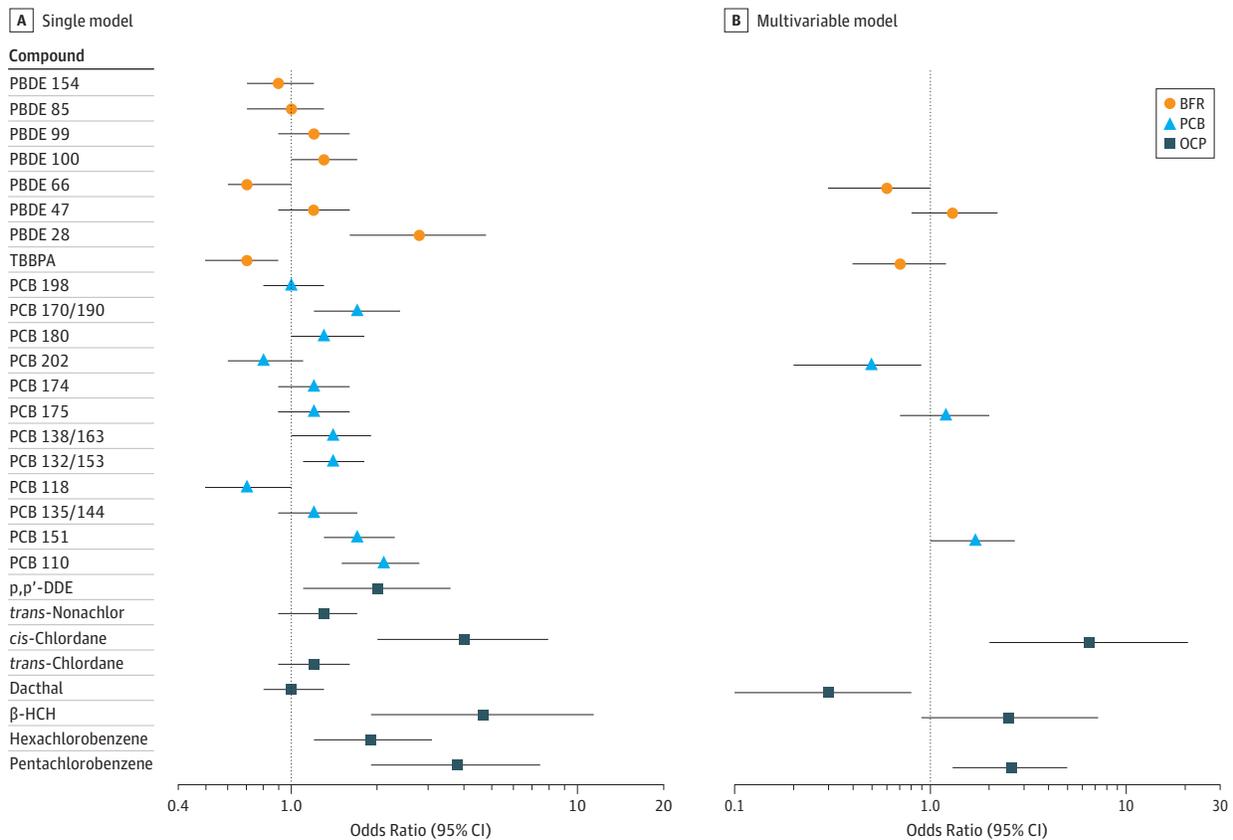
Abbreviation: OR, odds ratio.

<sup>a</sup> For imputed data, the sample size was 284 participants (156 cases and 128 controls) for 10 imputations; for observed data, the sample size was 244 participants (126 cases and 118 controls).<sup>b</sup>  $P < .01$ .<sup>c</sup>  $P < .05$ .

pected risk factors include environmental exposures, multiple vaccinations, physical activity, and traumatic injury.<sup>24,25</sup> Similar to our initial report of survey data,<sup>9</sup> we again found associations of ALS with reported occupational pesticide use and lower educational attainment. Unexpectedly, lead showed a statistically significant protective effect across the entire occupational history. Lead has received extensive attention as an ALS risk factor<sup>26</sup> and may increase survival,<sup>27</sup> indicating this topic deserves further attention.

Associations between persistent organic pollutants and ALS are strengthened using biological exposure measurements. Prior studies used a small set of exposure measurements to assess ALS risk. Our approach of testing a large number of chemicals contrasts to studies testing single compounds. Applying multipollutant models, 6 of 10 model-selected compounds were significantly associated with ALS (3 OCPs, 2 PCBs, and 1 BFR). Results between the single-compound models and multiple-compound model were similar and agree with studies demonstrating an increased ALS-pesticide association.<sup>28-31</sup> Surprisingly, 3 chemicals (Dacthal, PCB 202, and PBDE 66) showed protective effects. The positive association between ALS and these 3 compounds may result from correlations among co-existent chemicals and weak associations between the chemicals themselves and the disease. The analysis of a large number of pollutants and other exposure metrics can increase the likelihood of such effects as well as false associations resulting from multiple comparisons. The confidence in our results, particularly for OCPs, is increased by the agreement between pollutant and survey data.

Figure 1. Results of Logistic Regression Models for Amyotrophic Lateral Sclerosis and Exposure Pollutants Using Single and Multiple Chemicals



Odds ratios and 95% CIs were examined using standardized chemical concentrations from the imputed sample (n = 284, 10 imputations) and adjusted for age, sex, and educational levels. A, Single model shows the results of 28 regression models for individual compounds. B, Multivariable model shows the results of 1 regression model for 10 selected compounds. β-HCH

indicates β-hexachlorocyclohexane; BFR, brominated flame retardant; OCP, organochlorine pesticide; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; p,p'-DDE, p,p'-dichlorodiphenyldichloroethylene; and TBBPA, tetrabromobisphenol A.

Our approach is important because as persons are likely to be exposed to combinations of pollutants, such toxins may work in concert to activate several disease mechanisms. Similar methods in cognitive research show varying associations of disease risk.<sup>32-37</sup> Environmental pollutants cause biological effects that overlap with hypothesized ALS mechanisms, suggesting biological plausibility. For example, p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) causes sustained depolarization, leading to release of neurotransmitters (including glutamate) and hyperexcitability.<sup>5</sup> Polychlorinated biphenyls, which accumulate in the brain, impair neurotransmitter reuptake (including glutamate), calcium homeostasis, and signal transduction and accelerate cell death.<sup>38,39</sup> That PCBs can modify glutamate levels is consistent with proposed ALS disease mechanisms,<sup>40</sup> and both PCBs<sup>41</sup> and PBDEs<sup>42</sup> result in neuronal apoptosis in vitro. Finally, persistent environmental pollutants alter global DNA methylation,<sup>43</sup> which may play a role in ALS pathogenesis.<sup>44</sup> Further work is needed to understand the aforementioned potential pathogenic implications in ALS.

Because survey data and blood measurements both implicated pesticide exposure in ALS, we examined the concordance

between survey responses addressing cumulative occupational exposure of pesticides and actual chemical measurements of OCPs. The fact that we found only a weak concordance is not surprising. First, pollutant measurements reflect not only occupational exposures but also residential exposures and diet. Second, one-time collected pollutants may not represent lifetime cumulative occupational exposures owing to temporal variation, the finite biological half-lives of the chemicals, and sampling variation. Third, job-exposure matrix-based estimates along with self-reported exposures vary in validity and reliability, despite widespread use in occupational case-control studies.<sup>45</sup> Fourth, many currently used pesticides are short-lived organophosphate compounds, and while exposure to these compounds was likely included in responses to the occupational survey, these short-lived compounds were not measured in this study. Finally, individuals may also falsely believe they experienced pesticide exposures.

The lack of concordance between survey data and actual measurements of blood pollutants supports the use of direct pollutant assessments when possible, particularly for compounds that have long half-lives, and thereby reflect both current and historical exposures. For example, OCPs have environmental half-lives that potentially reach hundreds of years

for dichlorodiphenyltrichloroethane (DDT) and DDE<sup>5</sup> and, in humans, longer than 7 years for p,p'-DDE<sup>46</sup> and  $\beta$ -hexachlorocyclohexane.<sup>47,48</sup> While most OCPs have been banned or restricted in the United States, exposure still occurs, primarily through diet. As the relative contribution of diet on environmental exposures increases, agreement between occupational survey and pollutant data will likely even further decrease.

Given the case-control study design, selection and recall biases are possibilities in our study and varied validity and reliability of self-reported data and single biological measurements may occur. Seventy percent of controls lived less than 20 km from UM owing to the recruitment strategy, although a sensitivity analysis of a smaller geographic region supported the association of pollutants with ALS. Controls had higher educational levels, which contrasts to National ALS Registry data showing a higher rate of ALS in more educated persons.<sup>49</sup> While other ALS studies do have control groups with higher educational attainment,<sup>28</sup> the use of a primarily internet-based recruitment strategy for controls may have biased the group toward higher socioeconomic levels. Every model adjusted for education to control for potential confounding. Additionally, conditional logistic regression models on a frequency-matched data set, matching cases with controls for age, sex, and education, did not show changes in effect associations (eTable 8, eTable 9, and eTable 10 in the Supplement). Next, self-reported data typically involve recall bias that can lead to exposure misclassification and affect risk estimates.<sup>50</sup> Owing to a protocol change, the current pollutant measurements also used both plasma and whole-blood samples, although method- and chemical-specific partition coefficients were measured and used to determine unified measures with very high validity (data not shown). Last, pollutant measurements are weighted toward more recent exposures; however, most chemicals measured in this study have long half-lives and thus their measurements are a good indicator of historical exposure.<sup>5-8</sup>

## Conclusions

Our findings identify classes of pollutants that increase the likelihood of ALS and therefore are modifiable disease risk factors. Relative to the previous literature, the present study includes (1) a thorough survey assessment of occupational exposure risk factors over time, (2) neurotoxic occupational pollutant measurements, and (3) concordance testing between the survey and pollutant concentrations. We report that military service increases ALS risk throughout an individual's lifetime, and survey data reveal an association with pesticide exposure and ALS. Measured blood pollutants, particularly OCPs, increased the odds of ALS across all reported time exposure windows, although there was a low concordance between survey reports of pesticide exposure and actual blood measurements. These results highlight differences in reported vs measured exposures and underscore the need to understand how survey responses relate to actual exposures; this understanding is essential in many applications, particularly large-scale efforts to identify ALS risk factors such as the Na-

**Table 3. Results of Logistic Regression Models for Amyotrophic Lateral Sclerosis Using Stepwise Selection of Exposure Pollutants**

Compound	OR (95% CI) <sup>a</sup>	
	Imputed	Observed
<b>OCPs</b>		
Pentachlorobenzene	2.57 (1.31-5.02) <sup>b</sup>	3.87 (1.54-9.75) <sup>b</sup>
Hexachlorobenzene	NS <sup>c</sup>	
$\beta$ -HCH	2.50 (0.86-7.25)	3.00 (0.87-10.39)
Dacthal	0.34 (0.15-0.79) <sup>d</sup>	0.25 (0.10-0.62) <sup>b</sup>
<i>trans</i> -Chlordane	NS <sup>c</sup>	
<i>cis</i> -Chlordane	6.51 (2.05-20.73) <sup>b</sup>	9.10 (2.18-37.96) <sup>b</sup>
<i>trans</i> -Nonachlor	NS <sup>c</sup>	
p,p'-DDE	NS <sup>c</sup>	
<b>PCBs</b>		
110	NS <sup>c</sup>	
151	1.66 (1.03-2.67) <sup>d</sup>	1.63 (0.99-2.67)
135/144	NS <sup>c</sup>	
118	NS <sup>c</sup>	
132/153	NS <sup>c</sup>	
138/163	NS <sup>c</sup>	
175	1.19 (0.70-2.04)	1.16 (0.67-2.02)
174	NS <sup>c</sup>	
202	0.47 (0.25-0.87) <sup>d</sup>	0.40 (0.21-0.78) <sup>b</sup>
180	NS <sup>c</sup>	
170/190	NS <sup>c</sup>	
198	NS <sup>c</sup>	
<b>BFRs</b>		
TBBPA	0.72 (0.42-1.22)	0.66 (0.36-1.23)
PBDE 28	NS <sup>c</sup>	
PBDE 47	1.30 (0.77-2.17)	1.46 (0.90-2.38)
PBDE 66	0.57 (0.33-0.99) <sup>d</sup>	0.58 (0.33-1.03)
PBDE 100	NS <sup>c</sup>	
PBDE 99	NS <sup>c</sup>	
PBDE 85	NS <sup>c</sup>	
PBDE 154	NS <sup>c</sup>	

Abbreviations:  $\beta$ -HCH,  $\beta$ -hexachlorocyclohexane; BFRs, brominated flame retardants; NS, not selected; OCPs, organochlorine pesticides; OR, odds ratio; PBDE, polybrominated diphenyl ether; PCBs, polychlorinated biphenyls; p,p'-DDE, p,p'-dichlorodiphenyldichloroethylene; TBBPA, tetrabromobisphenol A.

<sup>a</sup> The logistic regression models were adjusted for age, sex, and educational levels and used standardized concentrations. For imputed data, the sample size was 284 participants (156 cases and 128 controls) for 10 imputations; for observed data, the sample size was 204 participants (97 cases and 107 controls).

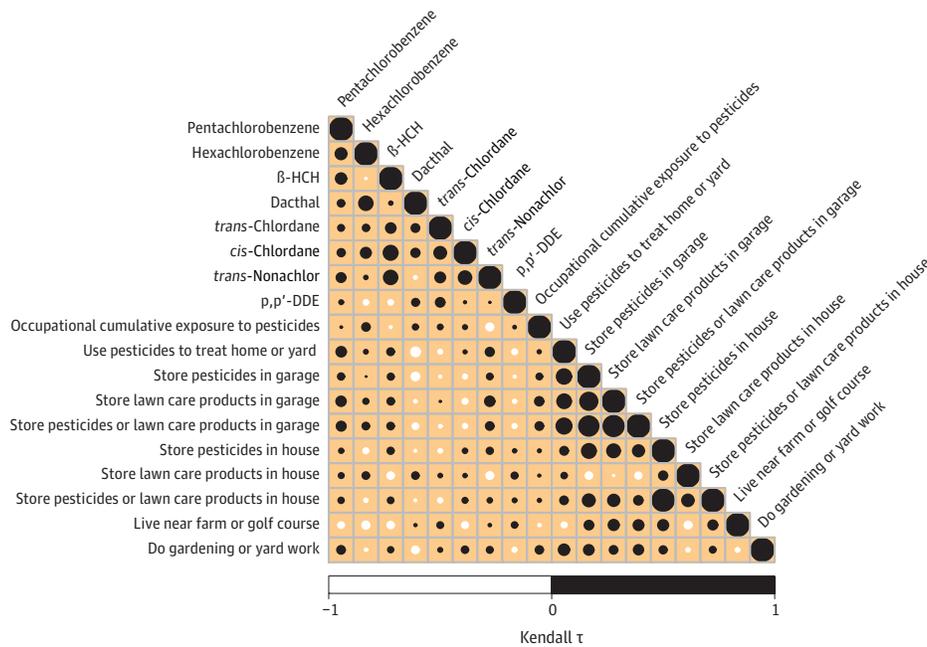
<sup>b</sup>  $P < .01$ .

<sup>c</sup> Eighteen compounds were not selected into the model under the criteria of significance levels for entering and removing effects ( $P = .10$  and  $P = .20$ , respectively).

<sup>d</sup>  $P < .05$ .

tional ALS Registry. Finally, as environmental factors that affect the susceptibility, triggering, and progression of ALS remain largely unknown, we contend future studies are needed to evaluate longitudinal trends in exposure measurements, assess newer and nonpersistent chemicals, consider pathogenic mechanisms, and assess phenotypic variations.<sup>51</sup>

Figure 2. Matrix Plot of Kendall  $\tau$  Correlation Coefficients for Self-reported and Exposure Pollutant Data of Pesticide Exposures



The organochlorine pesticide concentrations were partitioned into 5 groups from the lowest to highest levels; the occupational cumulative exposure to pesticides was partitioned into 4 groups from the lowest to highest levels; and the residential variables of pesticide exposures were yes or no questions. Larger circles indicate larger correlation coefficients. There was weak agreement between organochlorine pesticide concentrations and survey-based cumulative occupational exposure to pesticides (depending on organochlorine pesticide, Kendall  $\tau = -0.11$  to 0.05;  $P > .05$ ). For residential variables, "use pesticides to

treat home or yard" was positively correlated with pentachlorobenzene (Kendall  $\tau = 0.19$ ;  $P = .005$ ) and negatively correlated with Dacthal (Kendall  $\tau = -0.18$ ;  $P = .01$ ); "store lawn care products in garage" was positively correlated with pentachlorobenzene (Kendall  $\tau = 0.15$ ;  $P = .02$ ) and trans-nonachlor (Kendall  $\tau = 0.24$ ;  $P < .001$ ); and "store pesticides or lawn care products in garage" was positively correlated with pentachlorobenzene (Kendall  $\tau = 0.16$ ;  $P = .02$ ).  $\beta$ -HCH indicates  $\beta$ -hexachlorocyclohexane; p,p'-DDE, p,p'-dichlorodiphenyldichloroethylene.

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