RESEARCH ARTICLE

Brain Organochlorines and Lewy Pathology: The Honolulu-Asia Aging Study

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ABSTRACT: Although organochlorines have been reported more frequently in Parkinson's disease (PD) brains than in controls, the association with brain Lewy pathology is unknown. Honolulu-Asia Aging Study (HAAS) participants, exposed to organochlorines from a variety of sources during midlife, represent a population well suited to determining the relationship of brain organochlorines with Lewy pathology in decedents from the longitudinal HAAS. The study design included the measurement of 21 organochlorine levels in frozen occipital lobe samples from HAAS decedents. Alpha-synuclein immunostaining performed on 225 brains was used to identify Lewy bodies and Lewy neurites. With the potential for spurious associations to appear between Lewy pathology and 17 organochlorine compounds found in at least 1 brain, initial assessments identified heptachlor epoxide isomer b, methoxychlor, and benzene hexachloride b as being most important. The prevalence of Lewy pathology was 75% (6 of 8) among brains with any 2 of the 3 compounds, 48.8% (79 of 162) among those with 1, and 32.7% (18 of 55) for those with neither ($P = .007$ test for trend). Although findings persisted after removing cases with PD and dementia with Lewy bodies and after adjustment for age at death, body mass index, pack-years of cigarette smoking, and coffee intake ($P = .013$), the results were insignificant when correcting for multiple testing. Although consistent with earlier accounts of an association between organochlorines and clinical PD, associations with Lewy pathology warrant further study.

Key Words: Parkinson's disease; Lewy body; organochlorines; pesticides

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Evidence supporting a role for organochlorine pesticides in the etiology of Parkinson's disease (PD) comes from a variety of sources. Epidemiological studies have demonstrated an association of organochlorine exposure with PD including a dose–effect relationship,[1,2] and elevated serum levels of some organochlorines are associated with higher odds of developing PD.[3,4] Pathological studies have also shown an association between brain organochlorines and PD.[5,6] The organochlorine dieldrin induces proteasome dysfunction in rat mesencephalic dopaminergic cells overexpressing human alpha-synuclein, promoting apoptotic cell death.[7] It also leads to increased oxidative stress, increased expression of striatal alpha-synuclein, and impaired dopamine metabolism in exposed mice.[8] Last, exposure to dieldrin and lindane combined causes more dopaminergic neuron toxicity through induction of oxidative stress and mitochondrial dysfunction than does exposure to only 1 agent.[9]

Lewy pathology in human brains is considerably more prevalent than clinical PD. Demonstrating an association of brain organochlorine levels with brain Lewy pathology would strongly support a role for organochlorine exposure in the etiology of PD. The Honolulu-Asia Aging Study (HAAS) cohort is an ideal population to examine this association for 2 reasons. First, HAAS participants were exposed to organochlorine insecticides in a number of ways during the period from the early 1940s, when these compounds were introduced commercially in Hawaii, until 1988, when the ban on sales of organochlorines was instituted in the United States. Second, the HAAS has an autopsy component with a large brain archive. The aim of this study was to determine if there is an association of brain organochlorine levels with the presence of Lewy pathology in brains of deceased participants in the Honolulu-Asia Aging Study (HAAS) and to determine if the presence of multiple organochlorines confers greater odds of having Lewy pathology.

Materials and Methods

Honolulu-Asia Aging Study Design

The Honolulu Heart Program (HHP) is a longitudinal study of heart disease and stroke in a cohort of 8006 Japanese American men born between 1900 and 1919 and living on the island of Oahu, Hawaii, in 1965, when the study began.[10] Follow-up has continued through reexaminations and surveillance of hospital and death records. With the establishment of the HAAS at the 1991 examination, research on diseases of aging including dementia and PD was initiated.[11] Previously described standardized methods were used to identify all cases of PD in the cohort, both at the 1991 examination and in follow-up examinations every 2–3 years.[12,13] The study was approved by the Kuakini Medical Center Institutional Review Board, and participants signed informed consents at all examinations.

Assessment of Lewy Pathology

The autopsy component was initiated in 1991.[14] Autopsy was discussed with all HAAS participants, and consent for autopsy was given by the closest living family member according to Hawaii state law.

Standardized gross and microscopic examinations of multiple brain regions were performed. Details have been published.[15,16] These included immunohistochemical staining for alpha-synuclein[17] on sections of olfactory bulb, medulla, pons, midbrain, hippocampus, amygdala, and striatum at the level of the nucleus accumbens, basal forebrain, anterior cingulate gyrus, insula, and midfrontal, midtemporal, inferior parietal, and occipital regions. Semiquantitative pathology density analyses were carried out for each region examined, and staging was performed with some modifications to the methods of Braak and colleagues.[18] For this analysis, the presence of Lewy bodies or neurites was considered positive for the presence of Lewy pathology.

Measurement of Organochlorine Levels

Frozen occipital lobe samples were analyzed at the Research Triangle Institute for 21 organochlorines.[19,20] Approximately 0.025–0.030 g of tissue was weighed, transferred to a culture tube, and dried by grinding with 1 g of anhydrous sodium sulfate, then extracted 3 times with 5 mL of hexane. Extracts were combined, and extract volume was adjusted to 15 mL. A known aliquot of the extract was then removed for lipid analysis, whereas the remainder was concentrated to 1 mL. The extract was cleaned while being eluted with 3 solvent systems on a column of activated Florisil (partially deactivated before use with water). The first eluate (25 mL of hexane) contained DDE and other nonpolar pesticides. The second eluate (25 mL of 10% ether in hexane) contained the more polar pesticides, including dieldrin and endrin. The third eluate (25 mL dichloromethane) contained pesticides such as endosulfan II. Fractions were concentrated individually to 1.0 mL each. A quantitation standard (PCB congener 119) was added, and the extracts were analyzed by high-resolution gas chromatography using an electron capture detector. Concentration was reported in parts per billion for 21 organochlorines. Organochlorine levels were considered not present for this analysis if they were reported to be "not detected" or "less than the level of calibration."

Statistical Methods

Two-sample *t* tests were used to compare the average characteristics between autopsied and nonautopsied

TABLE 1. Average characteristics in the autopsied decedents versus all other decedents

Characteristic	Decedents: Autopsied (n = 225)[a]	Decedents: Others (n = 2838)
Age at death (y)	86.2 ± 5.1[b]	86.7 ± 5.5
Cigarette smoking (pack-years)	28.1 ± 29.3	28.2 ± 28.1
Coffee intake (oz/day)	12.9 ± 11.8	13.6 ± 13.0
Body mass index (kg/m^2)	23.1 ± 3.2	23.3 ± 3.2

[a]Sample size; [b]mean ± standard deviation.
Note: There were no significant differences in average characteristics between the autopsied decedents versus all other decedents using 2-sample *t* tests.

decedents and between decedents with and without Lewy pathology (Tables 1 and 2). Associations between an organochlorine compound and Lewy pathology were initially assessed using Fisher's exact test, with Lewy pathology categorized as absent (Braak stage = 0) or present (Braak stage ≥ 1); see Table 3. The actual Braak staging was not used for the latter assessments because the level of Braak staging was too limited for 10 of the 17 compounds, which were detected in fewer than 10 of the 225 decedents. Subsequent stepwise analyses that considered associations with more than 1 compound, however, permitted the use of the actual Braak stage as a measure of Lewy progression (eg, see Fig. 1). For these analyses, the effect of combinations of organochlorines that appeared to have a relationship with Lewy pathology was examined (eg, presence of none, 1, 2, or 3 compounds). The most important compound was first identified based on the initial results from the Fisher's exact test. Additional compounds were considered through a 2-step process that began with the second compound with the strongest association with Lewy pathology. In such instances, ordinal logistic regression was used to model Braak staging as a function of the number of compounds detected (0, 1, or 2).[21] After adjustment for this compound, a third compound was selected based on its having the strongest association with Lewy pathology among the remaining compounds. The process was stopped after a third compound was identified because the number of decedents exposed to more than 3 compounds was small. To help confirm the association between the selected compounds and Lewy pathology, adjustments were made for the confounding effects of age at death, body mass index, midlife pack-years of cigarette smoking, and coffee intake. Further testing was based on removing cases of PD and dementia with Lewy bodies (DLB). Separate adjustment for each of the remaining compounds was made to help determine if the association between the 3 selected compounds and Lewy pathology was independent of the other compounds. Findings were further examined through the use of permutation tests that account for multiple testing, correlation, and non-normal distributions.[22] For descriptive purposes (see Fig. 1), analysis of covariance models provided estimates of the prevalence of Lewy pathology (Braak stage ≥ 1) by the number of detectable compounds after age and risk factor adjustment.[23] Body mass index was included among the possible confounders, as fat is a potential depot for organochlorine compounds. All reported *P* values were based on 2-sided tests of significance.

TABLE 2. Average characteristics of the sampled decedents

Characteristic	Lewy pathology: Absent (n = 122)[a]	Lewy pathology: Present (n = 103)
Age death (y)	85.6 ± 5.2[b]	86.8 ± 5.0
Cigarette smoking (pack-years)	29.6 ± 28.5	26.3 ± 30.3
Coffee intake (oz/day)	13.6 ± 12.5	12.1 ± 11.0
Body mass index (kg/m^2)	23.2 ± 3.3	23.0 ± 3.1

[a]Sample size; [b]mean ± standard deviation.
Note: There were no significant differences in average characteristics between those with and without Lewy pathology using 2-sample *t* tests.

TABLE 3. Relationship of organochlorines with Lewy pathology using Fisher's exact test

Organochlorine	Percentage with Lewy pathology: Not detected	Percentage with Lewy pathology: Detected	*P* value
Aldrin	46.9% (98 of 209)[a]	31.3% (5 of 16)	.300
Benzene hexachloride b	44.8% (99 of 221)	100.0% (4 of 4)	.043
Benzene hexachloride g	46.2 % (103 of 223)	0.0% (0 of 2)	.501
a-Chlordane	43.2% (73 of 169)	53.6% (30 of 56)	.216
DDD	45.4 % (98 of 216)	55.6% (5 of 9)	.734
DDE	47.7% (21 of 44)	45.3% (82 of 181)	.866
DDT	45.5% (102 of 224)	100.0% (1 of 1)	.458
Dieldrin	45.7% (101 of 221)	50.0 % (2 of 4)	1.00
Endrin	46.8% (103 of 220)	0.0% (0 of 5)	.064
Hexachlorobenzene	45.5% (102 of 224)	100.0% (1 of 1)	.458
Heptachlor epoxide isomer b	36.2% (21 of 58)	49.1% (82 of 167)	.095
Heptachlor	46.1% (82 of 178)	44.7% (21 of 47)	1.00
Isobenzan	46.0% (103 of 224)	0.0% (0 of 1)	1.00
Mirex	45.5% (102 of 224)	100.0% (1 of 1)	.458
Methoxychlor	45.0% (98 of 218)	71.4% (5 of 7)	.251
t-Nonochlor	39.1% (18 of 46)	47.5% (85 of 179)	.325
Oxychlordane	45.8% (97 of 212)	46.2% (6 of 13)	1.00

[a]Number with Lewy pathology/sample size.

Results

There were 225 brains with available organochlorine levels and Braak Lewy body staging. Of these, 122

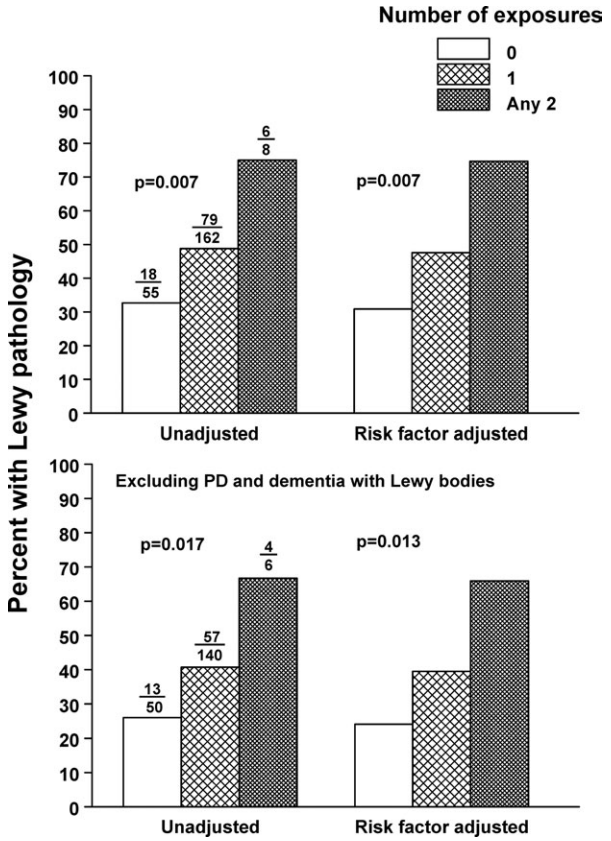

FIG. 1. Percentage of decedents with Lewy pathology by detectable levels of benzene hexachloride b, heptachlor epoxide isomer b, and methoxychlor. For descriptive purposes, analysis of covariance models provided estimates of the prevalence of Lewy pathology (Braak stage ≥ 1) by the number of detectable compounds after age and risk factor adjustment.[23] The *P* values are for a test for trend between the increasing number of exposures and the percentage of decedents with Lewy pathology. Numbers above the unadjusted bars are the cases with Lewy pathology/sample size. Risk factor–adjusted percentages are adjusted for age at death, body mass index, and midlife cigarette smoking and coffee intake.

were without Lewy pathology, 29 had PD or DLB, and 74 had incidental Lewy bodies (Braak stage > 0 and no clinical history of PD or DLB). Mean age at death was 86.2 years (range, 75–99 years).

Table 1 compares characteristics of the autopsied sample for which organochlorine levels were available and deceased participants without an autopsy. There were no significant differences in mean age at death, pack-years of smoking, coffee consumed, or body mass index.

Characteristics of individuals with and without brain Lewy pathology were similar (Table 2). Although those with Lewy pathology were slightly older, smoked less, and drank less coffee, differences were not statistically significant.

Among the 21 organochlorine compounds assessed, there were 3 that were not detected in any brains. These were benzene hexachloride a, endosulfan 1, and endosulfan 2. Data on g-chlordane were inadequate for analysis because of technical problems, resulting in large numbers of missing values. Among the remaining 17, clear positive associations with the presence of Lewy pathology were noted for 9. There were negative associations for 4 and very little relationship with the other 4 (Table 3). The only statistically significant association was with benzene hexachloride b ($P = .043$). Although only 4 brains had detectable levels, all had Lewy pathology, whereas only 45% of those without detectable levels (99 of 221) had Lewy pathology.

For the remaining compounds positively associated with Lewy pathology, prevalence of heptachlor epoxide isomer b seemed important. Nearly half those exposed had Lewy pathology versus 36.2% in those without detectable levels ($P = .095$). Although detection of methoxychlor was far less common ($n = 7$), in its presence, 71.4% had Lewy pathology versus 45% in its absence ($P = .251$).

Effects of DDT, hexachlorobenzene, and mirex may also be important, but detection of these compounds was noted in only a single decedent. Compounds with an inverse association were benzene hexachloride g, endrin, isobenzan, and aldrin. Individually or in combination, these compounds did not have significant relationships with Lewy pathology.

In the stepwise process to select compounds with the strongest association with Lewy pathology, benzene hexachloride b was selected first because of its association in Table 3 ($P = .043$). The second was heptachlor epoxide isomer b. Here, the prevalence of Lewy pathology was 33.9% (19 of 56) in the absence of both compounds, 49.1% (82 of 167) when 1 compound was present, and 100% (2 of 2) in the presence of both compounds ($P = .019$). The third selected was methoxychlor, although improvement in predicting the prevalence of Lewy pathology was modest. None of the decedents had detectable levels of all 3. Figure 1 shows that the percentage of Lewy pathology increased as the number of compounds detected increased ($P = .007$). Findings remained significant after adjustment for multiple potential confounders including age at death, body mass index, and midlife cigarette smoking and coffee intake ($P = .007$) and while also removing cases of PD and DLB ($P = .013$) The independent association between the 3 selected compounds and Lewy pathology also persisted ($P < .05$) after separate adjustment for the remaining unselected compounds. In each instance, the percentage of decedents with Lewy pathology was more than doubled when there was exposure to any 2 of the organochlorines versus no exposure. Findings were similar when examining associations with the 29 cases of clinical PD or DLB, although results were not significant because of the limited sample size ($P = .08$). Findings failed to persist after a permutation test, leaving the possibility that positive results could be due to chance related to multiple comparisons.

Most of the findings seem to be a result of the presence of heptachlor epoxide isomer b (possibly because

of its high frequency). For it to have a significant relationship with Lewy pathology, however, it needed to be in the presence of at least 1 of the other organochlorines ($P = .018$ for benzene hexachloride b and $P = .018$ for methoxychlor after risk factor adjustment). Overlap among these 3 compounds was not a large factor, given that only 8 of 170 decedents with detectable levels of any 1 compound also had detectable levels of a second compound (Fig. 1). Combinations involving the presence of other compounds were less informative, largely because of weaker associations, their rare detection, or associations that seemed largely determined by associations with the 3 compounds (Fig. 1).

Analyses were also performed to examine the association of brain levels of the organochlorines with Lewy pathology. No clear dose effect was found, possibly related to the small number of brains with detectable levels of some compounds.

Discussion

The original study design was to examine 21 organochlorine compounds and their association with Lewy pathology. In the absence of prior evidence favoring one compound over another, each compound was considered with equal weight, requiring multiple testing with a high risk for uncovering spurious associations. Findings highlight the difficulties in designing and analyzing studies based on the selection of several risk factors perceived to be important in the assessment of a single hypothesis and the need to be as focused as possible.

Nevertheless, findings based on the multiple stepwise approach in a search for compounds related to Lewy pathology followed by adjustment for concomitant information and confirmation in a subset of decedents without PD or DLB are at least consistent with other reports of an association with clinical PD.

Most organochlorines belong to 1 of 3 main classes: cyclodienes, including heptachlor and dieldrin; dichlorodiphenylethanes, including DDT and methoxychlor; and cylohexanes, including benzene hexachloride b and g. These classes differ in toxic effects. No particular class dominated the association with Lewy pathology in the HAAS brains. Experimental evidence is strongest for the cyclodiene group, which has been linked to alpha-synuclein aggregation, enhanced oxidative stress, and disruption of the ubiquitin-proteasomal system.[24] Among the cyclodienes in this analysis, the presence of aldrin was inversely associated with Lewy pathology whereas others, such as heptachlor epoxide isomer B, had positive associations.

Organochlorines were widely and heavily used in Hawaii on pineapple plants through the 1960s and applied around homes and other buildings as termiticides. Hawaii is particularly sensitive to groundwater contamination from agricultural pesticides owing to the high permeability of volcanic soil.[25] In 1982 the commercial milk supply on the island of Oahu was found to be contaminated with heptachlor, and the source for this contamination was chopped pineapple leaves fed to dairy cattle.[26,27] Interestingly, a previous HAAS publication found that those consuming greater than 16 ounces of milk per day were significantly more likely to develop PD than nondrinkers (relative risk, 2.6; 95% confidence interval, 1.1–6.4).[13] Organochlorine use has been restricted in the United States since the mid-1970s and banned since 1988. That organochlorine residues are detectable in the brains of deceased HAAS participants over 30 years following exposure may be related to bioaccumulation of these fat-soluble chemicals with very long half-lives.[25] Alternatively, brain levels in HAAS decedents could reflect more recent exposure to persistent organochlorines in Oahu fish, water, and soil.

A recent US Geological Survey report on fish from Oahu streams found that in 1998 tissue concentrations of several organochlorine compounds exceeded guidelines.[28] Although concentrations have decreased since the 1970s, levels remain high for some fish.

Earlier studies with small numbers of cases found that PD brains were more likely to have detectable levels of dieldrin[6] and higher mean concentrations of lindane and dieldrin[5] than Alzheimer's disease (AD) or normal brains. In a recent small case–control study of serum organochlorines and PD, beta hexachlorocyclohexane was more often detected in PD cases than in normal and AD controls.[3] In a larger follow-up study, the odds of PD were significantly higher among subjects with beta hexachlorocyclohexane levels above the detectable interquartile range.[29] In another case–control study, using prospectively collected serum, a higher mean concentration of only dieldrin of 5 analyzed organochlorine pesticides was associated with higher odds of PD.[4]

Epidemiological studies have reported associations between self-reported pesticide exposures and clinical PD.[1,2,30–33] A recent case–control study reported that professional use of organochlorines was associated with more than twice the odds of PD compared with those not exposed.[1]

Exposure to combinations of 2 pesticides has been associated with significantly more dopaminergic neuron injury than exposure to only 1 in animal models examining lindane and dieldrin[9] and maneb and paraquat.[34] In humans, exposure to both maneb and paraquat increased the risk of PD by 75% in a case–control study.[31]

Although the means by which organochlorines may result in Lewy pathology are unknown, current lines of evidence suggest several potential mechanisms. Dieldrin is selectively neurotoxic to dopaminergic

neurons in primary mesencephalic cultures[35] and accelerates the rate of alpha-synuclein fibril formation in vitro.[36] Exposure to dieldrin in mice is associated with a decrease in dopamine metabolites, an increase in markers of oxidative stress in the striatum and an increase in alpha-synuclein protein expression in the striatum.[8] Alternatively, Lewy pathology may result from neuronal damage caused by organochlorines. For example, heptachlor alters dopamine transport in mice, and it is speculated that this may increase susceptibility to other dopaminergic toxins.[37]

There are limitations to this study. The HAAS cohort is all male and of Japanese ancestry. Generalizations of findings to women and other ethnic groups cannot be made with certainty. However, studies including women and persons of European ethnicity have also reported associations between organochlorines and PD. Because organochlorine measurements were performed well after the initiation of processes leading to Lewy pathology, it is possible that these processes affect the organochlorine levels. For example, Lewy pathology may impede organochlorine clearance from the brain. However, to our knowledge, this has not been reported. The presence of other pesticides that may also be associated with Lewy pathology such as paraquat was not assessed. Detectable levels of most of the organochlorines in this report were often exceedingly rare, imposing an important limitation on our findings. Considering that exposures to organochlorines may have occurred more than 30 years ago, it is noteworthy that compounds were detected frequently enough to demonstrate a relationship.

There are several strengths. Sampled brains were from a population-based longitudinal study with well-characterized information on potential confounders. Organochlorine measurement in frozen brain tissue provided an objective measure of the presence of organochlorines. All the participants lived on the island of Oahu during the period that organochlorines were in heavy use. Finally, state-of-the-art immunohistochemical staining for alpha-synuclein was used.

In summary, prevalence of Lewy pathology in brains from HAAS participants was higher in those brains with detectable levels of most organochlorines assessed, although only 1 relationship was statistically significant. In particular, having detectable levels of any 2 of heptachlor epoxide b, methoxychlor, or b-BHC was associated with a high prevalence of Lewy pathology after adjustment for age at death, body mass index, cigarette smoking, and coffee intake; and, in a subset of decedents without PD or DLB. We cannot rule out the possibility that these results are a result of chance based on multiple comparison analyses. Although these findings support a role for organochlorines in the etiology of PD, further study is needed. Results might also provide direction in designing future studies with a more careful focus on selected compounds that seem most important in the current report. ■

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