

Levels of Organochlorine Pesticides Are Associated with Amyloid Aggregation in Apex Avian Brains

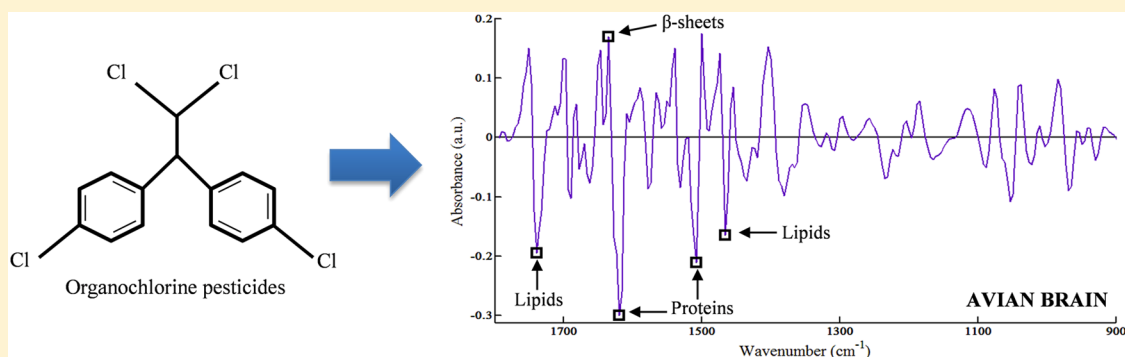
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Supporting Information



ABSTRACT: Organochlorine (OC) pesticides pose a significant environmental risk to wildlife and humans and have been associated with Alzheimer's disease (AD). This study aims to spectroscopically analyze brains from free-flying birds and link the results to OC exposure and consequent amyloid aggregation. As long-lived apex predators, predatory birds represent a sentinel species similar to humans. Therefore, the results have implications for both species and may also add to our understanding of the role OC pesticides play in the development of AD. Brains of wild Sparrowhawks were analyzed using ATR-FTIR and Raman spectroscopy and Congo red staining; results were correlated with OC pesticide concentrations in livers. Effects of OC exposure were sex- and age-dependent and associated alterations were seen in lipids and protein secondary structure. A shift from α -helix to β -sheet conformation of proteins indicated that concentrations of OC pesticides $>7.18 \mu\text{g/g}$ may lead to cerebral amyloid aggregation.

INTRODUCTION

Organochlorine (OC) insecticides are a large and diverse class of compounds, many of which are highly lipophilic and persistent in the environment. They have attracted a lot of attention in the past due to widespread usage between the 1940s and 1970s and the ensuing recognition that some OCs have significant detrimental effects on the environment.^{1,2} OC insecticides were banned in many countries during the 1990s due to environmental and human health concerns but are still used in many developing countries, particularly to control malaria.³ Since the global OC usage decreased due to substance control, there have been many questions regarding the long-term effects of environmental OC exposure. Even in the past three years, OC concentrations have been detected and measured in human blood samples from around the world^{4,5} as well as in many species of fish^{6,7} which is thought to represent a significant source of dietary OC intake. Recently, OCs have also been determined in environmental compartments including air,⁸ water,⁹ and soil¹⁰ and have been detected in various environmental species such as dolphins,¹⁰ bears¹¹ and mussels.¹² Such studies demonstrate that these persistent

chemicals are still a current environmental concern, both for wildlife and for the human population.

OCs are known neurotoxins which is the mechanism by which they are able to control pest populations. They alter sodium and potassium channels, in particular causing persistent opening of sodium channels which allows constant firing of action potentials.¹³ The brain is particularly vulnerable to their effects as it is rich in lipids and has a low capacity for detoxification. Thus, OCs have been implicated in the development of neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD). AD patients have been reported to have higher serum levels of dichlorodiphenyldichloroethylene (DDE, a metabolite of DDT), while elevated levels of β -hexachlorocyclohexane have been found in patients with PD.¹⁴ AD is characterized by the presence of aggregated amyloid- β protein plaques as well as

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neurofibrillary tangles composed of phosphorylated tau protein in the brain.¹⁵ The associated cognitive decline is caused by neuronal death and loss of synapses due to the presence of these protein aggregates. Many factors have been attributed to the causation of AD including genetic factors, aging and exposure to chemicals such as OCs.¹⁶ Environmental exposure to a number of chemicals, including OC pesticides, is thought to cause the aggregation of amyloid- β proteins by inducing cellular oxidative stress. OCs may directly alter NADH/NAD⁺ levels or interact with the respiratory chain in mitochondria leading to an increase in cellular reactive oxygen species (ROS) levels.¹⁷ This results in oxidation and aggregation of cellular proteins including amyloid- β .

AD is a major health issue in aging Western populations so research to further our understanding of the disease is essential. Although many studies aim to link chemical exposure with the etiology of AD, investigations are often limited by availability of post-mortem brain tissue and environmental exposure data for those samples.¹⁸ In this study, we have obtained Sparrowhawk (*Accipiter nisus*) brain samples that have been analyzed for concentrations of various OC pesticides, (see [Supporting Information \(SI\) Table S1](#)) allowing for further analysis of potential OC-mediated amyloid aggregation. Birds of prey, such as sparrowhawks, are apex predators and occupy a niche at the top of their food chain in the same way that humans do. Also, free-flying predatory birds encounter cumulative, real world OC exposures making them ideal sentinels in which to study environmental pollutants. Many predatory bird species have been previously used as sentinel species to study environmental contaminant such as OCs, PBDEs, PCBs and heavy metals in tissue samples as well as using feathers, blood, and eggs.^{19,20} Using vibrational spectroscopy techniques that are able to identify alterations at the biomolecular level, we aim to determine cellular alterations caused by high and low OC exposures. In line with current thinking, we will also investigate whether exposure to OC pesticides can be linked with amyloid aggregation in Sparrowhawk brain samples using spectroscopy, staining and immunoassay methodologies. To our knowledge, this is a unique study that attempts to couple chemical exposure data and spectral data with cerebral amyloid aggregation in birds.

MATERIALS AND METHODS

Brain Samples. Sparrowhawk (*Accipiter nisus*) brain samples were obtained from the Predatory Bird Monitoring Scheme (<http://pbms.ceh.ac.uk>) which receives dead birds for analysis from members of the public in the UK. On receipt, the Sparrowhawk brains were removed from the carcasses and stored in a tissue archive at -18°C . The brain samples for this study were chosen from the archived tissues from Sparrowhawks that died between 1979 and 1990 in the UK. The Sparrowhawk livers had been previously analyzed for wet weight OC pesticide concentrations using previously reported analysis methods and so the brain samples used were ranked according to the total OC pesticide concentrations found in the livers of the same birds. Total OC pesticide concentrations ranged from 0.56 to 82.31 $\mu\text{g/g}$ in liver (see [SI Table S1](#)). Brain samples ($n = 58$) were ranked and selected so that there were two main sample groups; 30 with “high” total OC concentrations (15 male, 15 female) and 28 with “low” total OC concentrations (15 male, 13 female). Details of samples used are included in [SI Table S1](#).

Spectral Signal of Amyloid Fibril. In order to investigate the spectral signal from amyloid, A β 1:42 fibers were interrogated using Raman spectroscopy. Fifty μM of A β 1:42 was aggregated for 1 week in 10 mM phosphate buffer and then spun down in an airfuge system (Beckman Coulter, (UK) High Wycombe, UK) for 1 h at 125 000g to pellet fibers. The pellet was resuspended in 100 μL of distilled water and 10 μL was deposited onto gold coated glass slides (Platypus Technologies, WI).

Spectroscopy Analysis of Amyloid Fibrils. For each sample, 1 g of brain material was spectroscopically analyzed on an infrared-reflective, low-E slide (Kevley Technologies, Chesterland, OH). For ATR-FTIR spectroscopy, five spectra were obtained per slide using a Bruker TENSOR 27 FTIR spectrometer with Helios ATR attachment containing a diamond IRE of $250\ \mu\text{m} \times 250\ \mu\text{m}$ (Bruker Optics, Coventry, UK). The ATR-FTIR was set to attain spectra with $8\ \text{cm}^{-1}$ spectral resolution and 32 coadditions, allowing $3.84\ \text{cm}^{-1}$ spectral data spacing. Mirror velocity was set to 2.2 kHz. After each sample was analyzed, the diamond was cleaned with distilled water and a new background was taken to account for environmental deviations. For Raman spectroscopy, samples were interrogated using an InVia Renishaw Raman spectrometer, containing a 785 nm excitation laser, coupled with a charge-coupled device (CCD) and Leica microscope systems (Leica Microsystems, Milton Keynes, UK). Before each session of taking spectra, the spectrometer was calibrated using a silicon source. Seven spectra were taken per sample using 100% laser power, 30 s exposure time and 2 accumulations with a $1200\ \text{mm}^{-1}$ grating. Spectra of amyloid fibers were taken at 100% laser power, 35 s exposure time and three accumulations. Spectra were preprocessed and analyzed using the IRootLab toolbox (<http://trevisanj.github.io/irootlab/>) with Matlab 2013a (The Maths Works, MA). First, all spectra were cut to the fingerprint region of $900\text{--}1800\ \text{cm}^{-1}$. ATR-FTIR spectra were preprocessed by baseline correction using second order differentiation, vector normalization and then mean centered. Spectral data from Raman were preprocessed in the same manner but spectra were wavelet denoised before mean centering. In order to extract features from the large spectral data set, principal component analysis (PCA) and linear discriminant analysis (LDA) were used. PCA was used as a data reduction technique and optimal number of PCs was input into LDA to minimize intraclass variation. Leave-one-out cross-calculation was employed to avoid overfitting data. Tentative wavenumber assignments were given to loadings using FTIR and Raman assignments reported.^{21,22} In order to investigate the secondary structure of proteins in brain samples from high and low OC concentration groups, deconvolution of the mean Amide I peak was performed using PeakFit v4.12 software (Systat Software Inc., San Jose, California). Using the software, spectra underwent baseline correction and were cut to the Amide I region of $1600\text{--}1700\ \text{cm}^{-1}$. Second derivatives of spectra were used to identify subpeaks that were “hidden” within the Amide I peak. Final deconvolution of subpeaks was performed using Gaussian peak-fit functions with 20% smoothing. The r^2 value of each fitted curve was maintained above 0.999.

Staining Amyloid Fibrils. All reagents were purchased from Sigma (Sigma-Aldrich, Dorset, UK) unless stated otherwise. A Congo red stock solution was made up in 100 mL of 80% ethanol using 0.3 g of Congo red and 0.3 g NaCl. This was diluted to a working solution with the addition of 1

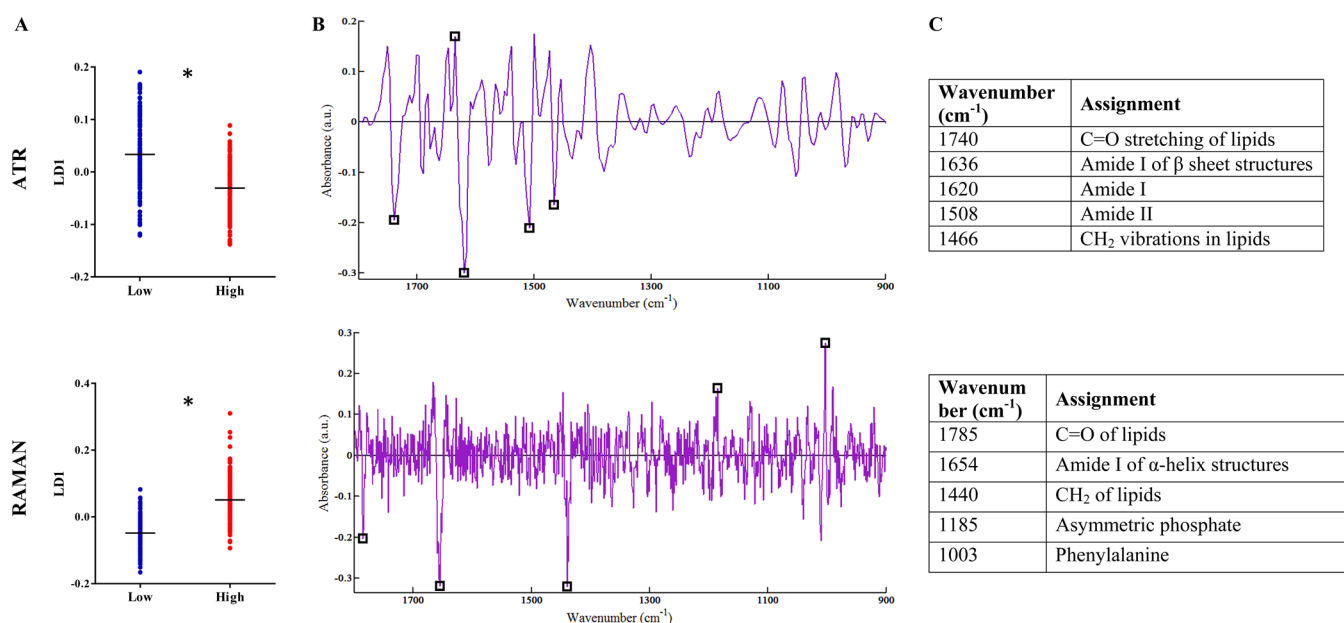


Figure 1. ATR-FTIR and Raman spectral comparison of brains samples from high and low OC exposure groups. (A) One-dimensional scores plots from cross-calculated PCA-LDA. Group means are represented by a black line and significance at the $P < 0.01$ level as determined by unpaired, two-tailed t tests is indicated by an asterisk. (B) PCA-LDA loadings along LD1 with top five peaks highlighted and (C) Tentative biochemical assignments for the top five wavenumber peaks.

mL of 1% NaOH. For each sample, 1 g of brain material was transferred onto a glass microscope slide and stained with Congo red working solution for 15 min. Slides were then rinsed in dH₂O before differentiation in alkaline alcohol and counterstaining with hematoxylin. Following this, they were briefly exposed to blueing reagent and rinsed with tap water. Slides were viewed using a 15 \times Replachromat objective on a Thermo Nicolet Continuum microscope with cross-polarizing filters, fitted with an Olympus U-TV0.5XC-3 video camera. Microscopy image contrast was adjusted and then sharpened with a 0.55 weight Unsharp mask using ImageJ software (<http://imagej.nih.gov/ij/>).

Quantifying Amyloid A β 1:42. To obtain tissue lysates suitable for ELISA, 100 mg of brain tissue was homogenized, on ice, in 500 μ L of TBS with 1% triton X-100 and 2 mM EDTA. Tissue homogenates were then centrifuged for 20 min at 13000 rpm. The supernatant was transferred to a fresh tube and stored at -80°C . The ELISA was performed using a colorimetric BetaMark x-42 ELISA kit from Biolegend (London, UK). The reagents, standards and test samples were diluted and prepared as outlined in the manufacturer's instructions. Fifty μ L of sample was loaded into each well along with 50 μ L of horseradish peroxidase detection antibody and incubated overnight at 4°C . The next day, wells were washed five times and incubated, in the dark, with the tetramethylbenzidine substrate for 50 min at room temperature. The plate was read at 620 nm using a Tecan Infinite 200 Pro microplate reader (Tecan, Männedorf, Switzerland). Results were analyzed by construction of a 4PL standard curve and interpolation using Graphpad Prism 4.

Statistical Analyses. GraphPad Prism 4 (GraphPad Software Inc., CA) was used to carry out statistical tests unless otherwise stated. Two-tailed, unpaired t -tests in order to compare PCA-LDA scores from two sample groups (High vs Low) or to compare absorbance at specific peak locations in the case of CySS:Protein ratio and amyloid peak analysis. Two-way ANOVAs with Sidak multiple comparison tests were performed

to compare PCA-LDA scores and account for interactions with other independent variables (Male vs Female or Adult vs Juvenile). T -tests and ANOVAs were done using sample spectral means rather than on individual spectra to avoid pseudoreplication. Normality of data was checked using D'Agostino-Pearson omnibus normality tests. Canonical correspondence analysis (CCA) was performed using XLSTAT (Addinsoft, New York). CCA is a direct gradient analysis technique and can be used to detect species variation patterns, which are caused by a provided set of environmental variables and thus explain variation in samples. Data was arranged into a species data table composed of spectral data and a table of environmental variables composed of OC concentration data. The CCA was run using 1000 random permutations.

RESULTS

Spectral Analysis of OC Exposed Brain Samples. To assess the effects of high and low OC pesticide exposure, Sparrowhawk brain samples were interrogated using ATR-FTIR and Raman spectroscopy and analyzed using cross-calculated PCA-LDA. High and low OC exposure groups were found to have significantly different spectral features. Figure 1A shows one-dimensional (1D) scores plots from PCA-LDA of ATR-FTIR and Raman spectra which illustrate the difference between the two exposure groups along linear discriminant (LD) 1. In scores plots distance denotes dissimilarity, thus we can see that diverse spectral alterations were induced by low (below $2.03 \mu\text{g/g}$) and high (above $7.18 \mu\text{g/g}$) OC concentrations as demonstrated by the differences between group means and in distribution patterns. Unpaired, two-way t tests using sample means (rather than spectral replicates) verified that the high and low exposure groups were significantly different at the $P < 0.01$ level. Analysis of corresponding ATR and Raman LD1 loadings (Figure 1B) was performed to detect biospectral alterations that were responsible for the separation observed between groups in the

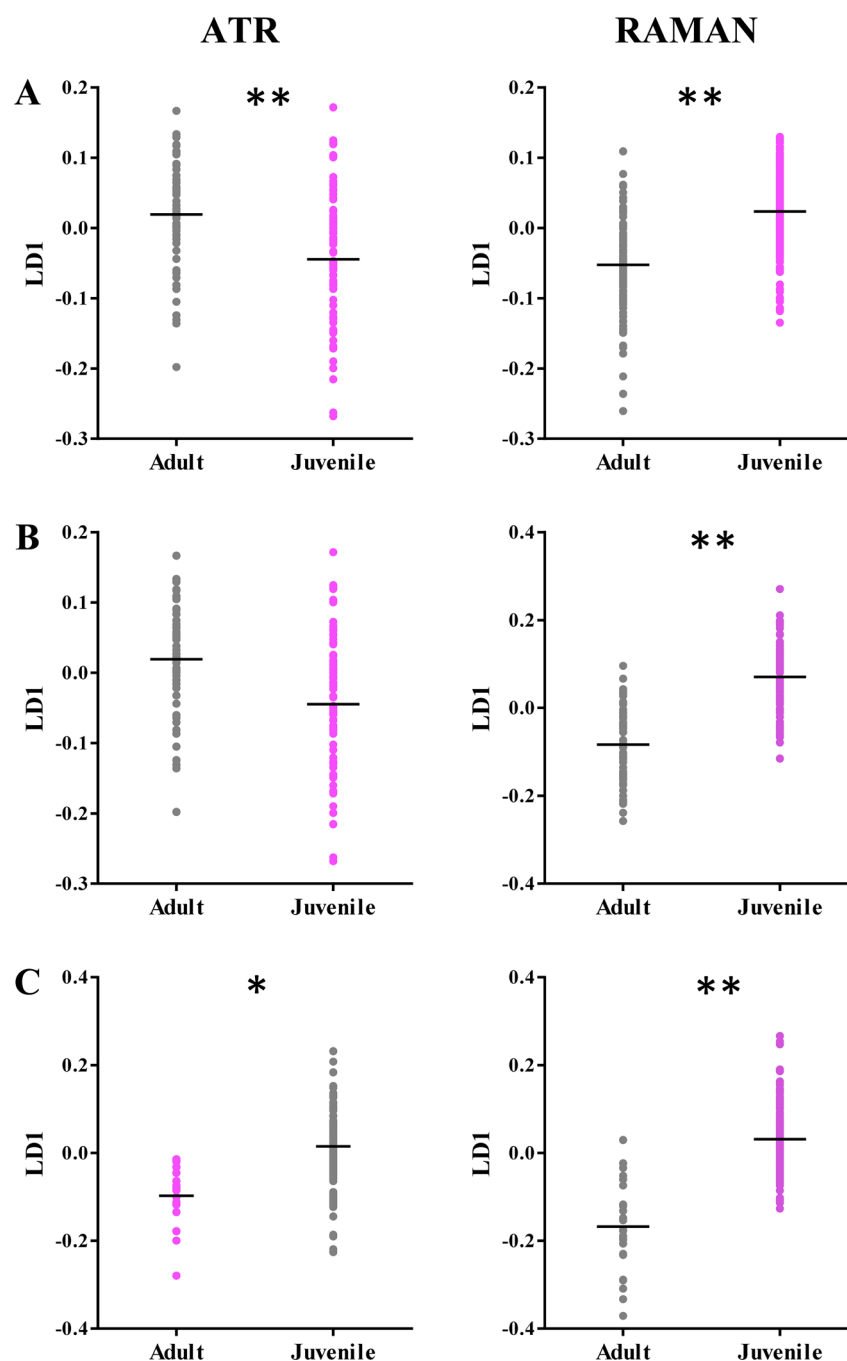


Figure 2. One-dimensional PCA-LDA scores plots from ATR-FTIR and Raman spectroscopy of brain samples showing age-dependent effects of OC exposure. (A) All samples, (B) high OC exposure group samples, and (C) low OC-exposure group samples. Group means are represented by a black line. Significance at the $P < 0.05$ level as determined by two-way ANOVA with Sidak multiple comparison test is indicated by one asterisk and significance at the $P < 0.01$ level is indicated by two asterisk.

scores plot. The top five peaks that contributed with the most magnitude to the observed variation were identified and tentative wavenumber alterations were assigned (Figure 1C). ATR-FTIR identified the top five wavenumber alterations in areas associated with C=O stretching and CH₂ vibrations of lipids (1740 cm⁻¹; 1466 cm⁻¹) and in regions associated with alterations in protein secondary structure. These comprised of changes in Amide I and Amide II spectral areas (1620 cm⁻¹; 1508 cm⁻¹) as well as alterations in β -sheet structures within the Amide I region (1636 cm⁻¹). Wavenumber alterations detected by Raman spectroscopy confirmed that variation

between the two exposure classes was due to alterations in C=O and CH₂ lipid regions (1785 cm⁻¹; 1440 cm⁻¹) and spectral regions corresponding with protein secondary structure as changes in the α -helix structures of Amide I (1654 cm⁻¹) were detected. Raman spectral analysis also determined that alterations in asymmetric phosphate stretching vibrations from DNA (1185 cm⁻¹) and the amino acid phenylalanine (1003 cm⁻¹) contributed importantly to the difference between low and high OC pesticide exposed brain samples.

Information was available on the age and sex of the birds from which brain samples were obtained so spectra were

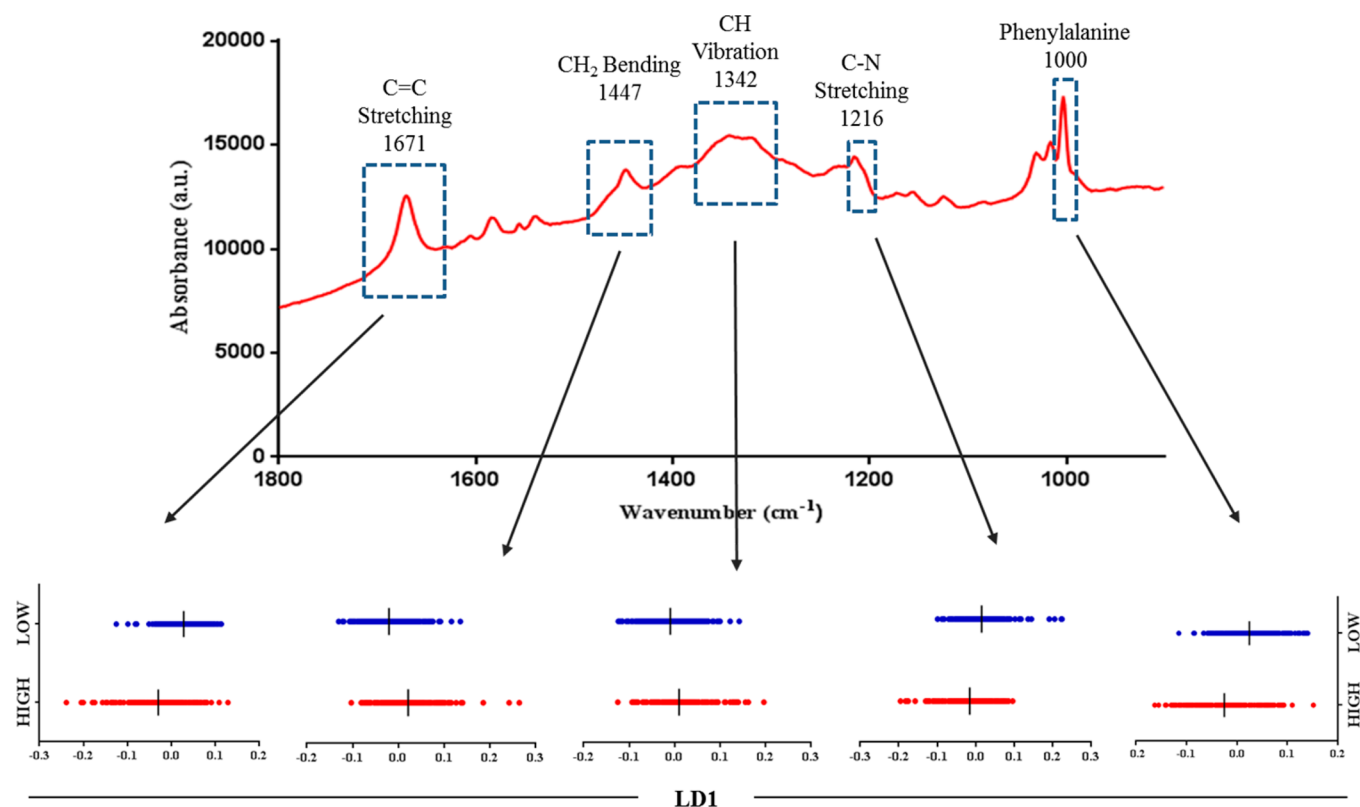


Figure 3. Raman spectra of amyloid A β 1:42 fibrils with main vibrational peaks highlighted in blue. ID PCA-LDA scores plots underneath show separation of high and low OC-exposed brain samples at these vibrational nodes. Group scores are significantly different at the $P < 0.05$ level as assessed by unpaired, two-way t tests.

reclassified accordingly so that possible age- and sex-dependent effects of OC exposure could be investigated. The significance of differences between PCA-LDA scores along LD1 was assessed using two-way ANOVAs with Sidak multiple comparison tests in order to account for the other independent variables (sex, age, OC concentration). P -value results from these analyses can be seen in SI Table S2. ATR-FTIR and Raman PCA-LDA scores plots that were classed by sex (SI Figure S2A) show that there were significant ($P < 0.01$) differences between male and female scores. Group means are significantly separated and distribution of spectra is altered along LD1. Sex-dependent effects were also analyzed individually in high and low OC exposure groups. Raman scores from samples exposed to high OC concentrations were significantly separated by sex along LD1 at the $P < 0.01$ level (SI Figure S2B). However, there was no significant difference found between male and female ATR-FTIR scores in the high OC group as there was a large amount of overlap between the two classes along the LD1 axis. Significant ($P < 0.01$) separation between male and female scores was revealed in the low OC exposure group using both spectral methods (SI Figure S2C). The effects of OC exposure were also found to be influenced by the age of the bird. Significant age-related differences at the $P < 0.01$ level were found in both high and low OC exposure classes when analyzed with Raman and at the $P < 0.05$ significance level for ATR-FTIR scores from the low OC exposure class (Figure 2B and C). Significant differences between adult and juvenile PCA-LDA scores were detected along LD1 using ATR-FTIR ($P < 0.01$) and Raman ($P < 0.01$) (Figure 3A). Therefore, the spectral results of exposure to OC pesticides are influenced by sex and age of the Sparrowhawk.

Contribution of Amyloid to Separation of High and Low OC-Exposed Samples.

To investigate the involvement of amyloid in the difference observed between high and low OC-exposed brain samples, amyloid A β 1:42 fibrils were interrogated using Raman spectroscopy (Figure 3). The five major vibrational peaks were identified as 1671, 1447, 1342, 1216, and 1003 cm^{-1} which are wavenumbers associated with C=C stretching, CH₂ bending, CH deformation, C–N stretching and phenylalanine, respectively. Some of these peaks are similar to those responsible for the separation of high and low Raman scores, in particular those related to regions of Amide I (C=C stretching), CH₂ vibrations and phenylalanine. The Raman spectral results were analyzed to see if the two OC exposure groups were separated at wavenumbers associated with amyloid spectral peaks. It was found that scores from high and low OC exposure brains were significantly dissimilar ($P < 0.05$) along LD1 at all the major peaks found in the amyloid spectrum. To further investigate, the Amide I peak was deconvoluted to reveal “hidden” subpeaks (Figure 4A). Deconvolution of the Amide I peak from both high and low OC exposure classes resulted in five peaks related to secondary protein structure. Analysis of subpeaks from high OC exposure spectra showed that there was a lesser proportion of α -helix than observed in the low OC exposure subpeaks. Conversely, more β -sheet was contributing to the Amide I peak in the high group than seen in the low group (see SI Table S3). As it is difficult to assess whether percentages are significantly different without additional values (numerator/denominator), we cannot state a definite significance but this should be explored in future work.

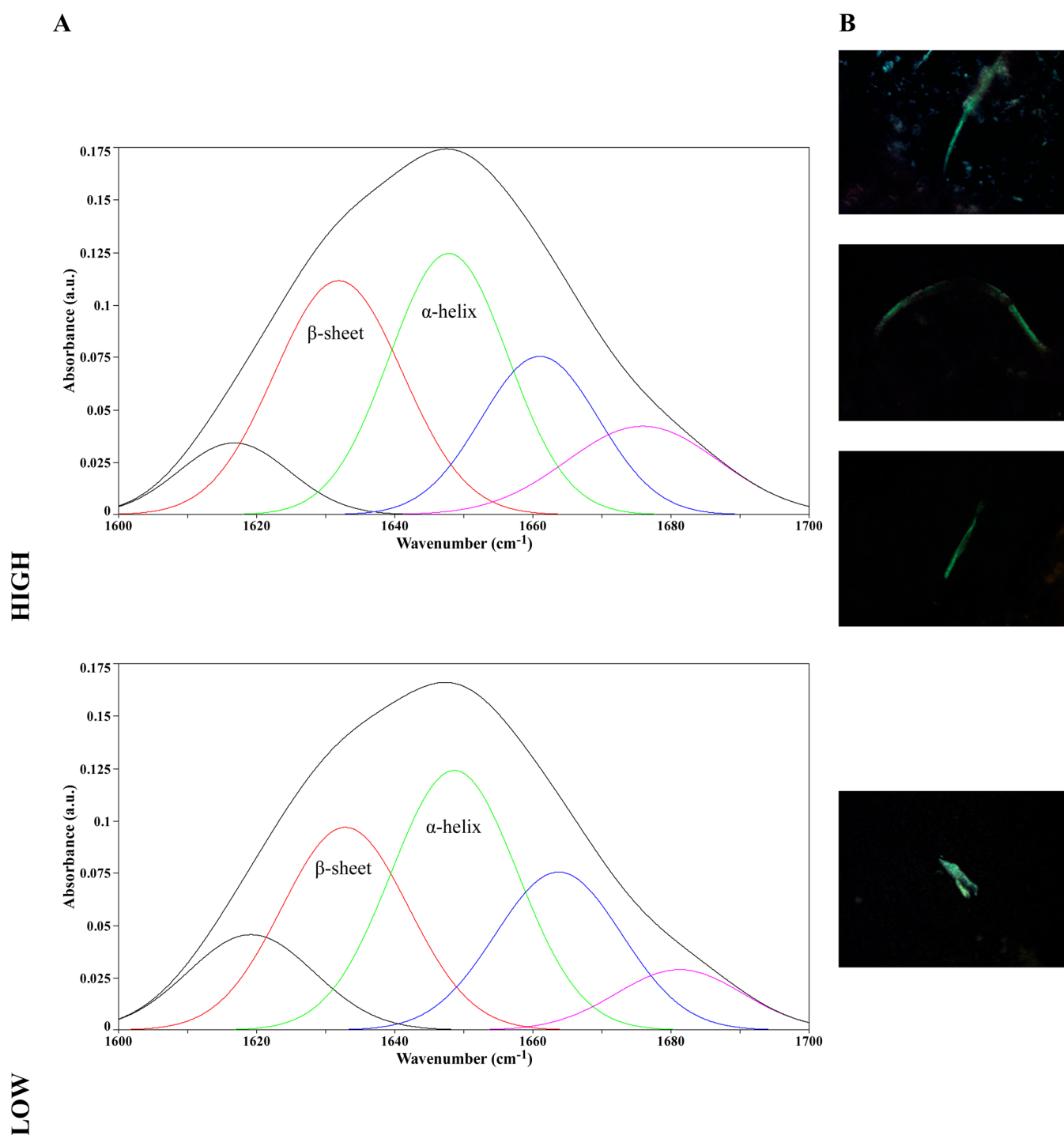


Figure 4. (A) Deconvolution of the Amide I peak derived from ATR-FTIR spectroscopy of high and low OC exposure groups, using second derivatives and Gaussian peak-fitting. (B) Microscopy images of Congo red stained brain samples, from high and low OC-exposure groups, which exhibited apple green birefringence when viewed under crossed polarizers.

Further to this, brain samples were stained with Congo red. All samples were stained and examined and four samples displayed the characteristic apple green color (Figure 4B). Three samples which were thin and fibrillar in appearance were from the high OC exposure group and one was from the low group. The three samples from the high group were all from male Sparrowhawks (two adults and one juvenile) and the sample from the low group was from a female. Finally, to confirm if amyloid was present in any of the samples, an ELISA was performed using lysates of the brain tissues (see SI Figure

S1). Amyloid A β 1:42 was detected in two of the brain samples but the points did not fall within the linear portion of the standard curve and so quantification would not be accurate. The two samples which contained amyloid were both from the low OC exposure group and both from juvenile female birds.

Correlation of Spectral Results and OC Chemical Exposure. A CCA was performed to determine if total OC pesticide exposure was having an effect on the variation seen in the spectral results between the high and low OC exposed brain samples. The analysis showed that the constrained variables

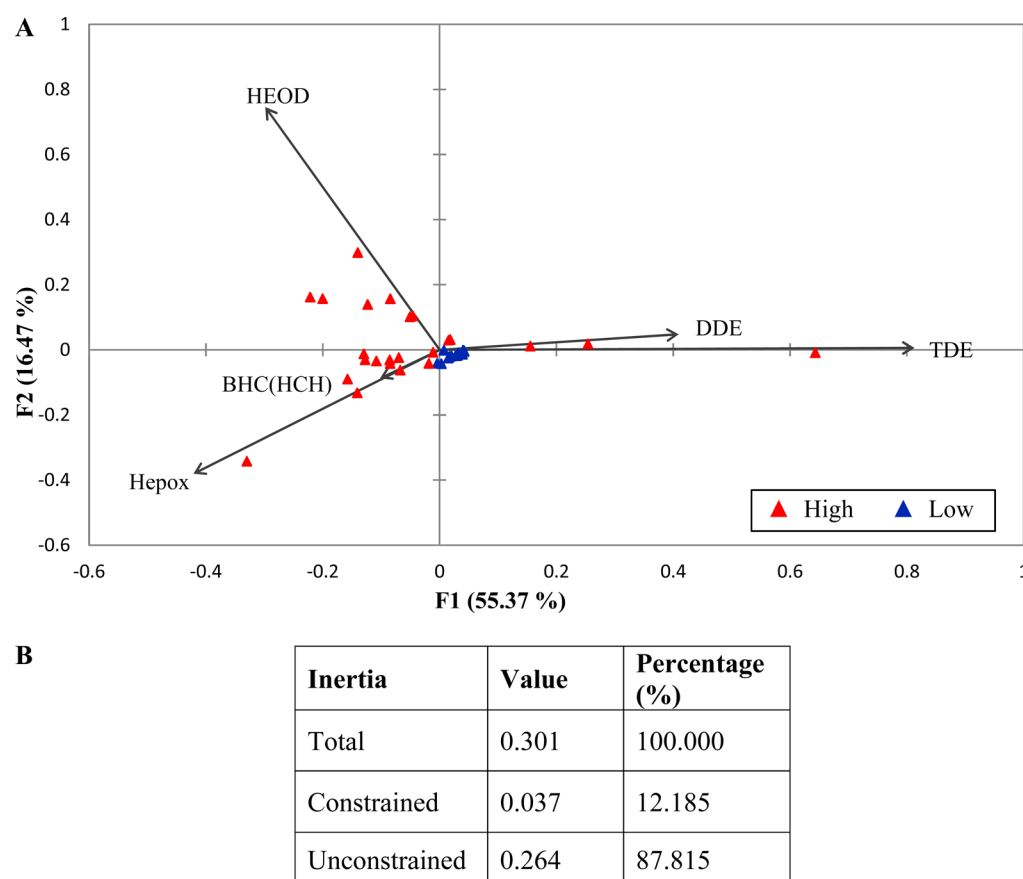


Figure 5. Correlation of chemical concentrations and ATR-FTIR spectral results from brain samples. (A) CCA ordination plot and (B) Inertia (variation) table showing correlation of spectral and chemical data from brain samples. Abbreviations as follows: BHC(HCH), (β)Hexachlorocyclohexane; TDE, tetrachlorodiphenylethane; Hepox, Heptachlor epoxide; DDE, dichlorodiphenyldichloroethylene; HEOD, Dieldrin.

explained 12.185% of the inertia observed (Figure 5B). This means that the imputed variables, that is, the chemical OC concentration data explain 12% of the variation seen in the spectral data. The remaining variance is due to other uncontrolled factors. The ordination plot (Figure 5A) showed that points from the low OC exposure group are more strongly coclustered than those from the high group, which exhibit more scattering and variability. There is a small amount of overlap but largely, the two groups are well separated and clustered along the F1 axis. Assessment of the length and position of chemical directional arrows shows that TDE, heptachlor epoxide and HEOD are the OC pesticides that are most important and influential for the ordination. It also shows that DDE and TDE exposures are correlated as are BHC(HCH) and heptachlor epoxide exposures.

DISCUSSION

The category of vibrational spectroscopy is composed of many techniques including FTIR and Raman methodologies which allow fast yet detailed analysis of biological samples. Such techniques are powerful tools which can be used to reveal alterations in biochemical composition and structure at the molecular level.²³ Infrared spectroscopy has previously been used in the monitoring and interrogation of environmental samples including assessment of the effect of water quality on tadpoles,²⁴ identifying biomarkers of water contamination in English sole²⁵ and detection of chemical exposure profiles in

Egret feathers to use as a monitoring tool.²⁶ Here, we have used ATR-FTIR and Raman spectroscopy to assess the effects of OC pesticide exposure and the implication of such exposures to amyloid aggregation in Sparrowhawk brains. Computational analysis of spectra was carried out to allow examination of spectral scores and loadings. We have identified that the major wavenumber associated alterations that occur in response to exposure to OC pesticides arise in lipids and the secondary structure of proteins. This is consistent with what we understand about the toxic mechanism of OC pesticides which can induce alterations in a range of biomolecules including proteins and lipids by modifying endocrine and apoptotic pathways.²⁷ The toxicity of strongly lipophilic chemicals such as OCs is often enhanced in the brain which is lipid rich and has poor detoxification mechanisms. As modifications in lipids and the secondary structure of proteins were identified by both ATR and Raman, these alterations may represent spectral markers of OC pesticide exposure in avian brain tissue.

The spectral results of OC pesticide exposure were found to be influenced by the sex and age of the bird. Sex-related differences in the body burdens of OC chemicals as well as other contaminants have previously been reported.²⁸ Research suggests that female body concentrations may be lower than their male counterparts due to loss of body mass during breeding and through the maternal transfer of chemicals into eggs.²⁹ The reported figures regarding the amount that is passed into eggs vary greatly but it is thought that as much as a

third of a female's OC burden could be transferred in this way and incorporated into the lipid-containing yolk.³⁰ Maternal transfer is also a relevant consideration for humans as secretion of OC pesticides into the breast milk of mammals including humans has been reported.^{31,32} The impact of age on the consequences of OC pesticide exposure has been well studied due to reports of neurodegeneration in aged individuals who are known to have been occupationally exposed to OCs during their lifetime.^{33,34}

Exposure to OC pesticides is regarded as an important environmental risk factor in the development of AD.³⁵ This study investigates the presence of amyloid in wild avian brains and attempts to link this to "real world" OC pesticide exposures. Using ATR-FTIR and Raman spectroscopy, we have identified that alterations in proteins, primarily changes in α -helix and β -sheet content, are induced by exposure to OCs. This suggests that a significant modification in secondary protein structure occurs in response to elevated OC pesticide concentrations (those above 7.18 $\mu\text{g/g}$). This is typically observed in amyloid- β protein aggregation which is characterized by a shift from α -helix to β -sheet formation as the peptide changes to an insoluble form.³⁶ Congo red staining also indicated that amyloid was present in four brain samples, three of which were exposed to high concentrations of OCs. This again suggests that exposure to higher concentrations of such pesticides may lead to amyloid aggregation in brain tissue. An exploratory A β 1:42 ELISA detected the peptide in two brain samples. Interestingly, the two birds from which these samples came from were both juvenile females and were both from the low OC pesticide exposure group. This suggests that other factors are also important in the process leading to amyloid aggregation and that possibly there are genetic factors involved. In humans, an increase in cerebral A β 1:42 has been associated with early onset AD.³⁷ As proposed by some "two-hit" hypotheses, genetic influences may act as a predisposition to amyloid aggregation that is mediated by other factors such as pesticide exposure. There are, however, many other factors involved in disease progression such as smoking, brain trauma and diet.³⁸ As there are numerous implicated factors, a CCA was performed to understand the relationship between the OC pesticide exposure and spectral results from the brain samples. This established that 12% of spectral variation was due to the influence of OCs, which is a reasonable amount as environmental and spectral data sets are complex with multiple factors to consider. In conjunction with spectral analysis, this lends evidence to the argument that exposure to elevated levels of OC pesticides are implicated in the aggregation of amyloid and possibly in the development of AD. Multiple studies have also found elevated OC pesticide levels in humans with AD.^{14,39}

Despite large scientific and financial input, there is currently no cure for AD and we still do not fully understand many aspects of the disease.⁴⁰ It is therefore imperative that research efforts continue to further our understanding of AD. However, many studies focusing on the role of chemical contaminants are often limited by the availability of chemical data and brain tissue from the same test subjects. Studies such as this one, aim to increase our knowledge base of the etiology of AD as predatory birds occupy a very similar position in the food chain as humans do. Being apex predators and relatively long-lived makes birds of prey ideal surrogates in lieu of human experimental subjects. The use of tissue and data from wild free flying birds, via initiatives such as the PBMS, allows us to study the effects of "real world" exposure profiles. Recent

reports have also suggested that the brains of birds are more similar to those of humans than previously thought and so the avian brain has been recognized as a potential model for human neuroscience.^{41,42} The results of studies like this may also be significant for avian research as well. Although wild birds do not usually live as long as their captive counterparts, many predatory bird species such as eagles and owls are remarkably long-lived and experience similar chemical exposures. If there are genetic predisposing factors as there are in humans, then younger birds and those with shorter lifespans may also be vulnerable to amyloid aggregation. Previously, an amyloid plaque has been identified in the brain of an aged captive woodpecker so there is a precedent of amyloid aggregation in the avian brain.⁴³ This also demonstrates that if birds live long enough, aggregation of the amyloid protein into cerebral plaques is possible. In homing pigeons, neurodegeneration due to age related synaptic loss has been associated with reduced spatial cognitive function.⁴⁴ If predatory birds develop amyloid aggregations and suffer synaptic loss, as has been demonstrated in humans, this could affect navigation and hunting behaviors, which require spatial cognition. Predatory birds may represent a new opportunity to study amyloid aggregation and AD risk factors, which has significant research implications for both humans and wildlife. This study also demonstrates that vibrational spectroscopy could be a useful tool in the investigation of OC pesticide exposure and potentially to identify markers of amyloid aggregation in brain tissue.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b00840.

Results that show findings of human anti-amyloid A β 1:42 calorimetric ELISA assay on Sparrowhawk brain lysates, sex-dependent effects of OC exposure, the panel of chemicals measured as wet weight concentration of contaminant in liver, and significance tests of PCA-LDA LD1 scores with age and sex (PDF)

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Notes

The authors declare no competing financial interest.

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