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Environmental exposure to organochlorine pesticides and its association with the risk of hearing loss in the Chinese adult population: A case-control study

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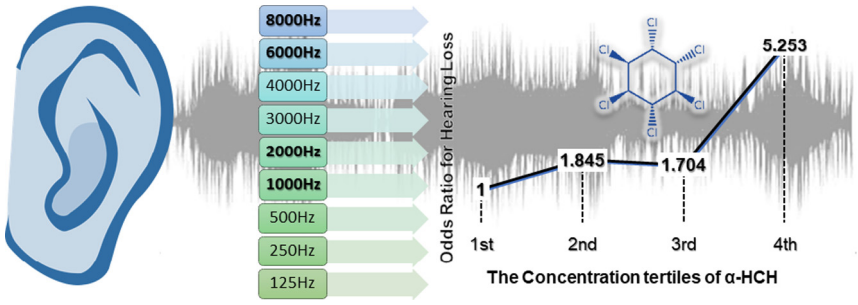
HIGHLIGHTS

- The association between serum OCPs and auditory impairment was evaluated in adults.
- Serum α-HCH level was a risk factor of increased prevalence of hearing loss.
- Correlation of α-HCH with worse hearing at mid- and high-frequencies.

GRAPHICAL ABSTRACT



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ABSTRACT

Hearing loss is a common chronic sensory deficit that has become a major public health concern worldwide. Hearing loss is well documented to be induced by noise and ototoxic drugs, and the association of hearing loss with environmental pollutants has received increasing attention. Organochlorine pesticides (OCPs) are an important group of environmental pollutants that exist ubiquitously in the human body and continue to represent a significant environmental health concern. Our case-control study was performed to explore the association between serum levels of OCPs and the risk of hearing loss in China, including 87 case-control pairs. Serum concentrations of 15 OCPs were measured. Pearson's correlation analysis and principal component analysis of frequently detected (>80%) OCPs showed a different distribution pattern, indicating possible exposure sources/scenarios for the case-control adult population. A higher α-hexachlorocyclohexane (α-HCH) level was a risk factor for an increased prevalence of hearing loss. The risk of hearing loss was increased by approximately 5.25-fold in the highest tertile compared with the lowest tertile. Furthermore, a significant association of the α-HCH level with an increased hearing threshold was observed at mid/high frequencies. This study provided the first evidence indicating that exposure to α-HCH might be a potential risk factor for hearing loss.

1. Introduction

Hearing loss, which is characterized by a decrease in hearing sensitivity, is a common chronic sensory deficit in humans and often occurs as the result of aging (Lin et al., 2019). Approximately 360 million people worldwide are estimated to suffer from an auditory impairment, and the number is increasing (Olusanya et al., 2014). Based on the United

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Nations World Health Organization (WHO) report published in 2017, the global annual cost of unaddressed hearing loss ranges from 750 to 790 billion dollars (WHO, 2017). A large number of studies have been conducted to support the connection between hearing impairment and ototoxic drugs and occupational ototoxicant exposure. However, the association between ambient pollutant exposure and hearing deficits has been less frequently explored.

Organochlorine pesticides (OCPs) are an important group of environmental organochlorine pollutants that are ubiquitously detected in the environment and biota. Although these chemicals have been banned or restricted for decades, their extensive usage, long half-life, and bioaccumulation still make them the most frequently detected xenobiotics in the human body. For instance, the fourth national report on environmental chemical burden from the U.S. Centers for Disease Control and Prevention (CDC) reported high serum concentrations of *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), a metabolite of the organochlorine pesticide dichlorodiphenyltrichloroethane (DDT), in U.S. residents (CDC, 2019). These OCPs are a cause for global concern because their existence is linked to various types of adverse health effects, such as endocrine disruption, immune dysfunction, and neurobehavioral impairment (Zhang et al., 2016).

Recently, researchers have expressed increasing interest in environmental ototoxicants as a risk factor contributing to hearing impairment (Fabelova et al., 2019). Animal studies have suggested a potential link between exposure to organochlorine pollutants and auditory impairment (Crofton et al., 2000; Powers et al., 2006). An early experiment showed that developmental administration of the commercial PCB mixture Aroclor 1254 induced permanent low- and mid-frequency auditory impairment (Herr et al., 1996). The results from subsequent studies revealed mechanistic insight that PCBs potentially function as endocrine disruptors in the thyroid hormone pathway and cause damage to outer hair cells (Crofton et al., 2000; Lasky et al., 2002). Only a few studies have investigated the ototoxicity of organochlorine pollutants in humans. An epidemiological survey indicated that serum PCB levels in school-age children were positively related to the hearing threshold at low frequencies and negatively related to the amplitude of the transient evoked otoacoustic emission response (Trnovec et al., 2008). Although many studies have implied the ototoxicity of PCBs, investigations that explore the association between other organochlorine pollutants, such as OCPs, and hearing loss are insufficient. One study reported that multiple OCPs, including *p,p′*-DDE and β-hexachlorocyclohexane (β-HCH), may affect the cochlear status during infancy (Sisto et al., 2015). As hearing loss is a chronic condition affecting all ages (Min et al., 2014), an evaluation of whether long-term exposure to OCPs at the environmental level is associated with a diagnosable auditory impairment in the general population is worthwhile.

In this study, we evaluated the serum concentration of 15 OCPs and investigated the association between serum OCP levels and auditory impairment. Recognizing the environmental risk of hearing loss may broaden the spectrum of environmental health outcomes, facilitate an improved understanding of how environmental pollutants might be deleterious to hearing function, and therefore help to establish prevention strategies for the population.

2. Method and materials

2.1. Study participant recruitment and serum sample collection

Research subjects were recruited from Hangzhou, Zhejiang Province, China, from September 2016 to October 2018. All related procedures were approved by the Hangzhou Normal University Ethics Committee (No. 2017LL107).

The audiometric tests were performed by a trained technician in a soundproof room at medical centers. A Madsen Itera clinical diagnostic audiometer (AT235, Interacoustics, Assens, Denmark) and TDH39 headphones (Telephonics Corporation, Farmingdale, New York, USA) were used, and the noise floor of the soundproof room was lower than 25 dB (A). The pure-tone audiometric thresholds of the left and right ears were measured at 125 Hz, 250 Hz, 500 Hz, 1000 Hz, 2000 Hz, 3000 Hz, 4000 Hz, 6000 Hz, and 8000 Hz in all subjects. These frequencies are standardly used in clinical practice and are an extension of the standards for hearing loss recommended by the World Health Organization (WHO-1997) (Murinova et al., 2016; WHO, 2013). The diagnostic criteria of hearing loss were an average hearing threshold of the standard frequency band (500 Hz, 1000 Hz, 2000 Hz, 4000 Hz) in the hearing-impaired ears greater than 25 dB (A) (WHO, 2013). Patients with ear diseases, such as drug-induced deafness and congenital deafness, or known occupational noise exposure were excluded.

We recruited 100 residents with worse-hearing ears in the standard band with an average hearing threshold of 25 dB (A) as the case group and performed 1:1 random matching based on age (±1 year) and gender. Information on demographic characteristics, smoking history, noise exposure, and disease history was collected through face-to-face interviews. The questionnaires of 13 case-control pairs were incomplete/missing; thus, these case-control pairs were excluded, and 87 case-control pairs were finally included in this study.

Each enrolled participant signed an informed consent form before blood collection. Venous blood was collected at the time of the interview by a certified nurse. The blood sample was centrifuged at 4000 rpm for 10 min, and the serum was stored at −80 °C until further analysis.

2.2. Measurements of organochlorine pesticides

Serum concentrations of 15 OCPs were measured, including aldrin, endrin, hexachlorobenzene (HCB), α-hexachlorocyclohexane (α-HCH), β-HCH, γ-HCH, δ-HCH, heptachlor epoxide isomers (HCEX A and B), *o,p′*-dichlorodiphenyldichloroethane (*o,p′*-DDD), *o,p′*-DDE, *o,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDT, and tetrachlorvinphos (TCVP). The internal standard (IS) tetrachloro-m-xylene (TCMX) was purchased from J&K Chemical, Beijing, China. The pretreatment of samples was performed as previously reported by Xu et al. (2017) and Yin et al. (2019), and validated according to the European Medicine Agency (EMA) method validation protocol (EMA, 2012). Briefly, 0.3 mL of the serum sample was spiked with 10 μL of premixed internal standards and then mixed with 0.5 mL of formic acid, 2.5 mL of ethanol, and 10 mL of n-hexane and dichloromethane (DCM) (1:1, *v*/v). The mixture was vortexed and ultrasonically extracted for 10 min, followed by centrifugation at 2000 rpm for 10 min. The organic phase was collected. The extraction procedure was repeated three times for better recovery. The extracts were evaporated to approximately 1 mL and then cleaned with a column filled with activated silica gel and Na_2SO_4. The target compounds were recovered by elution with 70 mL n-hexane and dichloromethane (DCM) (1:1, *v*/v) after a precleaning elution step. The eluent was evaporated to near dryness, redissolved in 50 μL of n-nonane and stored at −4 °C until quantification. All chemicals used in this experiment were purchased from J&K Chemical, Beijing, China.

The quantitative analysis of serum OCP congeners was performed using a gas chromatography-triple quadrupole mass spectrometer (Agilent 7890B GC/7000C, Agilent Inc. Santa Clara, USA). The sample was quantified using a previously described method (Yin et al., 2019). Detailed information is provided in the Supporting Information.

2.3. Quality control and quality assurance

Procedural blank and spiked blank samples were analyzed with each batch of samples to monitor potential external contamination. A premixed solution of OCP standards and internal standards in n-nonane was injected into every set of samples to inspect the condition of the instrument. Instrument maintenance was executed if the value of relative standard deviations (RSD) was >20%. The limit of detection for each OCP was calculated as a 3:1 signal to noise value (S/N). The

internal standard and method detection limit (MDL) of OCPs are provided in the Supporting Information (Supporting Information Table S1). A calibration curve was included at both the start and the end of each sequence and was used for quantification.

2.4. Statistical analysis

The OCP levels in serum samples were described using descriptive statistics. Extreme outliers, which were 3 times the SDs of the mean values, were excluded from the analysis. The detection frequencies (DFs%) of γ-HCH, *o,p′*-DDD, *o,p′*-DDT, TCVP, HECX B, and HECX A were lower than 20% in all samples and were excluded from further analysis. In this study, values below the MDL were assigned as MDL/√2 in the statistical analyses, while those values were considered not detected in the descriptive statistical analysis.

Total cholesterol (CHOL) and triglycerides (TG) were used to calculate the total lipid concentration using the equation TL (g/L) = 1.12 × CHOL + 1.33 × TG + 1.48 (Covaci et al., 2006). The concentrations of OCPs are reported in this paper as lipid-adjusted concentrations (ng/g lipid).

The concentrations of OCPs were analyzed for a normal distribution with the Kolmogorov-Smirnov test. The concentrations of the OCPs were then log-transformed to achieve a normal distribution. Pearson's correlation analysis was used to investigate the associations between OCPs. The OCPs (DF% higher than 80%) were further analyzed for the exposure source/scenario by performing a principal component analysis (PCA) between the case group and the control group.

The associations between OCPs and hearing loss were estimated using binary logistic regression models as odds ratios (ORs) and 95% confidence intervals (CIs). Age, body mass index (BMI), education, and stress (self-assessment from questionnaire) were selected as covariates based on the possible relationships with pollutants, and hearing loss was adjusted (Eskenazi et al., 2005).

All statistical analyses were conducted using SPSS (version 20.0 for Windows, IBM, Chicago, IL, USA), and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Participant characteristics

Characteristics of cases and controls stratified according to the presence or absence of hearing loss are summarized in Table 1. No significant differences were observed between the two groups in the distribution of age, education, BMI, smoking, and family history of hearing loss. However, compared with the control group, cases had lower annual household income and work/life stress.

3.2. Serum concentrations of organochlorine pollutants

Table 2 shows the detection frequency and mean concentrations of organochlorine pollutants in the serum samples of cases and controls. Among the 15 targeted OCPs, the detection frequency of *p,p′*-DDE was the highest, reaching 90.8%, similar to previous studies (Pan et al., 2019). The overall detection frequencies of 9 OCPs, including HCB, α-HCH, β-HCH, *p,p′*-DDD, *o,p′*-DDE, *p,p′*-DDT, *p,p′*-DDE, aldrin, and endrin, were higher than 80%, indicating persistent OCP exposure in the general population. The low detection frequencies of *o,p′*-DDD and *o,p′*-DDT were partially attributed to the higher detection limit of the method (Supporting Information Table S1).

We excluded 6 organochlorine pesticides (γ-HCH, *o,p′*-DDD, *o,p′*-DDT, TCVP, HECX B, and HECX A) from further analysis because of their low detection frequency (<20%). As described in the methods, the concentrations of chemicals were adjusted by calculating the total lipid concentration (Table 3). HCB was the compound with the highest geometric mean (GM) concentration, reaching 199 ng/g lipid, followed by *p,p′*-DDE. However, no significant difference in the serum concentrations of HCB or *p,p′*-DDE was observed between cases and controls. On the other hand, α-HCH was present at significantly higher levels in the cases ($p < 0.05$).

Table 1
General characteristic of hearing loss cases and controls.

Characteristic	Cases n (%)	Controls n (%)	*p*
Gender			1.000[b]
Male	45 (51.7)	45 (51.7)	
Female	42 (48.3)	42 (48.3)	
Age	51.3 ± 9.15	51.0 ± 9.27	0.799[a]
Education			0.896[b]
Elementary school	54 (62.1)	50 (57.5)	
High school	18 (20.7)	21 (24.1)	
College or more	15 (17.2)	16 (18.4)	
Monthly income per capita (RMB yuan)			0.007[b]
<4000	57 (65.5)	36 (41.4)	
4000–6000	21 (24.1)	38 (43.7)	
>6000	9 (10.3)	13 (14.9)	
Life stress			0.005[b]
High	4 (4.60)	11 (12.6)	
Medium	22 (25.3)	37 (42.5)	
Low	61 (70.1)	39 (44.8)	
Work stress			0.027[b]
High	4 (4.60)	12 (13.8)	
Medium	28 (32.2)	38 (43.7)	
Low	55 (63.2)	37 (42.5)	
Cigarette			0.767[b]
Never smoked	67 (77.0)	64 (73.6)	
Former smoker	7 (8.05)	6 (6.90)	
Current smoker	13 (14.9)	17 (19.5)	
Alcohol intake			0.721[b]
Never/few	65 (74.7)	64 (73.6)	
Former drinker	2 (2.30)	2 (2.30)	
Current drinker	20 (23.0)	21 (24.1)	
Family history of hearing loss			1.000[c]
Yes	22 (25.3)	23 (26.4)	
No	65 (74.7)	64 (73.6)	
Body mass index (BMI), kg m^{-2}			0.324[b]
<24	36 (41.4)	47 (54.0)	
24–28	39 (44.8)	32 (36.8)	
>28	12 (13.8)	8 (9.20)	
History of hypertension			1.000[c]
No	68 (78.2)	69 (79.3)	
Yes	19 (21.8)	18 (20.7)	
History of hyperlipidemia			0.815[c]
No	76 (87.4)	78 (89.7)	
Yes	11 (12.6)	9 (10.3)	

Statistical analysis applied: [a]*t*-test; [b]χ^2, [c] binomial distribution.

Table 2
Detection frequency and mean concentrations of OCPs.

Chemicals	DF_T (%)	Case ($n = 87$)		Control ($n = 87$)	
		DF (%)	Mean (ng/ml)	DF (%)	Mean (ng/ml)
Aldrin	89.7	88.5	0.846	90.8	1.45
Endrin	82.8	87.4	3.75	78.2	3.81
HCB	82.8	86.2	10.7	79.3	11.4
α-HCH	87.9	88.5	0.65	87.4	0.560
β-HCH	88.5	86.2	1.77	90.8	1.54
δ-HCH	79.3	78.2	0.42	80.5	0.504
o,p′-DDE	82.8	78.2	5.74	87.4	6.35
p,p′-DDD	80.5	82.8	1.38	78.2	1.26
p,p′-DDE	90.8	87.4	10.2	94.3	12.1
p,p′-DDT	88.5	86.2	0.330	90.8	0.410
γ-HCH	11.5	5.75	2.42	17.2	1.52
o,p′-DDD	2.30	1.15	0.407	3.45	0.449
o,p′-DDT	6.90	6.90	0.400	6.90	0.402
TCVP	16.7	17.2	0.057	16.1	0.061
HECX B	4.02	2.30	0.034	5.75	0.042
HCEX A	4.02	4.60	0.966	3.45	0.553

DF (%), detection frequency, DF_T (%), the detection frequency in the whole population.

Table 3
Lipid adjusted concentration distribution of OCPs.

Chemicals	Case		Control		*p*
	GM	Median (IQR)	GM	Median (IQR)	
Aldrin	22.2	20.99 (11.3–34.6)	24.2	21.8 (11.7–37.4)	0.417
Endrin	98.9	108 (36.2–149)	102	84 (25.4–144)	0.318
HCB	199	236 (105–398)	287.5	239 (122–407)	0.796
α-HCH	14.4	15.7 (7.90–29.4)	12.2	10.4 (6.36–24.6)	0.049*
β-HCH	27.0	25.2 (7.57–66.7)	29.6	26.2 (8.40–75.1)	0.647
δ-HCH	8.98	8.29 (3.55–15.2)	9.54	7.09 (3.93–13.2)	0.813
o,p′-DDE	42.6	78.7 (4.41–245)	79.3	133 (9.42–269)	0.275
p,p′-DDD	36.1	36.0 (21.1–59.9)	33.0	32.7 (16.4–49.2)	0.221
p,p′-DDE	167	206 (59.4–435)	270	246 (146–532)	0.258
p,p′-DDT	9.03	8.89 (5.66–13.8)	10.0	9.63 (4.97–18.6)	0.238
∑DDTs	296	367 (129–768)	398	438 (232–930)	0.260
∑OCPs	389	451 (303–681)	414	386 (276–639)	0.783

* Significant difference at $p < 0.05$, one-way ANOVA.

The potential associations between the general characteristics of the study population and the concentrations of OCPs were calculated using one-way ANOVA. No obvious correlation was observed with gender, education, monthly income per capita, life stress, cigarette smoking, alcohol intake, family history of hearing loss, history of hyperlipidemia, or BMI. However, age and work-related stress were significantly related to the concentration of α-HCH (Supporting Information Table S2).

The associations among 15 OCPs were evaluated using Pearson's correlation analysis of the log-transformed concentration (Table 4). A significant correlation ($p < 0.05$) was observed among most of the OCPs, which suggests a similar exposure pattern and fate in cases. However, the associations among OCPs were weaker in the control group. For instance, HCB and HCHs were significantly correlated in the case group but not in the control group. A similar difference was also observed for the correlation between β-HCH and DDXs. A potential explanation for this finding is differences in metabolism or the exposure scenario for the case-control population.

Principal component analysis (PCA) was used to assess the multiple effects of OCPs on hearing loss in the two groups. As shown in Supporting Information Table S3, the first 9 principal component (PC) variables accounted for 72.2% of the variation in the original chemical data with eigenvalues > 1. The remaining 27.8% of the variation was explained by PC variables 10–20 with eigenvalues < 1. We selected the first 3 PC variables, PC-1, PC-2, and PC-3, which accounted for 11.9%, 10.5%, and 9.5% of the variation in total chemical information, respectively, and had a high positive loading for *o,p′*-DDE, *p,p′*-DDE, β-HCH, and δ-HCH (Supporting Information Table S4). The 3D scatter plot of the OCPs for PC1 to PC3 is shown in Fig. 1, and a 2-dimensional version is provided in the Supporting Information (Fig. S1). Interestingly, the DDEs for the case and control groups were distributed axisymmetrically or centrosymmetrically in the loading plots. The distribution of β-HCH and δ-HCH in loading plots for PC3-PC1 and PC3-

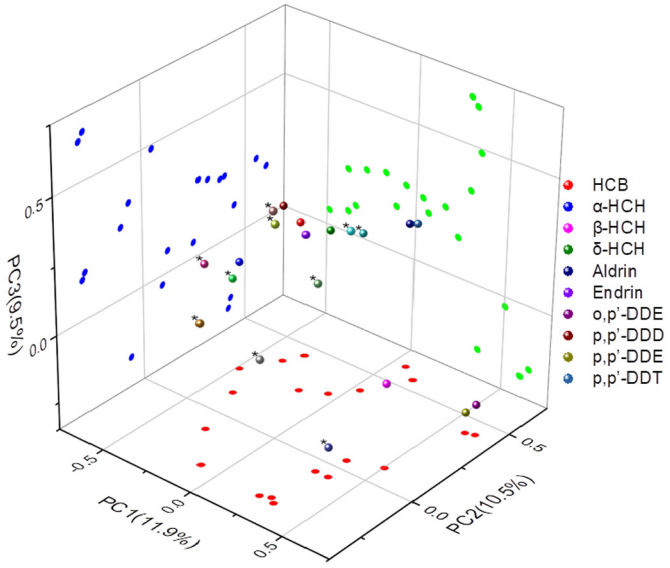

Fig. 1. Principal components analysis (PCA). Loading for OCPs for the three largest principal components (PCs). OCPs included in the PCA were detectable in ≥80% of samples. * represents the control group.

Table 4
Pearson correlation analysis of the log-transformed concentration of OCPs in the study population (cases and control).

	Aldrin	Endrin	HCB	α-HCH	β-HCH	δ-HCH	*o,p′*-DDE	*p,p′*-DDD	*p,p′*-DDE	*p,p′*-DDT
Case										
Aldrin	1									
Endrin	0.598[a]	1								
HCB	−0.463[a]	−0.630[a]	1							
α-HCH	−0.532[a]	−0.292[b]	0.683[a]	1						
β-HCH	0.637[a]	0.058	0.286[b]	0.008	1					
δ-HCH	−0.140	−0.304[a]	0.467[a]	0.295[b]	0.083	1				
o,p′-DDE	0.086	0.273[b]	−0.399[a]	−0.267[b]	−0.214	−0.691[a]	1			
p,p′-DDD	0.635[a]	0.659[a]	−0.463[a]	−0.339[a]	0.295[b]	−0.145	0.055	1		
p,p′-DDE	−0.376[a]	−0.651[a]	0.863[a]	0.589[a]	0.282[b]	0.761[a]	−0.583[a]	−0.439[a]	1	
p,p′-DDT	0.607[a]	0.457[a]	−0.306[a]	−0.328[a]	0.361[a]	0.028	−0.032	0.464[a]	−0.223	1
Control										
Aldrin	1									
Endrin	0.100	1								
HCB	0.189	0.313[a]	1							
α-HCH	0.001	0.264[b]	0.205	1						
β-HCH	0.082	0.552[a]	−0.145	0.158	1					
δ-HCH	−0.089	0.090	0.030	0.437[a]	0.021	1				
o,p′-DDE	−0.282[b]	−0.009	0.319[a]	0.316[b]	−0.256[b]	0.002	1			
p,p′-DDD	0.319[a]	0.244	0.277[b]	0.349[a]	0.222	0.028	0.061	1		
p,p′-DDE	0.644[a]	0.033	0.251[b]	0.047	−0.062	−0.019	−0.109	0.423[a]	1	
p,p′-DDT	0.316[a]	0.303[b]	0.395[a]	0.396[a]	0.124	−0.014	0.263[b]	0.589[a]	0.283[b]	1

[a] Correlation is significant at the 0.01 level.
[b] Correlation is significant at the 0.05 level.

Table 5
Association of α-HCH exposure with hearing loss in binary logistic regression models.

	Unadjusted		Adjusted[a]	
	OR (95% CIs)	*p*	OR (95% CIs)	*p*
Q1 (<7.32)	1		1	
Q2 (7.32–13.04)	1.63 (0.69–3.81)	0.265	1.85 (0.68–5.02)	0.230
Q3 (13.04–27.30)	1.32 (0.49–3.61)	0.586	1.70 (0.52–5.63)	0.382
Q4 (>27.30)	2.62 (0.94–7.35)	0.066	5.25 (1.45–19.0)	0.012*

[a] Adjusted by age, monthly income per capita, life, and work stress.
* Correlation is significant at the 0.05 level.

PC2 was also dramatically different. However, we were unable to identify factors that affect the distribution pattern. Many lifestyle factors that were not included in the present study may explain these differences, such as dietary habits, lifestyle, or other sociological factors. Further investigations are needed to explain the distribution pattern of the effects of environmental pollutants on hearing loss.

3.3. Association of organochlorine pollutant exposure with the hearing loss risk

The association between the risk of hearing loss and OCPs was analyzed using binary logistic regression models. As shown in Table 4, 10 OCPs had relatively higher detection frequencies (>75%) and were included in the subsequent analysis. Serum levels of DDT and its metabolites, especially *p,p′*-DDE, were detected in most of the samples but showed no significant differences between cases and controls. Only α-HCH was associated with an increased risk of hearing loss after lipid adjustment. As indicated above, age and work-related stress were proven to be correlated with the concentrations of α-HCH. The monthly income per capita and life stress showed a statistically significant difference between cases and controls. After further adjustment for these potential confounders, including age, monthly income per capita, life, and work stress, the correlation between α-HCH and hearing loss remained significant. A dose-response relationship of higher α-HCH levels with the risk of hearing loss was observed. When the lowest tertile was used as the reference in this adjusted model, an approximately 5.25-fold increase in the risk of hearing loss (95% CIs: 1.45–19.0) was observed for participants whose serum α-HCH levels were in the highest tertile (Table 5).

Similar analyses were performed on the correlations between OCP concentrations and hearing thresholds at different frequencies using a linear regression model. The hearing thresholds significantly increased with serum α-HCH concentrations at frequencies of 2000 Hz, 3000 Hz, 4000 Hz, and 6000 Hz in the hearing-impaired ears, but not for other OCPs (Supporting Information Table S2). After adjustment for confounders, a clear association was observed between the concentration of α-HCH and hearing impairment at mid-frequency (1000 Hz and 2000 Hz) and high-frequency (6000 Hz and 8000 Hz) ranges using binary logistic regression models (Table 6).

Table 6
Association of α-HCH exposure with hearing thresholds for different frequencies.

Frequency	β	OR (95%CIs)	*p*
125 Hz	−0.860	0.423 (0.094–1.90)	0.262
250 Hz	−0.418	0.658 (0.149–2.92)	0.582
500 Hz	0.134	1.14 (0.258–5.08)	0.860
1000 Hz	1.86	6.41 (1.20–34.2)	0.030*
2000 Hz	1.89	6.63 (1.28–34.3)	0.024*
3000 Hz	1.06	2.87 (0.635–13.0)	0.171
6000 Hz	2.06	7.87 (1.21–51.1)	0.031*
8000 Hz	2.05	7.80 (1.16–52.4)	0.035*

* Correlation is significant at the 0.05 level.

4. Discussion

Organochlorine insecticides are used worldwide for agricultural and public health. Due to the persistence and toxic effects on humans and wildlife, the use of OCPs has been restricted since the 1970s. Although these pesticides have been banned for decades, they remain ubiquitous in environmental samples, the food supply, and the human body and are therefore still a cause for global concern as a threat to human health (Niu et al., 2016).

Hearing loss in adults has become a major public health concern, and its prevalence is predicted to increase (Mathers et al., 2000). Chronic diseases, such as hypertension and diabetes, and some therapeutically used drugs with ototoxicity are involved in the pathogenesis of hearing deficits (Agrawal et al., 2009; Campo et al., 2013; Cruickshanks et al., 2003). To date, data regarding the adverse effects of environmental chemical exposure on hearing are insufficient, and recently, the recognition and evaluation of environmental ototoxicants has received increasing attention (Fabelova et al., 2019).

In the present study, higher serum α-HCH levels were positively associated with the prevalence of hearing loss. The confounding variables were evaluated among the general characteristics between the case and control groups to minimize bias induced by differences in the study population. The correlations between age and serum concentration of OCPs were possibly attributed to the longer environmental exposure period, which was also observed in previous studies (Kanazawa et al., 2012; Xu et al., 2017). The monthly income per capita was relatively higher in controls than in cases. This finding corresponded with previous studies indicating that hearing loss was positively related to decreased employment and income in the United States and Australia (Hogan et al., 2009; Jung and Bhattacharyya, 2012). Stress was also reported to be associated with hearing loss (Hasson et al., 2011; Jayakody et al., 2018). Thus, age, monthly income per capita, life stress, and work stress were considered potential confounding variables in subsequent analyses. After adjustment for these potential confounders, the association between the α-HCH concentration and hearing impairment was sustained, mainly at mid and high frequencies. Impairments in hearing mid and high frequencies may cause verbal cognitive deficits and reduce quality of life (Thomas et al., 1983).

HCHs are widely used as a mixture of isomers for insect control in agricultural applications. Before the official ban of HCHs in 1983, over 4.9 million tons of technical HCHs were produced in China, accounting for one-third of the total global production (Niu et al., 2013). Technical HCH is a mixture of α-HCH, β-HCH, γ-HCH, and other isomers. The most dominant isomer is α-HCH, with a proportion ranging from 60% to 70%. However, almost all of the insecticidal properties reside in γ-HCH, also known as lindane. γ-HCH is the most toxic isomer to mammals (Sudakin, 2007; Sujatha et al., 2001).

Consistent with previous observations, the median serum concentration of HCHs observed in this study was generally within the range reported in Tunisia (Ben Hassine et al., 2014). Many studies have indicated that HCHs pass through the blood-brain barrier and exert endocrine-disrupting effects (Khanna et al., 2002; Xue et al., 2010). A previous epidemiological study suggested that serum β-HCH levels in young children are associated with deficits in the cochlear status, but the mechanism was not explored (Sisto et al., 2015). However, no obvious association between the serum β-HCH concentration and hearing loss in the general population was reported previously or observed in this study.

Direct evidence for the ototoxicity of HCHs is rare (Fabelova et al., 2019). One possible hypothesis is that HCHs may interfere with hearing impairment by inducing oxidative stress (Sisto et al., 2015). Oxidative stress is a major cause of hearing loss (Becatti et al., 2017; Henderson et al., 2006). Experimental studies revealed that repeated administration of HCH at 10 mg/kg body weight/day induces oxidative stress in the rat cerebral hemisphere (Sahoo et al., 2000). α-HCH, γ-HCH and δ-HCH, but not β-HCH, were reported to stimulate superoxide anion

production in neutrophils (Kuhns et al., 1986; Tithof et al., 2000). Further studies designed to investigate whether long-term exposure to HCH may aggravate the prevalence of hearing impairment will be worthwhile in the future. Another potential target for HCH is the aryl hydrocarbon receptor (AhR) (Sisto et al., 2015). Activation of AhR has been shown to mediate a series of biological events resulting in damage to the cochlea and hearing loss (Safe and Luebke, 2016). HCHs exhibited potency in inducing the expression or activation of AhR (Jia et al., 2015; Wang et al., 2010).

The Fourth National Report by the U.S. Centers for Disease Control and Prevention indicated that the geometric mean serum *p,p′*-DDE concentration was 260 ng/g lipid in U.S. residents (CDC, 2019). In the present study, a high detection frequency and geometric mean concentration of *p,p′*-DDE were also observed, reaching 90.8% and 167 ng/g lipid, respectively. However, the serum levels of DDTs varied dramatically by region and exposure scenario and remarkably decreased over time (Wielsoe et al., 2017). The geometric mean concentrations of total DDTs and their metabolites evaluated here were 296.3 ng/g lipid, and the internal levels of individual or overall DDTs were not significantly differently between cases with hearing loss and the control group. Only a few studies have addressed the ototoxicity of DDT. Sisto et al. reported that *p,p′*-DDE exposure at environmental concentrations during infancy is significantly associated with deficits in cochlear status by measuring the amplitude of distortion product optoacoustic emissions (DPOAEs), which indicates the normal function of cochlear outer hair cells (Sisto et al., 2015). In two early studies, auditory potentials evoked in the cerebellum were impaired in rats with DDT administration (Woolley, 1968, 1976). However, the mechanism remains unclear. To the best of our knowledge, this study is the first to evaluate the association between DDTs and hearing loss in the general population. The serum DDT concentrations observed in this study are much lower than those in some agricultural countries with a larger historical DDT usage or countries that are still using DDTs for malaria control (Singh, 2001; Wong et al., 2008). Although our results indicated a weak association between DDTs and hearing loss, the potential effect of DDT exposure on the risk of auditory deficits in severely contaminated areas is worth evaluating.

To the best of our knowledge, this study is the first to evaluate the effects of OCPs on the risk of hearing loss in adults under ambient environmental conditions. However, the limitation of this study was evident. The relatively small sample size of 87 case-control pairs might limit the identification of statistically significant correlations. Further investigations with expanded sample sizes are required to validate the correlations observed in this study. Hearing loss was associated with income, and the monthly income per capita was relatively higher in controls than in cases in the present study. Although income was considered a confounding factor to be adjusted in the regression models, a source bias that cannot be ignored may still exist. Other unknown factors, such as coexposure to other environmental stressors, health conditions and lifestyles, may also contribute to hearing impairment.

In addition, environmental ototoxicants are generally accepted to target outer hair cells in the cochlea (Crofton et al., 2000; Safe and Luebke, 2016). Their state can be evaluated by measuring otoacoustic emissions (OAEs) (Murinova et al., 2016). In studies that evaluate auditory outcomes in relation to potential environmental ototoxicants, the examination of OAEs is becoming more prevalent. Thus, other tests, such as distortion product optoacoustic emissions (DPOAEs) and tympanometric examination, should also be evaluated in the future. Moreover, the proposed mechanisms of the adverse effect of α-HCH on hearing are insufficient, and related toxicological studies are worthwhile to obtain more evidence for potential mechanisms.

Furthermore, this study suggested the health risk of environmental ototoxicants and precautions, and further studies that link the human biomonitoring of environmental chemicals and epidemiological data of hearing impairment are required.

5. Conclusions

In the present study, we measured the serum concentrations of 15 OCPs and evaluated the potential association between the OCP burden and hearing loss. To the best of our knowledge, this study is the first to focus on the possible association between OCPs and hearing loss in the general population. Our results suggested an association between α-HCH exposure and an increased prevalence of hearing loss, indicating that environmental exposure to OCPs may contribute to the risk of hearing loss.

CRediT authorship contribution statement

Jianyun Zhang: Formal analysis, Writing – original draft, Visualization. **Chenhui Li:** Investigation, Formal analysis. **Shanshan Yin:** Methodology, Formal analysis, Writing – review & editing. **Yi Wang:** Formal analysis. **Yuanyuan Zhou:** Validation. **Shichang Wang:** Validation. **Xianrong Xu:** Formal analysis. **Weiping Liu:** Methodology. **Liangwen Xu:** Conceptualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Additional information concerning measurements of organochlorine pesticides, the association between the general characteristics of the study population, and the concentrations of OCPs and principal components analysis is available free of charge online in the Supporting Information (SI file). Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.145153.

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