의학석사 학위논문

유기염소 살충제와 비알코올 지방간 질환의 관련성

The association between organochlorine pesticides and nonalcoholic fatty liver disease

울산대학교 대학원

의 학 과

상 현 지

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이 논문을 의학석사 학위 논문으로 제출함

2022 년 2 월

울 산 대 학 교 대 학 원

의 학 과

상 현 지

상현지의 의학석사학위 논문을 인준함

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2022 년 2 월

Abstract

Background: Endocrine-disrupting chemicals (EDCs) are emerging as the cause of the nonalcoholic fatty liver disease (NAFLD). Widely used in the 20th century, organochlorine pesticides (OCPs) are absorbed into the body through food intake, respiratory tract, and skin contact and remain long even after discontinuing use. Recent studies have revealed a link between OCPs and metabolic syndromes such as obesity, diabetes, and dyslipidemia. However, little is known about their relevance with NAFLD. This study aimed to determine the association between exposure to OCPs and NAFLD prevalence.

Methods: We used National Health and Nutrition Examination Survey (NHANES) 2003–2004 data. Four substances, p,p'-DDE, oxychlordane, trans-nonachlor, and mirex, were analyzed. The exclusion criteria were as follows: age < 20 years, hepatitis B and C infection. The primary outcome was fatty liver index ≥ 60, a non-invasive marker for diagnosing NAFLD. We divided the serum concentration of OCPs into four quartiles and compared the adjusted odds ratios (OR) of NAFLD prevalence in each quartile.

Results: A total of 1512 participants was enrolled, and 579 were NAFLD patients who met the diagnostic criteria. We set the odds ratio (OR) to 1 as a reference in the fourth quartile for all substances. After adjusted logistic regression, oxychlordane had a higher risk of NAFLD with increasing serum concentrations (OR 0.288 in the first quartile, 95% CI 0.155–0.536). p,p'-DDE and trans-nonachlor did not significantly correlate with NAFLD, and mirex showed a higher prevalence of NAFLD at lower quartiles (OR 3.443 in the first quartile, 95% CI 2.072–5.721).

Conclusion: This study suggested that exposure to OCPs was associated with NAFLD prevalence, some of which showed a linear dose-dependent relationship. Periodic monitoring for NAFLD seems necessary for individuals with high exposure to OCPs in the general population.

Keywords: endocrine-disrupting chemicals, organochlorine pesticides, nonalcoholic fatty liver disease, fatty liver index

Contents

List of Tables and Figures

Introduction

Organochlorine pesticides, one of the endocrine-disrupting chemicals

Endocrine-disrupting chemicals (EDCs), also referred to as endocrine disruptors, disrupt various metabolisms and hormonal signaling pathways involved in the homeostasis in the body [1]. They are primarily synthetic and found in multiple substances such as pesticides, metals, additives, contaminants in food, and personal care products. Some representative substances include bisphenol A (BPA), phthalates, polybrominated diphenyl ethers (PBDEs), and parabens [2]. EDCs are exposed to the human body through ingestion of food, respiratory tract, and skin, which can have long-term health effects even after exposure has ceased.

Among EDCs, organochlorine pesticides (OCPs) belong to persistent organic pollutants (POPs), widely used in agriculture in the 1940s. Still, their use was banned by the US Environmental Protection Agency (EPA) and internationally by the Stockholm Convention because of their potential adverse effects on human health [1].

OCPs can be divided into two main groups: dichlorodiphenyltrichloroethane (DDT)-type compounds, including DDT and its metabolite, and chlorinated alicyclics, including aldrin, dieldrin, endrin, heptachlor, and chlordane [3]. OCPs have a chemical structure that represents a chlorine-substituted aliphatic or aromatic ring. They have properties of high persistence, low polarity, low aqueous solubility, and increased lipid solubility [4]. The half-life of organochlorine compounds varies from about 60 days to 10–15 years, depending on the type [3]. Even after discontinuation, OCPs have remained in the environment and accumulated through the food chain. Due to their fat-soluble properties, OCPs can be collected in the human body for a long time by consuming foods such as fatty dairy products and fish [1].

Organochlorine pesticides attracting attention as pathogenesis of metabolic diseases and nonalcoholic fatty liver disease

As industrialization progressed in the 20th century, exposure to EDCs became a social problem and attracted attention as it was known to affect metabolism and obesity as well as development, reproduction, and growth in humans. From animal experiments and epidemiological studies, it has been previously known that OCPs, polychlorinated biphenyls (PCBs), BPA, and phthalates in EDCs are associated with obesity. In addition, in epidemiological studies on humans, an association has been confirmed between the elevation of liver enzyme levels and EDCs, such as OCPs, PCBs, per- and polyfluoroalkyl substances

(PFAS), BPA, dioxin-based substances, and phthalates [2, 5-7].

Nonalcoholic fatty liver disease (NAFLD) encompasses a wide range of pathologies from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH), a condition that can progress to cirrhosis and hepatocellular carcinoma [8]. NAFLD is considered a sign of metabolic syndrome due to its association with obesity, insulin resistance, and type 2 diabetes [2]. According to a US study, the prevalence of NAFLD is 10–46%, with a median worldwide reported prevalence of NAFLD of 20% [9]. With the recent increase in NAFLD prevalence in Western countries, its etiology is also growing in importance, and EDCs are attracting attention as one of its possibilities. In particular, several studies have suggested the evidence that exposure to OCPs is associated with NAFLD, as well as obesity, insulin resistance, and metabolic syndromes such as type 2 diabetes and dyslipidemia [10, 11]. For instance, Al-Eryani et al. identified 123 chemicals associated with fatty liver, of which pesticides and their metabolites accounted for approximately forty-four percent [12].

Biochemical markers used to analyze the association between organochlorine pesticides exposure and nonalcoholic fatty liver disease

NAFLD's gold standard diagnostic test is a biopsy. However, most cases of NAFLD are asymptomatic or show nonspecific symptoms until they progress to decompensated liver cirrhosis or hepatocellular carcinoma, and their liver enzymes are usually in the normal range, so it is difficult to have opportunities to obtain biopsy results for the population exposed to EDCs.

For this reason, efforts to discover sensitive biochemical markers for early detection and prevention of NAFLD have continued. NAFLD is primarily diagnosed based on biochemical and imaging tests such as ultrasound. Recently, research on diagnostic biomarkers has been actively conducted. The predictive markers known so far include single biochemical parameters such as body mass index (BMI), waist circumference, aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), and scoring systems developed as predictive models such as fatty liver index (FLI).

ALT has been widely used as a non-invasive biomarker of NAFLD in several epidemiologic studies using National Health and Nutrition Examination Survey (NHANES) data. In particular, one of the previous studies investigated OCPs exposure and NAFLD prevalence using ALT in the US population based on NHANES 2003–2004 [13]. The cut-off for ALT can be affected by many variables such as age, gender, and race. Since the upper limit of normal ALT differs

according to age, race, and sex, the criteria for the ALT range to diagnose NAFLD are inconsistent [14]. In addition, the sensitivity for NAFLD might be lowered when ALT is used as a surrogate marker because NAFLD patients in the normal ALT range can be omitted [15]. Another limitation of ALT use is that ALT can be elevated not only by hepatic causes such as viral hepatitis, alcohol, autoimmune hepatitis, fatty liver, drugs, but also by nonhepatic causes such as metabolic syndrome, celiac disease, muscle damage, and hemochromatosis.

Recently, many studies have used FLI as a biomarker for NAFLD, one of the indices developed to predict NAFLD based on triglyceride, BMI, GGT, and waist circumference [16]. Although FLI is considered a more sensitive indicator for NAFLD than ALT, the evidence for the association between FLI and EDC exposure is still limited.

The purpose of this study was to determine the association between the exposure of OCPs, which is known to be related to various metabolic diseases among EDCs, and NAFLD prevalence using the fatty liver index. In addition, this study also investigated the relationship between OCP exposure and other NAFLD-related variables.

Design and Methods

Study design and participants

This study is a cross-sectional study based on the NHANES 2003–2004 census in the United States, which well-investigated exposure to EDCs in the body. NHANES is an annual survey to provide information on the health and nutritional status of the US population. Many of the existing studies investigating the effects of EDCs on the human body were based on the NHANES. The NHANES 2003–2004 is the most recent analysis of EDCs concentrations and NAFLD prevalence in the large populations in the United States.

The Centers for Disease Control and Prevention (CDC), in conjunction with NHANES, uses biomonitoring to provide an ongoing assessment of the US population's exposure to environmental chemicals. The NCHS Research Ethics Review Board approved data collection for NHANES 2003-2004. Analysis of de-identified data from the survey is exempt from the federal regulations for the protection of human research participants. The Institutional Review Board (IRB) of Asan Medical Center, Seoul, Korea reviewed the protocol of this study. It was exempted from the review because it included only secondary analyses for de-identified data (IRB number: 2021-1570).

Adults aged 20 years or older were enrolled, and among them, hepatitis B and hepatitis C positive patients and heavy drinkers were excluded. Here, heavy drinkers were defined as consuming more than 30 grams of alcohol per day for men and more than 20 grams for women [17]. Among them, subjects whose serum OCP concentration was measured were divided into two groups based on FLI 60.

Organochlorine pesticides

Serum levels of chlordane metabolites, including oxychlordane and trans-nonachlor, are still significant in the US population, according to the Fourth National Report on Human Exposure to Environmental Chemicals, published by the Centers for Disease Control and Prevention (CDC) utilizing the NHANES dataset. As subclasses of OCPs, p,p'-dichlorodiphenyldichloroethylene (DDE), a metabolite of DDT, oxychlordane, trans-nonachlor, and mirex, were selected. OCP substances not included in this study, such as hexachlorobenzene, heptachlor epoxide, aldrin, dieldrin, endrin, had serum concentrations that were too low below the detection limit, making it challenging to derive meaningful results.

OCP substances were measured in serum by high-resolution gas chromatography/isotope-

dilution high-resolution mass spectrometry (HRGC/ID-HRMS). Lipid-adjusted OCPs and routine biochemical profiles within NHANES 2003–2004 data were used. The description of the laboratory methodology was referred to the NHANES 2003–2004 codebook. [1].

Estimation of the prevalence of NAFLD using fatty liver index

The FLI was used as a surrogate marker for NAFLD, and it was calculated by the formula defined by Bedogni et al. [15]. It is already known that the fatty liver index has a better predictive ability than other known single biochemical parameters, such as BMI, waist circumference, ALT, and GGT [18].

According to previous references, if the FLI is less than 30, steatosis can be excluded, and if the FLI is greater than 60, it can be judged as hepatic steatosis. Since the sensitivity to fatty liver is known to be 61% and specificity to 86% when the FLI is 60 or higher [15], FLI 60 or higher was set as the outcome of NAFLD prevalence in this study in that it indirectly reflects the prevalence of NAFLD.

Outcome variables

The participants were divided into two groups for analysis according to the FLI cut-off value of 60; then, their demographic characteristics were analyzed. The questionnaire, examination, and laboratory data from the US population surveyed in the NHANES 2003–2004 were used.

Among the subjects in the NHANES census whose degree of exposure to OCPs was identified, the study analyzed whether the serum OCP concentration correlates with the odds ratio of NAFLD prevalence. Subjects whose lipid-adjusted serum OCP levels were measured were divided into quartiles according to the cumulative exposure ranking. The first quartile was defined as the subjects exposed to the lowest concentrations; otherwise, the fourth quartile referred to subjects exposed to the highest concentrations. The adjusted odds ratios of the NAFLD prevalence (FLI $\geq$ 60) of the remaining quartile groups were obtained by setting the fourth quartile group as a reference group. Separately, the adjusted means of other variables of NAFLD, such as AST, ALT, and GGT, were compared according to the OCP quartiles.

The fibrosis-4 (FIB-4) index, a variable used to predict hepatic fibrosis, consists of age, platelet, AST, and ALT and can be calculated relatively simply [19]. It is known that the FIB-4 index can predict advanced fibrosis in NAFLD when the cut-off is 2.67 or higher [20]. This study analyzed the association between hepatic fibrosis and OCPs by obtaining the adjusted odds ratio of FIB-4 index 2.67 or higher according to the degree of OCP exposure.

Statistical analyses

For the analysis of each group's clinical characteristics according to the FLI, a t-test was used for continuous variables, and a chi-square test was used for categorical variables. The association between OCPs and FLI and FIB-4 index was analyzed using logistic regression analysis. Pearson's correlation analysis was performed to evaluate the correlation with serum OCP concentration for variables constituting FLI—triglyceride, waist circumference, BMI, and GGT, respectively. ANCOVA was used for comparative analysis on the difference in adjusted means of other NAFLD-related variables (AST, ALT, GGT). Adjusted variables were set as age, gender, race, income, smoking, drinking, and physical activity. Statistical significance was defined based on $p < 0.05$. SAS (version 9.4, SAS Institute Inc., Cary, NC, US) was used for analysis.

Results

Demographic information

A total of 4861 adult subjects remained after applying the exclusion criteria. However, because not all contaminants were measured in all NHANES subjects, a total of 1515 subjects were measured their serum OCP concentrations, and they were divided into two groups based on FLI 60. Among them, 579 patients with NAFLD met the criteria for FLI of 60 or higher. Table 1 shows the clinical characteristics by dividing the participants into two groups based on the FLI 60.

Men accounted for more than women in the group of FLI above 60 (58.23% vs. 41.77%, $p < 0.0001$), whereas men had a lower proportion than women in the group of FLI less than 60 (39.09% vs. 60.91%, $p < 0.0001$). The mean age of the group with FLI 60 or higher was slightly older than that of the group with FLI less than 60 (47.94 years vs. 44.71 years, $p < 0.0001$). In the race, there was no significant difference in the composition ratio by the race between the two groups, and non-Hispanic white took the overwhelming majority (74.4% vs. 72.79%). There was no significant difference in the proportions between nonsmokers, ex-smokers, and current smokers between the two groups. The proportion of non-drinkers between the two groups did not significantly differ (11.71% vs. 11.27%). Low-income rates were also not significantly different between the two groups. Regarding physical activity, the group with FLI above 60 had relatively less activity than those below 60 (26.46% vs. 38.76%, $p < 0.0001$).

For obesity, the mean BMI was higher in the group with FLI more than 60 than the group with FLI less than 60 (33.4 kg/m^2 vs. 24.47 kg/m^2, $p < 0.0001$), Mean waist circumference was also longer in the group with an FLI of 60 or higher than with an FLI of less than 60 (110.96 cm vs. 88.1 cm, $p < 0.0001$). Mean serum glucose levels were higher in the FLI above 60 group than in the FLI below 60 group (107.81 mg/dL vs. 94.7 mg/dL, $p < 0.0001$), and mean serum triglyceride levels were also higher in the FLI group over 60 than in the FLI group below 60 (162.85 mg/dL vs. 86.19 mg/dL, $p < 0.0001$).

For liver enzymes, the mean AST of the FLI 60 or higher group was 24.87 mg/dL, slightly higher than the mean AST of 22.37 mg/dL in the FLI 60 or lower group ($p < 0.0001$). Mean ALT was also more elevated in the FLI above 60 group, 26.76 mg/dL in the FLI 60 or higher group, and 19.89 mg/dL in the below 60 group ($p < 0.0001$). The mean GGT was 28.22 mg/dL in the FLI 60 or higher group and 16.53 mg/dL in the FLI less than 60 group ($p < 0.0001$).

Summarizing the clinical characteristics of the NAFLD group defined based on FLI 60 or

higher, it was found that men, the elderly, obesity, hyperglycemia, high triglyceride levels, and high liver enzyme levels (AST, ALT, GGT) were the features of this group. There were no significant differences between the two groups for the race, smoking, alcohol drinking, and poverty income ratio.

The cut-offs of serum concentration by quartile section of each OCP subclass

Table 2 presents the cut-off values for each quartile when dividing participants into the quartiles according to the cumulative exposure rankings for each substance included in this study.

Relationship between OCPs and the prevalence of NAFLD estimated by fatty liver index

We showed the comparison of the adjusted odds ratios for FLI 60 or higher according to the concentration of each substance in OCPs (Table 3, Figure 1). Only two of the investigated OCP subclasses were significantly dose-dependently associated with the adjusted odds ratio for FLI 60. Oxychlordane had a higher risk of NAFLD with increasing serum concentrations (OR 0.288 in the first quartile, 95% CI 0.155–0.536, p = 0.0072). On the other hand, mirex showed a higher prevalence of NAFLD at lower quartiles (OR 3.443 in the first quartile, 95% CI 2.072–5.721, p = 0.0004). p,p'-DDE and trans-nonachlor did not significantly correlate with NAFLD.

Correlation with OCP exposure and each of the constituent variables of fatty liver index

Each variable constituting FLI was analyzed to see if there was a correlation with the serum concentration of OCPs. The p,p'-DDE showed a weak positive correlation with triglyceride concentration, waist circumference, and GGT but had no significant correlation with BMI (Figure 2A). Oxychlordane and trans-nonachlor showed weak positive correlations in all FLI parameters of triglyceride, waist circumference, BMI, and GGT (Figure 2B and 2C). On the other hand, mirex showed a weak negative correlation in triglyceride and BMI but showed a weak positive correlation in GGT and did not significantly correlate with waist circumference (Figure 2D).

Comparison of liver enzyme levels by OCP substance

The adjusted means of liver enzyme (AST, ALT, GGT) were compared for each OCP substance and exposure level among the blood chemistry data (Table 4). There was no

significant difference in the adjusted means for AST level according to the concentration of all four substances. In the case of p,p'-DDE, the means of serum AST, ALT, and GGT did not differ significantly with increasing concentration, which did not change the results before and after adjustment. For oxychlordane, there was a difference in the adjusted means of ALT and GGT according to the quartile divided by the exposure concentration, and it showed a tendency to increase as the concentration increased. Trans-nonachlor, similar to oxychlordane in the adjusted means of ALT, tended to grow in the high exposure group. However, there was no significant difference between quartiles in the adjusted means of GGT. For mirex, unlike other OCPs, the adjusted means for ALT and GGT tended to decrease in the higher quartile groups.

Association between OCP exposure and hepatic fibrosis

The analysis on whether the risk of developing hepatic fibrosis differs according to the concentration of OCPs was performed similarly. When hepatic fibrosis was evaluated when FIB-4 was 2.67 or higher, there was no significant association according to the concentration of all four substances for fibrosis (Table 5).

Table 1. Clinical characteristics of participants, grouped according to fatty liver index

	Fatty liver index		
	< 60 n = 783	≥ 60 n = 579	*p* value
Sex			< 0.0001
Male	39.09 (1.97) [c]	58.23 (1.31)	
Female	60.91 (1.97)	41.77 (1.31)	
Race			0.4884
Mexican American	7.32 (1.6)	8.07 (2.53)	
Other Hispanic	3.48 (0.82)	3.76 (1.48)	
Non-Hispanic White	72.79 (3.59)	74.4 (3.61)	
Non-Hispanic Black	9.89 (1.79)	10.06 (1.47)	
Other Race	6.52 (1.23)	3.7 (0.91)	
Smoking			0.14
Non	51.62 (2.9)	45.71 (2.87)	
Ex	22.83 (2.69)	29.64 (2.05)	
Current	25.56 (2.22)	24.65 (2.47)	
Never Drinker, yes	11.27 (1.23)	11.71 (1.63)	0.8262
Physical activity, yes [a]	38.76 (2.89)	26.46 (1.86)	< 0.0001
Low-income [b]	39.54 (3.25)	35.32 (2.66)	0.1908
Age (years)	44.71 ± 0.77 [d]	47.94 ± 0.77	< 0.0001
BMI (kg/m^2)	24.47 ± 0.09	33.4 ± 0.39	< 0.0001
Waist circumference (cm)	88.1 ± 0.43	110.96 ± 0.65	< 0.0001
Creatinine (mg/dL)	0.87 ± 0.01	0.9 ± 0.01	0.0026
Glucose (mg/dL)	94.7 ± 1.13	107.81 ± 2.04	< 0.0001
Total cholesterol (mg/dL)	199.53 ± 1.88	210.97 ± 1.7	0.0007
Triglyceride (mg/dL)	86.19 (81.87–90.74) [e]	162.85 (151.97–174.51)	< 0.0001
AST (mg/dL)	22.37 (21.75–23.02)	24.87 (24.27–25.48)	< 0.0001
ALT (mg/dL)	19.89 (19.17–20.63)	26.76 (25.75–27.81)	< 0.0001
GGT (mg/dL)	16.53 (15.56–17.55)	28.22 (26.71–29.82)	< 0.0001

Abbreviations: AST: aspartate transaminase, ALT: alanine transaminase, GGT: gamma-glutamyl transferase, CI: confidence intervals, SE: standard errors

[a] Physical activity: defined as moderate or vigorous activity over the past 30 days

[b] Low income: poverty income ratio < 2

[c] percent (SE)

[d] Mean ± SE

[e] Geometric mean (95% CI)

Table 2. Cut-off values according to quartile of organochlorine pesticide subclasses

Cut-off value (ng/g)	p,p'-DDE	Oxychlordane	Trans-nonachlor	Mirex
Q1	≤ 138	≤ 6	≤ 9.4	≤ 2
Q2	≤ 329	≤ 13	≤ 20.2	≤ 2.76
Q3	≤ 875	≤ 24.5	≤ 38.2	≤ 5
Q4	> 875	> 24.5	> 38.7	> 5

* First quartile (Q1): ≤ 25th percentile, second quartile (Q2): 25th–50th percentile, third quartile (Q3): 50th–75th percentile, fourth quartile (Q4): > 75th percentile

Table 3. Adjusted odds ratio (95% CI) for fatty liver index (≥ 60) by exposure quartile for organochlorine pesticides subclasses in adult NHANES 2003–2004

		% (SE)	OR (95% CI)		
			Model1 [a]	Model2 [b]	Model3 [c]
p,p'-DDE (ng/g)	Q1	35.47 (2.65)	0.678 (0.487, 0.946)	0.735 (0.449, 1.202)	0.71 (0.464, 1.087)
	Q2	39.93 (3.42)	0.82 (0.543, 1.241)	0.88 (0.577, 1.341)	0.895 (0.561, 1.428)
	Q3	50.68 (3.71)	1.268 (0.809, 1.988)	1.249 (0.822, 1.896)	1.259 (0.787, 2.015)
	Q4	44.76 (4.08)	1 (ref.)	1 (ref.)	1 (ref.)
	p value		0.0148	0.2375	0.2393
Oxychlordane (ng/g)	Q1	30.89 (3.09)	0.37 (0.261, 0.526)	0.298 (0.174, 0.512)	0.288 (0.155, 0.536)
	Q2	39.72 (3.26)	0.546 (0.36, 0.827)	0.475 (0.252, 0.894)	0.438 (0.236, 0.811)
	Q3	46.32 (4.8)	0.715 (0.439, 1.164)	0.653 (0.371, 1.152)	0.633 (0.343, 1.166)
	Q4	54.69 (3.12)	1 (ref.)	1 (ref.)	1 (ref.)
	p value		0.0002	0.0022	0.0072
Trans-nonachlor (ng/g)	Q1	31.94 (3.96)	0.504 (0.345, 0.736)	0.605 (0.35, 1.047)	0.591 (0.327, 1.066)
	Q2	40.53 (2.18)	0.732 (0.501, 1.07)	0.874 (0.521, 1.465)	0.839 (0.467, 1.504)
	Q3	49.67 (2.73)	1.06 (0.707, 1.588)	1.166 (0.716, 1.9)	1.096 (0.637, 1.888)
	Q4	48.22 (4.3)	1 (ref.)	1 (ref.)	1 (ref.)
	p value		0.0069	0.1592	0.1823
Mirex (ng/g)	Q1	54.27 (3.1)	2.086 (1.491, 2.919)	3.458 (2.216, 5.395)	3.443 (2.072, 5.721)
	Q2	32.55 (3.31)	0.848 (0.522, 1.377)	1.315 (0.788, 2.194)	1.22 (0.692, 2.15)
	Q3	44.44 (3.92)	1.406 (0.916, 2.157)	1.644 (1.048, 2.578)	1.51 (0.965, 2.364)
	Q4	36.26 (3.16)	1 (ref.)	1 (ref.)	1 (ref.)
	p value		0.0015	0.0002	0.0004

Abbreviations: OR: odds ratio, CI: confidence interval, SE: standard error, ref.: reference group

[a] Model1: Non-adjusted

[b] Model2: Adjusted for age, sex, race

[c] Model3: Adjusted for age, sex, race, poverty income ratio, smoking, drinking, physical activity

* First quartile (Q1): ≤ 25th percentile, second quartile (Q2): 25th–50th percentile, third quartile (Q3): 50th–75th percentile, fourth quartile (Q4): > 75th percentile

* Cut-off values according to quartile of organochlorine pesticides subclasses (see Table 2)

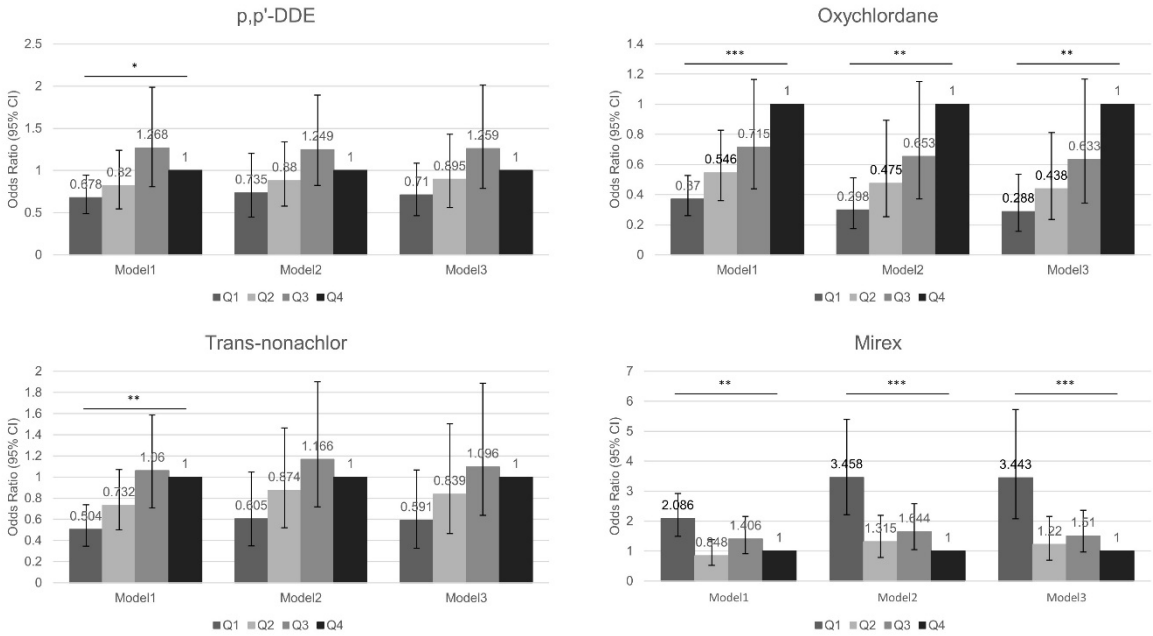
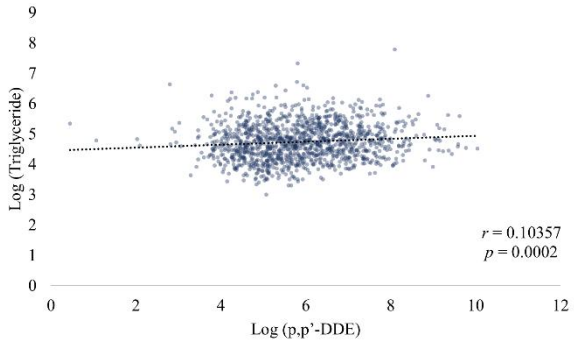
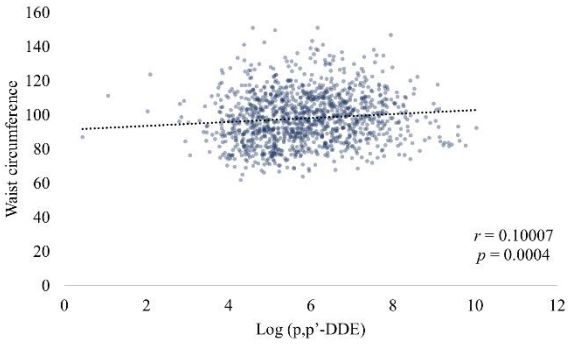


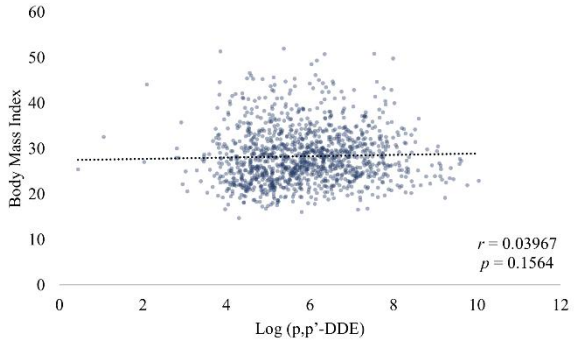
Figure 1. Adjusted odds ratio (95% CI) for fatty liver index (≥ 60) by exposure quartile for organochlorine pesticides subclasses in adult NHANES 2003–2004 (shown the contents of Table 3 as plots)

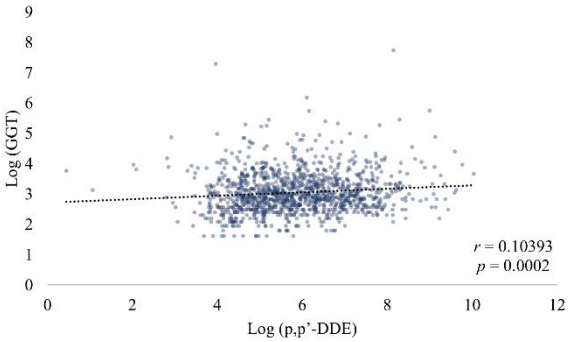
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

(A)

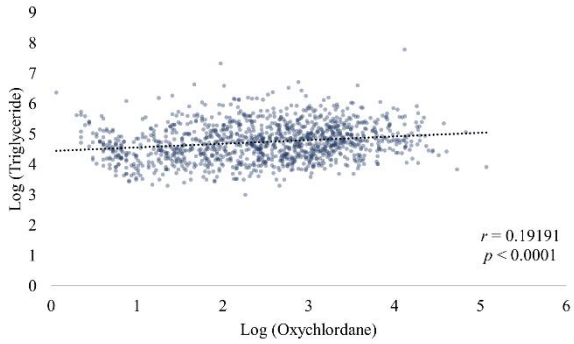

Log (Triglyceride)
Log (p,p'-DDE)
r = 0.10357
p = 0.0002

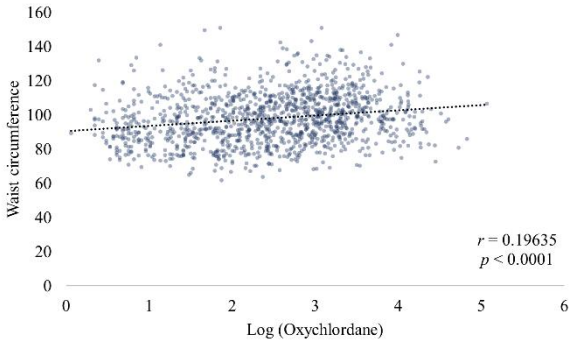

Waist circumference
Log (p,p'-DDE)
r = 0.10007
p = 0.0004

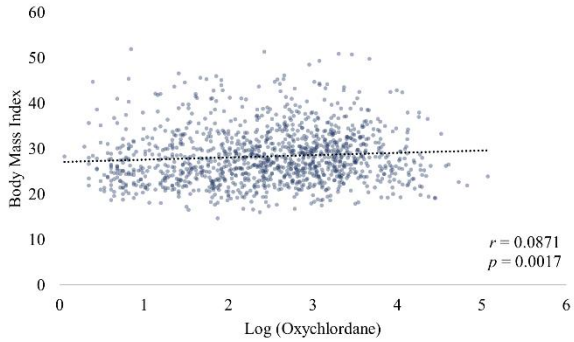

Body Mass Index
Log (p,p'-DDE)
r = 0.03967
p = 0.1564

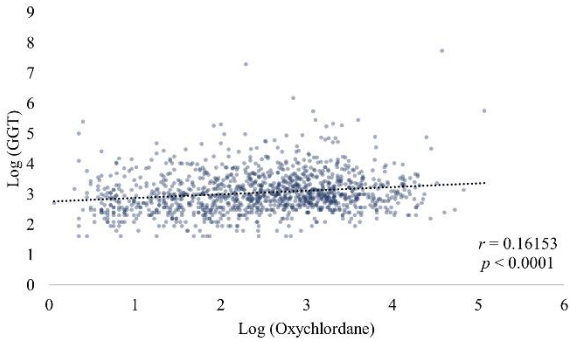

Log (GGT)
Log (p,p'-DDE)
r = 0.10393
p = 0.0002

(B)

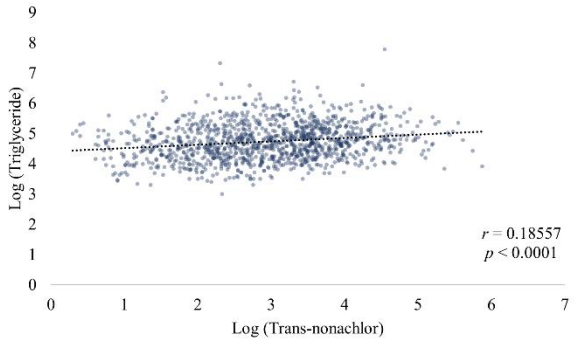

Log (Triglyceride)
Log (Oxychlordane)
r = 0.19191
p < 0.0001

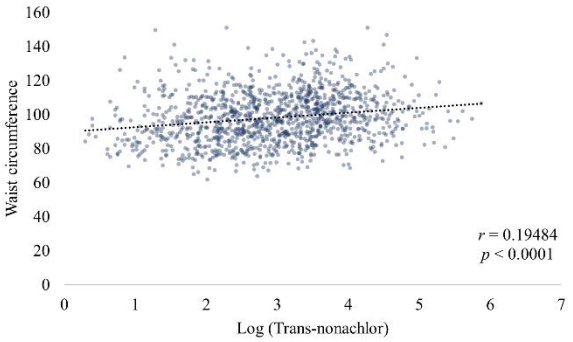

Waist circumference
Log (Oxychlordane)
r = 0.19635
p < 0.0001

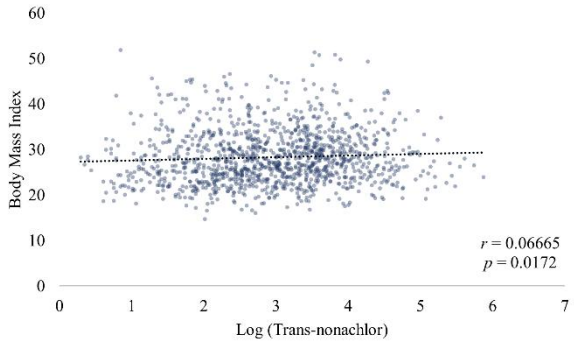

Body Mass Index
Log (Oxychlordane)
r = 0.0871
p = 0.0017

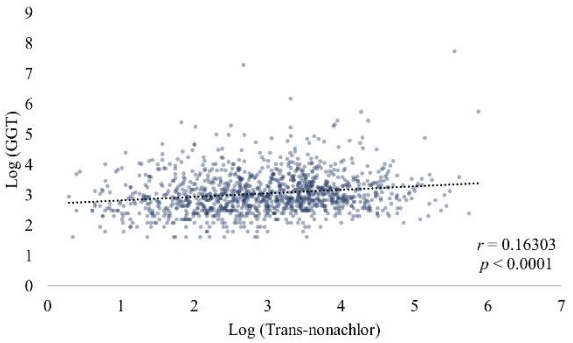

Log (GGT)
Log (Oxychlordane)
r = 0.16153
p < 0.0001

(C)

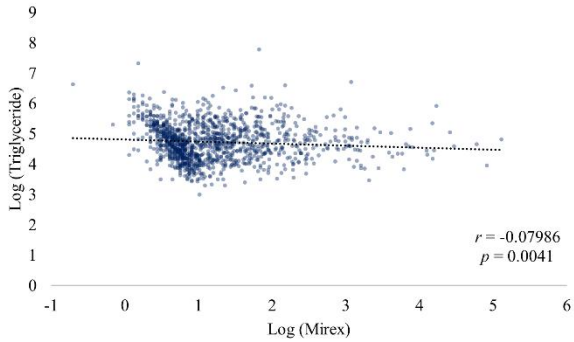

Log (Triglyceride)
Log (Trans-nonachlor)
r = 0.18557
p < 0.0001

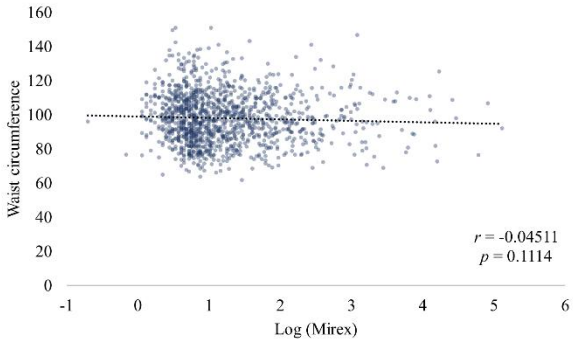

Waist circumference
Log (Trans-nonachlor)
r = 0.19484
p < 0.0001

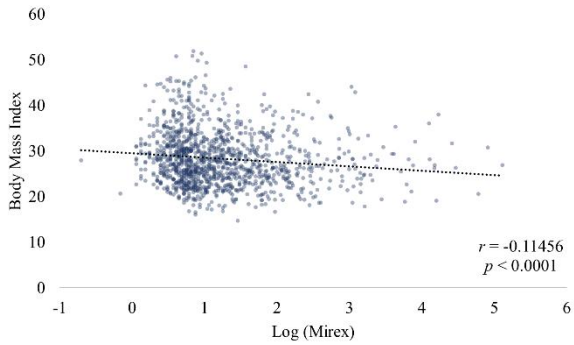

Body Mass Index
Log (Trans-nonachlor)
r = 0.06665
p = 0.0172


Log (GGT)
Log (Trans-nonachlor)
r = 0.16303
p < 0.0001

(D)


Log (Triglyceride)
Log (Mirex)
r = -0.07986
p = 0.0041


Waist circumference
Log (Mirex)
r = -0.04511
p = 0.1114


Body Mass Index
Log (Mirex)
r = -0.11456
p < 0.0001

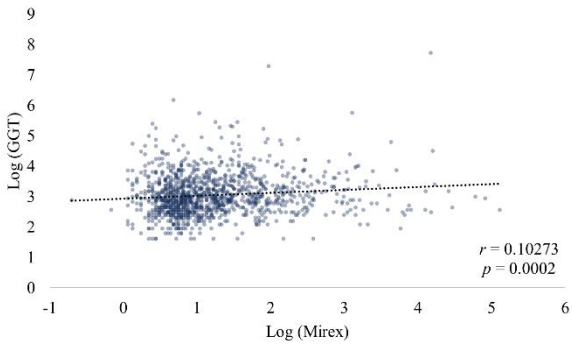

Log (GGT)
Log (Mirex)
r = 0.10273
p = 0.0002

Figure 2. Correlation analysis between log-transformed OCP serum concentration and FLI component variables. **(A)** p,p'-DDE **(B)** Oxychlordane **(C)** Trans-nonachlor **(D)** Mirex. The correlation coefficients (r) and p values are described in each scatter plot.

Table 4. Adjusted means (95% CI) for other NAFLD-related variables (AST, ALT, GGT) according to quartiles of organochlorine pesticide subclasses in adult NHANES 2003–2004

		Adjusted Mean (95% CI)								
		AST			ALT			GGT		
		Model1 [a]	Model2 [b]	Model3 [c]	Model1	Model2	Model3	Model1	Model2	Model3
p,p'-DDE (ng/g)	Q1	22.67 (21.76–23.62)	23.76 (22.41–25.2)	23.49 (22.19–24.87)	22.2 (21.26–23.17)	21.79 (20.58–23.07)	21.56 (20.51–22.67)	20.27 (18.72–21.95)	22.47 (19.94–25.33)	22.28 (19.74–25.16)
	Q2	23.65 (22.65–24.69)	24.51 (23.12–25.98)	24.34 (22.92–25.84)	22.88 (21.64–24.19)	22.67 (21.53–23.87)	22.99 (21.9–24.14)	20.27 (18.69–21.98)	21.79 (19.68–24.12)	22.08 (19.96–24.42)
	Q3	23.92 (22.92–24.97)	24.31 (23.15–25.54)	24.58 (23.44–25.77)	23.23 (22.01–24.53)	23.18 (21.59–24.9)	23.5 (21.95–25.15)	22.06 (19.82–24.56)	22.2 (19.31–25.52)	21.98 (19–25.42)
	Q4	24.05 (22.87–25.29)	24.38 (23–25.83)	24.43 (22.71–26.29)	21.94 (20.4–23.6)	22.85 (21.1–24.74)	22.96 (20.97–25.15)	21.24 (19.15–23.56)	21.21 (19.14–23.49)	21.37 (18.91–24.15)
	p value	0.1701	0.6128	0.3392	0.4735	0.3496	0.0756	0.4656	0.7648	0.9323
Oxychlordane (ng/g)	Q1	22.27 (21.13–23.47)	22.74 (20.9–24.74)	22.44 (20.64–24.4)	21.23 (20.03–22.51)	20.15 (18.76–21.64)	19.87 (18.72–21.09)	17.75 (16.24–19.41)	18.8 (16.68–21.18)	18.74 (16.86–20.83)
	Q2	23.64 (22.89–24.41)	24.53 (23.19–25.94)	24.22 (22.91–25.6)	23.61 (22.63–24.64)	23.51 (22.08–25.03)	23.26 (21.83–24.77)	21.62 (19.98–23.38)	23.23 (21.07–25.6)	22.67 (20.71–24.82)
	Q3	24.02 (23–25.09)	24.93 (23.85–26.05)	25.08 (23.99–26.22)	23.72 (21.99–25.58)	24.43 (22.59–26.42)	25.01 (23.16–27)	21.81 (19.11–24.88)	22.67 (19.3–26.64)	22.92 (19.46–26.98)
	Q4	24 (23.04–25)	24.98 (23.74–26.3)	25.43 (24.09–26.85)	21.04 (19.43–22.8)	22.78 (21.09–24.61)	23.33 (21.48–25.34)	22.45 (19.55–25.77)	22.94 (19.05–27.63)	23.46 (19.06–28.86)
	p value	0.04	0.1315	0.0888	0.0104	0.0003	0.0004	0.0087	0.0336	0.0218
Trans-nonachlor (ng/g)	Q1	22.67 (21.67–23.71)	23.39 (21.73–25.19)	23.09 (21.68–24.61)	21.62 (20.42–22.9)	20.78 (19.21–22.48)	20.47 (19.27–21.75)	18.66 (17.13–20.33)	20.33 (18.28–22.61)	20.03 (18.14–22.13)
	Q2	23.08 (22.18–24.02)	24.13 (22.68–25.67)	24.09 (22.61–25.68)	22.76 (21.77–23.79)	23 (21.52–24.58)	23.24 (21.85–24.72)	20.36 (18.79–22.07)	22.09 (20.07–24.32)	21.72 (19.79–23.83)
	Q3	24.4 (23.57–25.24)	25.1 (24.08–26.18)	25.13 (23.94–26.37)	24.06 (22.59–25.63)	24.6 (22.84–26.49)	24.81 (22.88–26.91)	22.38 (20.5–24.43)	22.92 (20.15–26.07)	23.27 (20.4–26.55)
	Q4	24.08 (23.1–25.11)	24.56 (23.33–25.86)	24.97 (23.67–26.34)	21.7 (20.31–23.18)	22.64 (21.37–24)	23.3 (21.8–24.9)	22.83 (20.83–25.03)	22.17 (19.2–25.6)	22.73 (19.26–26.82)
	p value	0.0062	0.341	0.1	0.0437	0.0217	0.0007	0.0097	0.4791	0.225
Mirex (ng/g)	Q1	23.74 (22.73–24.8)	24.87 (23.21–26.66)	24.95 (23.19–26.83)	23.87 (22.81–24.99)	24.43 (22.75–26.24)	24.54 (23.03–26.15)	21.45 (20.29–22.66)	24.17 (21.82–26.78)	24.43 (21.67–27.54)
	Q2	22.39 (21.92–22.87)	23.44 (22.22–24.72)	23.53 (22.31–24.82)	21.25 (20.11–22.45)	21.62 (19.85–23.55)	21.85 (20.03–23.83)	17.9 (16.81–19.07)	19.84 (18.17–21.66)	20.03 (18.22–22.02)
	Q3	23.41 (22.42–24.44)	23.75 (22.6–24.96)	23.91 (22.71–25.17)	22.73 (21.42–24.13)	22.3 (21.01–23.68)	22.58 (21.3–23.94)	23.15 (21.49–24.94)	23.3 (21.28–25.51)	23.11 (20.92–25.52)
	Q4	24.7 (23.93–25.49)	24.85 (23.95–25.79)	24.6 (23.63–25.61)	22.64 (21.36–24)	22.34 (21.12–23.63)	22.43 (21.13–23.81)	21.51 (18.71–24.73)	20.6 (17.83–23.81)	20.56 (17.67–23.93)
	p value	0.0003	0.0986	0.1897	0.0068	0.0062	0.0029	< 0.0001	< 0.0001	0.0012

Abbreviations: AST: aspartate transaminase, ALT: alanine transaminase, GGT: gamma-glutamyl transferase, CI: confidence intervals

[a] Model1: Non-adjusted

[b] Model2: Adjusted for age, sex, race

[c] Model3: Adjusted for age, sex, race, poverty income ratio, smoking, drinking, physical activity

Table 5. Adjusted odds ratio (95% CI) for fibrosis-4 (FIB-4) index (≥ 2.67) by exposure quartile for organochlorine pesticides subclasses in adult NHANES 2003–2004

		% (SE)	OR (95% CI)		
			Model1 [a]	Model2 [b]	Model3 [c]
p,p'-DDE (ng/g)	Q1	1.56 (0.67)	0.297 (0.102, 0.862)	1.717 (0.538, 5.481)	3.225 (0.855, 12.163)
	Q2	1.17 (0.67)	0.222 (0.052, 0.941)	0.975 (0.308, 3.082)	0.403 (0.088, 1.85)
	Q3	1.57 (0.68)	0.299 (0.091, 0.979)	0.516 (0.158, 1.679)	0.692 (0.19, 2.522)
	Q4	5.07 (1.31)	1 (ref.)	1 (ref.)	1 (ref.)
	p value		0.0714	0.4261	0.1905
Oxychlordane (ng/g)	Q1	1 (0.79)	0.165 (0.028, 0.985)	2.495 (0.34, 18.297)	3.05 (0.24, 38.803)
	Q2	1.29 (0.68)	0.213 (0.072, 0.631)	1.568 (0.515, 4.772)	0.81 (0.181, 3.616)
	Q3	1.14 (0.49)	0.188 (0.06, 0.592)	0.471 (0.153, 1.451)	0.467 (0.149, 1.467)
	Q4	5.79 (1.04)	1 (ref.)	1 (ref.)	1 (ref.)
	p value		0.0056	0.3209	0.4625
Trans-nonachlor (ng/g)	Q1	1.2 (0.81)	0.211 (0.048, 0.935)	4.21 (0.799, 22.168)	8.933 (0.511, 156.096)
	Q2	0.89 (0.6)	0.156 (0.039, 0.618)	0.979 (0.253, 3.789)	0.373 (0.047, 2.951)
	Q3	1.75 (0.44)	0.307 (0.155, 0.61)	0.697 (0.32, 1.519)	0.671 (0.29, 1.552)
	Q4	5.47 (0.81)	1 (ref.)	1 (ref.)	1 (ref.)
	p value		0.0022	0.155	0.3288
Mirex (ng/g)	Q1	1.57 (0.79)	0.405 (0.099, 1.656)	1.054 (0.29, 3.833)	1.231 (0.303, 4.997)
	Q2	0.62 (0.32)	0.158 (0.041, 0.611)	0.414 (0.11, 1.559)	0.715 (0.162, 3.163)
	Q3	2.52 (0.8)	0.655 (0.22, 1.949)	0.858 (0.233, 3.165)	1.203 (0.378, 3.834)
	Q4	3.79 (1.26)	1 (ref.)	1 (ref.)	1 (ref.)
	p value		0.1011	0.5976	0.8977

Abbreviations: OR: odds ratio, CI: confidence interval, SE: standard error, ref.: reference group

[a] Model1: Non-adjusted

[b] Model2: Adjusted for age, sex, race

[c] Model3: Adjusted for age, sex, race, poverty income ratio, smoking, drinking, physical activity

Discussion

This study found the association between NAFLD prevalence and the degree of OCP exposure in adults NHANES 2003–2004 through the fatty liver index. A significant association could be found for some OCP, such as oxychlordane.

According to literature published by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA), it presented the lowest-observed-adverse-effect-levels (LOAELs) (mg/kg/day) of chlordane for the health effect on animals. Here, the LOAEL for chlordane with intermediate to chronic effects on the liver is 0.1–0.273 mg/kg/day. In the case of mirex, the LOAEL for acute liver injury in animals is 1 mg/kg/day, and the LOAEL for intermediate to chronic effect is 0.25–0.75 mg/kg/day. Dose-response data have not yet been established in humans [21].

It can be estimated that the degree of OCP exposure will vary between the general population and those in specific regions. In areas with high pesticide use in the past, oral, inhalational, and dermal exposures are high due to high OCP concentrations in soil and water. Additional epidemiological studies might help determine whether the prevalence of NAFLD is indeed high within these populations compared to the general population.

In vivo experiments in mice have shown that exposure of hepatocytes to POPs containing an OCP mixture promotes hepatic steatosis, increases hepatic triglyceride levels, and reduces hypertriglyceridemia [22]. The mechanisms by which OCPs cause NAFLD are assumed to induce oxidative stress by increasing reactive oxygen species (ROS) production through activation of cytochrome P450 (CYP450) expression related to the detoxification pathway in the liver [23] or by affecting lipid metabolism [24]. For example, as a result of exposure in adult male C57BL/6 mice to low doses of p,p'-DDE and β-hexachlorocyclohexane (β-HCH) corresponding to human exposure for eight weeks, high accumulation of p,p'-DDE and β-HCH in the liver and mitochondrial damage were observed, with increased gene expression in fatty acid synthesis and decreased expression in mitochondrial fatty acid β-oxidation [25].

Among the subclass of OCPs, it can be seen that the adjusted odds ratio of FLI above 60 was higher as the serum concentration of oxychlordane was higher in this study. In addition, regarding variables constituting FLI, such as triglyceride, BMI, and waist circumference, the adjusted odds ratios for hypertriglyceridemia and obesity increased in the group exposed to the high concentration of oxychlordane. Oxychlordane is a major metabolite of chlordanes and nonachlors and has a higher toxicity and bioaccumulation potential than trans-nonachlor. Dose-

dependent accumulation of oxychlordane in the liver and adipose tissue was confirmed in female rats fed oxychlordane at doses ranging from 0.01 to 10 mg/kg/day for up to 28 days [26]. From these results, it can be interpreted that the dose-dependent NAFLD prevalence of oxychlordane in this study is due to its high bioaccumulation properties.

In the case of p,p-DDE and trans-nonachlor, although the adjusted odds ratio for NAFLD prevalence showed an increasing trend according to the concentration, it did not show a significant association. Previous studies have shown that p,p'-DDE is associated with increased BMI, triglycerides, insulin resistance, and reduced HDL cholesterol, while trans-nonachlor is related to the development of type 2 diabetes [10]. On the other hand, it is necessary to refer to the results that p,p'-DDE and trans-nonachlor significantly affect the liver in animal experiments. Previously, as a result of incubating trans-nonachlor in McArdle-RH7777 (MCA) hepatoma cells for 24 hours, it was found that neutral lipid accumulation increased in a concentration-dependent manner by increasing extracellular free fatty acid and de novo lipogenesis [27]. It has been demonstrated that trans-nonachlor has a pro-steatotic effect on hepatocytes.

Meanwhile, due to exposing trans-nonachlor and oxychlordane to murine macrophage, ROS production increased, and inflammatory markers such as tumor necrosis factor-α (TNF- α) and interleukin 6 (IL-6) were significantly elevated in a concentration-dependent manner, while the expression of cyclooxygenase 2 (*Cox-2*), an M1 pro-inflammatory marker, was decreased [28]. It suggests that trans-nonachlor and oxychlordane may have a concentration-dependent effect on the function of macrophages and may be involved in the immune response and metabolic process in metabolic diseases. However, unlike oxychlordane, the concentration-dependent association with trans-nonachlor was not significant for the prevalence of NAFLD in this study, and further studies are needed to clarify this.

Mirex showed opposite results with oxychlordane. The adjusted odds ratio of NAFLD prevalence increased in the lowest exposure group compared to the highest exposure group of mirex. A study analyzed the difference between each substance of OCPs for blood biochemistry results, including immune cells. It referred that white blood cell (WBC) levels were higher in the high oxychlordane and trans-nonachlor groups, and there was no significant difference in the p,p'-DDE groups. In contrast, WBC levels were lowest at the highest exposure to mirex [10, 29]. Interestingly, the trend of WBC level for each OCP substance is similar to that of NAFLD prevalence in this study, so it can be used as a reference when studying the pathogenesis of NAFLD.

The fatty liver index (FLI) shows high concordance with imaging and histological criteria for NAFLD [30]. In addition, FLI can also be used as a marker for diagnosing cardiovascular disease (CVD), as variables in FLI are also risk factors for CVD. Because of these advantages, FLI has been used in numerous prospective and epidemiologic studies and can predict the risk of type 2 diabetes, atherosclerosis, and CVD, helping to predict the prognosis of NAFLD [31, 32].

The correlation with the OCPs' concentration for each variable of FLI appeared similar pattern shown in the adjusted OR for NAFLD. Oxychlordane showed a positive correlation with increasing concentration in all FLI variables (triglyceride, waist circumference, BMI, and GGT), as well as in the adjusted OR of FLI 60 or higher. Although p,p'-DDE and trans-nonachlor did not show a significant correlation according to the concentration in the adjusted OR of FLI 60 or higher, p,p'-DDE correlated to triglyceride, waist circumference, and GGT; trans-nonachlor correlated to all variables of FLI. Mirex showed negative correlations for triglyceride and BMI, consistent with the trend of adjusted OR above FLI 60 (Figure 1 and 2).

These results show that the pattern seen in NAFLD prevalence for each OCP substance is similar to that of hypertriglyceridemia and obesity. Because OCPs are lipophilic, it can be inferred that people with high BMI will accumulate more OCPs in the body than people with low BMI when exposed to the same amount of pesticides [33]. However, few epidemiologic studies previously established an association between OCPs and obesity and hypertriglyceridemia [34]. Lee et al. found that only p,p'-DDE increased adjusted means of BMI as exposure increased, and there was no significant difference according to quartiles for the rest. The adjusted triglyceride levels of all OCPs but mirex increased in the upper quartiles [35].

Compared with a previous similar study in the liver enzyme levels for each OCP substance, this study showed slightly different results in the trend of liver enzyme levels in p,p'-DDE and mirex [29]. Contrary to this study, Serdar et al. did not exclude hepatitis B and C positive patients and heavy drinkers from the analysis and only adjusted age and gender. Since the effects of viral hepatitis and alcohol on liver enzyme elevation were excluded in this study, the results of this study could be more reliable for the purely NAFLD-induced liver enzyme elevation. The reason why the trends observed in FLI and the adjusted means of ALT and GGT according to the serum concentrations of oxychlordane and mirex were similar might be inferred that ALT and GGT have been used as conventional markers of NAFLD.

On the other hand, it was revealed that there was no significant difference in the prevalence of hepatic fibrosis according to the concentration of all four OCP substances. Since the final

diagnostic method for confirming fibrosis is a liver biopsy, there may be limitations in substituting the results of FIB-4 as it is, and statistical analysis is complex through the biopsy results. Therefore, it would be good to analyze the association with OCPs by finding other non-invasive markers with good predictive power for fibrosis in the future.

This is the first study to use the fatty liver index to analyze the association between OCPs and NAFLD. Previous studies have shown an association between OCP concentration and ALT elevation when the overall serum concentration of OCPs is analyzed. However, this study showed a dose-specific difference in NAFLD prevalence in the FLI criteria for each subclass of OCPs. Therefore, this study can support the evidence that OCPs are one of the possible etiologies of NAFLD. It is expected to provide clues to the discovery of NAFLD therapeutic targets.

This study has limitations in verifying causality over time due to the design nature of the cross-sectional study. Additionally, it is unknown whether participants were confirmed with NAFLD by ultrasound or biopsy, so cases other than actual NAFLD patients may have been included. Finally, this study did not separately consider the effects of simultaneous exposure to different types of OCPs and multiple kinds of EDCs other than OCPs on NAFLD.

Because little is known about the differences in chemical properties of each substance and the effects on NAFLD prevalence, further experiments are needed to elucidate the mechanism by which these differences occur. In this study, it was not clear whether there is a dose-dependent relationship between OCP exposure and the development of steatohepatitis and hepatic fibrosis in NAFLD. The reason is that the number of subjects that could obtain the FIB-4 index value was minimal (48 out of 1,515), so it was challenging to have meaningful results. Furthermore, newly emerging biomarkers such as cytokeratin 18 [36] have limitations in that they cannot be used for cross-sectional or retrospective studies using existing NHANES data. To predict the prevalence of steatohepatitis and fibrosis and to confirm the association with the degree of OCP exposure within the completed census data, it is necessary to conduct analysis using previously obtained variables or a scoring system, or if not, a prospective study using new biomarkers will be required.

In conclusion, the study suggested that OCP exposure was associated with NAFLD prevalence, some of which showed a linear dose-dependent relationship. Populations most likely to be exposed to chlordane include those who frequently use chlordane for termite control, live in structures treated with chlordane, or live in chlordane-rich soils [21]. Although most pesticides have been deprecated, periodic monitoring for NAFLD appears necessary in

populations in developing countries where pesticides are still used or in areas where pesticides have been used in the past. It is thought that further research is needed to clarify the mechanism of the influence of OCPs on the pathogenesis of NAFLD through *in vivo* experiments.

References

1. *NHANES 2003-2004: Pesticides - Organochlorine Metabolites - Serum (Surplus) Data Documentation, Codebook, and Frequencies*. Available from: https://wwwn.cdc.gov/Nchs/Nhanes/2003-2004/L28OCP_C.htm.
2. Cano, R., et al., *Role of Endocrine-Disrupting Chemicals in the Pathogenesis of Non-Alcoholic Fatty Liver Disease: A Comprehensive Review.* Int J Mol Sci, 2021. **22**(9).
3. Coats, J.R., *Mechanisms of toxic action and structure-activity relationships for organochlorine and synthetic pyrethroid insecticides.* Environ Health Perspect, 1990. **87**: p. 255-62.
4. Tsai, W.T., *Current status and regulatory aspects of pesticides considered to be persistent organic pollutants (POPs) in Taiwan.* Int J Environ Res Public Health, 2010. **7**(10): p. 3615-27.
5. Heindel, J.J., et al., *Metabolism disrupting chemicals and metabolic disorders.* Reprod Toxicol, 2017. **68**: p. 3-33.
6. Jin, J., et al., *Dioxin-like and non-dioxin-like PCBs differentially regulate the hepatic proteome and modify diet-induced nonalcoholic fatty liver disease severity.* Med Chem Res, 2020. **29**: p. 1247-1263.
7. Verstraete, S.G., et al., *Bisphenol a increases risk for presumed non-alcoholic fatty liver disease in Hispanic adolescents in NHANES 2003-2010.* Environ Health, 2018. **17**(1): p. 12.
8. Clark, J.M., *The epidemiology of nonalcoholic fatty liver disease in adults.* J Clin Gastroenterol, 2006. **40 Suppl 1**: p. S5-10.
9. Williams, C.D., et al., *Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study.* Gastroenterology, 2011. **140**(1): p. 124-31.
10. Lee, D.H., et al., *Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes.* PLoS One, 2011. **6**(1): p. e15977.
11. Mostafalou, S. and M. Abdollahi, *Pesticides: an update of human exposure and toxicity.* Arch Toxicol, 2017. **91**(2): p. 549-599.
12. Al-Eryani, L., et al., *Identification of Environmental Chemicals Associated with the Development of Toxicant-associated Fatty Liver Disease in Rodents.* Toxicol Pathol, 2015. **43**(4): p. 482-97.
13. Wahlang, B., et al., *Insecticide and metal exposures are associated with a surrogate*

biomarker for non-alcoholic fatty liver disease in the National Health and Nutrition Examination Survey 2003-2004. Environ Sci Pollut Res Int, 2020. **27**(6): p. 6476-6487.

14. Liu, Z., et al., *Alanine aminotransferase-old biomarker and new concept: a review.* Int J Med Sci, 2014. **11**(9): p. 925-35.
15. Bedogni, G., et al., *The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population.* BMC Gastroenterol, 2006. **6**: p. 33.
16. Motamed, N., et al., *Fatty liver index vs waist circumference for predicting non-alcoholic fatty liver disease.* World J Gastroenterol, 2016. **22**(10): p. 3023-30.
17. European Association for the Study of the, L., D. European Association for the Study of, and O. European Association for the Study of, *EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease.* J Hepatol, 2016. **64**(6): p. 1388-402.
18. Koehler, E.M., et al., *External validation of the fatty liver index for identifying nonalcoholic fatty liver disease in a population-based study.* Clin Gastroenterol Hepatol, 2013. **11**(9): p. 1201-4.
19. Sterling, R.K., et al., *Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection.* Hepatology, 2006. **43**(6): p. 1317-25.
20. Xu, X.L., et al., *The role of fibrosis index FIB-4 in predicting liver fibrosis stage and clinical prognosis: A diagnostic or screening tool?* J Formos Med Assoc, 2021.
21. Abadin, H., R. Baynes, and P.F. Goetchius, *Toxicological profile for chlordane.* 1994.
22. Mulligan, C., et al., *Exposure to an environmentally relevant mixture of organochlorine compounds and polychlorinated biphenyls Promotes hepatic steatosis in male Ob/Ob mice.* Environ Toxicol, 2017. **32**(4): p. 1399-1411.
23. Migliaccio, V., et al., *Mitochondrial Involvement in the Adaptive Response to Chronic Exposure to Environmental Pollutants and High-Fat Feeding in a Rat Liver and Testis.* Cells, 2019. **8**(8).
24. Ward, A.B., M.B. Dail, and J.E. Chambers, *In Vitro effect of DDE exposure on the regulation of lipid metabolism and secretion in McA-RH7777 hepatocytes: A potential role in dyslipidemia which may increase the risk of type 2 diabetes mellitus.* Toxicol In Vitro, 2016. **37**: p. 9-14.
25. Liu, Q., et al., *Organochloride pesticides impaired mitochondrial function in hepatocytes and aggravated disorders of fatty acid metabolism.* Sci Rep, 2017. **7**: p. 46339.
26. Bondy, G., et al., *Toxicity of the chlordane metabolite oxychlordane in female rats: clinical and histopathological changes.* Food Chem Toxicol, 2003. **41**(2): p. 291-301.
27. Howell, G.E., 3rd, et al., *"Trans-nonachlor increases extracellular free fatty acid*

accumulation and de novo lipogenesis to produce hepatic steatosis in McArdle-RH7777 cells". Toxicol In Vitro, 2018. **50**: p. 285-292.

28. Young, D., et al., *Alterations in macrophage phagocytosis and inflammatory tone following exposure to the organochlorine compounds oxychlordane and trans-nonachlor.* Toxicol In Vitro, 2020. **65**: p. 104791.
29. Serdar, B., et al., *Potential effects of polychlorinated biphenyls (PCBs) and selected organochlorine pesticides (OCPs) on immune cells and blood biochemistry measures: a cross-sectional assessment of the NHANES 2003-2004 data.* Environ Health, 2014. **13**: p. 114.
30. Yang, B.L., et al., *External validation of fatty liver index for identifying ultrasonographic fatty liver in a large-scale cross-sectional study in Taiwan.* PLoS One, 2015. **10**(3): p. e0120443.
31. Cuthbertson, D.J., et al., *External validation of the fatty liver index and lipid accumulation product indices, using 1H-magnetic resonance spectroscopy, to identify hepatic steatosis in healthy controls and obese, insulin-resistant individuals.* Eur J Endocrinol, 2014. **171**(5): p. 561-9.
32. Khang, A.R., et al., *The fatty liver index, a simple and useful predictor of metabolic syndrome: analysis of the Korea National Health and Nutrition Examination Survey 2010-2011.* Diabetes Metab Syndr Obes, 2019. **12**: p. 181-190.
33. Montgomery, M.P., et al., *Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural Health Study, 1993-2003.* Am J Epidemiol, 2008. **167**(10): p. 1235-46.
34. Xiao, X., J.M. Clark, and Y. Park, *Potential contribution of insecticide exposure and development of obesity and type 2 diabetes.* Food Chem Toxicol, 2017. **105**: p. 456-474.
35. Lee, D.-H., et al., *Low Dose Organochlorine Pesticides and Polychlorinated Biphenyls Predict Obesity, Dyslipidemia, and Insulin Resistance among People Free of Diabetes.* PLoS ONE, 2011. **6**(1): p. e15977.
36. Feldstein, A.E., et al., *Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: A multicenter validation study.* Hepatology, 2009. **50**(4): p. 1072-1078.

국문 요약

배경: 비알코올 지방간질환(NAFLD)의 원인으로 내분비 교란 물질(EDC)이 부상하고 있다. 20세기에 널리 사용된 유기염소 살충제(OCP)는 음식물 섭취, 호흡기, 피부 접촉을 통해 체내에 흡수되어 사용을 중단한 후에도 체내에 오래 잔류한다. 최근 연구에 따르면 OCP와 비만, 당뇨병 및 이상지질혈증과 같은 대사 증후군 사이의 연관성이 밝혀지고 있다. 그러나 NAFLD와의 관련성에 대해서는 알려진 바가 거의 없다. 이 연구는 OCP에 대한 노출과 NAFLD 발병률 사이의 연관성을 밝히는 것을 목표로 하였다.

방법: 미국 국민영양조사 NHANES (National Health and Nutrition Examination Survey) 2003-2004 데이터를 사용하였다. p,p'-DDE, oxychlordane, trans-nonachlor, 그리고 mirex의 4가지 물질을 분석하였다. 제외 기준은 연령 20세 미만, B형 및 C형 간염 감염이었다. Primary outcome은 NAFLD 진단을 위한 비침습적 지표인 지방간 지수(fatty liver index) 60 이상이었다. OCP의 혈청 농도를 4개의 사분위수로 나누고 각 사분위수에서 NAFLD 발생의 조정된 오즈비(OR)를 비교하였다.

결과: 총 1512명의 참가자가 등록되었으며 579명이 진단 기준을 충족하는 NAFLD 환자였다. 모든 물질에 대한 네번째 사분위수를 기준으로 오즈비(OR)를 1로 설정하였다. 보정된 로지스틱 회귀분석 후, oxychlordane은 혈청 농도가 증가함에 따라 NAFLD의 위험이 더 높았다(첫번째 사분위수에서 OR 0.288, 95% CI 0.155-0.536). p,p'-DDE와 trans-nonachlor는 NAFLD와 유의한 상관관계가 없었고, mirex는 낮은 농도의 사분위수에서 더 높은 NAFLD의 유병률을 보여주었다(첫번째 사분위수에서 OR 3.443, 95% CI 2.072-5.721).

결론: 본 연구는 OCP에 대한 노출이 NAFLD 유병률과 관련이 있으며 그 중 일부는 선형 용량 의존 관계가 있음을 보여주었다. 일반 인구 중 OCP에 많이 노출되는 개인에게서 NAFLD에 대한 정기적인 모니터링이 필요할 것으로 보인다.

중심단어: 내분비 교란 물질, 유기염소 살충제, 비알코올 지방간질환, 지방간 지수