Persistent organic pollutant exposures are associated with liver toxicity and decreased albumin in a highly exposed human cohort

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BACKGROUND

- Persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polybrominated diphenyl ethers (PBDEs) have been associated with hepatotoxicity and abnormal liver function.
- Ortho-substituted PCB exposures were associated with liver necrosis and circulating microRNAs (miRs) previously associated with liver toxicity in the Anniston Community Health Survey (ACHS) (PMID:29684222, 34989596).
- Other POPs (e.g., non-ortho-substituted PCBs, PCDDs, and PCDFs) potently active the aryl hydrocarbon receptor (AhR). These dioxin-like molecules have reproducibly been associated with metabolic disruption and NAFLD in animal models (PMID: 31134516).

OBJECTIVE

- Here, we validate the miR associations previously determined in (ACHS-I) (PMID:34989596) and determine cross-sectional relationships between persistent organic pollutants including dioxins and liver biomarkers in the follow-up ACHS-II cohort.

STUDY DESIGN AND METHODS

- The ACHS is a longitudinal cohort consisting of adult community participants living near a former PCB production facility in Anniston, Alabama.
- The design of the ACHS-II follow-up study was previously reported (PMID:25982988). A sample size of 345 subjects was utilized in this analysis, which was IRB-approved.
- Serum biomarkers of pollutant exposure included: [PCDDs (N=7), PCDFs (N=10), PCBs (N=40), and PBDEs (N=11)]. POPs were measured by gas chromatography isotope dilution high-resolution mass spectrometry (GC/ID-HRMS) as previously reported (PMID:29763869).
- Dioxin toxic equivalency (TEQ), a biomarker of aryl hydrocarbon receptor by dioxin-like molecules, was determined using World Health Organization toxic equivalency factors (PMID:16829543) for PCDDs, PCDFs and PCBs and their summed total (total dioxin TEQ). Total dioxin TEQ was previously reported to be significantly higher in ACHS-II than NHANES (PMID:29763869). Total dioxin TEQ was the primary exposure biomarker. Other secondary exposure biomarkers included the TEQ subgroupings, ΣPCBs, and the individual congeners, including the PBDEs, themselves.
- Serum liver biomarkers included: (*i*) liver enzymes (clinical chemistry analyzer); (*ii*) keratin 18 M30 & M65 (ELISA, DiaPharma); (*iii*) a panel of 68 hepatotoxicity miRs (FirePlex, Abcam).
- Raw mean fluorescent intensities (MFIs) of 35 highly expressed miRs (>LOD in 90+% of the sample) were quantile-normalized and log10-transformed.
- Categorical liver disease variables were created using K18 as follows: no liver disease (K18 M65<300 U/L & M30<200 U/L); necrotic liver disease (K18 M65>300 U/L & M30<200 U/L); and other (apoptotic) liver disease (K18 M30>200 U/L).
- Associations between log-transformed exposure and disease biomarkers were determined using generalized, confounder-adjusted linear models using SAS v9.4 and R and presented as beta coefficients. Here, these adjustments included: age, BMI, self-reported race, sex, total PCBs, total lipids, miR plate.
- Ingenuity Pathway Analysis (IPA) of associated miRs was performed.
- Statistical significance was set at a p-value ≤0.05 and/or a false discovery rate (FDR) of ≤0.10 for the primary outcome (associations between miRs and liver disease categories) and p<0.05 for secondary outcomes.
- More details regarding these methodologies can be found in our previous publications on liver disease, cytokines and miRs in ACHS-I (PMID: 29684222, 34989596).

SUBJECT CHARACTERISTICS

Table 1. Demographic characteristics by K18-categorized liver disease status (N=345).

Characteristic	Liver disease status[a, b] None (N = 131)		Necrotic (N = 158)		Apoptotic (N = 56)		P-value[c]
Age (years)	63.8	12.7	63.4	13.2	59.8	12.6	0.13
BMI (kg/m^2)	32.0	9.5	31.2	7.2	32.2	7.2	0.59
Keratin 18 M65 (U/dL)[d]	231.8	46.0	436.7	142.9	624.8	368.1	**<.0001**
Keratin 18 M30 (U/dL)[d]	84.1	26.5	111.6	36.3	380.1	269.8	**<.0001**
∑PCBs (whole weight)	6.0	6.4	5.4	7.2	5.6	9.8	0.17
Total TEQ (whole weight)	164.8	129.9	144.1	139.9	142.5	174.6	**0.04**
Total lipids (mg/dL)	611.1	143.7	632.5	162.1	622.1	154.8	0.47
Sex							0.07
Male	26	(19.9)	49	(31.0)	18	(32.1)	
Female	105	(80.2)	109	(69.0)	38	(67.9)	
Race/ethnicity							**0.006**
Non-Hispanic White	50	(38.2)	90	(57.0)	28	(50.0)	
Nonwhite	81	(61.8)	68	(43.0)	28	(50.0)	

Abbreviations: dL, deciliter; K18, Keratin 18; kg, kilograms; m, meters; mg, milligrams; PCB, polychlorinated biphenyl; SD, standard deviation; TEQ, toxic equivalency; U, units.
[a] Liver disease categories are defined as: none (K18 M30 < 200 and K18 M65 < 300), necrotic (K18 M30 < 200 and K18 M65 ≥ 300), and other abnormal K18 (K18 M30 ≥ 200).
[b] Units are in Mean±SD or N (%) for continuous or categorical values, respectively.
[c] P-values for continuous characteristics are based on a one-way ANOVA using $\log_{10}$-transformed values where ∑PCBs and total TEQ are adjusted for $\log_{10}$-transformed total lipids.
[d] Each pairwise comparison differed significantly: p<0.0001.

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RESULTS

A. Liver Disease Prevalence: Consistent with ACHS-I (PMID:29684222), there was a high prevalence of K18-categoraized liver disease (62.0%), with 61.2% of that (or 45.7% overall) being necrotic (**Table 1**). This was consistent with ACHS-I (PMID:29684222). The K18-categorized liver disease groups were associated with increased liver enzymes (**Table 2**), demonstrating validity of the categorization procedures.

Table 2. Mean Liver Enzymes Stratified by Liver Disease Status, ACHS II (n=345)

	None		Necrotic		Apoptotic			Total	
	Mean	SD	Mean	SD	Mean	SD	P-Value	Mean	SD
AST (U/L)	25.9	8.0	29.9	14.5	42.1	27.2	**<.0001**	30.4	16.4
ALT (U/L)	22.8	8.6	26.5	9.8	38.1	19.2	**<.0001**	26.9	12.6
Alkaline phosphatase (U/L)	85.4	22.8	91.1	38.7	105.7	57.9	**0.004**	91.3	38.3
Albumin (g/dL)	4.2	0.4	4.1	0.4	4.2	0.5	0.78	4.2	0.4
Total Protein	7.3	0.6	7.2	0.6	7.4	0.7	0.053	7.3	0.6
Total bilirubin (mg/dL)	0.4	0.2	0.5	0.5	0.5	0.2	**0.03**	0.4	0.4
Direct bilirubin (mg/dL)	0.2	0.1	0.2	0.2	0.2	0.1	0.47	0.2	0.2

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; dL, deciliters; g, grams; mg, milligrams; L, liters; SD, standard deviation; U, units.

B. Relationships Between Liver Injury/Disease Biomarkers: 35 of 68 circulating miRs were highly-expressed. Associations between these miRs and the other liver biomarkers was determined. Twenty-nine miRs were significantly associated with at least 1 other liver biomarker (not shown). MiR-122-5P was associated with M30, M65, AST, ALT, and ALP (all p<0.01) with a trend towards total protein (p=0.06). MiR-192-5P was associated with the same variables (all p<0.03) as well as direct bilirubin (p=0.003).

Using complete linkage analysis, miRs, K18 and liver enzyme disease biomarkers clustered into four groups (G): G1 (n=9 miRs), G2 (n=4), G3 (n=11), G4 (n=11). G2 miRs were associated with the liver enzymes, K18 and the synthetic function biomarkers (**Figure 1**). Using Ingenuity Pathway Analysis, miRs in the four groups were linked to various liver disease processes including: hepatic steatosis (G2), hepatitis (G4), cirrhosis (G4), liver hyperplasia (G1-G2), and hepatocellular carcinoma (G1-G3) (**Figure 2**).

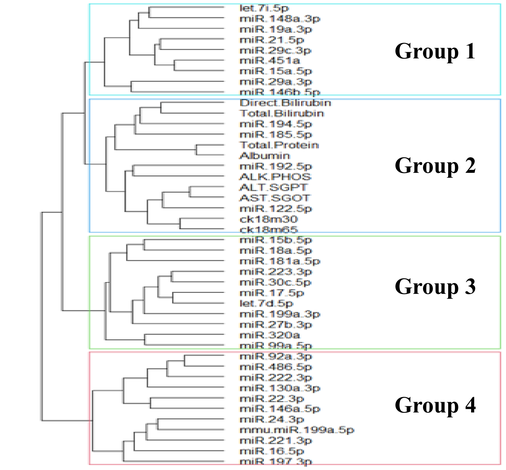

Figure 1. Using complete linkage analysis, serum miRs and liver tests clustered into four groups (G): G1 (n=9 miRs), G2 (n=4), G3 (n=11), G4 (n=11). G2 miRs were associated with liver enzymes and K18.

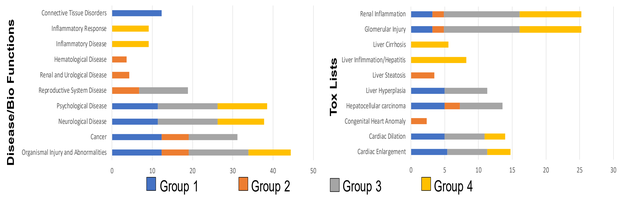

Figure 2. Ingenuity pathway analysis (IPA) of miRs from the four liver biomarker groups determined by complete linkage analysis (n=345). Top 5 pathways for diseases and biofunctions and top 2 pathways for liver, renal, and cardiac tox lists are shown. X-axis represents –log(p-value) of pathway enrichment.

C. Relationships miRs and Liver Disease Categories: Associations between differentially regulated highly-expressed serum miRs which were significantly associated with the liver disease categories in ACHS-II were determined (**Table 3**).

Table 3. Differentially regulated serum miRs and liver disease categories (vs. without liver disease) in a PCB-exposed residential cohort study (ACHS-I, n-738; ACHS-II, n=345).

miR	Differentially regulated miRs in necrotic liver disease (N=158) FC	SE	FDR	P_{raw}	Differentially regulated miRs in apoptotic liver disease (N=56) FC	SE	FDR	P_{raw}
Up-regulated miRs								
miR-122-5p	**1.36**	**0.16**	**0.09**	**0.01**	**2.69**	**0.42**	**<.0001**	**<.0001**
miR-192-5p	1.08	0.11	0.80	0.41	**1.61**	**0.21**	**0.01**	**0.0002**
miR-99a-5p	1.05	0.11	0.80	0.64	**1.43**	**0.19**	**0.09**	**0.01**
miR-22-3p[a]	1.03	0.06	0.80	0.59	**1.24**	**0.10**	**0.09**	**0.01**
Down-regulated miRs								
miR-18a-5p[a]	0.97	0.09	0.90	0.77	**0.74**	**0.08**	**0.09**	**0.01**
miR-199a-3p[a]	0.96	0.06	0.80	0.54	**0.78**	**0.07**	**0.09**	**0.01**
let7d-5p[a]	1.03	0.07	0.80	0.60	**0.80**	**0.07**	**0.01**	**0.01**
Differentially regulated miRs in ACHS-I[b]								
miR-320a	0.97	0.04	0.80	0.45	1.08	0.06	0.54	0.17
miR-221-3p	0.97	0.06	0.80	0.60	0.88	0.07	0.54	0.11
miR-24-3p	0.96	0.05	0.80	0.49	0.89	0.06	0.54	0.10
miR-17-5p	0.98	0.06	0.90	0.77	0.93	0.08	0.80	0.38
miR-197-3p	0.98	0.08	0.95	0.84	0.95	0.10	0.80	0.65

Note: Statistical significance was set at FDR <0.20 for the primary endpoint (categorical liver disease associations) and P-value <0.05 for all secondary endpoints. Bold font denotes statistically significant results. **Abbreviations:** ACHS-I, Anniston Community Health Survey I; ACHS-I, ACHS Survey II; FC, fold change (vs. the no liver disease category); FDR, false discovery rate; K18, Keratin 18; let, lethal; miR, microRNA; PCB, polychlorinated biphenyls; SE, standard error. **Model:** Adjusted for age, BMI, race sex, miR plate. [a] Significant in ACHS-II only. [b] Significant in ACHS-I only

D. Relationships between POP Exposures and Liver Toxicity Biomarkers.

- Total dioxin TEQ was significantly associated with liver toxicity biomarkers from group 1 (miR-29a-3p) and group 2 (miR-185-5p, albumin and total protein) (**Table 4**). The relationships were inverse.
- Albumin was also inversely associated with PCDD, PCDF, and NO-PCB TEQ sub-groupings and greater than half of abundant (>40% detection rate) dioxin-like congeners including 2,3,7,8-TCDD (p=0.001) and 2,3,4,7,8-PCDF (p=0.01). However, only one abundant non-dioxin-like POP (PCB 99) was associated with albumin (**Tables 4-5**).
- PCDD TEQ was significantly associated with liver toxicity biomarkers from group 2 (albumin) and group 3 (miR-27b-3p and miR-320a) (**Table 4**).
- PCDF TEQ was significantly associated with a total seven liver toxicity biomarkers coming from all four groups and including albumin and miR-320a (**Table 4**).
- Non-ortho PCB TEQ was inversely associated with miR-29a-3p and albumin. Other PCB biomarkers were also inversely associated with miR-29a-3p, although ΣPCBs was not associated with any liver toxicity biomarker (**Table 4**).
- Associations for selected individual congeners and liver biomarkers is given in **Table 5**. All five abundant PBDEs were inversely associated with miR-92a-3p. Three of these PBDEs were positively associated with miR-122-5p and two with K18 M65.

Table 4. Selected Significant Associations Between Dioxin Exposure and Liver Toxicity Biomarkers

Exposure Biomarker	Liver Toxicity Biomarker (Group 1)	Liver Toxicity Biomarker (Group 2)	Liver Toxicity Biomarker (Group 3)	Liver Toxicity Biomarker (Group 4)
Total Dioxin TEQ	**miR-29a-3p** (β= -0.16±0.05, p=0.0004)	**Albumin** (β= -0.02±0.01, p=0.03) **Total Protein** (β= -0.02±0.01, p=0.03) **miR-185-5p** (β= -0.07±0.04, p=0.049)	-	-
PCDD TEQ	-	**Albumin** (β= -0.02 ±0.01, p=0.03)	**miR-27b-3p** (β= -0.18±0.09, p=0.04) **miR-320a** (β= 0.08±0.04, p=0.04)	-
PCDF TEQ	**miR-146b-5p** (β= -0.21±0.07, p=0.003) **miR-451a** (β= -0.11±0.05, p=0.02)	**Albumin** (β= -0.02±0.01, p=0.04) **miR-194-5p** (β= 0.19±0.08, p=0.02)	**miR-320a** (β= 0.08±0.04, p=0.02) **let-7d-5p** (β= -0.13±0.06, p=0.03)	**miR-197-3p** (β=0.15±0.07, p=0.04)
Non-ortho PCB TEQ	**miR-29a-3p** (β= -0.08±0.03, p=0.01)	**Albumin** (β= -0.01±0.01, p=0.04)	-	-
Mono-ortho PCB TEQ	**miR-29a-3p** (β= -0.08±0.03, p=0.02)	-	-	-
∑Non-dioxin like PCBs	**miR-29a-3p** (β= -0.07±0.04, p=0.04)	-	-	-
ΣPCBs	-	-	-	-

CONCLUSIONS

- A high liver disease prevalence was confirmed in ACHS-II, a largely minority female population with comorbid obesity and high PCB exposures.
- Well-validated alternate indicators of liver injury (e.g., ALT and miR-122-5p) were significantly increased in the groups with K18-categorized liver disease.
- Recently reported associations between miRs (e.g., miR-122-5p and miR-192-5p) and liver disease category in ACHS-I (PMID:34989596) were confirmed.
- By complete linkage analysis, serum liver toxicity biomarkers (e.g., miRs, K18, and routine biochemistries) clustered into four groups associated with hepatic steatosis (G2), hepatitis (G4), cirrhosis (G4), liver hyperplasia (G1-G2), and hepatocellular carcinoma (G1-G3).
- Several hepatotoxicity miRs (including miR-29a-3p and miR-185-5p) were associated with POP exposure biomarkers. PCDF TEQ was associated with greatest number of liver toxicity biomarkers. Albumin was inversely associated with most dioxin-like-molecule exposures.
- While dioxin exposures were associated with decreased hepatic synthetic function (albumin), PBDEs were associated with increased hepatocyte death and different circulating miRs.
- miRs could be involved with the liver toxicity of, or adaptation to, environmental exposures.
- In this population with suspected NAFLD based on demographic factors, POP exposures were differentially associated with the regulation of liver metabolic function and cell death.
- The potential clinical impact of albumin suppression by dioxin exposures in liver disease patients warrants future investigation.
- Reverse causality cannot be excluded.

Table 5. Selected significant associations of serum POP congeners with liver biomarkers in ACHS-II (n-345)

POP Congener		G1 Liver Biomarkers miR-29a-3p	miR-29c-3p	G2 Liver Biomarkers K18 M65	miR-122-5p	Albumin	Total Protein	miR-451a	G4 Liver Biomarkers miR-92a-3p
Dioxin-like molecules	2,3,7,8-TCDD TEQ					-0.04±0.01	-0.02±0.01		
	1,2,3,7,8-PCDD TEQ					-0.02±0.01	-0.02±0.01	-0.12±0.06	
	1,2,3,4,7,8-HCDD TEQ					-0.03 0.01	-0.02 0.01	-0.11±0.05	
	1,2,3,6,7,8-HCDD TEQ							-0.13±0.06	
	1,2,3,7,8,9-HCDD TEQ								-0.17±0.07
	2,3,4,7,8-PCDF TEQ					-0.02±0.01		-0.11±0.05	
	1,2,3,4,7,8-HCDF TEQ					-0.03±0.01		-0.15±0.06	
	1,2,3,6,7,8-HCDF TEQ					-0.17±0.06		-0.17±0.06	
	2,3,4,6,7,8-HCDF TEQ			-0.12±0.06	-0.28±0.14				
	PCB 126 TEQ (0.1)					-0.01±0.00	-0.01±0.00		
Non-dioxin-like molecules	PCB 66	-0.07±0.03							
	PCB 99					-0.01 0.00			
	PCB 146	-0.06±0.03							
	PCB 153								
	PCB 177	-0.06±0.03							
	PCB 180	-0.08±0.04							
	PCB 187	-0.06±0.03							
	PBDE 28			0.06±0.03	0.16±0.06				-0.06±0.03
	PBDE 47				0.11±0.05				-0.05±0.02
	PBDE 99				0.10±0.04				-0.06±0.02
	PBDE 100		0.07±0.03	0.04±0.02					-0.04±0.02
	PBDE 153		0.07±0.03						-0.05±0.02

Beta coefficients +/- SE are provided. All p-values < 0.05 (not shown). Adjustments included: age, BMI, self-reported race, sex, total PCBs, total lipids, miR plate. All abundant dioxin-like molecules with WHO toxic equivalency factor ≥ 0.1 are provided.