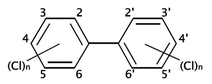
EVALUATION OF SEX-DEPENDENT EFFECTS CAUSED BY POLYCHLORINATED BIPHENYLS EXPOSURE ON CANCER ENDPOINTS USING AN OMICS APPROACH

Zayna Qaissi [1], Josiah E. Hardesty [1], Shesh N. Rai [2,3,4], Matthew C. Cave [1,3], Banrida Wahlang [1,3]

[1] Medicine, Division of Gastroenterology, Hepatology, and Nutrition, School of Medicine; [2] Department of Bioinformatics and Biostatistics, School of Public Health and Information Sciences; [3] UofL Superfund Research Center; [4] UofL JG Brown Cancer Center; University of Louisville, Louisville, KY, USA

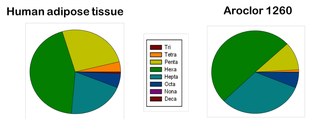
BACKGROUND & INNOVATION

- Polychlorinated biphenyls (PCBs) are polyhalogenated aromatic hydrocarbons consisting of up to 10 chlorine atoms attached to biphenyl (209 congeners). PCBs are classified as persistent organic pollutants that are detrimental to our ecosystem. PCBs were manufactured between the 1930s-1970s and primarily used as dielectric fluids in capacitors and transformers.
- In North America, PCBs were commercially produced as mixtures of congeners, under the trade name 'Aroclor' (Monsanto Corp.). While PCB production and use have been banned by the US Congress (1979) and worldwide (2001), they are still present in our environment due to PCBs' resistance to degradation and bioaccumulation in the food chain. PCB exposure in humans are associated with a range of diseases including numerous forms of cancer such as liver, thyroid, prostate and breast cancer; reproductive defects; immunosuppressive effects; and cardio-metabolic diseases (hypertension, stroke, fatty liver disease).



Why Aroclor 1260 ?

The distribution of PCB congeners in human fat resembles that of Aroclor 1260

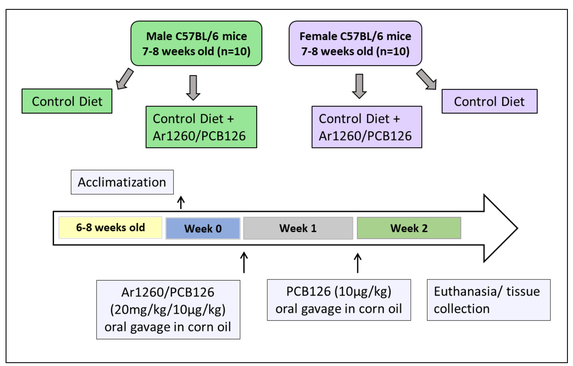


- Previous studies have also demonstrated evidence of sex-dependent effects with PCB-induced liver tumors in rodent models using different Aroclors at high doses. Importantly, epidemiologic studies also reported that women tended to have higher incidences of all forms of cancers in a historic population that was accidentally exposed to PCBs. Additionally, our laboratory group recently demonstrated that long-term exposures to Aroclor 1260 at doses that reflect current human bioaccumulation patterns led to live tumor incidences in male mice. However, similar studies are lacking in female models and need to be addressed.

RESEARCH OBJECTIVE

The objective of the current study is to evaluate sex-dependent effects of low-dose PCB exposures that are reflective of current human exposure levels, on carcinogenic endpoints, using a throughput Omics approach.

EXPERIMENTAL DESIGN

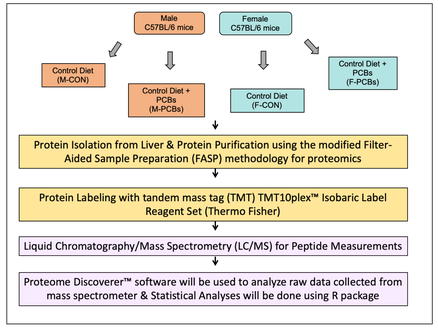


A mix of PCBs comprising of the commercial PCB mixture, Aroclor 1260 (mostly non-dioxin-like), and the dioxin-like congener, PCB 126, was used for exposures. The doses were designed to reflect current PCB serum levels in PCB-exposed populations and current environmental exposure patterns.

FUNDING SUPPORT & ACKNOWLEDGEMENTS

This research was supported, in part, by the National Institute of Environmental Health Sciences (R35ES028373, R01ES032189, P42ES023716, P30ES030283 and R21ES031510); National Institute of General Medical Sciences (P20GM113226); National Institute on Alcohol Abuse and Alcoholism (P50AA024337); and the National Cancer Institute NCI R25 University of Louisville Cancer Education Program (R25-CA134283). The authors would like to acknowledge Dr. Michael Merchant and Dr. Jianmin Pan for their assistance with the proteomics experiments and analysis respectively.

MATERIALS & METHODS



- Proteomics Study Design: 4 groups of mice (n=5) based on sex (Male or Female) and exposure (Control or PCBs).
- Liquid Chromatography/Mass Spectrometry (LC/MS) was used to measure tandem mass tag (TMT)-labeled peptides. Proteome Discoverer v2.2.0.388 was used to analyze the raw data collected from the mass spectrometer.
- Hepatic proteins that had significance abundance were imported into MetaCore software (Clarivate Analytics, Philadelphia, PA) for the following analyses: Enrichment Ontologies for Disease (by Biomarkers) and Transcription Factor Analysis (TFA).
- Additionally, hepatic gene expression of pro-inflammatory cytokines and chemokines, previously reported to play a role in liver-related carcinogenesis were examined using RT-PCR. GAPDH was used as the endogenous control.

RESULTS

PCB-mediated Alterations of The Hepatic Proteome was Sex-Dependent

Comparison	MCON *vs.* FCON		MPCB *vs.* FPCB		MCON *vs.* MPCB		FCON *vs.* FPCB	
Summary of results	*P*-Not Adj	*P*-Adj	*P*-Not Adj	*P*-Adj	*P*-Not Adj	*P*-Adj	*P*-Not Adj	*P*-Adj
# of proteins analyzed	3665	3665	3665	3665	3665	3665	3665	3665
# of DE proteins	1172	289	914	119	633	11	451	4
# of DE proteins (-0.5 <$\log_2$FC <0.5)	910	179	617	40	526	1	414	0
# of up-regulated proteins ($\log_2$FC ≥ 0.5)	26	**16**	100	**38**	54	**9**	20	**1**
# of down-regulated proteins ($\log_2$FC ≤-0.5)	236	**94**	197	**41**	53	**1**	17	**3**

Table 1. Effects of PCB exposures and sex on hepatic proteins using high-throughput proteomics analysis. A summary of the overall results is presented. The dataset consisted of 3770 proteins (corresponding to 9310 peptides). After running Statistical Analysis Tool for Proteomics (STAP), the number of differentially expressed (DE) proteins was obtained for each comparison group. *P*-Not Adj is *P* <0.05 and *P*-Adj is *P* <0.2, after correcting for false discovery rate. FC - fold change. M-males, F-females.

Enrichment Analysis Demonstrated Key Cancer Endpoints in PCB-exposed Females

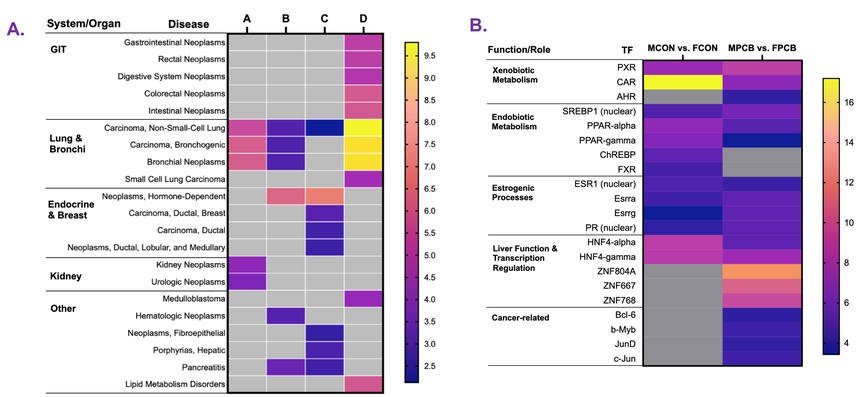


Figure 1.A. Sex and PCB effects on enrichment for disease (by biomarkers). Heat map showing cancer-related disease endpoints altered by sex and/or PCB exposures, according to the -log(*p*-value), obtained using MetaCore Software. A - MCON *vs.* FCON, B - MPCB *vs.* FPCB, C - MCON *vs.* MPCB, D - FCON *vs* FPCB. **B. Sex and PCB effects on transcription factor (TF) activation.** Heat map of selected TFs with their corresponding *z* scores, obtained from TFA.

Evaluation of Pro-inflammatory Cytokines and Chemokines Expression Levels

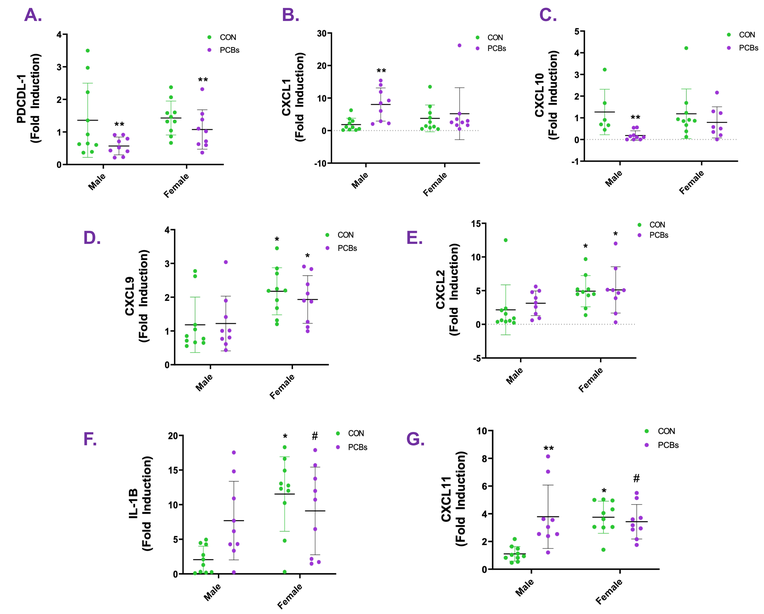


Figure 2.A-G. Hepatic gene expression was assessed using RT-PCR. Hepatic gene expression of pro-inflammatory cytokines, chemokines and liver injury markers were assessed using RT-PCR. 2-Way ANOVA was initially performed to determine effects of "sex" and/or "PCB"; followed by Tukey's post hoc test; *p* <0.05 is considered statistically significant, *denotes sex effect, **denotes PCB effect, #denotes interaction between sex and PCB exposure.

CONCLUDING REMARKS

- The current study demonstrated apparent sex differences in the mouse hepatic proteome due to PCB exposures.
- Disease biomarker analysis indicated that cancer outcomes were most impacted in PCB-exposed females, in concordance with activated TFs associated with oncogenic processes.
- Initial screening of cytokine/chemokine expression demonstrated that females had higher basal levels of CXCL-9, CXCL-2, and IL-1Beta; PCB exposure altered chemokine gene expression (CXCL-1, CXCL-10), only in males while most chemokine expression was suppressed in females.
- The preliminary data suggest that PCB-exposed females exhibited higher cancer endpoints based on the MetaCore findings. More extensive evaluation of cancer biomarkers is needed. Our follow-up studies will evaluate the effects of long-term PCB exposures on liver cancer endpoints.

IMPACT & RELEVANCE

- The focus of the current study is significant because it helps in identifying existing disparities in environmental health. Findings from these studies will aid in better risk assessment and identifying at-risk population. Importantly, such studies are relevant to PCB-exposed population, given that in a historical PCB-exposed cohort residing in Anniston, AL (former Monsanto Corp. PCB production plant), PCB body burdens are higher in women than men.

REFERENCES

1. Wahlang B *et al*., Identifying sex differences arising from polychlorinated biphenyl exposures in toxicant-associated liver disease. 2019. *Food Chem Toxicol*. 129:64-76.
2. Brunner, M.J *et. al*., An assessment of the chronic toxicity and oncogenicity of Aroclor-1016, Aroclor-1242, Aroclor- 1254, and Aroclor-1260 administered in diet to rats. Study No. SC920192. *Chronic toxicity and oncogenicity report*. Battelle, Columbus OH. 1996.
3. Ruder, Avima M *et al*., Mortality among 24,865 workers exposed to polychlorinated biphenyls (PCBs) in three electrical capacitor manufacturing plants: a ten-year update. *Int J Hyg Environ Health*. 2014. 217 (2-3); 176-87.