Funded by NIEHS EHS Core Center Grant #P30ES020957

WAYNE STATE UNIVERSITY

LEGACY AND EMERGING HALOGENATED POLLUTANTS MODULATE GUT MICROBIOTA AND ACCELERATE ATHEROSCLEROSIS

CURES

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Acknowledgments

- Petriello Lab
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P30 ES020957, K99/R00ES028734, R01DK106540, and Wayne State OVPR

ECHO
Environmental influences on Child Health Outcomes
A program supported by the NIH

CURES
Center for Urban Responses to Environmental Stressors

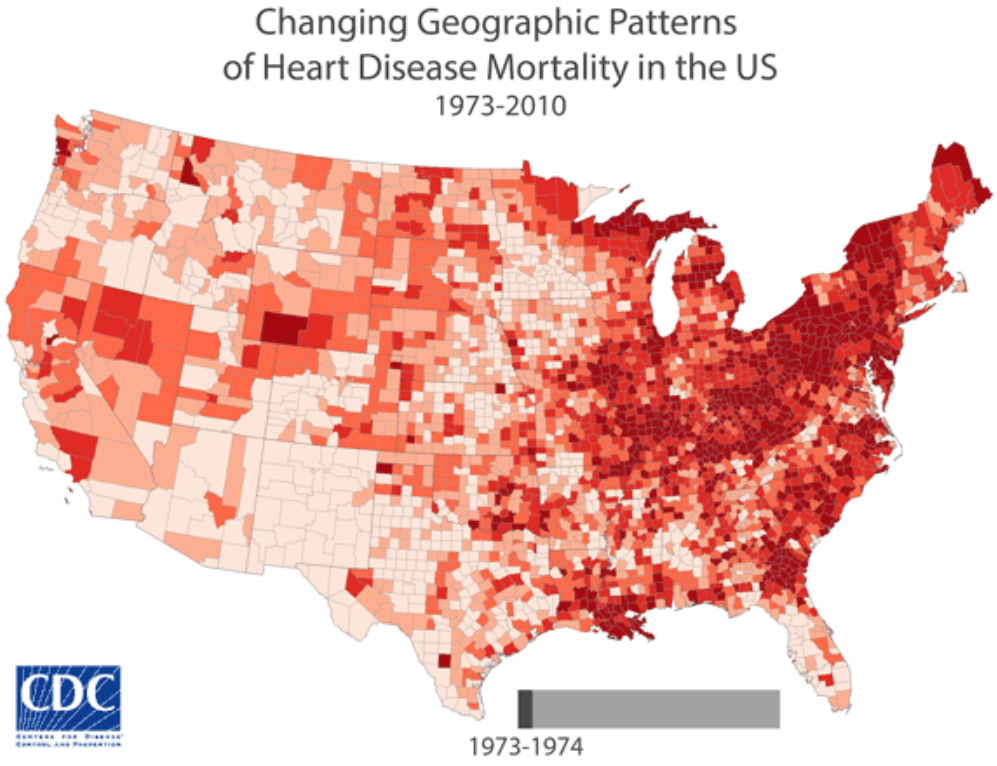
NIH
National Institute of Environmental Health Sciences
Superfund Research Program

Outline

- Background
  - Atherosclerosis and CVD
- Dioxin-like pollutants
- Per and polyfluoroalkyl substances

Funded by NIEHS EHS Core Center Grant #P30ES020957

Heart disease risk over time



"The sweeping geographic changes in heart disease mortality observed over a relatively short period of time…suggest that systematic changes may have occurred in a variety of biomedical, behavioral, and socioenvironmental factors. The accompanying regional disparities…highlight the importance of identifying conditions that are contributing to these disparities".

DOI: (10.1161/CIRCULATIONAHA.115.018663)

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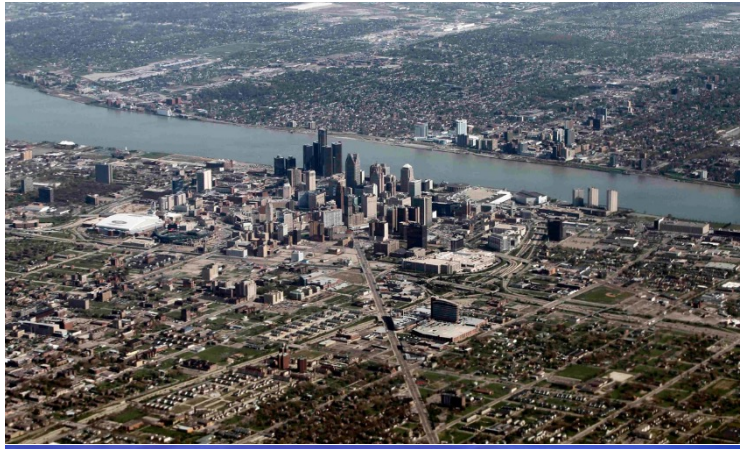

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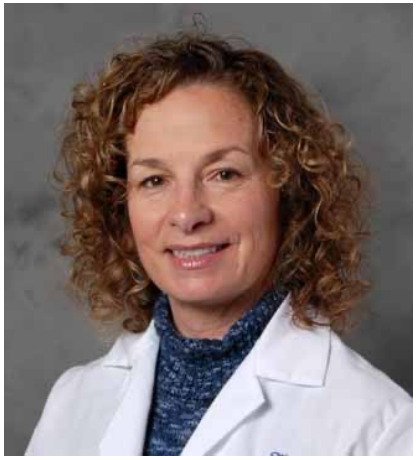
Detroit is a highly polluted industrial city



WAYNE STATE UNIVERSITY

Henry Ford HEALTH SYSTEM

CURES

Mechanistic toxicologists interacting with community engagement experts

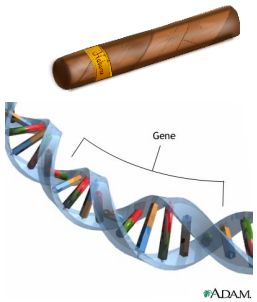
What determines cardiovascular risk?

Non-modifiable ?

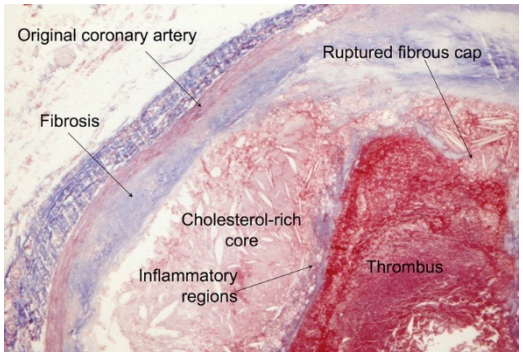
- **GENES**

Genetic (heritable) risk factors account for 30-60% of inter individual cardiovascular disease risk

- **Family History**
- **Gender**



Atherosclerotic Artery



Modifiable ?

- **ENVIRONMENT**
- **Smoking**
- **Environmental Pollutants**
- **Diet and lifestyle**

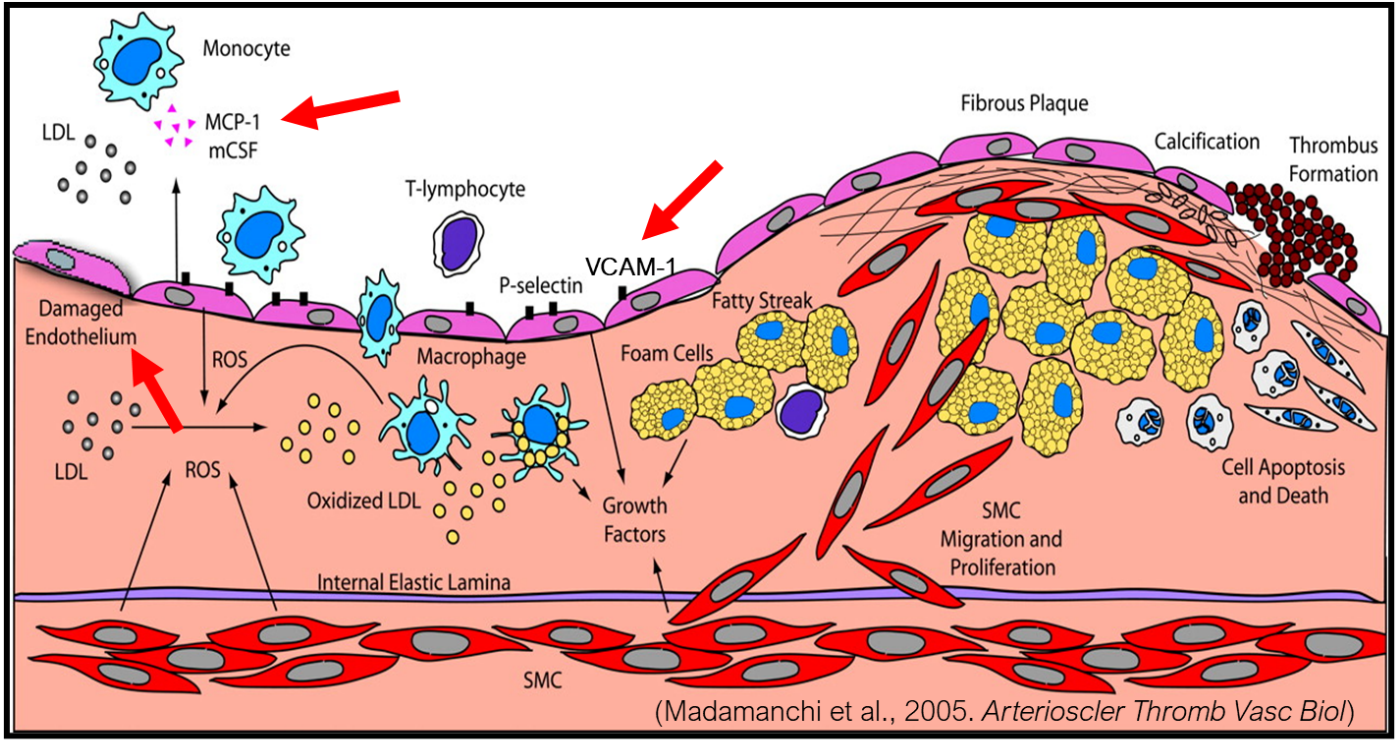
Diet-Dependent OBESITY

Hyperlipidemia

High Blood Pressure | **Diabetes** | **Inflammation**

Metabolic Syndrome

Development and progression of Atherosclerosis



(Madamanchi et al., 2005. *Arterioscler Thromb Vasc Biol*)

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Causative or associative biomarkers of atherosclerosis

Lipid markers of atherosclerosis:

- Total Cholesterol, VLDL, LDL, HDL
- Triglycerides
- Apolipoproteins

Markers of Inflammation:

- C-reactive protein
- IL-1β and other cytokines
- Vascular adhesion molecules and other proteins

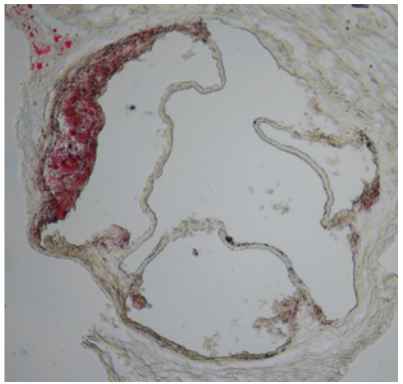
Emerging markers:

- Trimethylamine N-oxide

https://www.medscape.com/viewarticle/869226

https://www.hopkinsmedicine.org/health/healthy_heart/stay_healthy/3-myths-about-cholesterol-lowering-statin-drugs

Preclinical models to study atherosclerosis



•Mouse models of hypercholesterolemia are gold standards for cardiovascular medicine.

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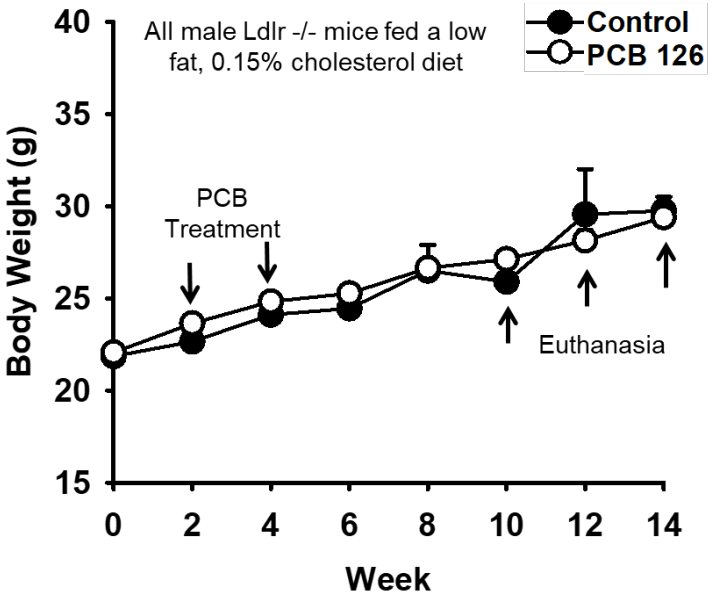
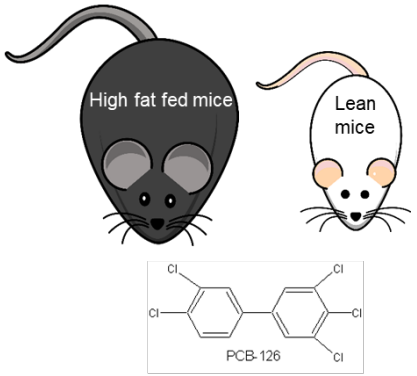
Model to study lipophilic pollutant-accelerated atherosclerosis



Petriello et al. 2017, *Toxicol Sci.*

LF Clinton-Cybulsky Atherogenic Diet	gm	kcal
Ingredient		
Corn Starch	375	1500
Maltodextrin 10	125	500
Sucrose	200	800
Cellulose BW200	50	0
Casein, Lactic	200	800
L-Cystine	3	12
Soybean Oil	25	225
Cocoa Butter	20	180
Cholesterol	1.6	0
Mineral Mix S10021	10	0
Dicalcium Phosphate	13	0
Calcium Carbonate	5.5	0
Potassium Citrate	16.5	0
Vitamin Mix V10001	10	40
Choline Bitartrate	2	0
	gm%	kcal%
Carbohydrate	67.2	70.0
Protein	19.2	20.0
Fat	4.3	10.0



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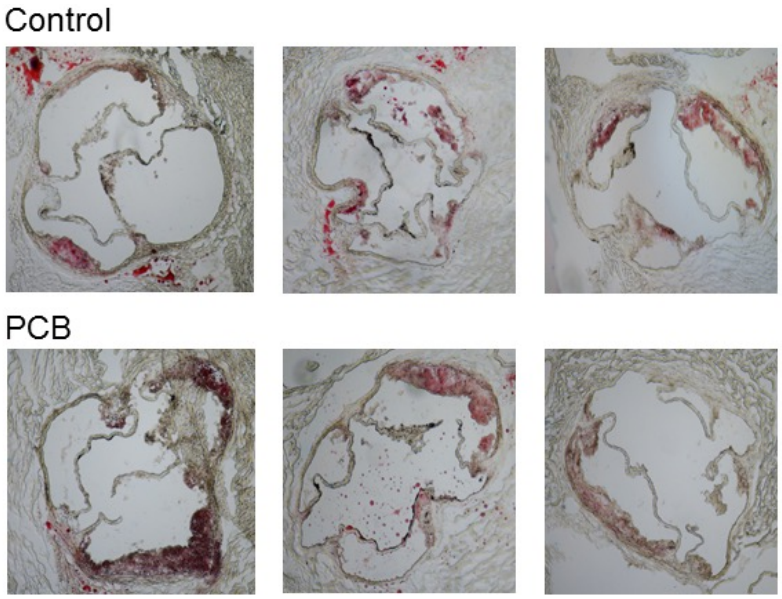
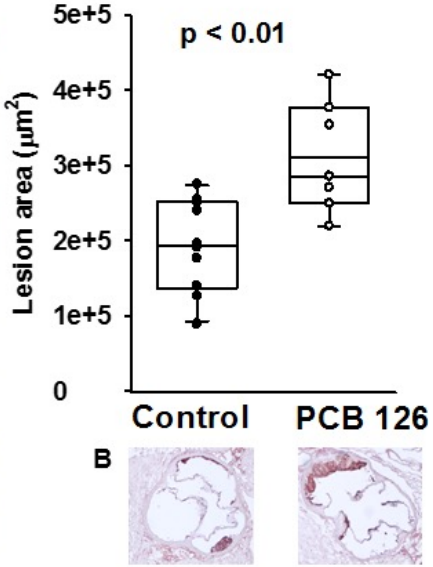
Model to study lipophilic pollutant-accelerated atherosclerosis



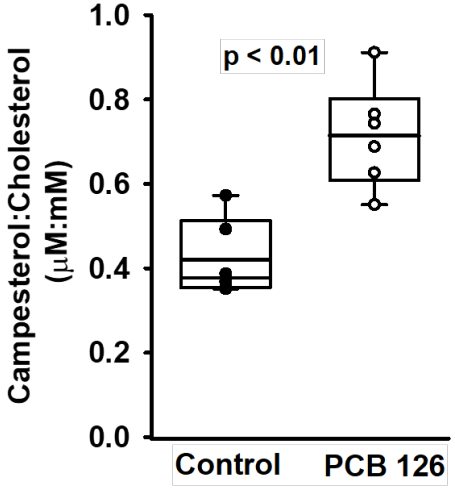


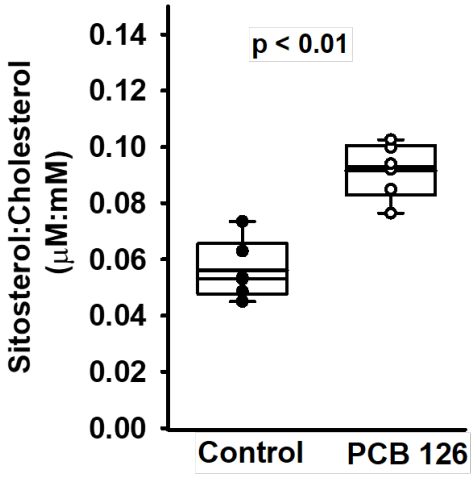
PCB 126 increases lesion formation, circulating cytokines, and inflammatory mediators

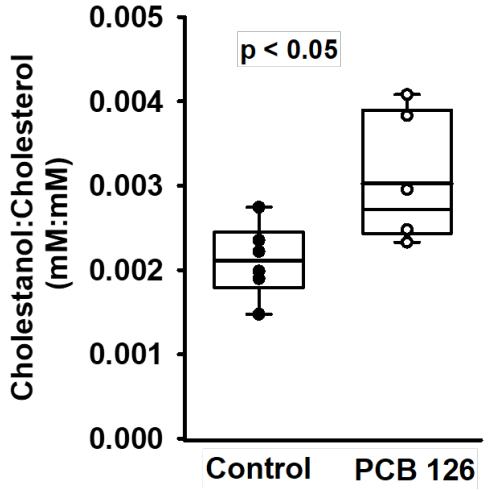
Petriello Toxicological Sciences, 2017

Funded by NIEHS EHS Core Center Grant #P30ES020957

PCB 126 treatment increases markers of cholesterol absorption







- According to the Framingham Offspring Study, people who are cholesterol hyperabsorbers are at increased risk of cardiovascular disease.

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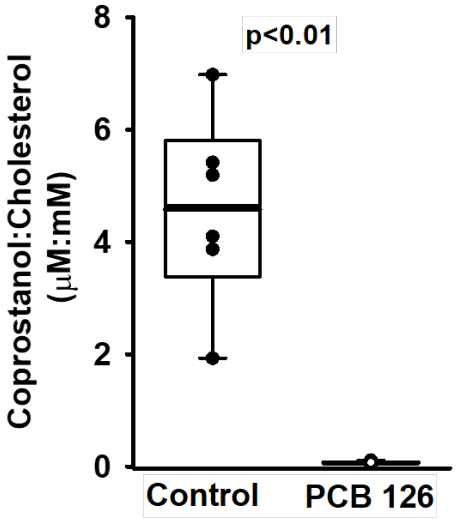
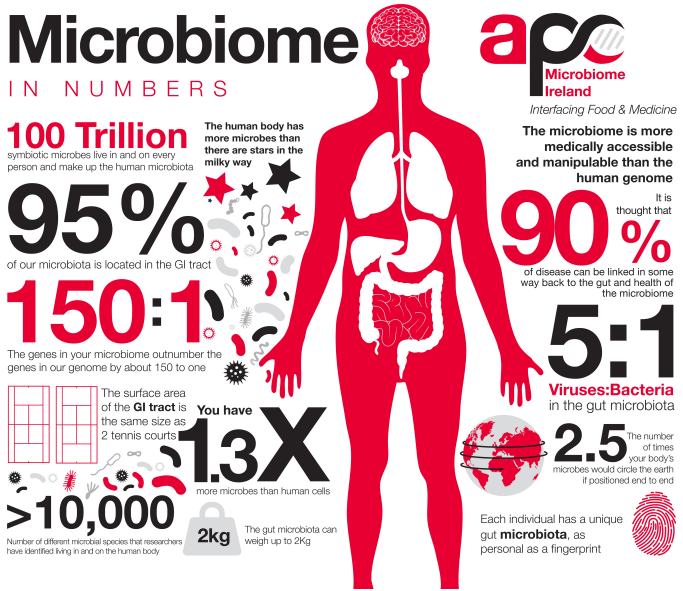
PCB 126 depletes a gut microbiota mediated metabolite of cholesterol





- The bacteria responsible for this function have mostly been classified within the Eubacterium genus

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Xenobiotics and microbiota interact

•Microbiota metabolize some pollutants and pollutants can impact on bacteria health

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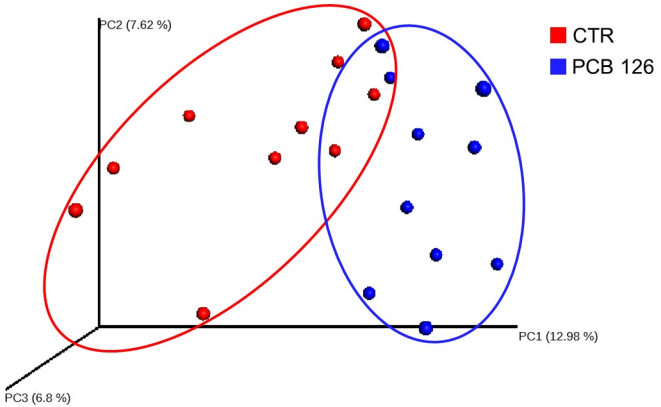
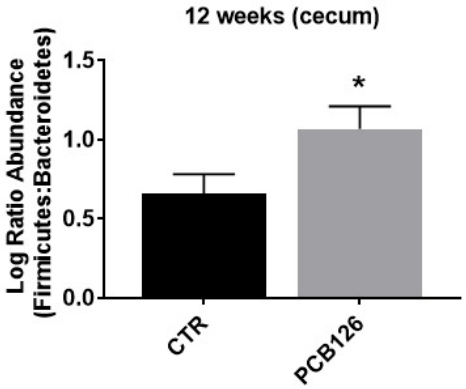
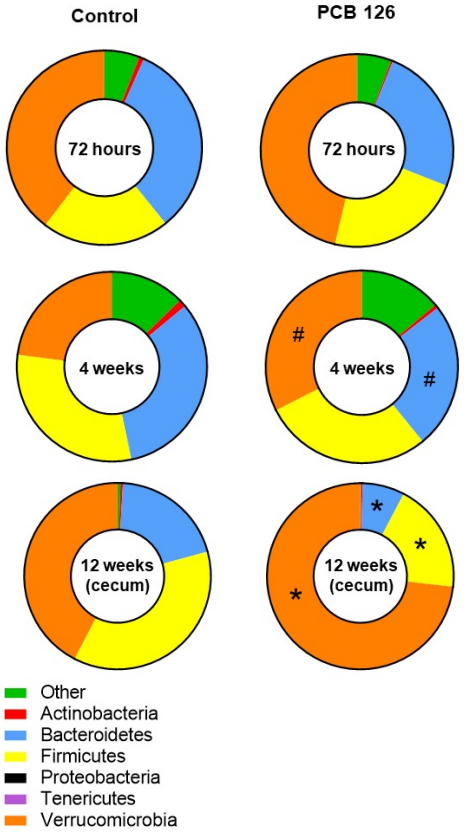
PCB 126 modulates microbiota form







16S rRNA sequencing allows us to examine changes at the diversity, phyla, and genera/species level

…and function

Hepatic Metabolomics Analysis [a]

Metabolite Category	Fold Change PCB vs. Vehicle	P-value	Pathway
Gut Microbiota Influenced Metabolites			
5-hydroxyindoleacetate	0.55	**<0.01**	Tryptophan metabolism
Retinol	0.58	**0.015**	Vitamin A metabolism
Retinal	0.52	**<0.01**	Vitamin A metabolism
Hypotaurine	0.47	**<0.01**	Methionine, cystine, SAM
1-methylhistamine	0.33	0.081	Histidine metabolism
N-acetylarginine	0.64	**<0.010**	Urea cycle
hypoxanthine	0.79	**0.012**	Purine metabolism
N-acetylphenylalanine	0.35	**<0.010**	Phenylalanine metabolism
Hippurate	0.52	0.067	Benzoate metabolism
Creatinine	0.52	0.086	Creatine metabolism
Argininate	0.72	**0.014**	Urea cycle
N-oleoyltaurine	1.74	**0.049**	Endocannabinoid
O-methyltyrosine	1.54	**0.050**	Tyrosine metabolism
Urate	2.13	**<0.01**	Purine metabolism
dimethylglycine	1.41	**0.022**	Glycine, serine, and threonine metabolism
glutarate	1.79	0.097	Fatty acid, dicarboxylate

[a] n=6 per group, statistically significant differences determined by Welch's two-sample t-tests.

Are there circulating metabolites we can use as biomarkers in humans?

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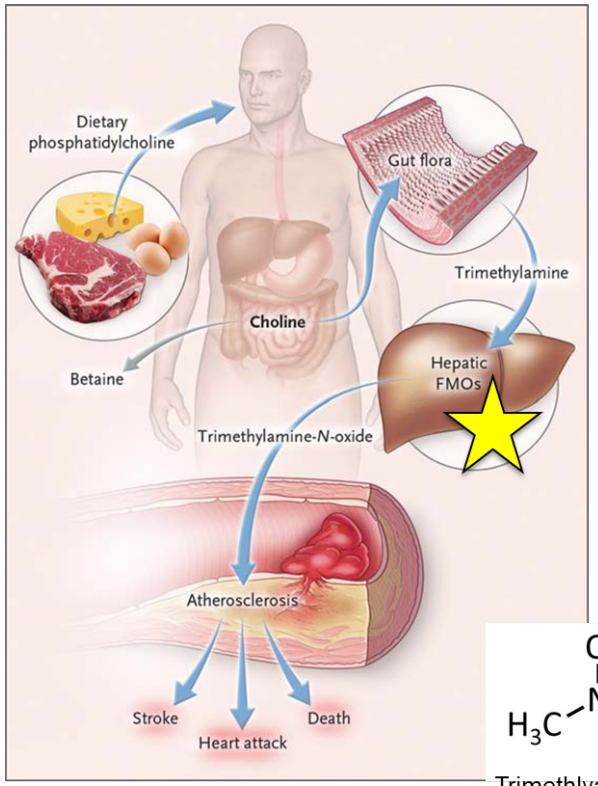
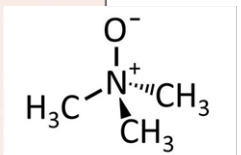
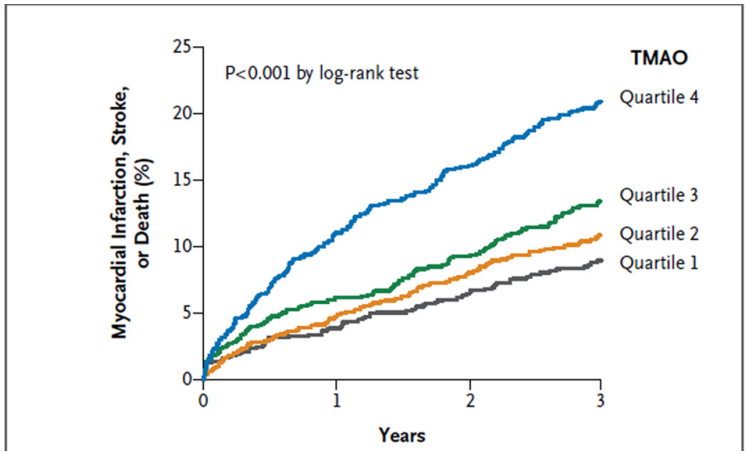
TMAO – an Emerging Biomarker of CVD



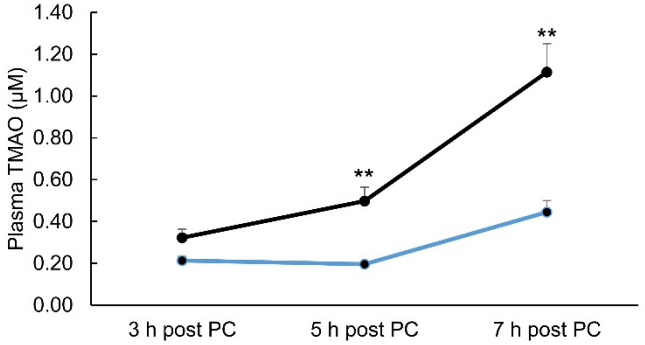


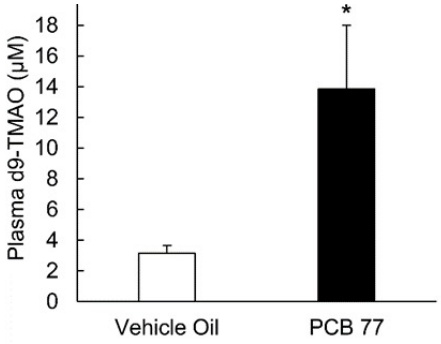
Trimethlyamine N-oxide



- Increased plasma levels of TMAO were associated with an increased risk of a major adverse cardiovascular event.
  - N Engl J Med. 2013 Apr 25; 368(17): 1575–1584.

Exposure to Dioxin-like PCBs Increased Diet-derived TMAO





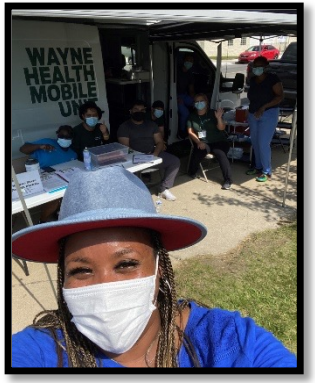
- The association between DLPs and increased TMAO was confirmed in the Anniston Community Health Survey.

Petriello et al. 2018, *Environ Res.*

Petriello et al. 2016, *JNB.*

CURES IN THE COMMUNITY

- **Wayne State Health Mobile Unit** offered lead and Covid testing and wellness checks
- **Detroit Health Department** administered Covid-19 vaccines
- **E-Chats** working with Urban Neighborhood Initiatives. PFAS, lead, and air pollution are hot topics.



Outline

- Background
  - Atherosclerosis and CVD
- Dioxin-like pollutants
- Per and polyfluoroalkyl substances

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Background

- Per- and Polyfluorinated Alkyl Substances (PFAS)
  - Hydrophilic functional group + Hydrophobic tail
  - Functional groups commonly are carboxylate and sulfonates
- "Forever Chemicals"
- Over 4000 different PFAS
- PFAS used in a variety of common industrial and consumer products including carpets, cookware, food packaging, fire fighting foams.
- Teflon and Scotchgard

https://www.wateronline.com/doc/the-microplastics-and-pfas-connection-0001

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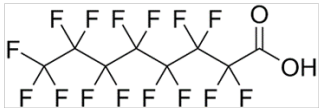
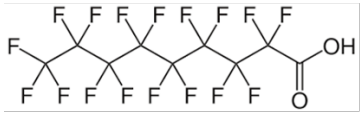
PFAS Subtypes and Structure

Legacy



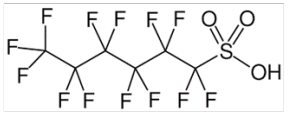
PFOA
Perfluorooctanoic acid

PFOS
Perfluorooctane sulfonate



PFNA
Perfluorononanoic acid

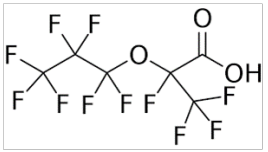
Alternative



PFHxS
Tridecafluorohexane-1-sulfonic acid

*PFOS alternative

Emerging



GenX
Ammonium perfluoro(2-methyl-3-oxahexanoate)

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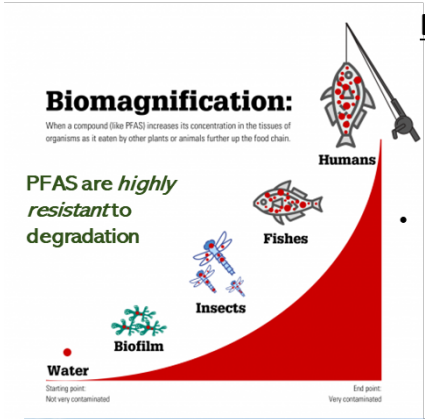
PFAS Levels in Humans and the Environment



Environment

- PFAS concentrations in environmental water sources range from pg/L to µg/L levels.

https://cals.ncsu.edu/applied-ecology/news/pfas-in-yadkin-pee-dee-river-food-chain/

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PFAS and cardiovascular diseases

American Journal of Epidemiology

Society for Epidemiologic Research | Johns Hopkins Bloomberg School of Public Health

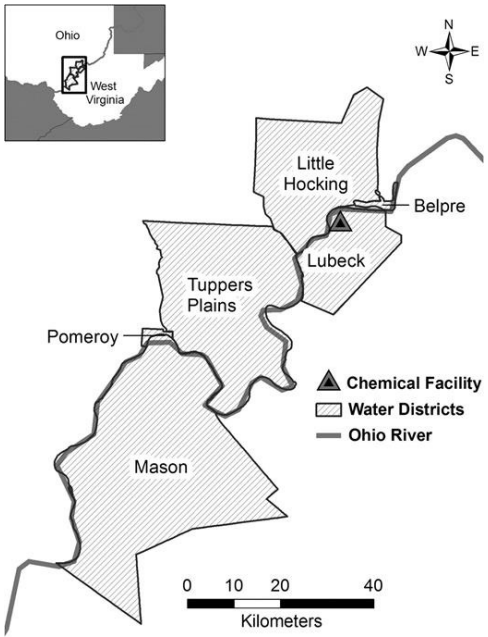
Association of Perfluorooctanoic Acid and Perfluorooctane Sulfonate With Serum Lipids Among Adults Living Near a Chemical Plant

Kyle Steenland, Sarah Tinker, Stephanie Frisbee, Alan Ducatman, Viola Vaccarino

American Journal of Epidemiology, Volume 170, Issue 10, 15 November 2009, Pages 1268–1278,
https://doi-org.proxy.lib.wayne.edu/10.1093/aje/kwp279

- Residential communities exposed to environmental PFAS contamination from a chemical plant in West Virginia

- Total cholesterol showed significant increasing trends by increasing decile of both PFOA and PFOS



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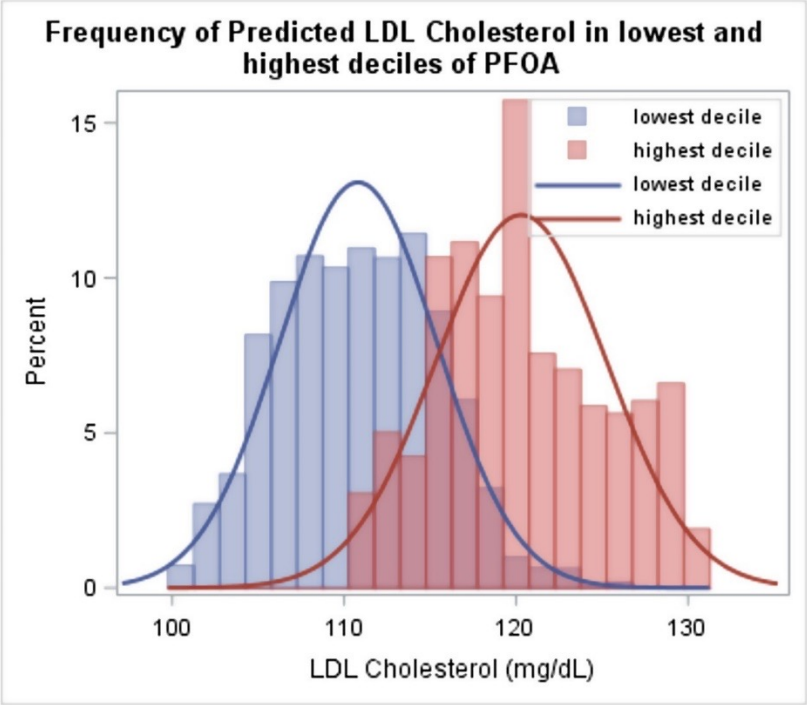
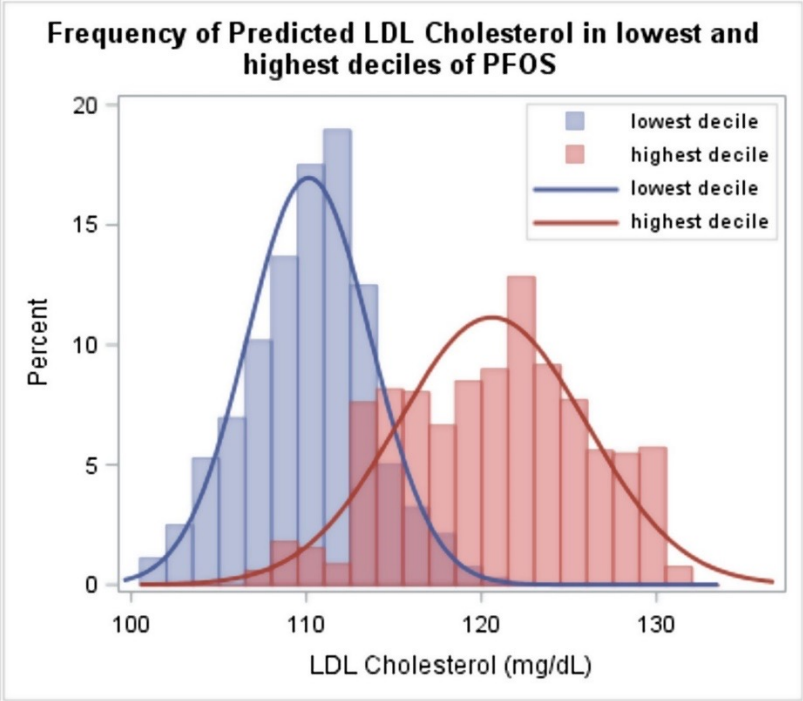
Distribution of LDL cholesterol among NHANES subjects 2003-2016.

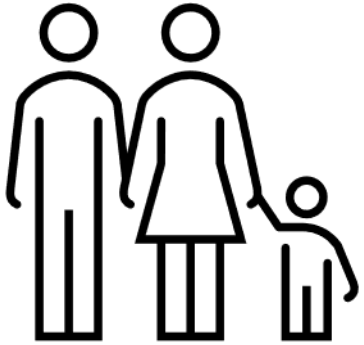
https://doi.org/10.1016/j.tox.2021.152845



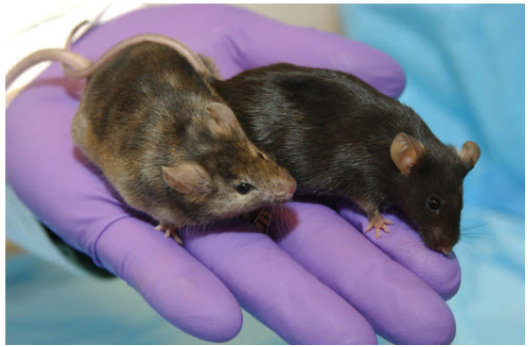


"LDL cholesterol (mg/dl) was adjusted for age, sex, ethnicity, and an index indicating survey wave. The deciles were determined for PFOA or PFOA based on the distribution of values after adjusting for age, sex, ethnicity, and an index indicating survey wave and calculated using the sampling parameters."

PFAS and cholesterol



In humans, PFAS exposure associated with INCREASED cholesterol levels.



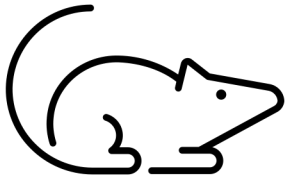
However, historically, PFAS exposure DECREASED cholesterol levels in rodent studies.

Funded by NIEHS EHS Core Center Grant #P30ES020957

Rodent studies

Why don't our animal models mirror impacts on cholesterol levels seen in humans?

- Diet?
- Genetic differences (PPARα)?
- Single exposure vs. mixture?
- Length of exposure?
- Differences in cholesterol fractions?



*More recent studies using hPPARα and HFD-feeding have found elevated serum cholesterol in PFAS-exposed mice.

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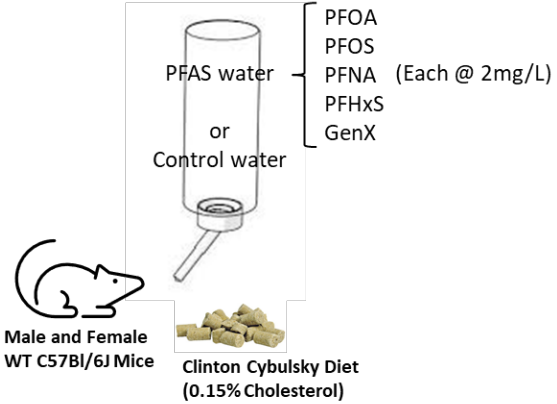
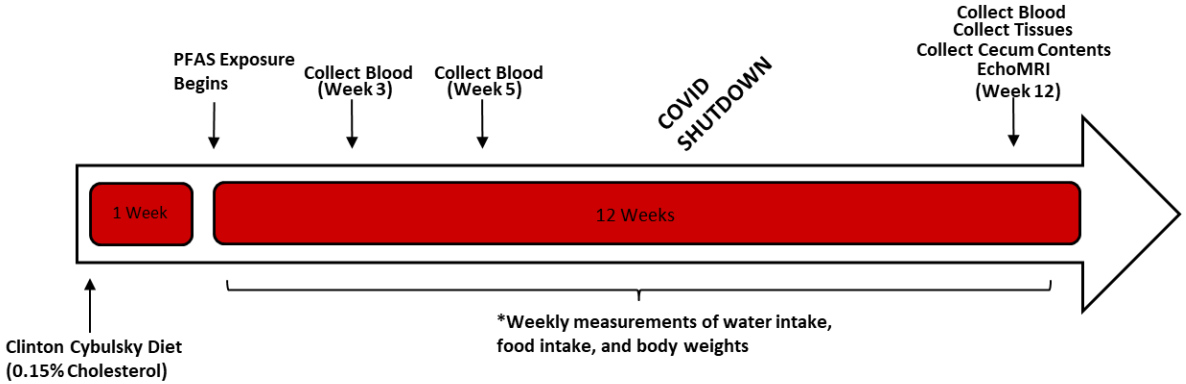
PFAS mixture study design





4 Treatment Groups:

1. Female Control Water
2. Female PFAS Water
3. Male Control Water
4. Male PFAS Water

•Exposure to this PFAS mixture resulted in circulating PFAS concentrations in the ug/mL range, similar to occupational exposures.

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A mouse model of PFAS mixture-increased circulating cholesterol

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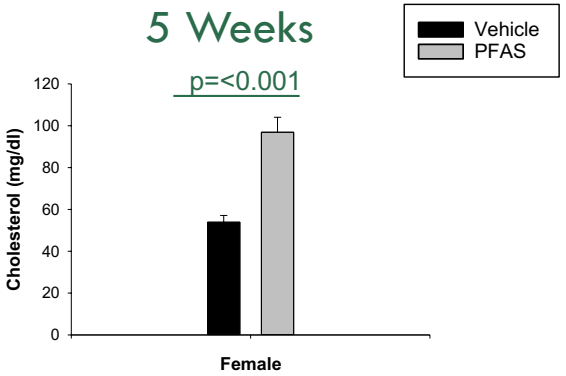
Exposure to a mixture of legacy, alternative, and replacement per- and polyfluoroalkyl substances (PFAS) results in sex-dependent modulation of cholesterol metabolism and liver injury

Katherine Roth [a], Zhao Yang [a], Manisha Agarwal [a,b], Wendy Liu [c], Zheyun Peng [d], Ze Long [d], Johnna Birbeck [e], Judy Westrick [e], Wanqing Liu [b,d], Michael C. Petriello [a,b,*]



- PFAS increased circulating cholesterol and bile acids and hepatic injury.
- PFAS-exposed females displayed increased lobular and portal inflammation compared to the males.
- Hepatic and circulating levels of PFOA were increased in exposed females compared to males.

Roth et al. 2021, *Environment International.*