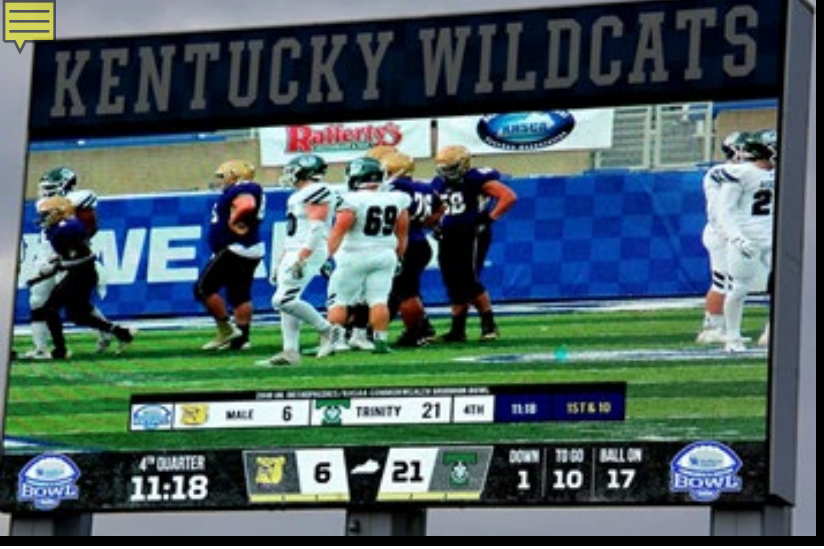

Environmental Liver Disease

UK-CARES 2/6/20

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Center for Integrated Environmental Health Sciences
Hepatobiology and Toxicology COBRE
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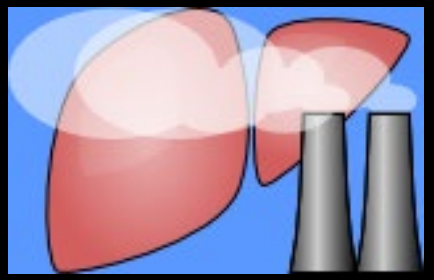
Disclosures



"Do unto those downstream as you would have those upstream do unto you."

- Wendell Berry

Industry relationships:
Lakeside Biosciences & Diapharma Inc., Gilead, Abbvie, Dova, Merck, Galmed, Intercept, Conatus, Durect, Hightide, Genfit, Allergan.

Investigational medications and biomarkers will be discussed.

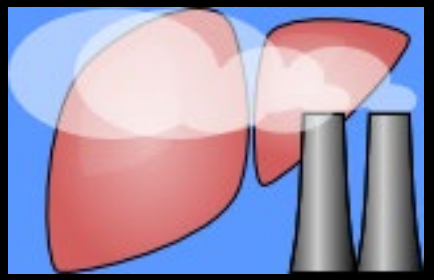
Case presentation: a veteran with cirrhosis and a family history of liver cancer

Figure 1. Location of U.S. Marine Corps Base, Camp Lejeune, North Carolina.

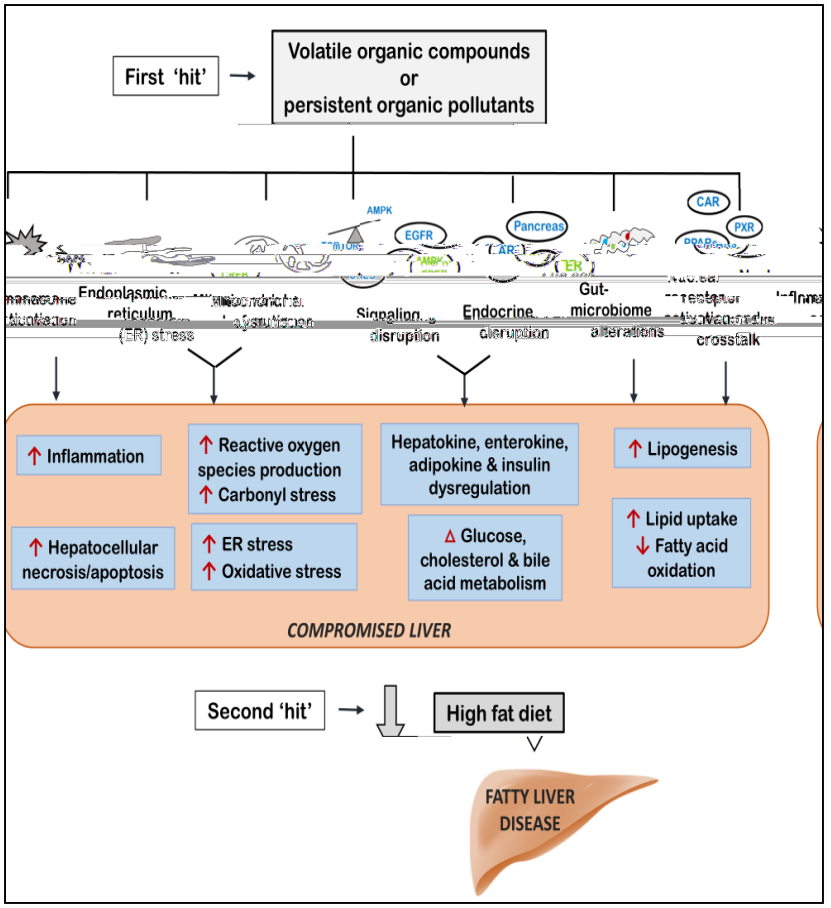
- Paul is a retired marine with steatohepatitis and cirrhosis.
- Exposure history: volatile organics (including **vinyl chloride**) in drinking water at USMC Base Camp Lejeune (1976-1978).
- Risk factors: diabetes & obesity.
- Family history: Paul's brother died of cirrhosis and hepatocellular carcinoma. He was a **polyvinyl chloride** production worker in Louisville's Rubbertown chemical manufacturing complex.
- Question: What's the contribution of vinyl chloride exposures?

• Cernansky, R. A Double Whammy: Exposure to Certain Chemicals May be A Quiet Culprit in the Global Increase In Liver Disease. Discover Magazine, July/August 2018.
• Institute of Medicine. Review of the VA Clinical Guidance for the Health Conditions Identified by the Camp Lejeune Legislation. 2015. Washington, DC: The National Academies Press.

Objectives



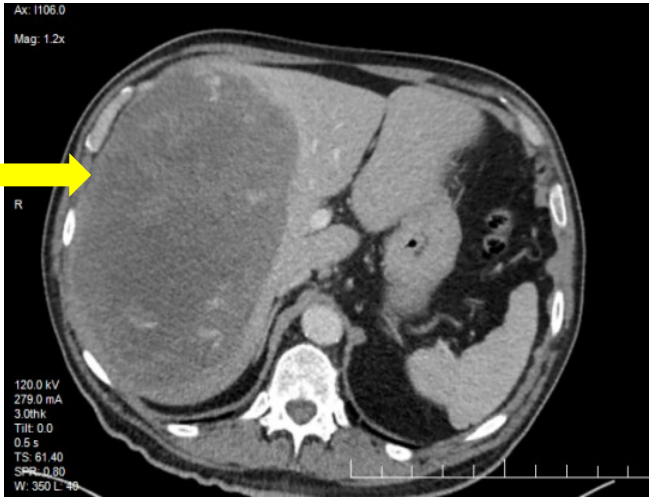
1) **Occupational and environmental hepatology overview**
2) **Toxicant associated steatohepatitis (TASH) related to volatile organic compounds (VOCs) and persistent organic pollutants (POPs)**
3) **Key environmental health concepts impacting digestive diseases:**
   - Endocrine disrupting chemical (EDC) hypothesis
   - Obesogens
   - Metabolism disrupting chemical (MDC) hypothesis
   - Signaling disrupting chemicals (SDCs)
   - Two 'hit' models



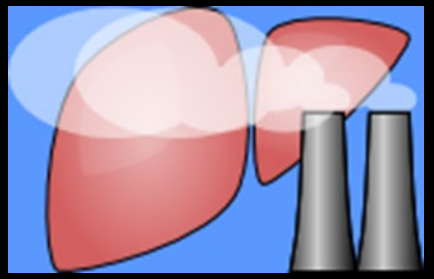
Low level pollutions may serve as a 'first' hit compromising the liver to hypercaloric diets thereby promoting fatty liver disease.

Historical perspective on chemical liver diseases - poisoning events

- 1960, England: aflatoxin and liver necrosis - **turkey X disease**. Blount. Turkeys (1961), pp. 52-55.
- 1965, England: methylene dianiline-contaminated flour - **Epping jaundice**. PMID: 15538614
- 1974, Louisville, KY: Polyvinyl chloride production workers and **hepatic hemangiosarcoma**. PMID: 4856325
- 1978, Taiwan: PCB-contaminated cooking oil and cirrhosis - **Yucheng event**. PMID: 23026800
- 1981, Spain: aniline contaminated cooking oil and cholestasis / steatohepatitis - **toxic oil syndrome**. PMID: 3609665



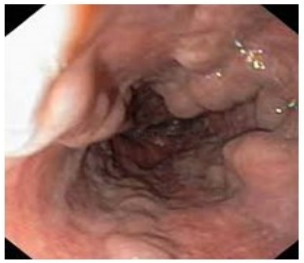
What is the environmental contribution to the current liver disease epidemic?

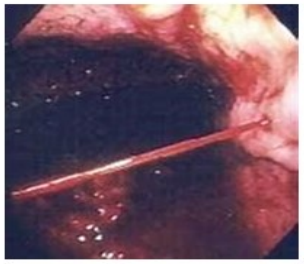


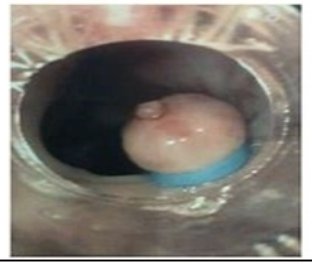
US cirrhosis and liver cancer-related death rates increased 65% and 50% respectively (1999-2016). PMID: 30021785

Ascites

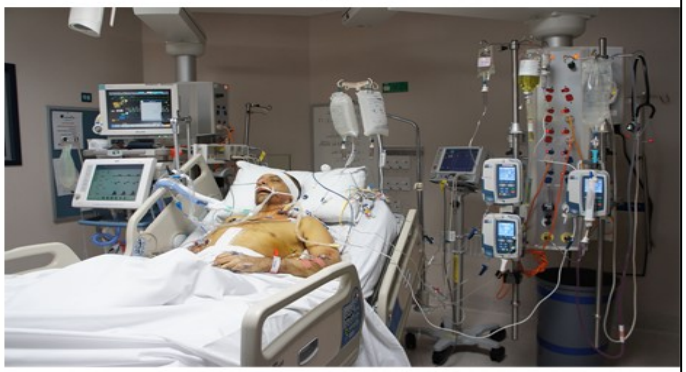
Esophageal varices





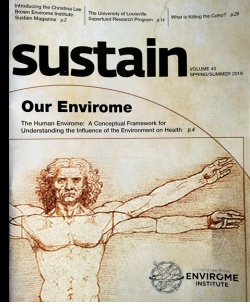


Multi-organ failure



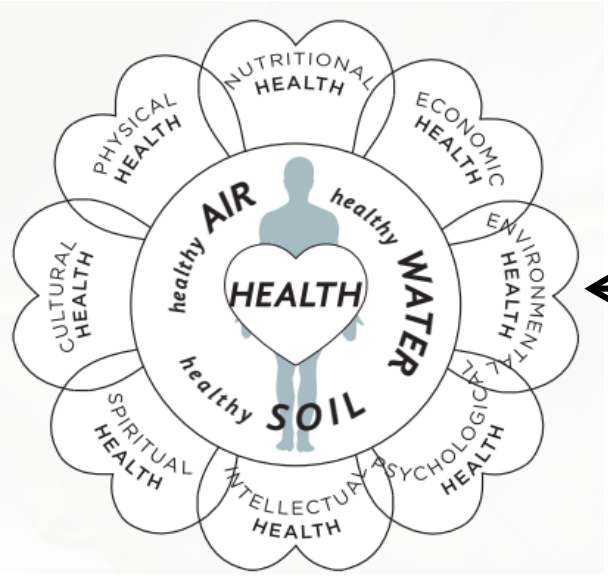
Liver transplantation

UofL's Environmental health vision



The **Envirome Institute** pioneers a new interdependent vision of health; supports research on the effects of the environment on health; and promotes holistic scholarship. https://enviromeinstitute.com/

The Envirome Institute (Bhatnagar)

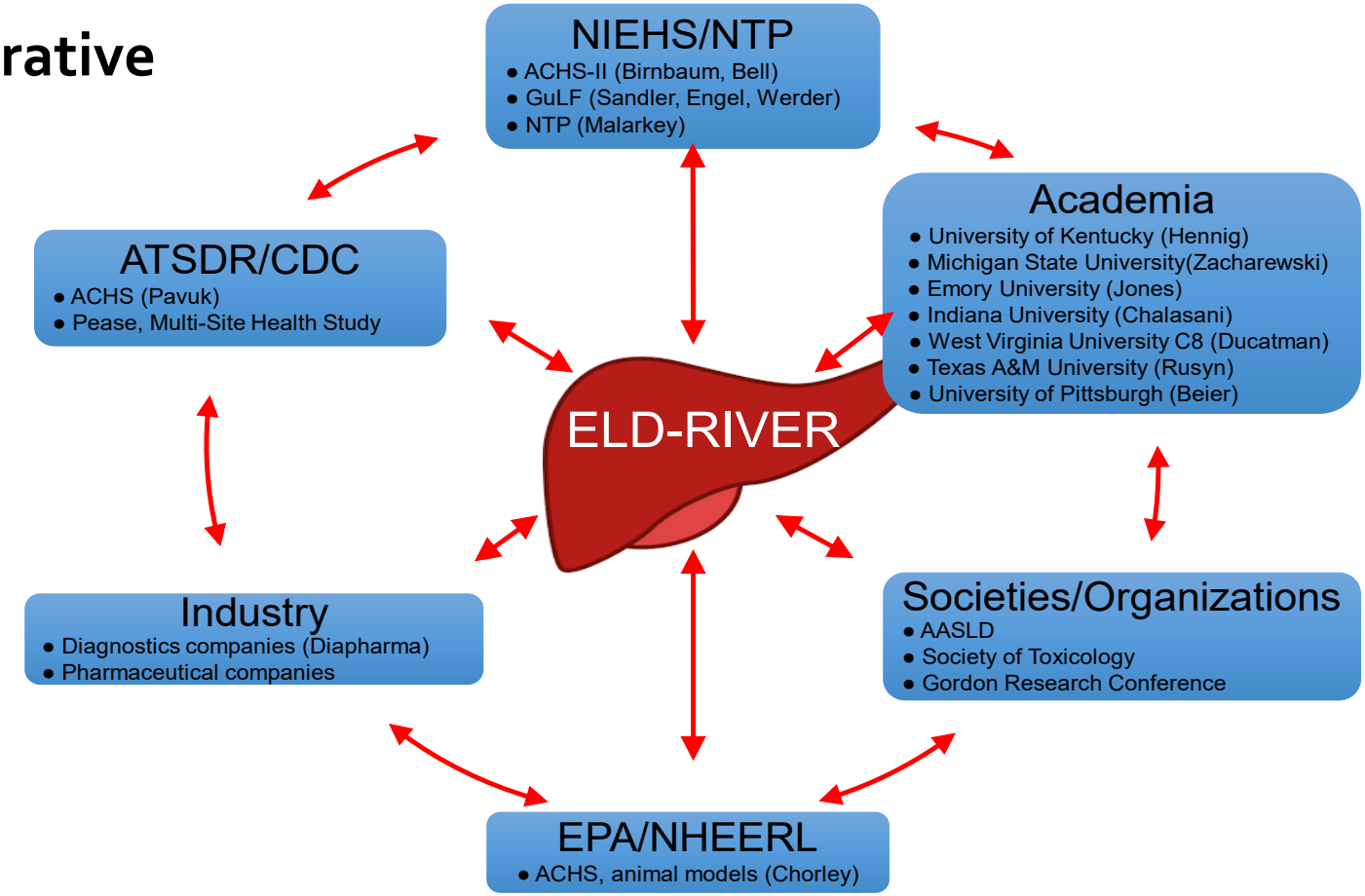


Selected Major EHS Projects Interacting with Envirome

- NIEHS P42 Superfund Research Center (Srivastava)
- NIEHS Training Grants (T32 - Hein, T35 - States)
- NIGMS P20 Hepatobiology and Toxicology COBRE (McClain)
- NIEHS P30 Center for Integrated Environmental Health Sciences (States)
- **NIESH R35 Environmental Liver Disease RIVER (ELD-RIVER) (Cave)**
- NIEHS R01's and other grants

The R35's approach to environmental hepatology

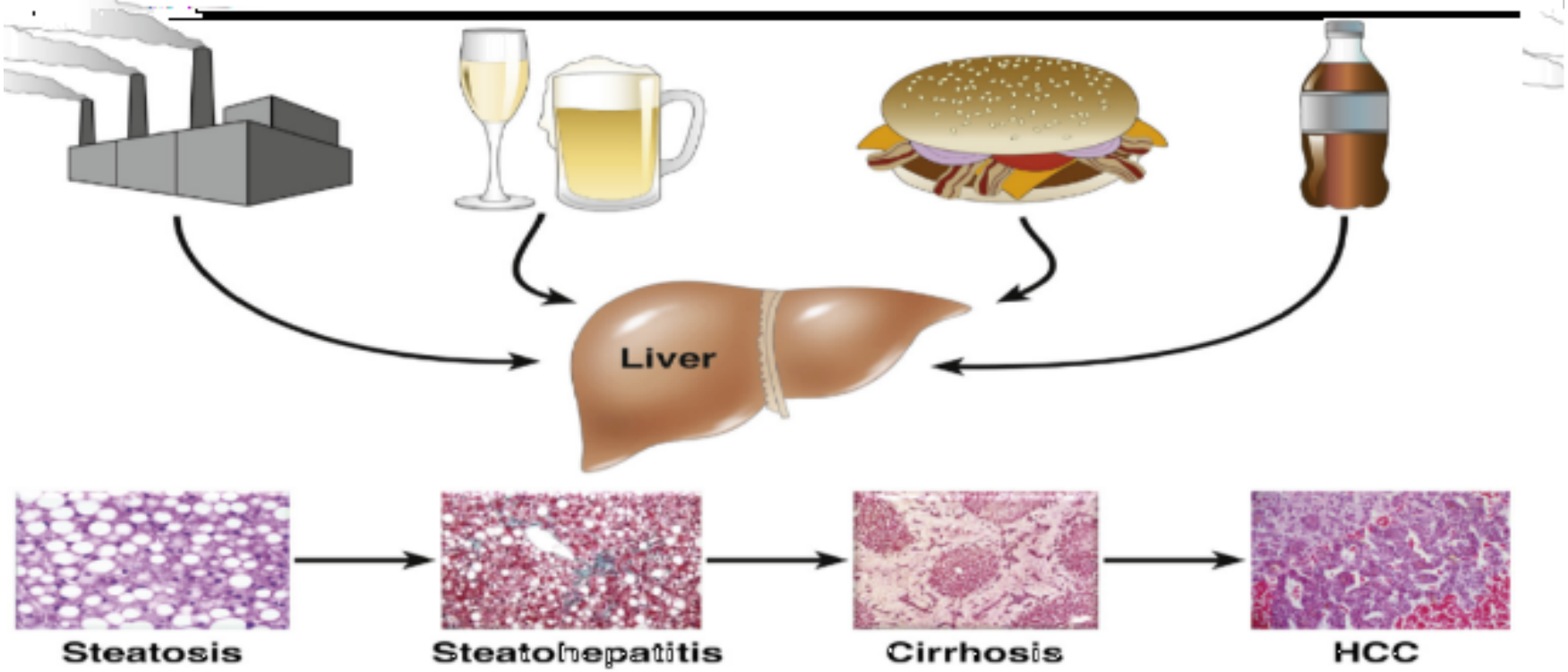
Collaborative



Translational

Epidemiology → Bench → Bedside

Which liver diseases do we study?

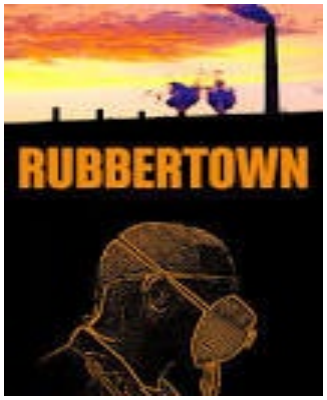


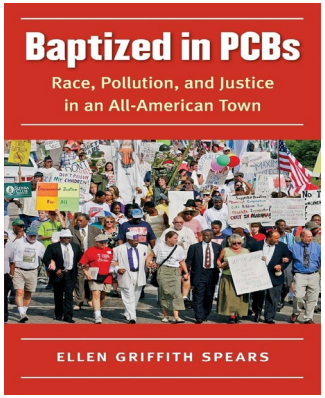
The current etiologies, nomenclature, and pathologic spectrum of the fatty liver diseases. TAFLD/TASH, toxicant associated fatty liver disease and toxicant associated steatohepatitis; ALD/ASH, alcoholic liver disease and alcoholic steatohepatitis; NAFLD/NASH, nonalcoholic fatty liver disease and nonalcoholic steatohepatitis.

PMID: 30021785, 26707365, 27373606, 25291348,19224838, 23262638, 19902480, 21915069

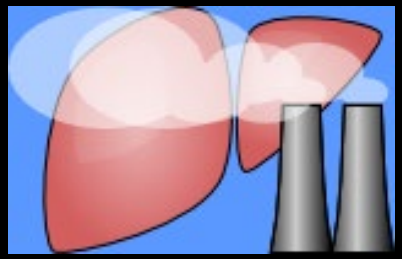
What pollutants do we study?

The Cave Laboratory's Occupational and Environmental Liver Disease Cohort Studies (mostly cross-sectional, * = longitudinal)				
Type	**Subtype**	**Population**	**Company/Exposure**	**Reference**
Special Exposure	Occupational	Polyvinyl chloride polymerization workers	BF Goodrich / VOCs	27765658, 19902480
		Elastomer and polymer workers	Zeon / VOCs	21915069
		GuLF Study (Deepwater Horizon disaster response)	B.P. / VOCs & metals	under review
		Vietnamese electronic waste recyclers	None / POPs & metals	SOT abstract
	Residential			
	↑ Industrial Emissions	C8 Health Study	DuPont / PFAS	30823334
		Anniston Community Health Survey I	Monsanto / PCBs	29684222
		Anniston Community Health Survey II*	Monsanto / dioxins	AASLD abstract
		Former Black Leaf chemical plant brownfield site	Black Leaf / Insecticides	SOT abstract
	↓ Urban Green Space	HEAL Study (UofL SRC)	VOCs	Superfund abstract
General Population	United States	NHANES	None / Exposome	31873887, 21126940
Precision Medicine	Clinical Population	IU NAFLD clinic cohort	None / Exposome	AASLD abstract
		NASH Clinical Research Network (FLINT Study)*	None / Exposome	Proposed





Chemicals associated with TAFLD/TASH



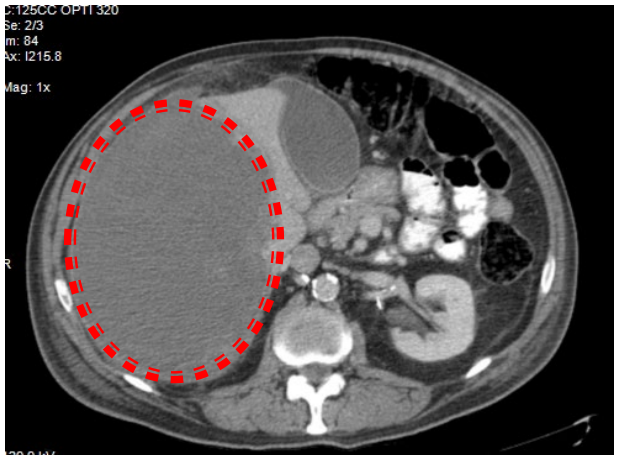
Selected chemicals associated with fatty liver disease			
Chemical / Chemical Group	Laboratory animals	Epidemiology/Clinical Evidence	ATSDR SPL Rank
Arsenic	[97-100]	[101]	#1
Atrazine	[102, 103]		
Benzo[a]pyrene	[104-105]		#8
Bisphenol A	[106-109]		
Cadmium	[110, 111]	[112, 113]	#7
DEHP	[114-116]		#77
TCDD/dioxins	[117-120, 62]	[121-123]	#72
Fungicides	[117]		
Lead		[124, 125]	#2
Mercury	[126]	[124]	#3
Organochlorine insecticides	[127, 128]	[129, 121, 123]	#13
Particulate matter	[92-95]	[96]	
PBDEs	[130]		#144
PFAS	[78, 80, 131. 135-138]	[132, 83, 133, 134, 84, 132]	#143
PCBs	[117, 56, 139, 23, 65]	[124, 129, 122, 121, 140-142]	#5
Smoking/nicotine	[143, 144]	[145, 146]	
Tributyltin	[147-150]		
Vinyl chloride	[151]	[4]	#4
PMID: 31134516			

TASH was discovered using UofL's unique Occupational Health Biorepository

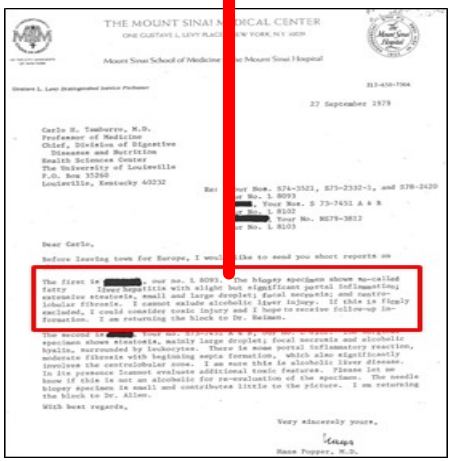
- 1942: Polyvinyl chloride production began in **Rubbertown**.
- 1974: **Hepatic hemangiosarcoma** reported in four workers (*JAMA)*. Carlo Tamburro, MD, initiated medical surveillance & biorepository.
- 2014: 26th cancer case.

The first is [redacted], our no. L 8093. The biopsy specimen shows so-called fatty liver hepatitis with slight but significant portal inflammation; extensive steatosis, small and large droplet; focal necrosis; and centrolobular fibrosis. I cannot exlude alcoholic liver injury. If this is firmly excluded, I could consider toxic injury and I hope to receive follow-up information. I am returning the block to Dr. Reiman.

Louisville's Rubbertown chemical complex.

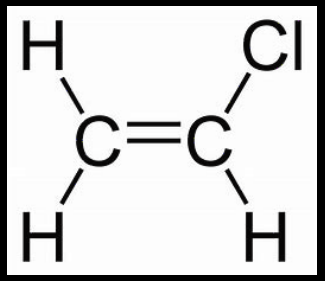


CT scan of retired Rubbertown worker demonstrating hepatic hemangiosarcoma.

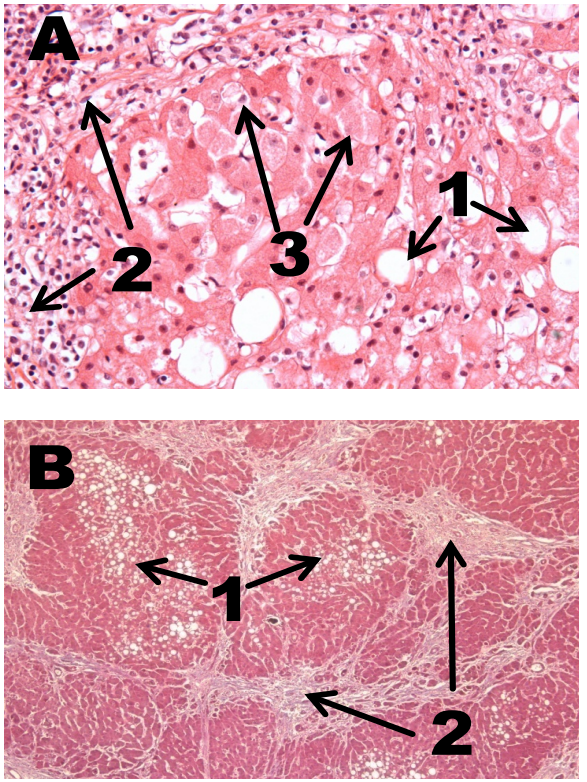


Letter from the pathologist Hans Popper, MD to Carlo Tamburro, 1979.

TASH in Rubbertown PVC workers



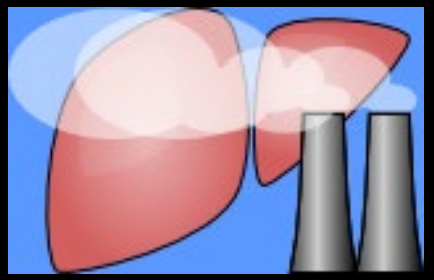
- In 25 workers with high-level vinyl chloride exposures, the prevalence of biopsy-proven steatosis was **84%** and steatohepatitis was **80%**. Not explained by obesity or EtOH.
- Fibrosis was present in 55% of cases.
- The term, TASH, was initially coined to describe this observation.
- Consistent with results from Brazilian petrochemical worker studies (reversible).
- A urine vinyl chloride exposure biomarker was recently associated with increased odds for NAFLD in children living near a petrochemical complex.



Liver biopsies from vinyl chloride workers with TASH. Panel A: steatosis (1) with inflammatory infiltrate, fibrosis (2) and Mallory-Denk bodies (3), (H&E stain, 200X). Panel B: steatosis (1) with extensive fibrosis (2) consistent with cirrhosis. (trichrome stain, 40X).

PMID: 16003133, 15354062, 2499459, 6539291, 19902480, 31325714

Circulating biomarkers of hepatocyte death

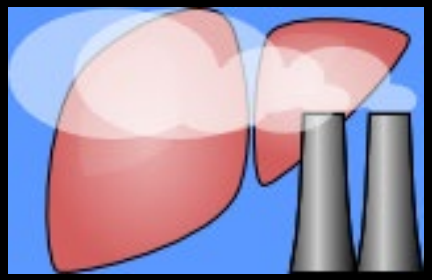


Lab variable (SD)	Unexposed controls (n = 11)	Chemical worker controls (n = 26)	TASH (n= 21)
ALT (U/L)	14.0 ± 8.6	28.4 ± 11.3	29.0 ± 48.3
AST (U/L)	20.8 ± 3.3	21.5 ± 7.3	19.9 ± 8.0
CK-18 M30® (U/L)	164.1 ± 26.3	150.9 ± 74.6	183.7 ± 88.6
CK-18 M65® (U/L)	215.8 ± 98.6	272.7 ± 71.3	**583.4 ± 319.2[a,b]**

[a] p<0.001 *vs.* unexposed controls; [b] p<0.001 *vs.* chemical worker controls.

PVC workers with TASH had histologix biochemical evidence of hepatocellular necrosis despite normal ALT. PMID:19902480

Circulating biomarkers of inflammation, oxidative stress and metabolism



Lab variable	Unexposed controls (n = 11)	Chemical worker controls (n = 26)	TASH (n = 21)
TNFα (pg/ml)	4.1 ± 1.5	3.0 ± 1.2	**11.2 ± 18.0**[b]
IL-1β (pg/ml)	0.1 ± 0.1	0.4 ± 0.6	**9.1 ± 11.9**[a,b]
IL-6 (pg/ml)	1.4 ± 1.6	3.5 ± 3.0	**10.9 ± 10.6**[a,b]
IL-8 (pg/ml)	2.7 ± 1.9	3.7 ± 1.6	**12.0 ± 12.9**[a,b]
Antioxidants (mM)	4.1 ± 0.3	3.5 ± 0.8	**2.6 ± 0.3**[a,b]
Glucose (mg/dL)	89.3 ± 16.8	89.4 ± 11.9	**112.0 ± 26.3**[a,b]
Insulin (pg/ml)	517.8 ± 440.5	327.3 ± 372.6	**1155.8 ± 1500.4**[b]
Adiponectin (µg/ml)	54.9 ± 50.4	**29.5 ± 17.6)**[a]	**14.4 ± 8.3**[a]
Triglycerides (mg/dL)	123.0 ± 71.2	145.4 ± 101.6	128.8 ± 47.2

[a] $p<0.05$ *vs.* unexposed controls; [b] $p<0.05$ *vs.* chemical worker controls

Highly exposed PVC workers with TASH had ↑ hepatocyte necrosis, ↑ serum pro-inflammatory cytokines, ↑ insulin resistance with ↓ anti-oxidant defenses & ↓ adiponectin. These results were replicated in a cohort of Rubbertown elastomer workers with ABS exposures. PMID: 21915069, 19902480

Altered plasma metabolome in PVC workers suggests abnormal energy homeostasis

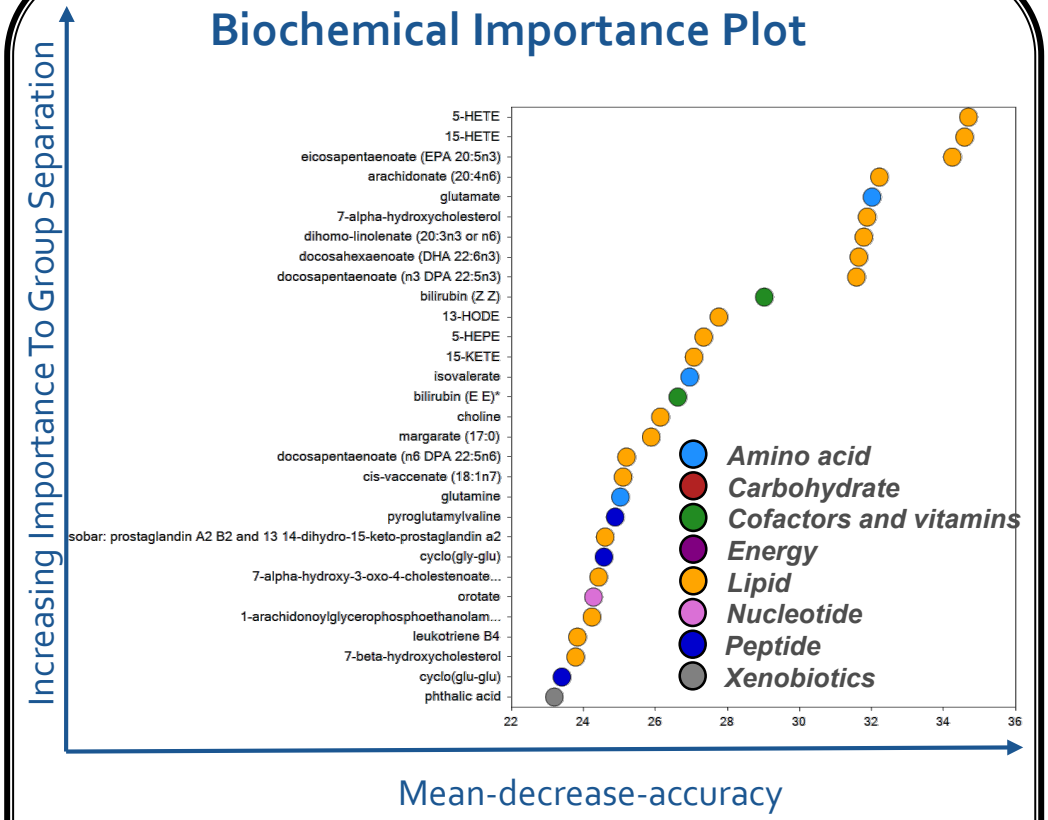
- 17 highly exposed workers
  27 unexposed controls
- Serum GC/MS &LC/MS/MS

The 30 top ranking biochemicals in the importance plot suggest key differences in:

- Lipid metabolism
- Cofactors & vitamins
- Amino acid metabolism

Metabolite profiling and IPA demonstrated mitochondrial dysfunction with altered AMPK and Akt signaling.

Biochemical Importance Plot



Random Forest classification had 98% predictive accuracy for predicting exposure group.

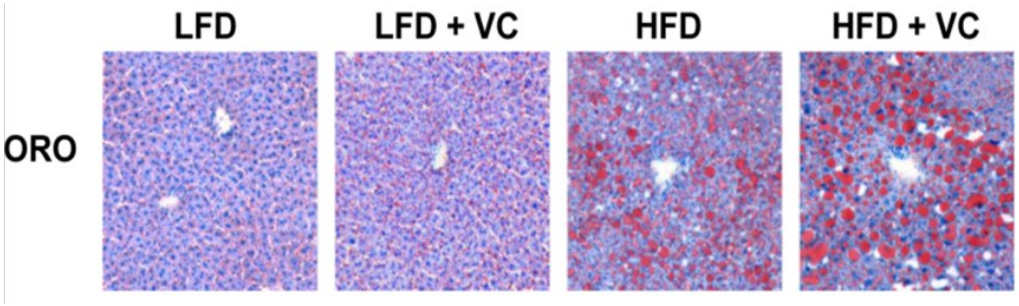
PMID: 27765658

Current key hypotheses in the field

- The metabolism disrupting chemical (MDC) hypothesis: "Environmental chemicals have the ability to promote metabolic changes that can result in obesity (obesogens), T2D or **fatty liver**." PMID: 27760374.
- These changes can be independent of effects on hormone action (the endocrine disrupting chemical (EDC) hypothesis). PMID: 27760374
- Two 'hit' hypothesis: The liver is generally tolerant to a single insult. First hit sensitizes to the second ('double whammy'). PMID: 9547102
- "Increased susceptibility to obesity/diabetes/metabolic syndrome may result directly from exposure to the **metabolic disruptor** or in other cases may require a second 'hit', for example, increased fat or sugar in the diet…." PMID: 26092037

COBRE-generated animal models of low-dose vinyl chloride-related steatohepatitis reproduce clinical observations and inform mechanism

Experimental design: C57Bl/6 mice fed control or HFD ±0.8 PPM VC (sub-OSHA) for 12 weeks.



Vinyl chloride worsened diet-induced NASH. The ALDH2 activator, ALDA-1, protected, suggesting the involvement of **reactive aldehyde metabolites** (e.g., chloroacetaldehyde) in VC toxicity.

In model systems, vinyl chloride exposures impactd NASH mechanisms:

- Insulin resistance / diabetes ☑
- Hepatocyte apoptosis (in contrast to necrosis in highly-exposed workers) ✗
- Inflammasome activation ☑
- Oxidative stress, carbonyl stress, ER stress ☑
- Δ energy homeostasis: Mitochondrial dysfunction, simultaneous AMPK & mTOR activation with hepatic glycogen depletion ☑
- Obesity (**second 'hit'**) and sex interactions ☑

PMID: 29507902, 26962056, 27693805, 31026768

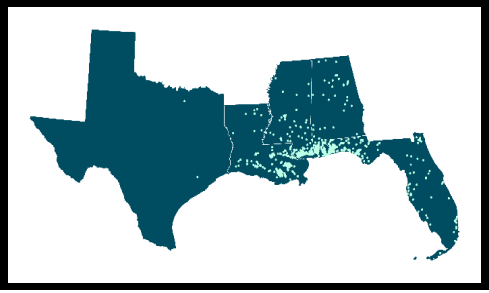


- Background: The GuLF Study enrolled 1,055 *Deepwater Horizon* disaster response workers in a chemical biomonitoring study (CBS). Blood *BTEXS* (benzene, toluene, ethylbenzene, xylene, styrene) were assessed 2-3 years after the spill, and do not reflect oil spill-related exposures.
- Purpose: This cross-sectional sub-study evaluates associations of blood BTEXS & metal exposure biomarkers with serum biomarkers of liver injury, systemic inflammation and endocrine function in 214 CBS participants.
- Inclusion criteria: male, nonsmokers, no previous liver disease/hepatitis, ≥ 3 alcoholic drinks per day, stratified across range of toluene levels.
- Methods: Confounder-adjusted beta coefficients were determined by linear models including ones with a multiplicative interaction term (exposure and BMI), allowing for estimation of stratum-specific exposure-outcome associations for obese (n=108) and non-obese (n=106) participants.

Werder EJ, Beier JI, Sandler DP, Falkner KC, Gripshover T, Wahlang B, Engel L, Cave MC. Blood BTEXS and Heavy Metal Levels Are Associated with Liver Injury and Systemic Inflammation in Gulf States Residents. *Food Chem Toxicol*. 2019. (under review).

Demographics	%
>45 years old	47
BMI ≥30	51
White	57
≤High school diploma/GED	54
Employed	66
Worked on *DWH* spill response ≥1 day	90
Diabetes	13

Results



GuLF STUDY

Benzene exposures interacted with obesity and were positively associated with hepatocyte apoptosis and systemic inflammation in obese participants. Similar associations occurred with heavy metal exposures.

Disease Biomarker	Exposure	Overall (N=214)[1] β (SE)	p-value	Obese (n=108)[2] β (SE)	p-value	Not obese (n=106)[2] β (SE)	p-value	Interaction p-value[3]
CK18 M30	Benzene	59.3 (63.7)	0.4	253.6 (112.8)	0.03	-19.5 (73.5)	0.8	0.04
	Cadmium	51.8 (28.8)	0.07	100.8 (41.8)	0.02	15.7 (36.1)	0.7	0.1
	Lead	21.7 (6.0)	0.0004	57.7 (8.4)	<.0001	-3.2 (7.3)	0.7	<.0001
IL-1β	Benzene	98.6 (58.2)	0.1	336.1 (100.5)	0.001	-4.9 (65.5)	0.9	0.004
	Cadmium	77.8 (26.3)	0.003	150.6 (37.2)	<.0001	21.4 (32.1)	0.5	0.01
	Lead	32.8 (5.2)	<.0001	76.3 (6.5)	<.0001	-0.6 (5.6)	0.9	<.0001
IL-6	Benzene	131.6 (194.9)	0.5	685.3 (338.7)	0.04	-123.9 (220.8)	0.6	0.04
	Cadmium	117.3 (88.9)	0.2	307.4 (126.3)	0.02	-36.4 (109.0)	0.7	0.03
	Lead	72.8 (18.3)	0.0001	169.6 (25.4)	<.0001	-2.6 (21.9)	0.9	<.0001
IL-8	Benzene	431.6 (443.9)	0.3	1713.0 (769.8)	0.03	-125.5 (501.9)	0.8	0.04
	Cadmium	419.5 (201.2)	0.04	822.8 (285.9)	0.004	140.3 (246.7)	0.6	0.1
	Lead	140.8 (42.2)	0.001	360.9 (58.5)	<.0001	-18.4 (50.5)	0.7	<.0001

[1] Adjusted for age (<30, 30-45, >45), race (white, nonwhite), typical alcohol consumption (0, 1, 2 drinks/day), serum cotinine (continuous), BMI (<25, 25-<30, ≥30 kg/m²), diabetes diagnosis, education (<high school diploma, high school diploma/equivalent, some college, college degree); sample size is 211 for all associations with cadmium due to missing exposure data for three participants

[2] Adjusted as above, except BMI is dichotomized at the threshold for obesity (<30 vs ≥30 kg/m²) and an interaction term is added between the exposure of interest and the dichotomous obesity term; sample size is 103 for all associations with cadmium among non-obese sample due to missing exposure data for three participants

[3] P-value associated with interaction term (exposure x obesity)

No associations between benzene, cadmium, lead and CK18 M65, TNF, MCP-1, adiponectin, or resistin. Lead inversely associated with leptin in obese only.

Toluene was positively associated with IL-1 in obese subjects, otherwise, no associations between toluene, ethylbenzene, xylenes, or styrene with any biomarker.

Werder EJ, Beier JI, Sandler DP, Falkner KC, Gripshover T, Wahlang B, Engel L, Cave MC. Blood BTEXS and Heavy Metal Levels Are Associated with Liver Injury and Systemic Inflammation in Gulf States Residents. *Food Chem Toxicol*. 2020. (in revision).

Clinical Practice Guidelines

JOURNAL OF HEPATOLOGY

EASL Clinical Practice Guideline: Occupational liver diseases☆

European Association for the Study of the Liver*

Table 1. Pathological patterns and morphological features of liver disease associated with workplace-related toxicants.

Pathological patterns	Morphological features	Toxicants
	Acute damage	
PCBs, chloronaphthalene,	Hepatocellular	Hepatocellular necrosis ± lobular inflammation	CCl_4, chloroform, toluene, TNT,
	DMF	Microvesicular steatosis
	Methylenedianiline	Cholestatic/mixed	Cholestasis, cholangitis
microvesicular)	Chloroalkenes (PCE, TCE), VCM, chloroform, CCl_4, volatile	TAFLD	Steatosis (macro
(steatosis + lobular	organic compounds (benzene, toluene, styrene, xylene),		Steato-hepatitis
	pesticides, and some nitro-organic compounds		
obstruction syndrome	VCM, dioxin, pyrrolizidine alkaloids, arsenic, copper sulfate	Vascular	Sinusoidal
	VCM		Peliosis
		Chronic damage	
fibrosis	VCM, PCBs, chloronaphthalene, Tetrachloroethane	Fibrosis	Periportal
Vascular	Porto-sinusoidal vascular disease (previously hepatoportal sclerosis)	VCM, sprays containing copper sulfate and lime	
	Tumors		
	Epithelial		
	Hepatocellular carcinoma	Arsenic, dimethylnitrosamine	
	Cholangiocarcinoma	1,2-Dichloropropane, dichloromethane	
	Vascular		
	VCM, Arsenic	Angiosarcoma	
	VCM	Epithelioid hemangioendothelioma	

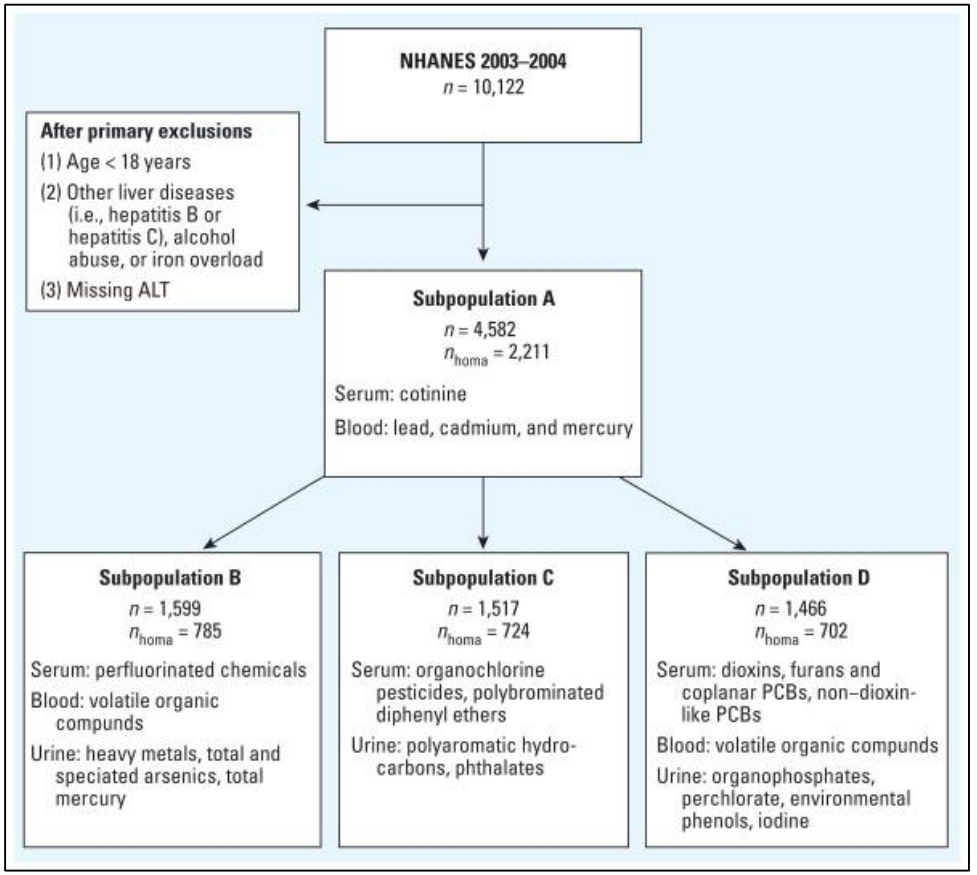
ts; TCE, trichloroethylene; VCM, vinyl chloride monomer.

DMF, dimethylformamide; PCBs, polychlorinated biphenyls; POPs, persistent organic pollutan

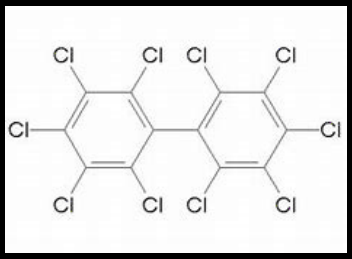
Environmental liver disease: TASH in the National Health and Nutrition Examination Survey 2003-2004

- **Analyzed Pollutants:** 196 pollutants from17 subclasses.
- **Primary Outcome Variable:** Dose-dependent, multivariate-adjusted odds ratios for 'unexplained ALT elevation' (NASH biomarker) across exposure quartiles.
- **Adjustments:** Age, sex, race, poverty income ratio, HOMA-IR, BMI, multiple comparisons.
- 2 ALT cutoffs used: >48 or 30 U/L men, >31 or 19 U/L women.

PMID: 21126940



Environmental polychlorinated biphenyls (PCBs), pesticides and metals were associated with suspected fatty liver disease in NHANES 2003-2004



Pollutant	Adjusted* Odds Ratio for 'Unexplained ALT Elevation' by Exposure Quartile				$p_{trend-adj}$
	1st	2nd	3rd	4th	
PCBs (non-dioxin-like)[1]	Ref	0.8	2.4	4.5	**0.001**
PCBs (dioxin-like)[1]	Ref	2.2	4.4	7.6	**<0.001**
Heptachlor epoxide[2]	1.4	1.3	1.9	2.6	**0.001**
Dieldrin[2]	1.6	1.8	2.2	3.1	**0.007**
Trans-nonachlor[2]	0.7	1.6	1.7	1.6	**0.050**
Mercury[1,2]	1.1	2.0	2.2	1.6	**0.014**
Lead[1,2]	Ref	1.2	1.5	1.6	**0.014**

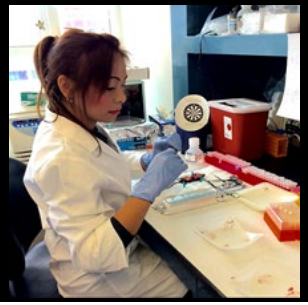
- 1 = ALT cutoff 1 (>48 M 31F), 2 = ALT cutoff 2 (>30 M, 19 F).
- Results were confirmed by 3 other groups. PMC4290093, PMID25173059, PMC3713174.

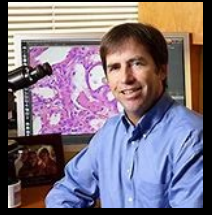
Excerpt from the Monsanto PCB MSDS (1988).

The consistent finding in animal studies is that PCBs produce liver injury following prolonged and repeated exposure by any route, if the exposure is of sufficient degree and duration. Liver injury is produced first, and by exposures that are less than those reported to cause cancer in rodents. Therefore, exposure by all routes should be kept sufficiently low to prevent liver injury.

PMID:31873887, 21126940

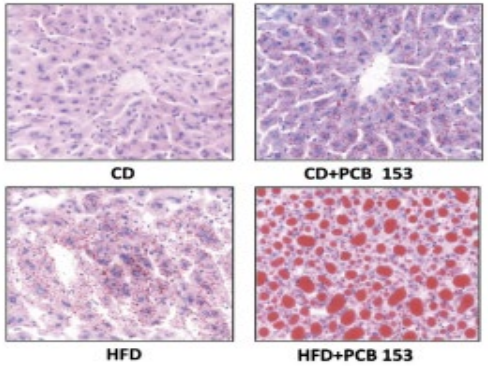
Animal models of non-dioxin-like PCB exposures develop NAFLD

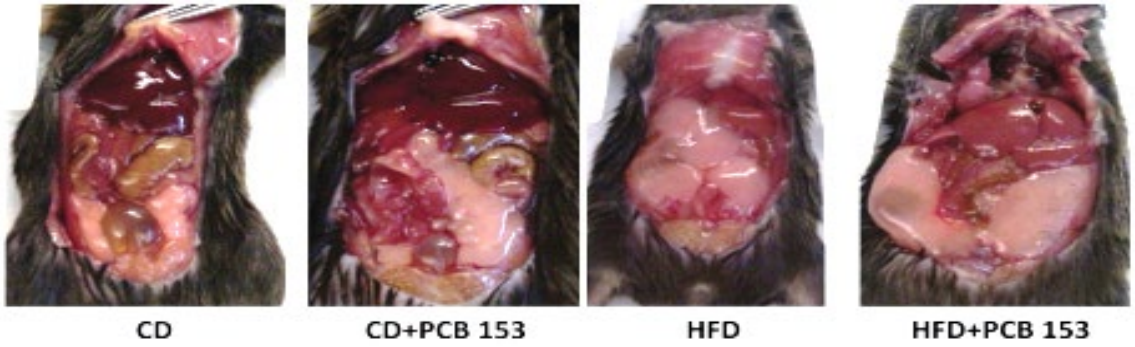




National Toxicology Program
U.S. Department of Health and Human Services

- **Single congener – PCB 153 & diet interaction** (C57Bl/6 mice 12weeks)





- **<u>PCB mixture – Aroclor 1260 & diet interactions</u>** (C57Bl/6 mice 12 weeks)
  Promotes transition of diet-induced steatosis to steatohepatitis
- **<u>Fundamental observations</u>**
  Diet interaction
  Necrotic hepatocyte death
  Altered xenobiotic (P450) & intermediary metabolism (↓ insulin & leptin)
  Increased pro-inflammatory cytokines (IL-6 and PAI-1)
  Worse in females.

Wahlang B, Hardesty JE, Jin J, Falkner KC, Cave MC.. Polychlorinated Biphenyls and Nonalcoholic Fatty Liver Disease. *Curr Opin Toxicol*. 2019; 14:21-28. https://doi.org/10.1016/j.cotox.2019.06.001

Validation of PCB (non-dioxin-like) mechanisms in the Anniston Community Health Survey-I

ATSDR Agency for Toxic Substances and Disease Registry

Background: PCBs were previously produced in Anniston, AL. ACHS-I participants have 2-3 fold increased mean ortho-PCB levels *vs.* NHANES, a high prevalence of overweight / obesity (80.3%) and diabetes (27%).

Analytes measured in archived serum samples (n=738):

- Disease biomarkers: CK18, adipocytokines, glucose, lipids
- Exposure biomarkers: 35 ortho-substituted PCB congeners (mostly non-dioxin-like)

Liver disease categorization procedures:

- No liver disease: CK18 M65<300; M30<200 U/L
- Necrotic liver disease: CK18 M65>300; M30<200 U/L
- Apoptotic liver disease: CK18 M30>200 U/L

Statistical methods: Cross-sectional. Log-transformed analytes/PCBs, multivariable linear regression models adjusted for lipid levels, age, BMI, gender, race/ethnicity, diabetes, and alcohol use.

PMID:29684222, 21915069 24374590, 25982988, 30300668

Validation of PCB (non-dioxin-like) mechanisms in the Anniston Community Health Survey-I

ATSDR Agency for Toxic Substances and Disease Registry

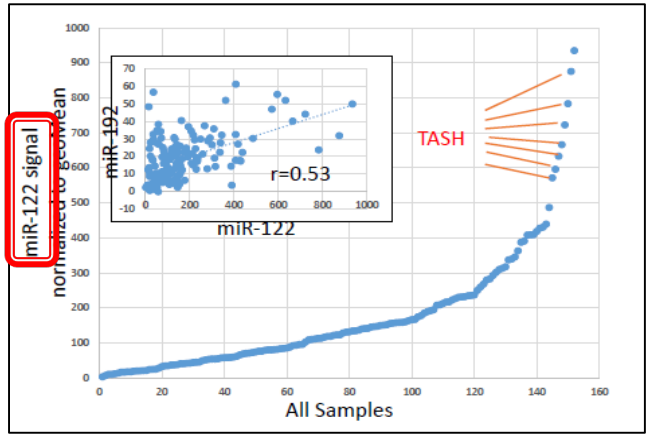
- Liver necrosis was positively associated with 15 of 35 ortho-PCBs tested.
- Necrotic liver disease was positively associated with HOMA-IR and pro-inflammatory cytokines (IL-1β, IL-6, PAI-1).
- ΣPCBs was inversely associated with insulin and leptin. PMID:29684222

PCB congener	Necrosis *vs.* No Liver Disease β	S.E.	P-Value	CK18 M65 β	S.E.	P-Value
28	0.24	0.11	0.03	0.07	0.02	<0.001
44	0.45	0.23	0.04	0.11	0.04	0.01
49	0.66	0.23	0. 004	0.10	0.04	0.01
52	0.37	0.14	0.01	0.11	0.03	<0.001
66	0.29	0.09	0.002	0.07	0.02	<0.001
101	0.20	0.10	0.05	0.05	0.02	0.02
105				0.04	0.02	0.03
110	0.36	0.13	0.004	0.05	0.03	0.04
128	0.22	0.10	0.02			
149	0.24	0.10	0.02			
151	0.25	0.09	0.01	0.05	0.02	0.01
172				0.04	0.02	0.02
178				0.04	0.02	0.03
187				0.04	0.02	0.04
195				0.04	0.02	0.04

- The necrotic liver disease positively associated with ortho-substituted PCB congeners, insulin resistance and pro-inflammatory cytokines is consistent with PCB-induced TASH. These data are concordant with the animal models.
- The inverse associations between PCBs and insulin/leptin are consistent with PCB-induced endocrine disruption.

'Liquid liver biopsy' (serum miRNA panel) demonstrated steatohepatitis in ACHS-I subjects categorized as having TASH by the CK18 serum biomarker

Methods: ACHS-I participants (76 with TASH and 76 without liver disease, stratified by sex) were evaluated. 68 targeted hepatotoxicity miRNAs (Fireplex assay, Abcam) were measured in serum. Adjusted β coefficients examined relationships between miRNAs and liver disease category. Ingenuity pathway analysis was performed (liquid liver biopsy).



TASH & MiRNA Associations

Table 2. Differentially regulated miRNAs in TASH.

Probe	Quantile-Normalized Data Fold Change[a]	FDR[b]	Raw-P
Up-regulated miRNA			
hsa-miR-122-5p	4.88	**0.01**	**0.003**
hsa-miR-22-3p	2.97	**0.06**	**0.04**
hsa-miR-320a	2.98	**0.02**	**0.01**
hsa-miR-375	3.25	**0.03**	**0.02**
Down-regulated miRNA			
hsa-miR-21-5p	0.33	**0.08**	0.07
hsa-miR-223-3p	0.33	**0.06**	**0.048**
hsa-miR-410-3p	0.33	**0.11**	0.11
hsa-miR-92a-3p	0.34	**0.01**	**0.003**

Abbreviations: FDR, false discovery rate.
[a] Fold change is based on quantile normalized MFI values adjusted for plate effect, age, race, BMI, log(10)-sum of 35 ortho-substituted PCBs, and log(10)-lipids.
[b] FDR<0.15 is considered significant. Significant FDR and p-values are bolded.

Liquid Liver Biopsy

Table 6. Enriched Pathways Associated with Altered Serum MiRNAs

Enriched Diseases/Functions	P-Value	miRNAs (#)
Hepatotoxicity/Top Tox Functions		
Steatosis	**0.00030**	2
Liver hyperplasia/hyperproliferation	**0.00035**	4
Hepatocellular carcinoma (HCC)	**0.00037**	4
Decreased albumin	**0.0049**	1
Diseases and Disorders/Top Networks		
Cancer	**6.4E-06**	7
Organismal injury and abnormalities	**6.4E-06**	8
Inflammatory response	**9.0E-06**	5
Cell cycle, embryonic development, cell death/survival		27 (score)

Cave et al. In preparation.

Nuclear receptor dysregulation in the Aroclor 1260 TASH mouse model

A TASH model was developed: Aroclor 1260±HFD in C57Bl/6 male mice (12 week). PMID: 30807179

Proteomics with transcription factor analysis was performed. PMID: 30807179

Aroclor 1260 (154↑, 93↓), HFD (239↑, 137↓), and their interaction (60↑, 179↓) altered protein expression and transcription factor function.

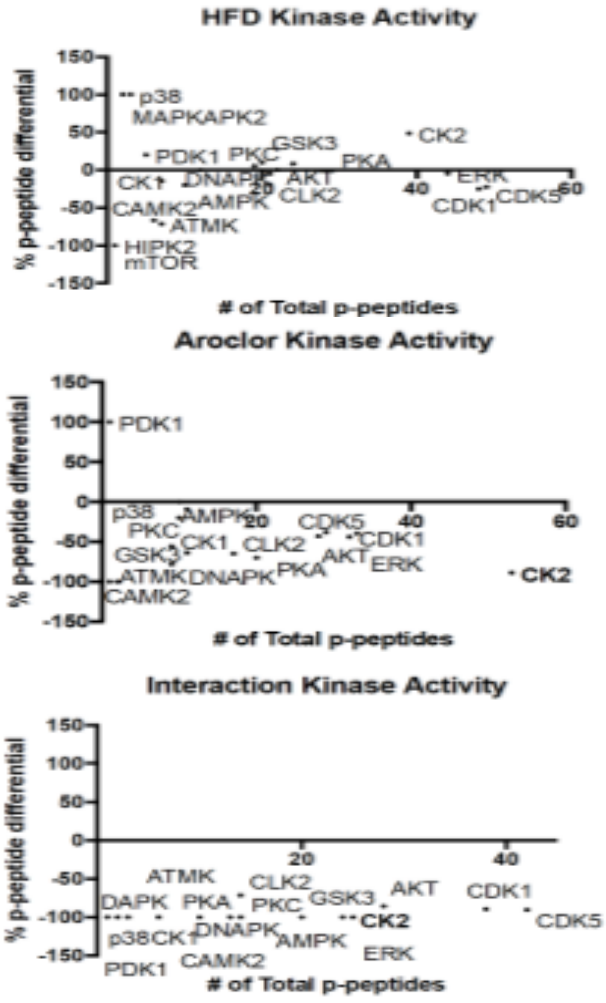
Target	PCB effect	NASH therapeutic landscape
NRF2	↓	vitamin E - available
PPARγ	↓	pioglitazone (agonist) – off label
PPARα/δ	↓	elafibranor (dual agonist) – Phase 3
FXR	↓	obeticholic acid (agonist) – Phase 3
TRα	↓	MGL-3196 (TRβ agonist) – Phase 2
HNF4α	↓	HTD1801 (agonist)? – Preclinical
CREB1	↓	Pentoxifylline – off label
ESR1	↓	Females protected against NAFLD
FGF-21*	↓	BMS-986036 (Peg-FGF21) – Phase 2

- Many PCB effects of transcription factors were likely indirect and due to decreased receptor expression or activity (e.g., post-translational modification).
- Many of the down-regulated transcription factors are currently being targeted with agonists in NASH clinical trials. PMID: 26612838, 24812009, *30312631

Hepatic signaling disruption by non-dioxin-like PCBs

- Hepatic **phosphoproteomics** with **kinase activity analysis** was performed using the Aroclor 1260 TASH mouse model.
- The abundance of detected phosphopeptides was altered (588/1760). Aroclor 1260 and its interaction with HFD reduced nearly **25%** of phosphopeptides.
- Aroclor 1260 significantly decreased kinase activity and interacted with HFD. Phosphatases unchanged.
- Pathway analysis demonstrated liver necrosis and altered endocrine signaling (leptin and insulin – consistent with ACHS-I).

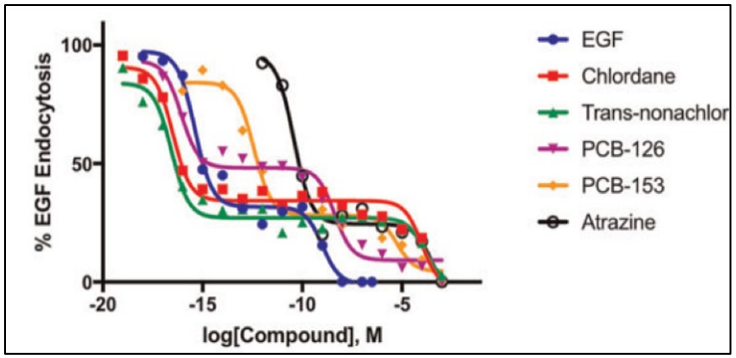
Aroclor 1260 is a signaling disrupting chemical. It reduces kinase activity to decrease the abundance of hepatic phosphoproteins impacting pathways involved in liver metabolism, cell survival, and fibrosis.



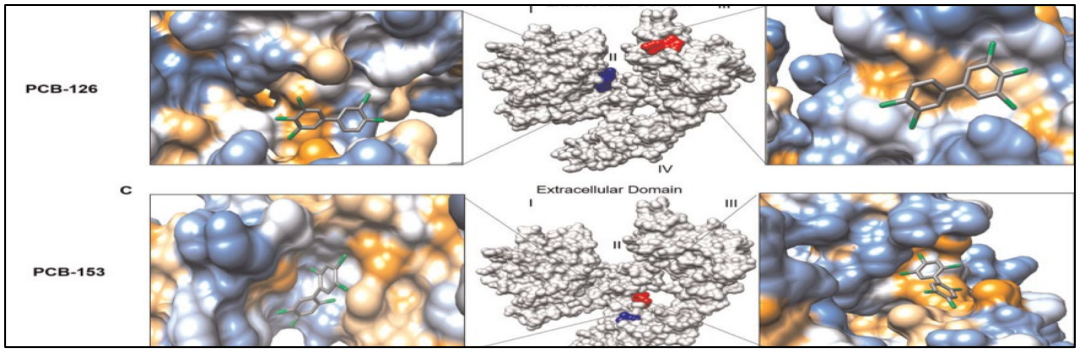
Hardesty et al. Cellular Signaling (in press).

PCB-induced phosphoprotein signaling disruption and TASH may be due, in part, to inhibition of epidermal growth factor receptor

Background: The EGFR is a receptor tyrosine kinase. Hepatocytes abundantly express EGFR, and most labeled EGF traffics to the liver. Placental EGFR phosphorylation was reduced following PCB poisoning (Yucheng). PMID: 3119985



PCBs and pesticides potently (fM) inhibited EGFR signaling *in vitro* (mouse and human) and *in vivo.*

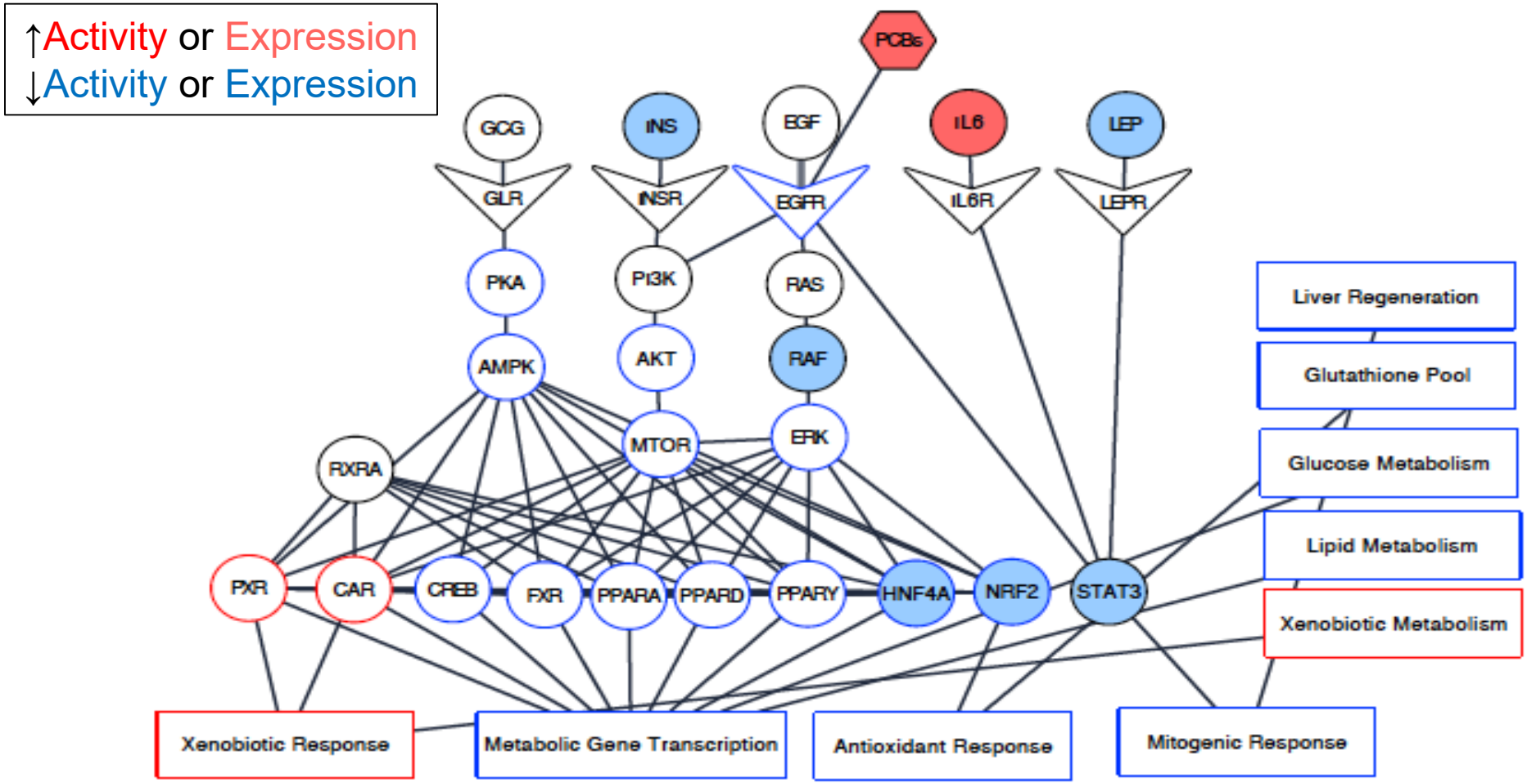


In silico models determined high affinity hydrostatic PCB binding sites on the EGFR.

EGF treatment (daily i.p. injection of 0.2 µg/g EGF during wks 10-12) attenuated hepatic inflammation and fibrosis while redistributing hepatic free fatty acids to the adipose tissue in the mouse Aroclor1260/HFD TASH model (Hardesty in preparation).

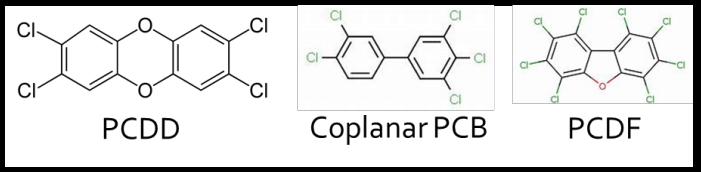
PMID: 29329451, 27458090

Hepatic signaling disruption by non-dioxin-like PCBs leads to transcriptional reprogramming to promote diet-induced steatohepatitis



Wahlang B, Hardesty JE, Jin J, Falkner KC, Cave MC.. Polychlorinated Biphenyls and Nonalcoholic Fatty Liver Disease. *Curr Opin Toxicol*. 2019; 14:21-28. https://doi.org/10.1016/j.cotox.2019.06.001

Dioxins as NAFLD modifiers

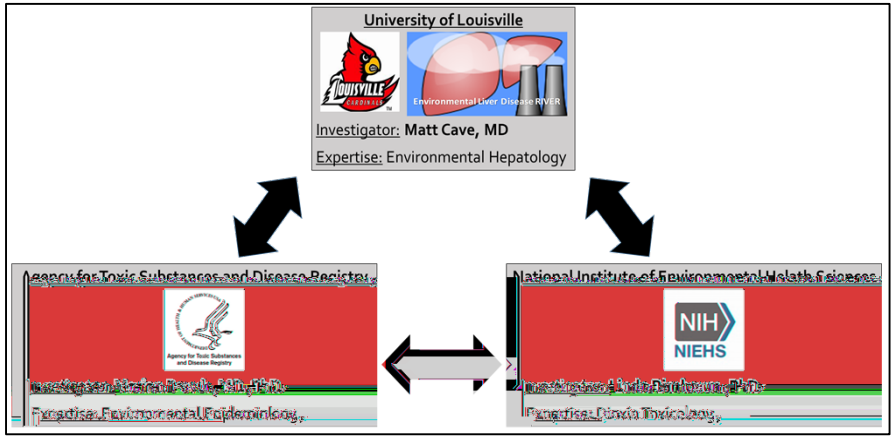


- Exposures to dioxins [polychlorinated dibenzo-p-dioxins (PCDDs), coplanar polychlorinated biphenyls (PCBs), and polychlorinated dibenzofurans (PCDFs)] have been associated with nonalcoholic fatty liver disease (NAFLD) and diabetes. Dioxins were the most potent pollutants associated with steatosis in rodents (PMID:25326588, 31134516, 27760374).
- Environmental epidemiologic cohort studies reported positive associations between PCB exposures and NAFLD (reviewed in *https://doi.org/10.1016/j.cotox.2019.06.001*). These cohort studies include the Anniston Community Health Survey-I (ACHS-I) (PMID:29684222).
- Dioxins activate xenobiotic receptors including the aryl hydrocarbon receptor (AhR). Xenobiotic-induced AhR activation may be modeled using the total dioxin toxic equivalency (TEQ) using World Health Organization toxic equivalency factors (PMC2290740).
- Hypothesis: Total dioxin TEQ is associated with liver metabolism, injury, inflammation and fibrosis in subjects suspected to have NAFLD.

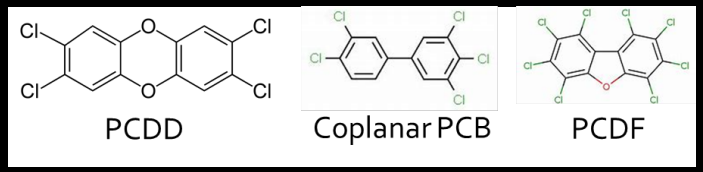
Dioxins as NAFLD modifiers (multi-pollutant model)

- We previously demonstrated in ACHS-I (n=776) that ortho-PCB exposures were associated with liver injury and endocrine disruption. Liver injury was categorized by the serum CK18 biomarker and was consistent with NAFLD by a 'liquid liver biopsy' consisting of a panel of serum microRNAs (PMID:29684222, *Gastroenterology* 2018(S1);7110A).
- The re-contact study, ACHS-II (n=359), was designed, in part, to include additional dioxin exposure biomarkers and liver disease biomarkers (PMC4648703).
- In ACHS-II, CDC previously performed serum exposure assessments. The total dioxin TEQ (ww) was determined by summing the individual TEQs for the non-ortho **PCBs (n=3)**, the **PCDDs (n=7)** & the **PCDFs (n=10)**. Dioxin exposures were estimated to be approximately 2-fold higher than the general US population.

Objective: The purpose of this cross-sectional analysis of the subgroup of ACHS-II participants with liver injury is to determine associations between total dioxin TEQ and serological biomarkers of liver metabolism, injury, inflammation and fibrosis.



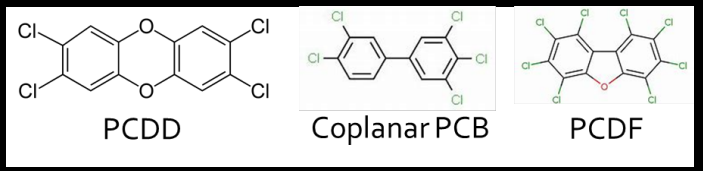
Dioxins as NAFLD modifiers



Materials and Methods

- The study design of ACHS-II was previously reported (PMID:25982988). IRB-approval and informed consent were obtained.
- Disease biomarkers: Serologic disease biomarkers were previously measured by (multiplexed)-ELISA or clinical chemistry analyzer. Disease biomarkers included routine clinical chemistries and biomarkers of liver injury/cell death (CK18), inflammation (TNFα), fibrosis (hyaluronic acid), and liver metabolic function (VLDL, HOMA, and albumin).
- Participants were categorized by liver injury/disease (by CK18). The subgroup with liver injury/disease was subsequently analyzed.
- Linear regression models were used to determine adjusted (confounders ± lipids) beta coefficients for associations between total dioxin TEQ (ww) and the disease biomarkers. The confounder-adjusted models were the primary outcomes. Statistical significance was set at $P<0.05$ and $P\text{-adj}<0.1$ (Holm-Bonferroni multiple comparison test).

Liver disease categorization procedures



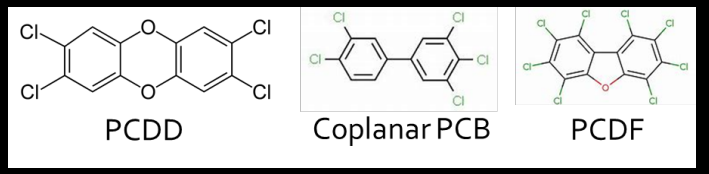
- ACHS-II participants (**n=338**) were first categorized according to liver injury (no liver disease/liver disease) using the serum CK18 hepatocyte cell death biomarker (cutoffs M65>300 or M30>200 U/L) as previously published (PMID:29684222).
- No liver disease subgroup (n=129), liver disease subgroup (n=209).
- Serum liver enzymes were then compared across categories.

	No Liver Disease (n=129)				Liver Disease (n=209)				
	Mean	SD	Median	Range	Mean	SD	Median	Range	P-Value
AST (U/L) [a] ↑	**25.9**	8.1	24	(11-52)	**32.5**	18.4	28	(11-158)	**<.0001**
ALT (U/L) [b] ↑	**22.8**	8.6	21	(7-86)	**29.7**	14	25	(13-81)	**<.0001**
Alkaline phosphatase (U/L) [c] ↑	**85.5**	22.7	83	(44-154)	**94.7**	45	86.5	(43-438)	**0.046**
Total bilirubin (mg/dL)	0.36	0.16	0.3	(0.1-1.1)	0.43	0.24	0.4	(0.0-1.7)	0.08
Albumin (g/dL)	4.2	0.4	4.2	(3.4-5.2)	4.2	0.4	4.2	(2.6-5.2)	0.93
Cytokeratin 18 M65 (U/L) [d]	231.8	46.4	241	(112-299)	486.1	240.5	412	(218-1879)	**<.0001**
Cytokeratin 18 M30 (U/L) [e]	83.6	26	78	(34-174)	182.4	185.2	125	(54-1872)	**<.0001**

[a] Normal range: ≤50 U/L. A single female patient with normal CK 18 levels has a slightly elevated AST value of 52 U/L. [b] Normal range: ≤70 U/L. A single male patient with normal CK 18 levels has an elevated ALT of 86 U/L. [c] Normal range: ≤126 U/L. Six female patients with normal CK 18 levels have elevated alkaline phosphate values (range: 127-154 U/L). [d] Normal range: ≤200 U/L. [e] Normal range: ≤300 U/L.

Mean AST, ALT, & ALP were higher in subjects with ↑ CK18, supporting the categorization procedures.

Dioxins as NAFLD modifiers



Demographics

Characteristic	Liver disease status: No Liver Disease (N=129) Mean	SD	Liver Disease[b] (N=209) Mean	SD	P-value	Total (N=338) Mean	SD
Age (years)	63.6	12.7	62.2	13.2	0.34	62.7	13.0
BMI (kg/m²)	32.1	9.5	31.4	7.2	0.44	31.7	8.2
	N	**%**	**N**	**%**		**N**	**%**
Gender					**0.02**		
Male	26	20.2	67	32.1		93	27.5
Female	103	79.8	142	67.9		245	72.5
Race/ethnicity					**0.003**		
Non-Hispanic White	50	38.8	116	55.5		166	49.1
African/American	79	61.2	93	44.5		172	50.9
Diabetes Status					0.09		
Ever Diabetic	44	34.1	91	45.5		135	39.9
Non-diabetic	85	65.9	118	56.5		203	60.1
Typical Number of Drinks Per Week					0.44		
No drinks	98	76.0	146	69.9		244	72.2
≤7 F / ≤14 M	22	17.1	42	20.1		64	18.9
>7 F / >14 M	9	7.0	21	10.0		30	8.9
Current Smoker					0.59		
No	103	80.5	163	78.0		266	78.9
Yes	25	19.5	46	22.0		71	21.1
Missing	1					1	

- ↑ **liver disease prevalence** consistent with ACHS-I (61.8% *vs.* 60.2%) (PMID:29684222).
- ↑ **liver disease prevalence** in males *vs.* females (72.0% *vs.* 60.0%, p=0.02) & in non-Hispanic whites *vs.* African Americans (69.9% *vs.* 54.0%, p=0.003) c/w ACHS-I (PMID:29684222).
- Trend towards ↑ **diabetes prevalence** in the liver disease subgroup (45.5% *vs.* 34.1%, p=0.09).
- The liver disease subgroup had NAFLD risk factors including obesity (mean BMI 31.4 kg/m²) and a 45.5% diabetes prevalence.

Dioxins as NAFLD modifiers

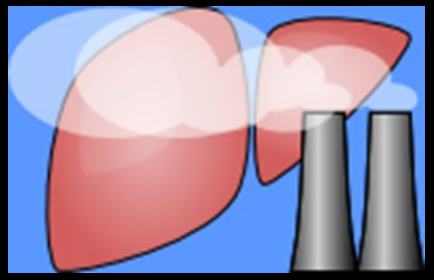
Adjusted beta coefficients were determined for associations between exposure and disease biomarkers for the liver disease subgroup. Adjustments included: age, race, sex, BMI, smoking, alcohol consumption, ± lipids.

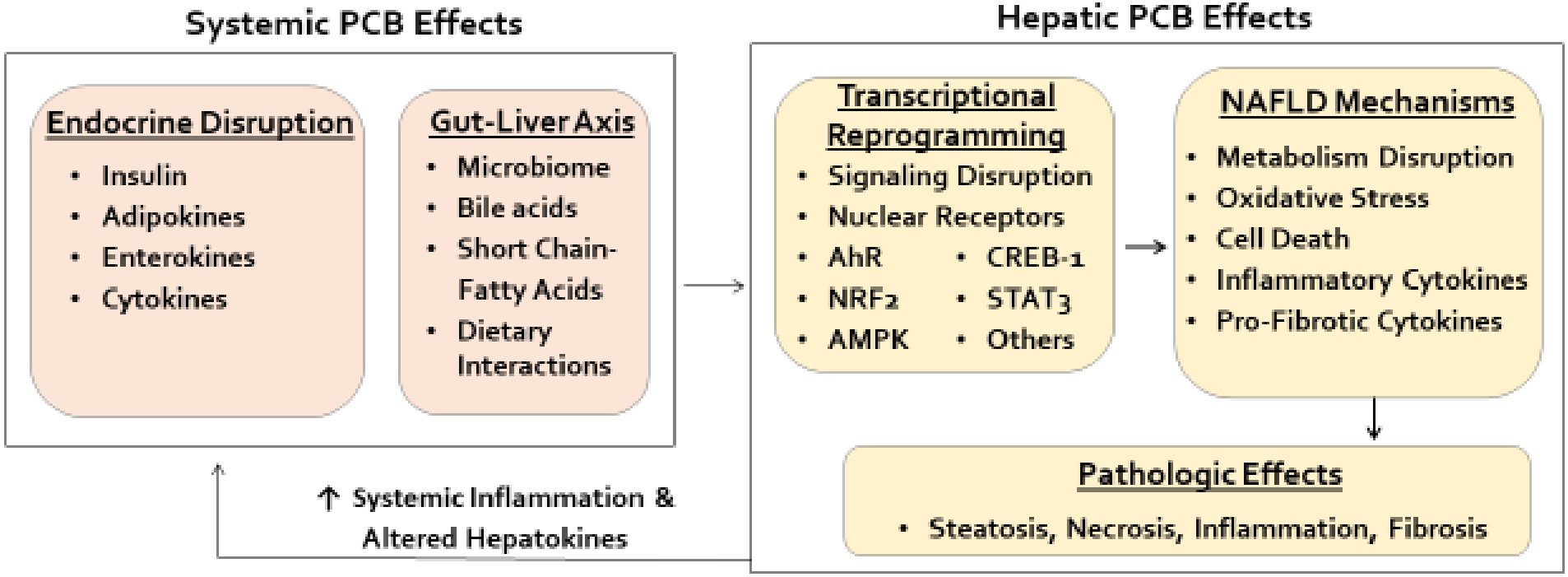
Associations of total dioxin TEQ[a] with disease biomarkers in ACHS-II participants (N=209) with liver disease[b]

	Unadjusted				Adjusted[c]			
Biomarker (Disease Outcome)	**Beta**	**SE**	**P**	**P-adj[d]**	**Beta**	**SE**	**P**	**P-adj[d]**
Lipid Metabolism: **VLDL** ↑	0.12	0.04	**0.004**	**0.02**	0.26	0.05	**<.0001**	**<.0001**
Glucose Metabolism: **HOMA-B** ↓	-0.25	0.09	**0.004**	**0.02**	-0.31	0.12	**0.01**	**0.04**
Protein Metabolism: **Albumin** ↓	-0.03	0.01	**0.001**	**0.01**	-0.03	0.01	**0.004**	**0.02**
Inflammation: **TNFα**	0.15	0.06	**0.02**	**0.03**	0.08	0.09	0.38	0.76
Fibrosis: **Hyaluronic Acid** ↑	0.43	0.07	**<.0001**	**<.0001**	0.23	0.09	**0.02**	**0.07**
Hepatocyte Death: **CK18 M65**	-0.05	0.03	0.08	**0.08**	-0.06	0.04	0.20	0.59

Total dioxin TEQ was associated with ↑ VLDL & hyaluronic acid; and ↓ albumin & HOMA-B. **Dioxins may be pro-fibrotic metabolism disrupting chemicals capable of exacerbating NAFLD.**

PCB modes of action in fatty liver disease

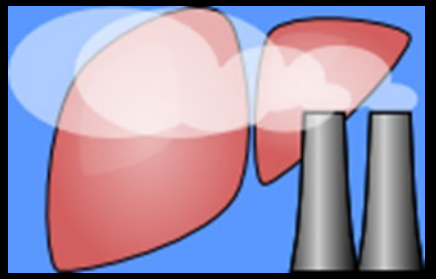




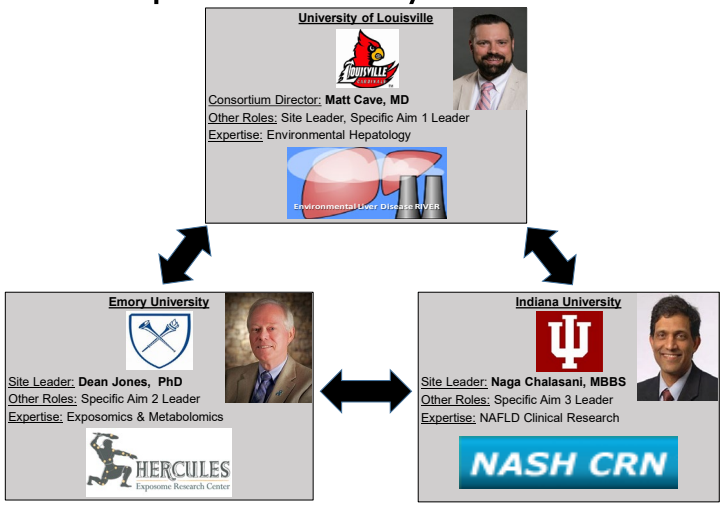
The UK-SRC has made significant contributions to the understanding of the mechanisms of dioxin-like PCBs

Wahlang B, Hardesty JE, Jin J, Falkner KC, Cave MC.. Polychlorinated Biphenyls and Nonalcoholic Fatty Liver Disease. *Curr Opin Toxicol*. 2019; 14:21-28. https://doi.org/10.1016/j.cotox.2019.06.001

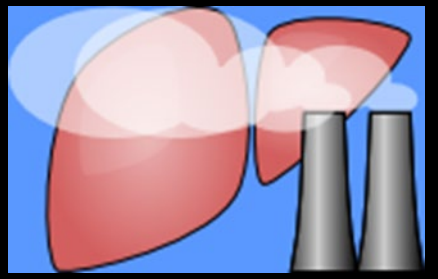
Precision medicine



- Few studies investigate exposures in unselected NAFLD patients. However, **PFHxS** was positively associated with metabolic disruption, lobular inflammation (NASH) and fibrosis on liver biopsy in 74 children with NAFLD. PMID:31744629
- Precision medicine refers to the tailoring of medical treatments to the individual characteristics of each patient including environmental chemical exposures.
- The exposome is the 'omics-scale characterization of the nongenetic drivers of health and disease including chemical pollutants. PMID:3009531
- Hypothesis: NAFLD is a multifactorial disease associated with abnormal hepatic metabolism. Environmental exposures may influence NAFLD disease severity.



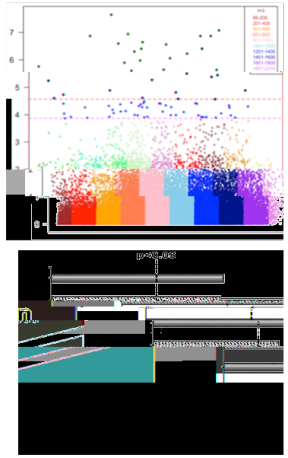
Precision medicine



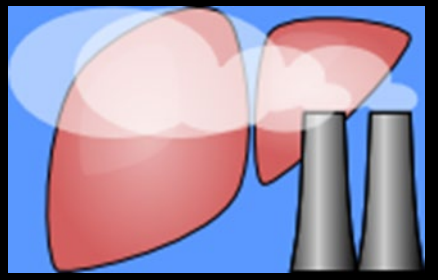
- Objective: To determine (*i*) if the exposome is associated with the severity of steatosis/fibrosis; and (*ii*) if steatosis & fibrosis share any metabolic pathways.
- Study Design: Cross-sectional EWAS/MWAS in 150 adult NAFLD patients.
- NAFLD Biomarkers: **Steatosis** (controlled attenuation parameter, CAP) and **fibrosis** (liver stiffness measurement, LSM) were determined by Fibroscan®.
- Exposure & Metabolite Biomarkers: Untargeted plasma HRE/HRM.

LC-MS/MS **Analysis** **Outputs**

Exposome- & metabolome-wide association studies



Subjects



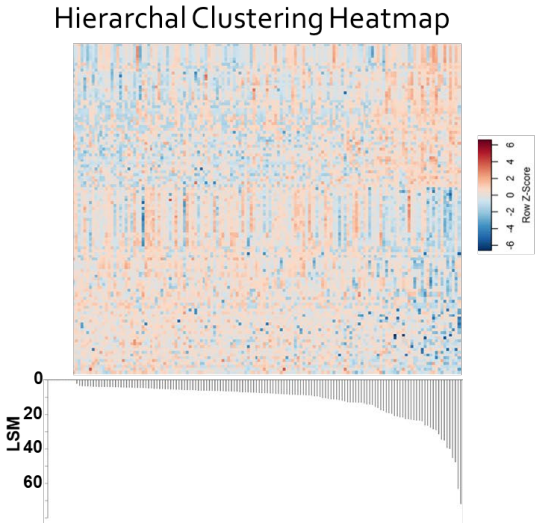
Research Subjects: A pre-existing cohort of 150 well-characterized, adult NAFLD patients with archived, de-identified data and plasma samples from IU was utilized. Informed consent and IRB approval were obtained.

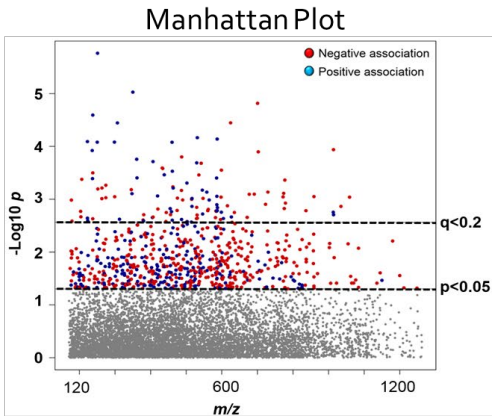
Fibroscan® VCTE

Variable	n=150
Age	51.4±12.4
BMI (kg/m^2)	36.0±7.4
LSM (kPa)	12.2±11.1
CAP (dB/m)	325.8±53.5
ALT (U/L)	60.6±47.4
Diabetes	51%
Female	65%

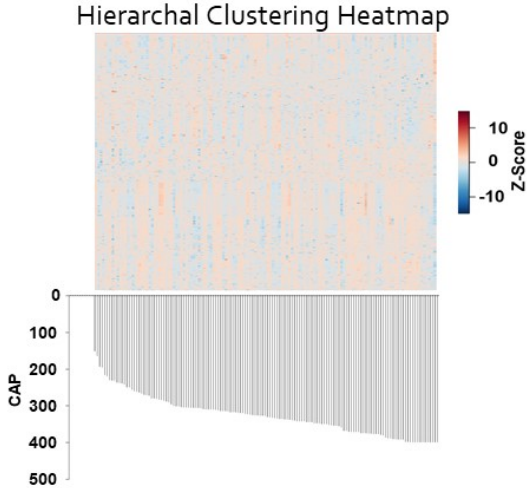
Cave MC, Smith MR, Go YM, Uppal K, Samala N, Falkner KC, Jones DP, Chalasani N. Identification of Environmental Exposures and Metabolic Pathways Associated with Fibrosis in Human Subjects with Nonalcoholic Fatty Liver Disease. AASLD 2019.

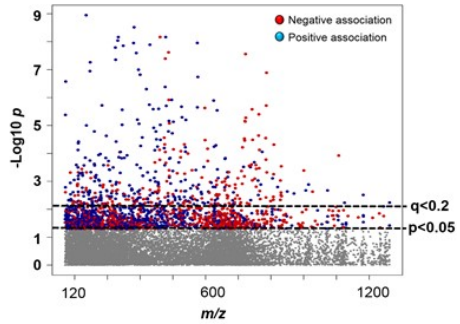
EWAS/MWAS *vs.* LSM. ≈31,000 features were detected. 130 features were significantly associated with LSM (FDR<0.2) with nearly equal numbers being up-regulated and down-regulated.



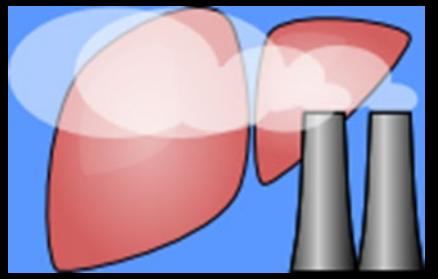


EWAS/MWAS *vs.* CAP. 508 features were significantly associated with LSM (FDR<0.2) with approximately 60% being up-regulated and 40% being down-regulated.





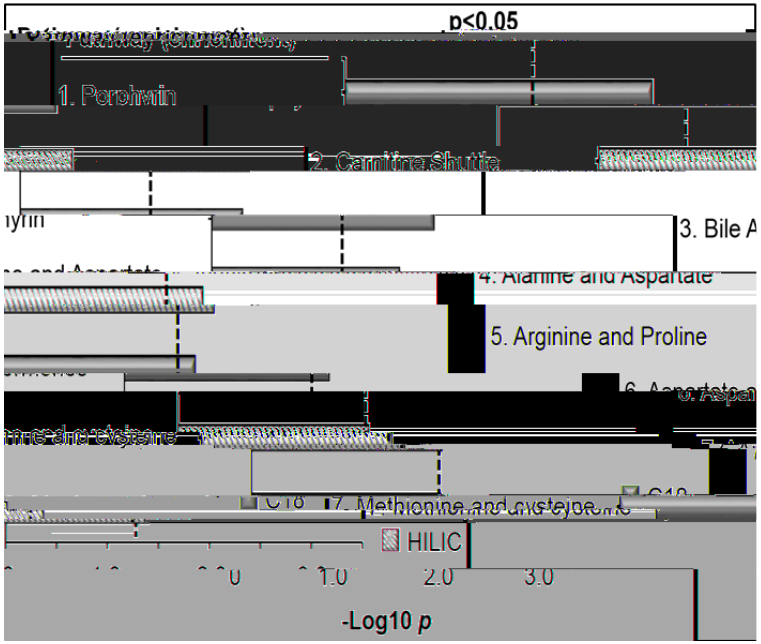
Precision medicine



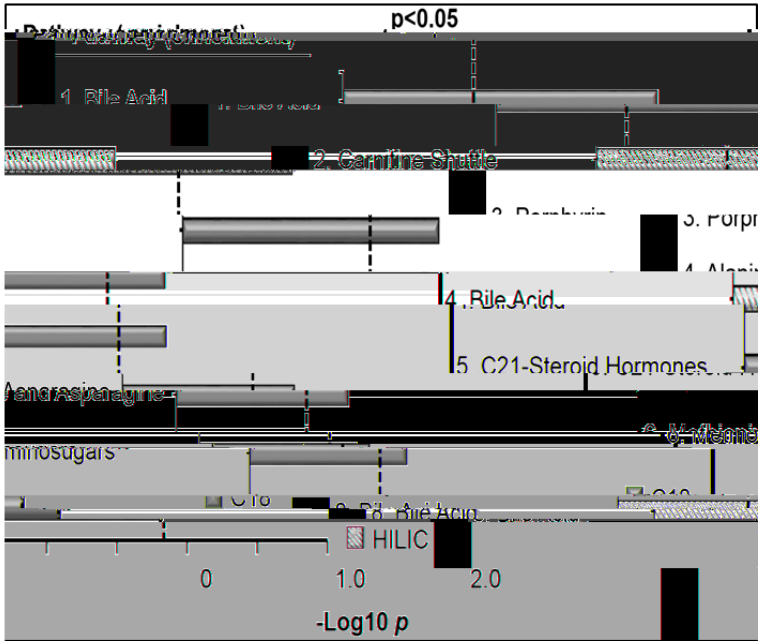
Pathways enriched with LSM & CAP

- Porphyrin, carnitine shuttle, and bile acid pathways were enriched with both LSM and CAP.

LSM (fibrosis)



CAP (steatosis)



Cave MC, Smith MR, Go YM, Uppal K, Samala N, Falkner KC, Jones DP, Chalasani N. Identification of Environmental Exposures and Metabolic Pathways Associated with Fibrosis in Human Subjects with Nonalcoholic Fatty Liver Disease. AASLD 2019.

Precision medicine: EWAS *vs.* LSM or CAP

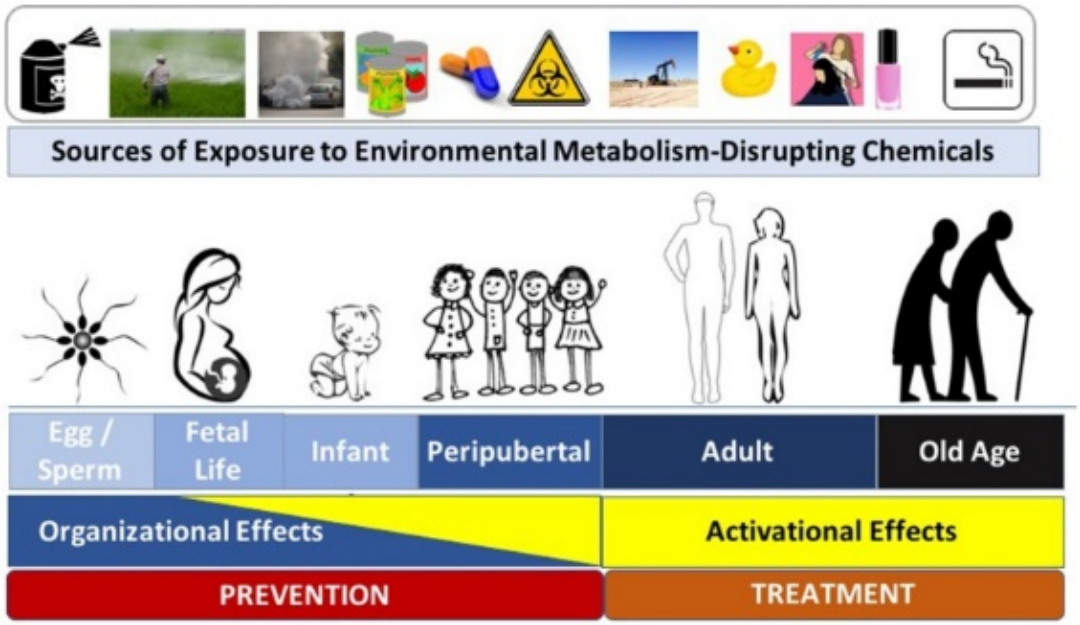
Chemicals Positively Associated with the Severity of Fibrosis (LSM) or Steatosis (CAP) in Adult NAFLD Patients (n=150)					
Chemical Class	**Subclass**	**Chemical**	**LSM**	**CAP**	**Literature**
Pesticide	Carbamate Insecticide	Methomyl*		+	(51, 52)
		Metolcarb	+		
	Azole Fungicide	Bromuconazole		+	(53, 54)
	Other	Nitromethylidene-hydrazinylbenzoic Acid	+		
Dioxin	Dibenzofuran	Hexabromodibenzofuran		+	(11)
Food Preservative	Antimicrobial	Copper Benzoate	+		
Medication	Antihistamine	Olopatadine	+		
Drug of Abuse		Nicotine*	+		(55, 56)
* = confirmed by authentic standard. Putatively annotated chemicals are being confirmed. The cited publications are for NAFLD animal model studies for the identified chemical or related chemicals from its class/subclass, supporting the potential causality of the identified associations.					

Future direction: new R01 under review to investigate NASH CRN (FLINT) and other samples.

Cave MC, Smith MR, Go YM, Uppal K, Samala N, Falkner KC, Jones DP, Chalasani N. Identification of Environmental Exposures and Metabolic Pathways Associated with Fibrosis in Human Subjects with Nonalcoholic Fatty Liver Disease. AASLD 2019.

Strategies to mitigate the impact of environmental MDCs

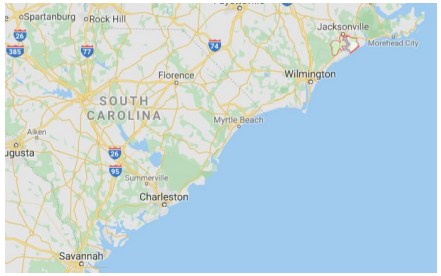
1) Empower individuals to reduce their exposures to MDCs.
2) Reduce the burden of persistent pollutants that bioaccumulate.
3) Therapeutic/dietary approaches to antagonize the deleterious effects of exposures.



Sargis et al. Interventions to Address Environmental Metabolism-Disrupting Chemicals: Changing the Narrative to Empower Action to Restore Metabolic Health. PMID: 30778334

Case Presentation: federal legislation on VOCs and fatty liver in Veterans

- "Honoring America's Veterans and Caring for Camp Lejeune Families" Public Law 112-154
- Industrial solvents (VC, benzene, etc.) contaminated drinking water at the barracks.
- Increased liver cancer mortality (Lejeune *vs.* Pendleton). PMC394337



VA | U.S. Department of Veterans Affairs
Veterans Health Administration

IB 10-449
July 2019

CAMP LEJEUNE: PAST WATER CONTAMINATION

From the 1950s through the 1980s, people serving or living at the U.S. Marine Corps Base Camp Lejeune, North Carolina, were potentially exposed to drinking water contaminated with industrial solvents, benzene, and other chemicals. This chemical exposure may have led to adverse health conditions.

YOU MAY BE ELIGIBLE FOR VA HEALTH BENEFITS IF YOU SERVED ON ACTIVE DUTY (VETERANS) OR RESIDED (FAMILY MEMBERS) AT CAMP LEJEUNE FOR 30 DAYS OR MORE BETWEEN AUGUST 1, 1953 AND DECEMBER 31, 1987:

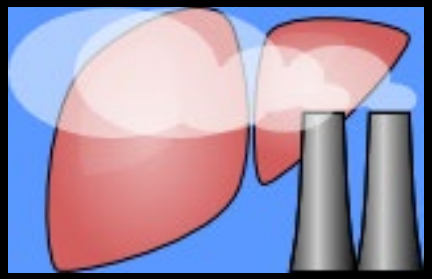
- Veterans who are determined to be eligible will be able to receive VA health care. In addition, care for qualifying health conditions is provided at no cost to the Veteran.
- Eligible family members will receive reimbursement for out-of-pocket medical expenses incurred from the treatment of qualifying health conditions, after all other health insurance plans are applied.

QUALIFYING HEALTH CONDITIONS INCLUDE:

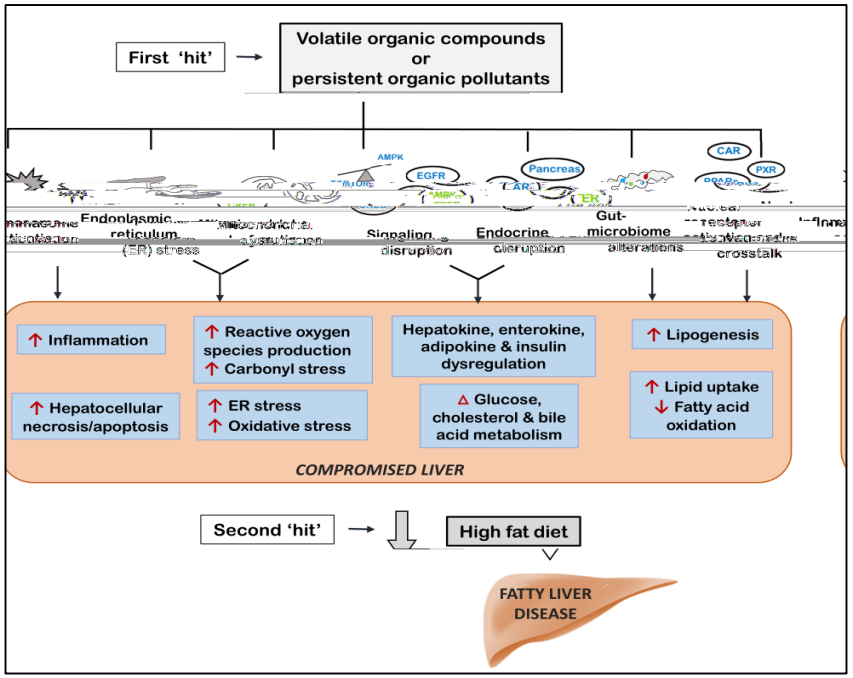
- Bladder cancer
- Breast cancer
- Esophageal cancer
- Kidney cancer
- Leukemia
- Lung cancer
- Myelodysplastic syndromes
- Neurobehavioral effects
- Non-Hodgkin's lymphoma

yeloma • Scleroderma • Hepatic steatosis • Multiple m

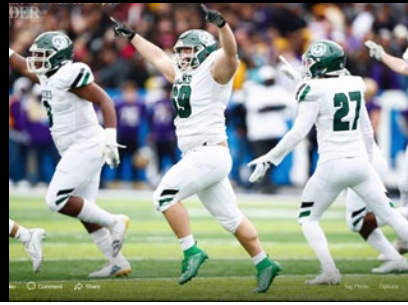
Conclusions



- Occupational and environmental chemical exposures contribute to liver diseases.
- Discussed the collaborative and translational approach to the identification of pollutants contributing to liver disease and the elucidation of mechanisms.
- VOCs and POPs are implicated in TASH, a new liver disease.
- Reviewed key concepts including EDCs, MDCs, SDCs, obesogens and two 'hits'.



Acknowledgments



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