KUMC Industrial Toxicology Lecture Series
February 22, 2023

Life as Researcher at the US EPA

A little about me, the Agency, and some data..

Brian N. Chorley, PhD

Office of Research and Development

Center for Computational Toxicology and Exposure

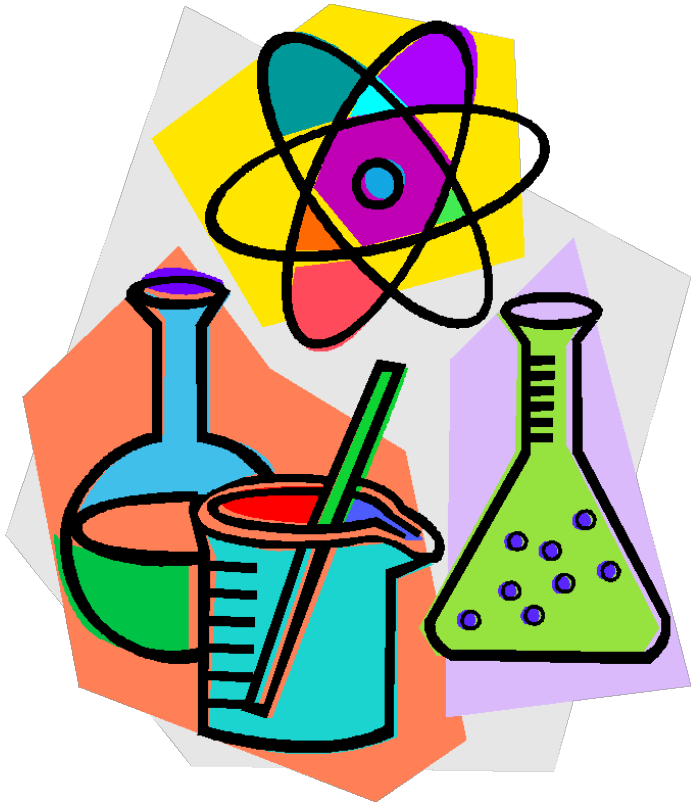
Research Triangle Park, NC, USA

Disclaimer

This presentation does not necessarily reflect EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

About me…

Where did you first learn to love science?



1976-1994

Jacksonville High School, NC

Camp Lejeune Marine Corps base

1994-1998

Polk Hall, Animal Science

1999-2005

College of Veterinary Medicine, Comparative Biomedical Sciences

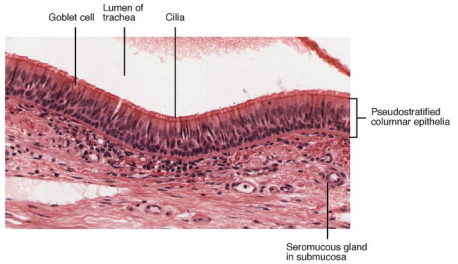
Pylon Park

Ken Adler

DIFFERENTIAL MUCIN SUBTYPE REGULATION AND ANTI-INFLAMMATORY EFFECTS OF INDUCIBLE NITRIC OXIDE SYNTHASE IN STIMULATED AIRWAY EPITHELIAL CELLS *IN VITRO*

by

BRIAN NORRIS CHORLEY

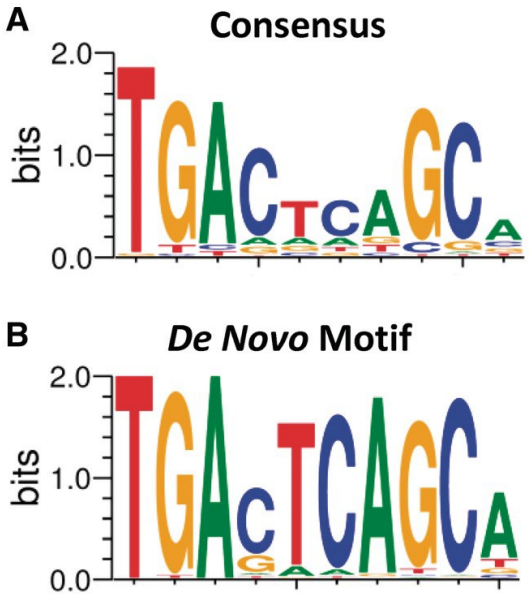


By OpenStax College - Anatomy & Physiology, Connexions

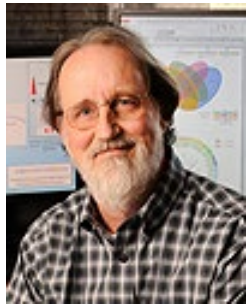
2005-2010

NIEHS
National Institute of Environmental Health Sciences

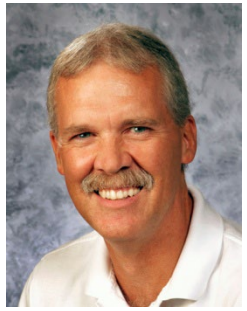
NIEHS, Postdoctoral IRTA fellow



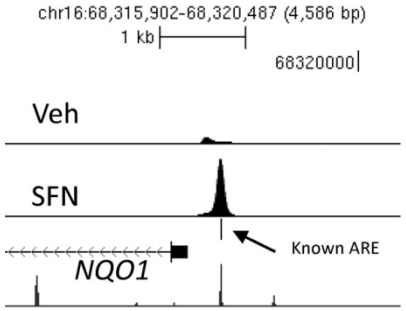
NFE2L2 binding motif, ChIP-seq



Doug Bell



Steve Kleeberger



Chorley et al. NAR 2012

Lessons Learned

A love for the molecular

Independence

A way of thinking

Importance of mentorship

Translation and application

2010-present

US EPA-RTP, Research Biologist

Gail Nelson, Biologist

Gleta Carswell, Biologist

Post-docs
David Gallegos
Alysa Suen
Jason Franklin
Michelle Angrish
Natalia Ryan (VanDuyn)
Jenna Guynn (Currier)
April Lake

Grad students
Bryanna Vacca
Maureen Malloy
John Chamberlin
Javaughn Baker
Patrice Cagle

Post-bacs
Ivy Guyotte
Nyssa Tucker
Emily Woolard

Undergrads/High School
Arjun Keshava
Ry Gibson
Malik Ko
David Bullock

About the Agency…

Our mission is to protect human health and the environment.

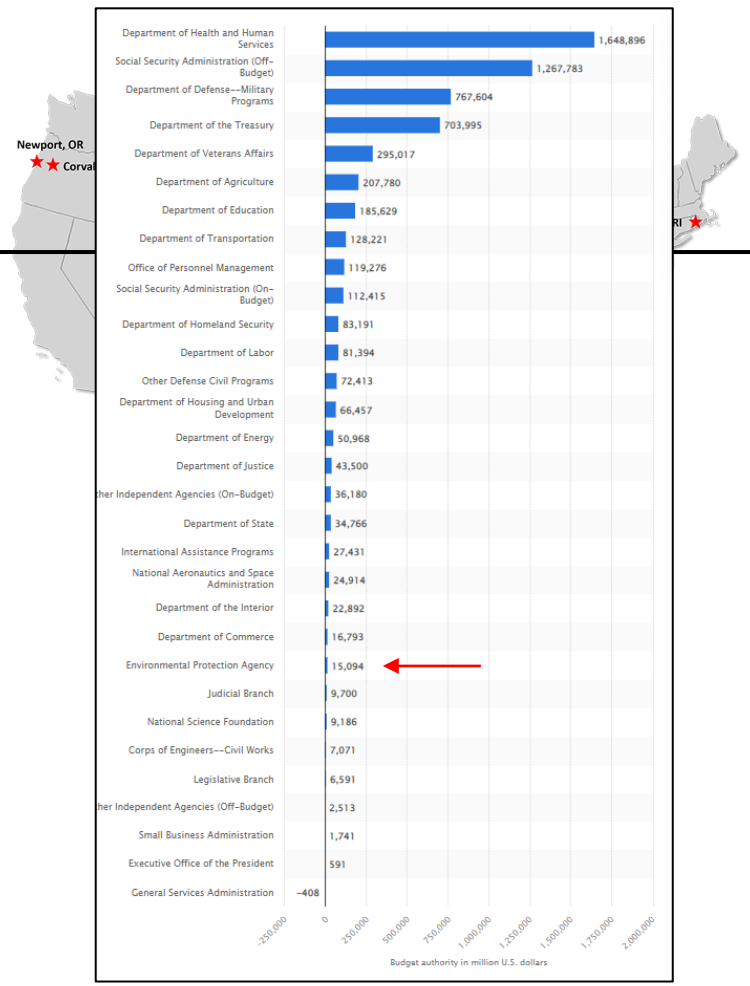
To accomplish this mission, we:

- Develop and enforce regulations
- Give grants
- Study environmental issues
- Sponsor partnerships
- Teach people about the environment
- Publish information

Michael S. Regan
EPA Administrator

- Office of the Administrator (AO)
  - Headquarters Offices
    - Office of Air and Radiation (OAR)
    - Office of Chemical Safety and Pollution Prevention (OCSPP)
    - Office of the Chief Financial Officer (OCFO)
    - Office of Environmental Justice and External Civil Rights (OEJECR)
    - Office of General Counsel (OGC)
    - Office of Inspector General (OIG)
    - Office of International and Tribal Affairs (OITA)
    - Office of Land and Emergency Management (OLEM)
    - Office of Mission Support (OMS)
    - Office of Research and Development (ORD)
    - Office of Water (OW)
  - Regional Offices
    - Region 1 / Boston
    - Region 2 / New York
    - Region 3 / Philadelphia
    - Region 4 / Atlanta
    - Region 5 / Chicago
    - Region 6 / Dallas
    - Region 7 / Kansas City
    - Region 8 / Denver
    - Region 9 / San Francisco
    - Region 10 / Seattle

~14,500 employees
$10.1 Billion Fiscal Year 2023 budget



- Office of Research and Development (ORD) Immediate Office of the Assistant Administrator
  - Office of Resource Management (ORM)
  - Office of Science and Information Management (OSIM)
  - Office of Science Advisor, Policy, and Engagement (OSAPE)
  - Center for Computational Toxicology and Exposure (CCTE)
  - Center for Environmental Measurement and Modeling (CEMM)
  - Center for Environmental Solutions and Emergency Response (CESER)
  - Center for Public Health and Environmental Assessment (CPHEA)

Strategic Research Action Plan (StRAP)

Air, Climate and Energy

Homeland Security

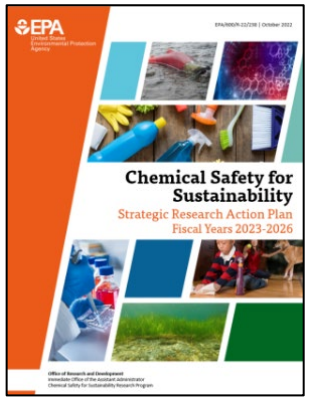
Chemical Safety & Sustainability

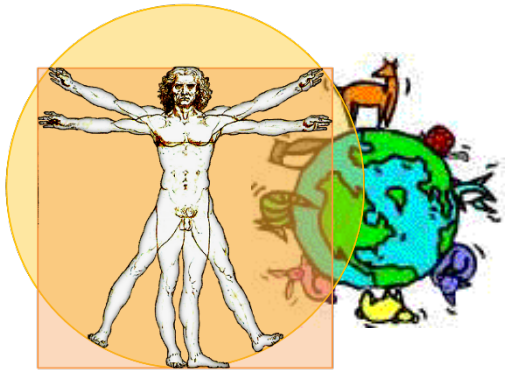
National Research Programs

Sustainable & Healthy Communities

Health and Environmental Risk Assessment

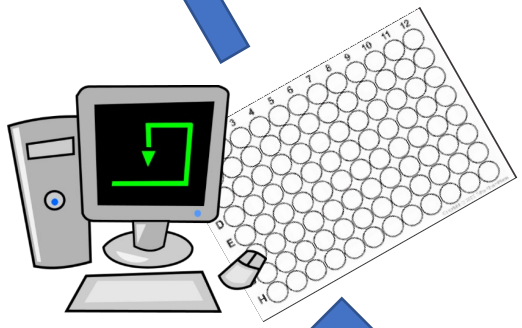
Safe and Sustainable Water Resources



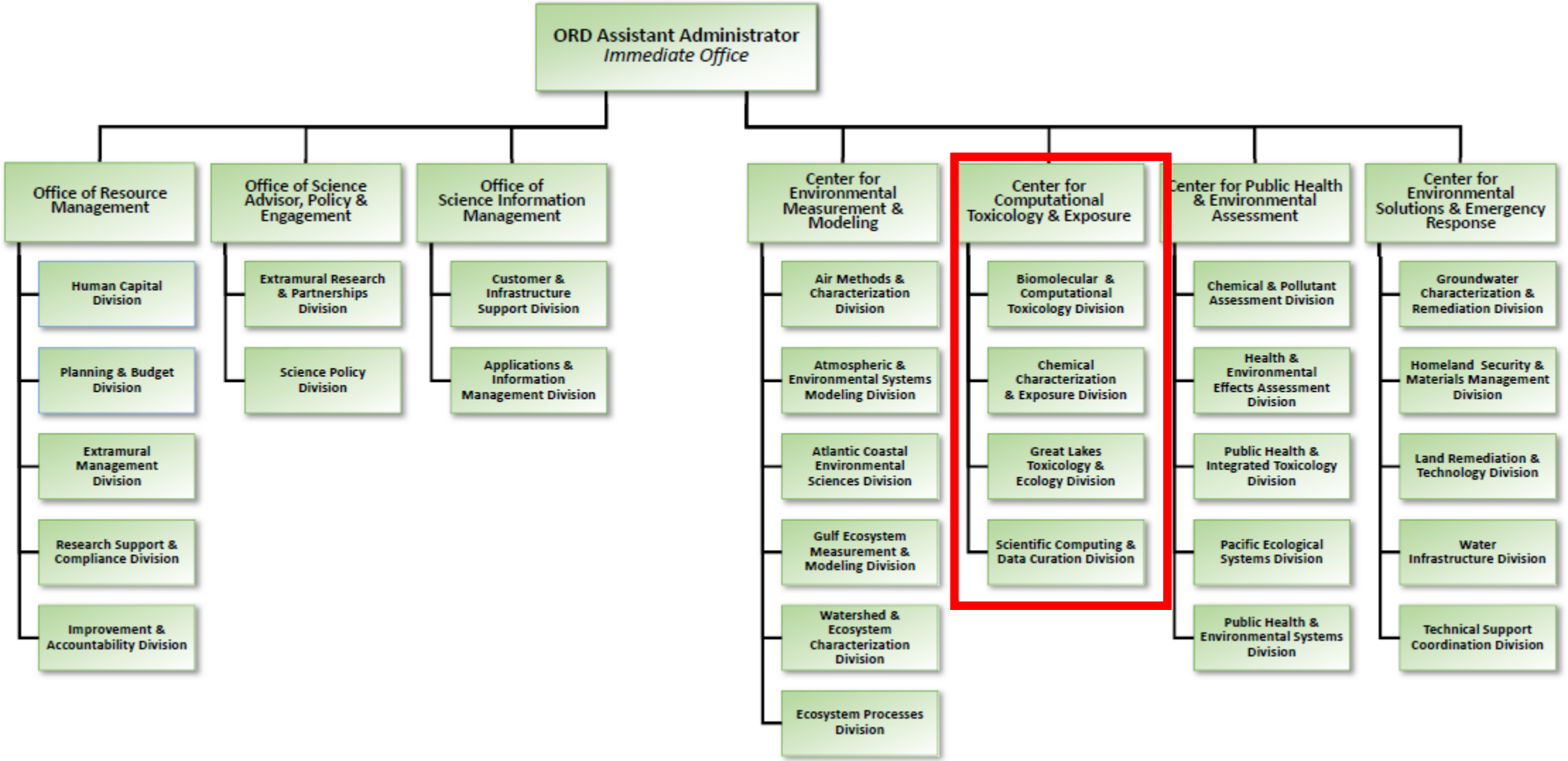


Human and ecological health

Pollution, toxins, chemicals



Office of Research and Development



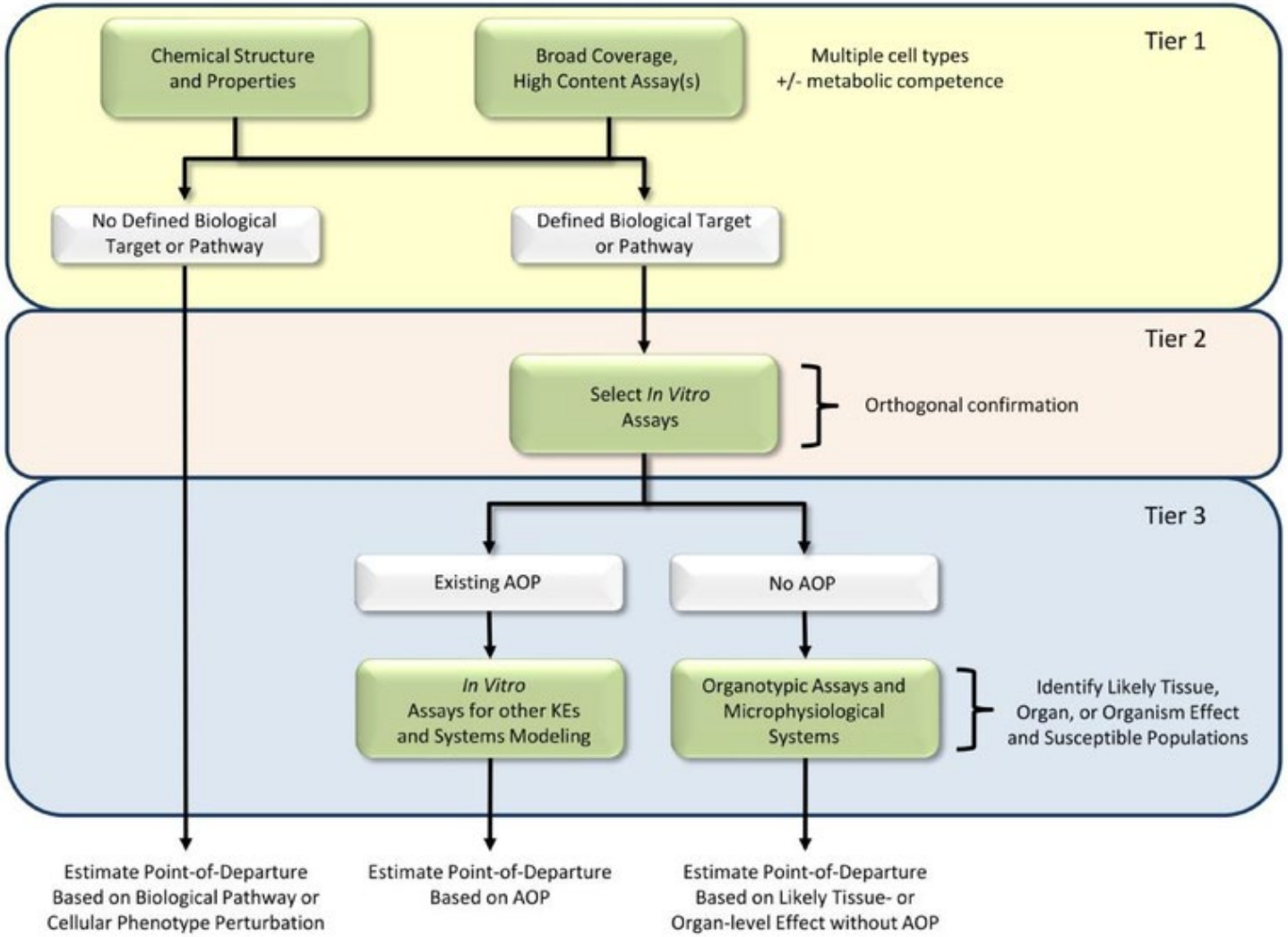
ORD-CCTE

Rusty Thomas
CCTE Center Director

The Center for Computational Toxicology & Exposure (CCTE) research strives to:

- Reduce the time required to test chemicals
- Expand our understanding exposures for thousands of chemical substances
- Develop a comprehensive information system
- Demonstrate translation of data into regulatory decisions

"The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency"



Advanced Experimental Toxicology Models Branch (AETMB)

Computational Toxicology and Bioinformatics Branch (CTBB)

Rapid Assay Development Branch (RADB)

Thomas et al. *Tox Sci* 2019

Brian Chorley, PhD
CCTE/BCTD
Laboratory studies

Chemical Safety & Sustainability

Safe and Healthy Communities

FY19-22

Adverse Outcome Pathways

- Noninvasive biomarker development
- Epigenetic biomarkers of liver disease
- Second generation sequencing tool development for archived samples

FY23-26

Adverse Outcome Pathways

- Refining Developmental Neurotoxicant-based AOPs on single cell transcriptomic characterization
- Assessing early epigenetic signatures of cancer susceptibility in archived tissues

High Throughput Testing

- Nondestructive epigenetic assay for chemical screening and prioritization
- Function genomic characterization of poorly defined mechanisms-of-action

Virtual Tissues Modeling

- System state trajectory and development modeling based on single cell data

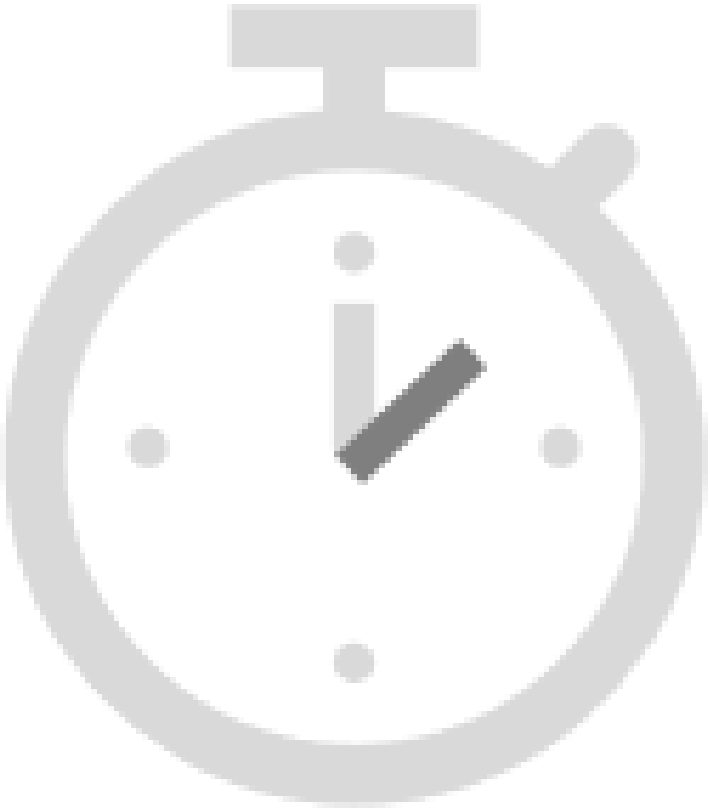
Environmental Justice and Children's Health

- Noninvasive biomarker development
- Pollutant effects on development

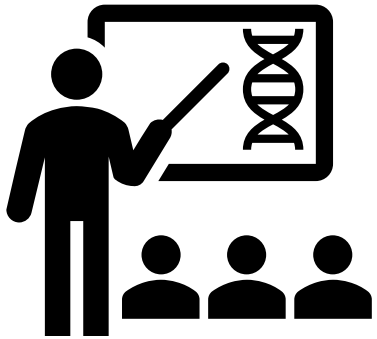
ORD/CCTE work in summary

- A lot of research that we do at the EPA for Office of Research and Development furthers “next-generation” toxicology
- This simply means we are taking a new approach to increase information, decrease cost, decrease time, and make better informed decisions
- This is influenced by:
  - Improving knowledge
  - New tools and technology (NAMs)
  - New and complex challenges for the Agency

A break for questions…

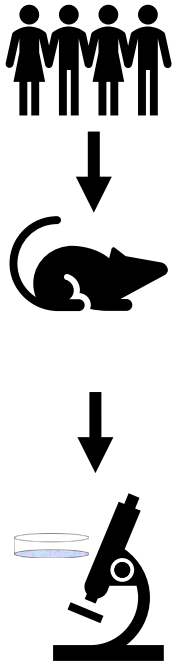


About my research…



1. Scientific drivers for transcriptomic biomarkers
   a) Why microRNA?

2. Background studies
   a) Biofluid-based indicators of liver disease in an PCB-exposed residential cohort
   b) Dose-responsive microRNA biomarkers of chemical mode-of-action



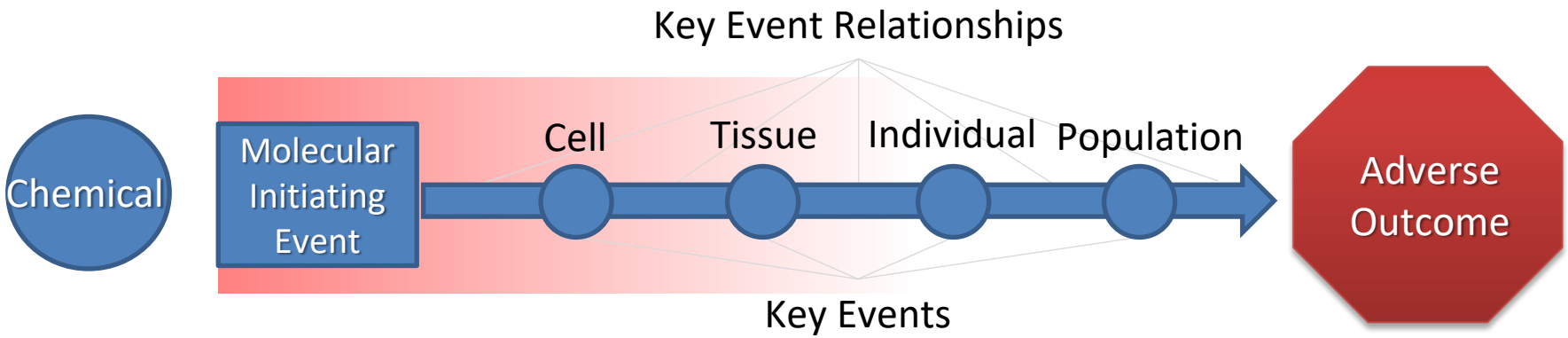
3. In vitro screening development using microRNA biomarkers
   a) Initial optimization
   b) Identification of microRNAs in media with sequencing
   c) Chemical exposure study design and preliminary results

4. Conclusions/Future directions

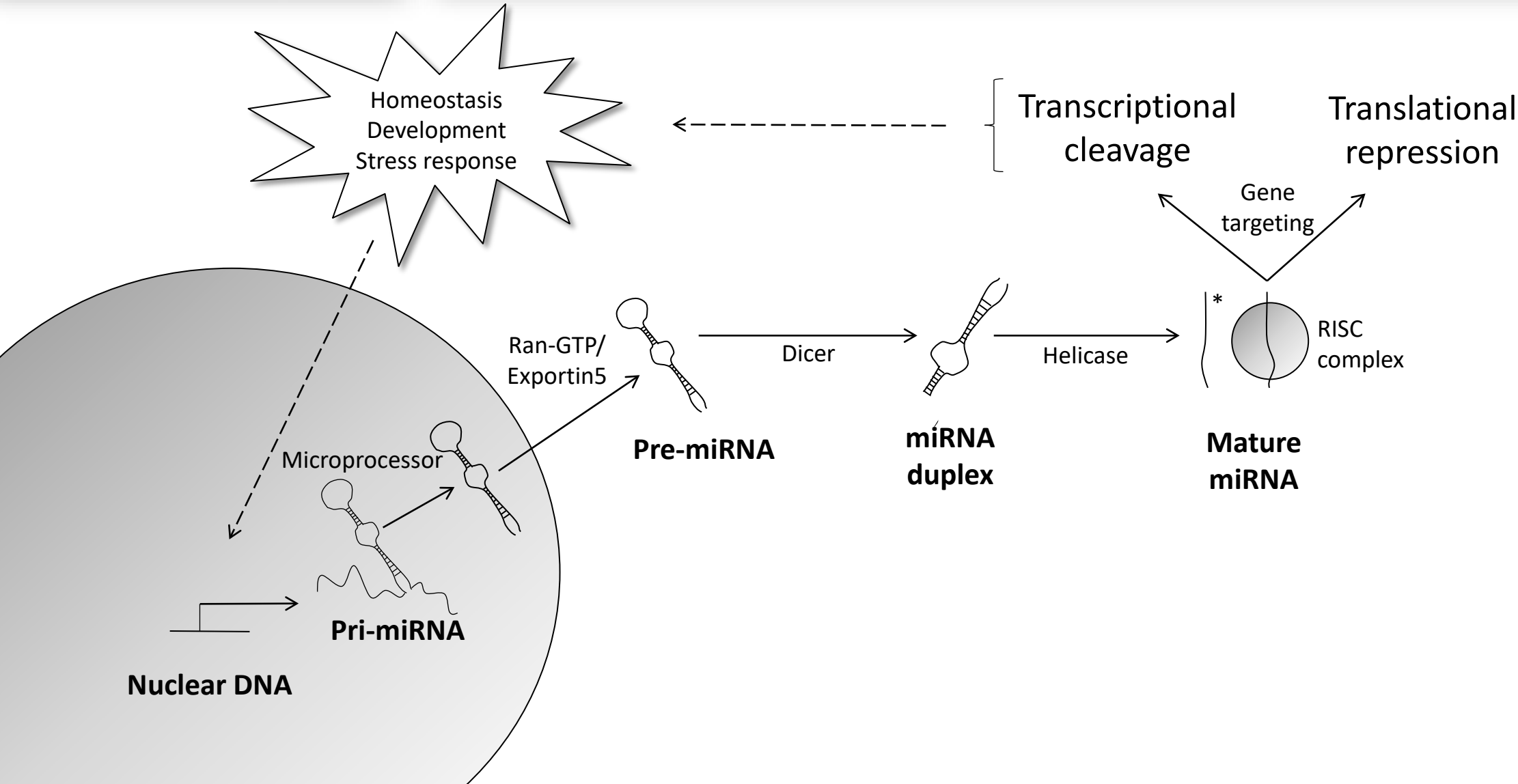
Transcriptomic biomarkers in toxicology

- Many thousands of chemicals without data to provide a reference value
- Costly and time consuming to generate apical data

- Early transcriptional biomarkers may be sensitive measure of chemical perturbation and link to mechanism of adverse outcome of regulatory interest



MicroRNAs as biomarkers in toxicology



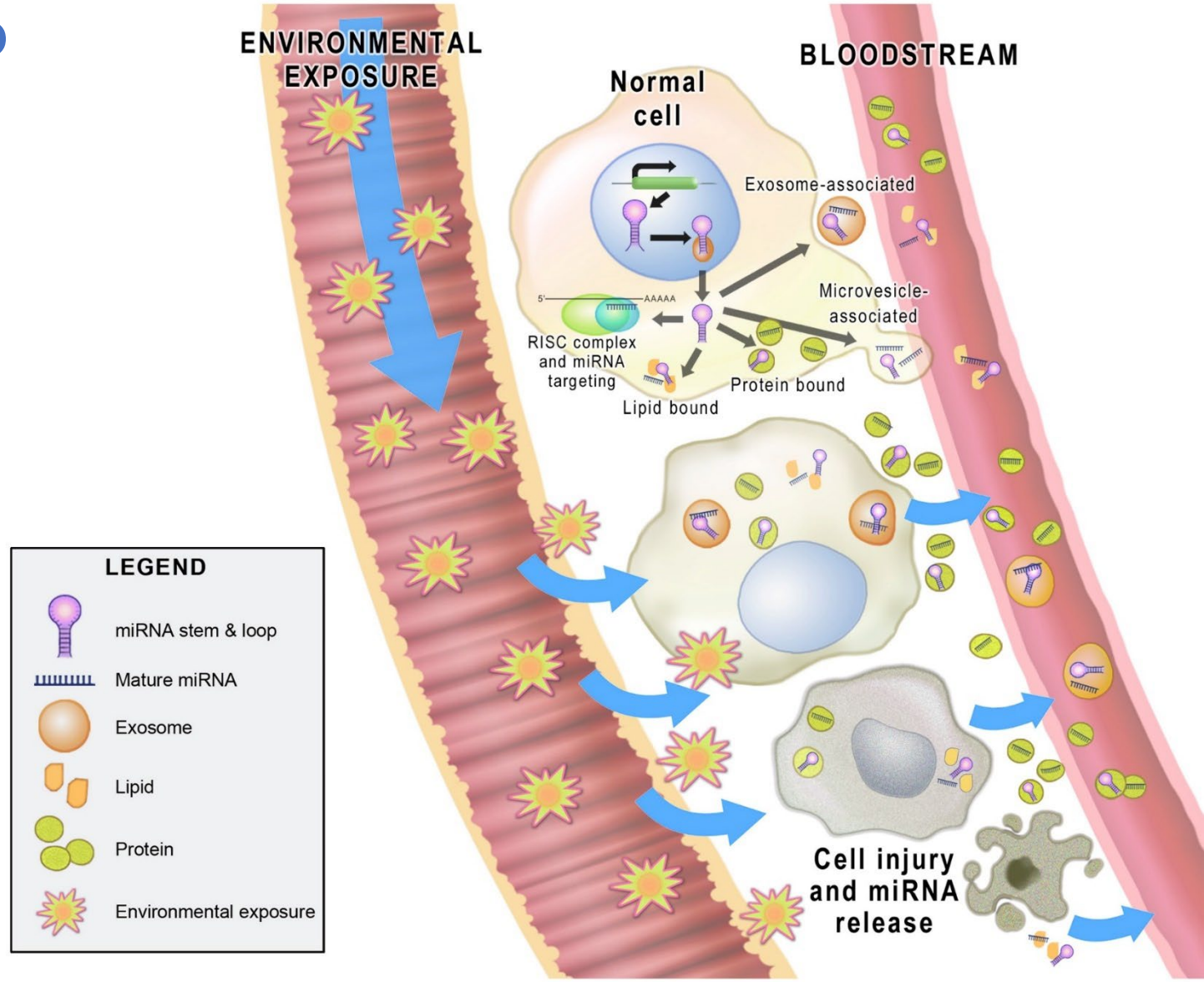
MicroRNAs as biomarkers in toxicology

- **MicroRNAs are responsive to exogenous exposures**
- **Regulatory nodes for transcriptional networks**
- **MicroRNAs present in biofluids**

MicroRNAs in Biofluids
Non-invasive biomarkers?

Predictive and non-invasive

- Passive secretion of microRNA
  - Associated with cell death and toxicity
- Active secretion of microRNA
  - Potentially vesicle-associated and involved in cell-to-cell signaling



Harrill *et al. Toxicological Sciences* 152(2):264-272, 2016

MicroRNAs as biomarkers in toxicology

Are biofluid-based miRNA biomarkers informative for health effects due to environmental exposure?

Serum microRNA associated with toxicant-associated liver disease

Hypothesis: Previously identified individuals with toxicant-associated fatty liver disease will exhibit an altered liver microRNA profile in serum.

- **Method:** Use targeted panel to directly measure microRNA in archived serum and correlate with other metrics in cohort.

Anniston Community Health Survey (ACHS)

- PCB (polychorinated biphenyls) mixtures produced at a chemical plant from 1929-1971 in Anniston, Alabama
- Large, cross-sectional epidemiological study of residential population:
  - Increased PCB levels compared to NHANES reference (2-3 fold)
  - High prevalence of obesity (54%)
  - Associations between PCB exposures and hypertension, diabetes, and dyslipidemia (conditions commonly seen in metabolic syndrome)

Anniston, AL near former Monsanto PCB plant

Linda Birnbaum (NIEHS)

Pavuk et al. Sci Total Environ 2014; Goncharov et al. J Hypertension 2010; Silverstone et al. EHP 2012; Aminov et al. Env. Health 2013; Cave et al. J Occ Env Med 2011; https://www.atsdr.cdc.gov/sites/anniston_community_health_survey/overview.html

Biomarker Evidence of TASH

- Toxicant-associated steatohepatitis (TASH) is a form of necrotic liver disease associated with both industrial and environmental chemical exposures.
- Cave *et al.* found evidence of TASH in 738 ACHS samples (Phase I)
    - Fragment analyses of CK18 in serum indicate oncotic necrosis or apoptotic death processes in hepatocytes
    - Can distinguish TASH from other liver disease
    - Positive associations of steatohepatitis with elevated pro-inflammatory cytokines, insulin resistance, hypertriglyceridemia and specific PCB congeners
        - Linked to environmental liver disease

Matt Cave (U. of Louisville)

Clair HB, Pinkston CM, Rai SN, et al. *Toxicol Sci*. 2018;164(1):39-49.

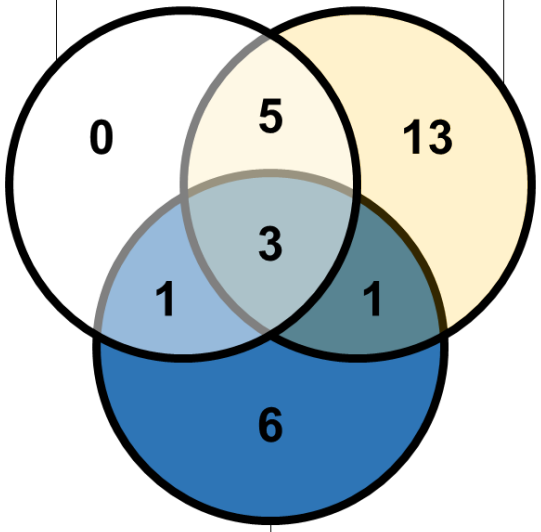
Profile screen: liver-associated miRNAs

Bullets indicate location/species of altered miRNAs in liver disease/toxicity, based on published literature

- human serum or plasma
- human liver/hepatocytes
- mouse/rat serum or plasma
- mouse/rat liver

Cave MC, Pinkston CM, Rai SN, et al. *EHP* 2022

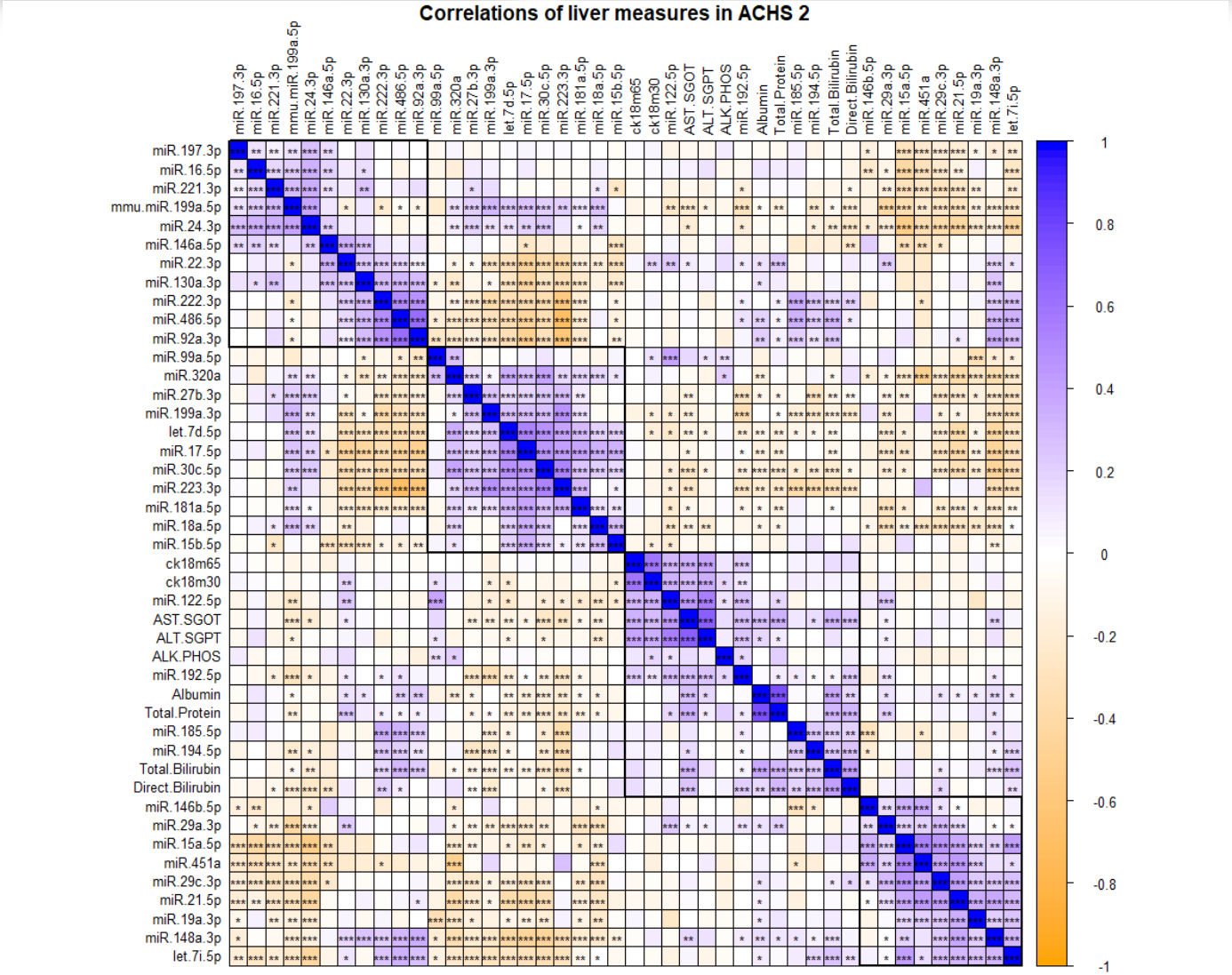
Serum miRNAs correlate with liver disease and PCB levels

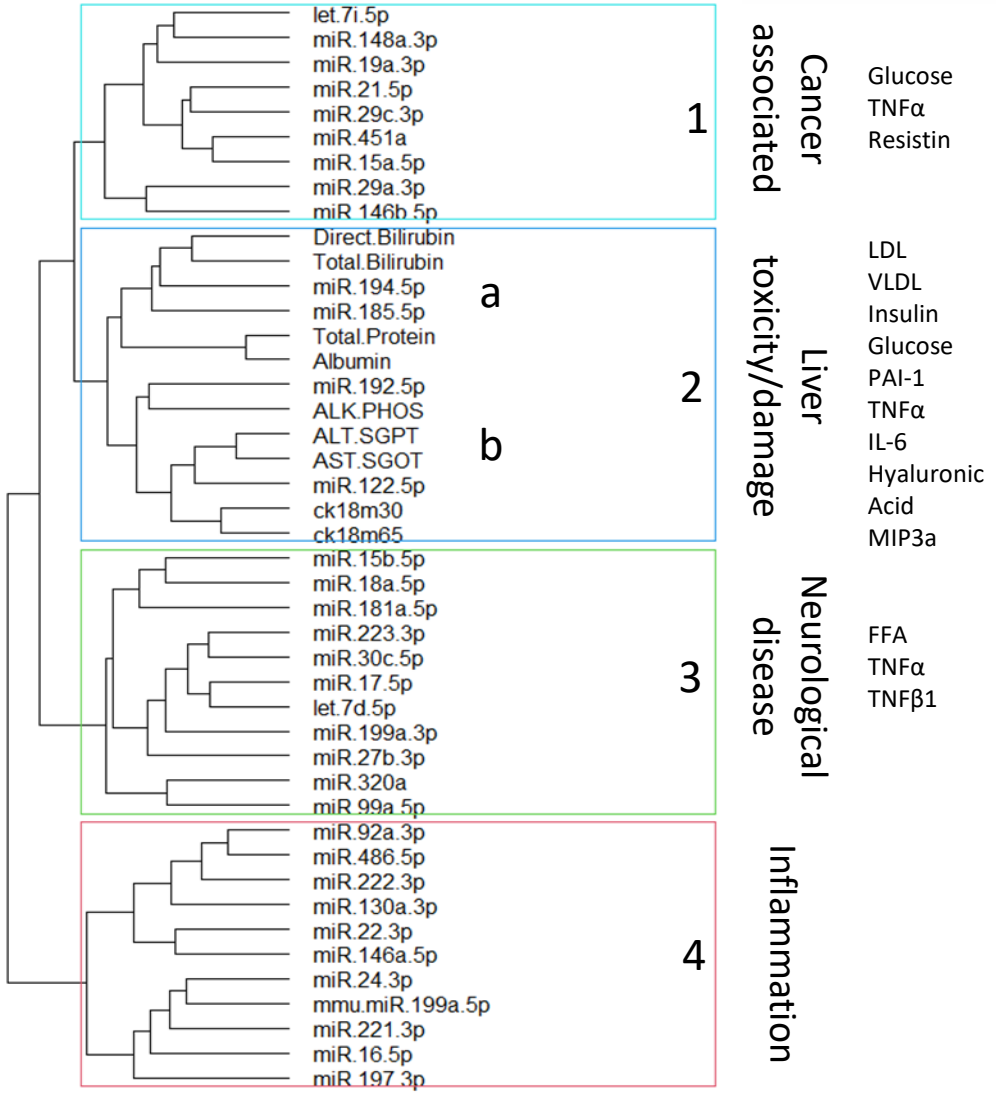


Cell death marker associated miRNAs	Liver disease associated miRNAs	PCB associated miRNAs
miR-122-5p	miR-122-5p	miR-122-5p
miR-192-5p	miR-192-5p	miR-192-5p
miR-99a-5p	miR-99a-5p	miR-99a-5p
miR-197-3p	miR-197-3p	miR-185-5p
let-7d-5p	let-7d-5p	miR-15a-5p
miR-221-3p	miR-221-3p	miR-22-3p
miR-17-5p	miR-17-5p	miR-21-5p
miR-24-3p	miR-24-3p	miR-29c-3p
let-7i-5p	miR-320a	miR-320a
miR-130a-3p		miR-130a-3p
miR-146a-5p		miR-451a
miR-148a-3p		
miR-15b-5p		
miR-181a-5p		
miR-18a-5p		
miR-194-5p		
miR-199a-3p		
miR-27b-3p		
miR-29a-3p		
miR-30c-5p		
miR-486-5p		
mmu-miR-199a-5p		

Cave MC, Pinkston CM, Rai SN, et al. *EHP* 2022

Candidate serum miRNAs correlate with liver toxicity biomarkers and other adverse processes





Unpublished results, please do not cite

Take home message: Anniston study

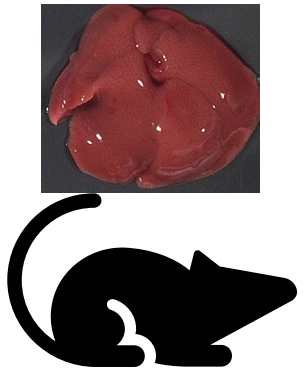
- Measured miRNA in biofluid correlated with specific liver injury biomarkers, but also indicated other adverse health processes
- *Are they indicative of adverse mechanisms beyond general toxicity?*
- *Can we link miRNA alterations to specific exposure-mediated mode-of-action?*

Tumorigenic Phthalate Exposure in Mice

Hypothesis: Dose-responsive microRNAs correlates with gene expression and toxicology data in a PPARα mouse model of liver tumorigenesis

Method: Use microRNA profiling after short-term exposure of liver tumorigen

tumorigenic	di(2-ethylhexyl) phthalate (DEHP)
non-tumorigenic	di-n-octyl phthalate (DNOP) n-butyl benzyl phthalate (BBP)



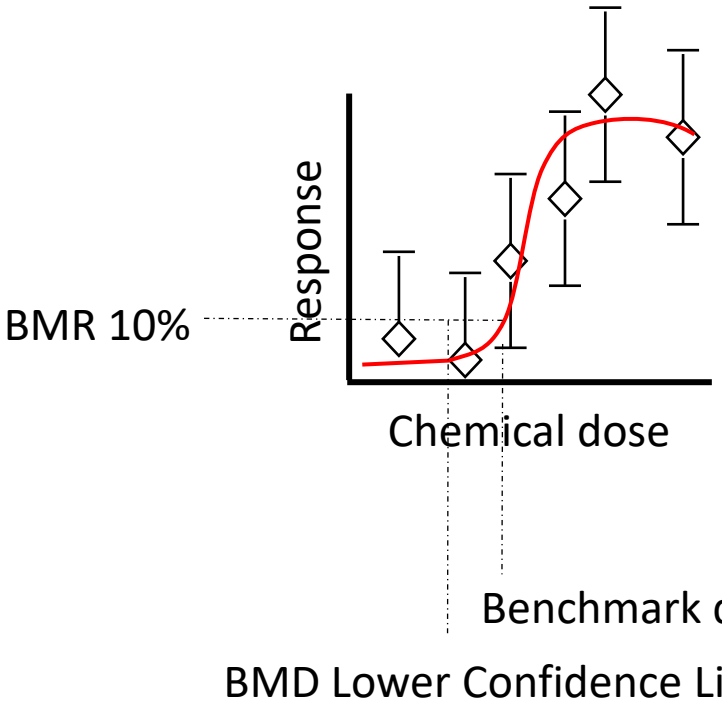
7 days (4 doses)
and
28 days (1 high dose)

Benchmark dose response (BMD) for EPA risk assessment

In the absence of available human data...

Non-cancer risk assessment

Short term, sub-chronic, and chronic animal studies



Uncertainty

- cross-species extrapolation
- pharmacodynamic pharmacokinetic variability
- sensitive subpopulations
- exposure duration

Reference values

Phthalate potency predicted using transcriptional measurements

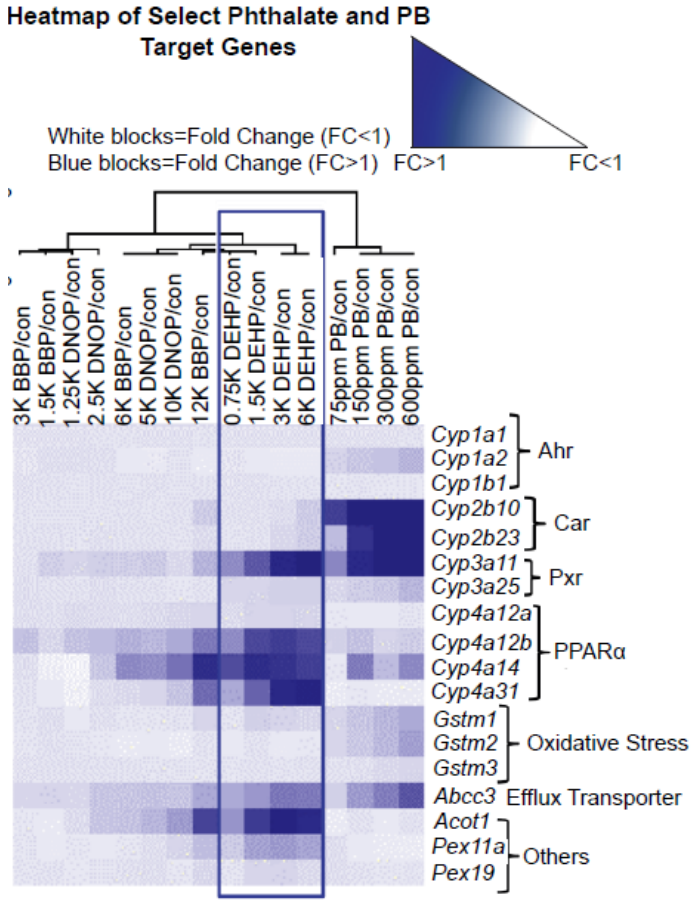


Table 3. Transcriptional (BMD_T) and apical (BMD_A) Benchmark Dose Estimates for DEHP DNOP and BBP Exposed Mouse livers.

Illumina Array Genes	DEHP (mg/kg-day) BMD_T	DNOP (mg/kg-day) BMD_T	BBP (mg/kg-day) BMD_T
Acot1	29	370	676
Cyp4a12b	61	64	210
Cyp4a31	57	912	851
Pdk4	150	>958	>1140
Abcc3	35	164	322
qPCR Genes	**DEHP (mg/kg-day) BMD_A**	**DNOP (mg/kg-day) BMD_A**	**BBP (mg/kg-day) BMD_A**
Abcc3	18	NA	NA
Acot1	77	NA	NA
Cyp4a12b	69	NA	NA
Cyp4a31	47	NA	NA
Pdk4	183	NA	NA
Functional Non-Genomic Markers	**DEHP (mg/kg-day) BMD_A**	**DNOP (mg/kg-day) BMD_A**	**BBP (mg/kg-day) BMD_A**
Ki67 (small cells only)	215	>958	>1140
PROD	42	>958	>1140
BROD	532	>958	892
Relative Liver weight	48	NA	311
Hepatocyte cytoplasmic alteration	116	NA	996
2-year Tumorigenic Potency	**DEHP (mg/kg-day) BMD_A**	**DNOP (mg/kg-day) BMD_A**	**BBP (mg/kg-day) BMD_A**
HCC	71	>1268	>1600
HCA+HCC	35	431	>1600

Potency Rank

DEHP>DNOP>BBP

DEHP>BBP>DNOP

DEHP>DNOP>BBP

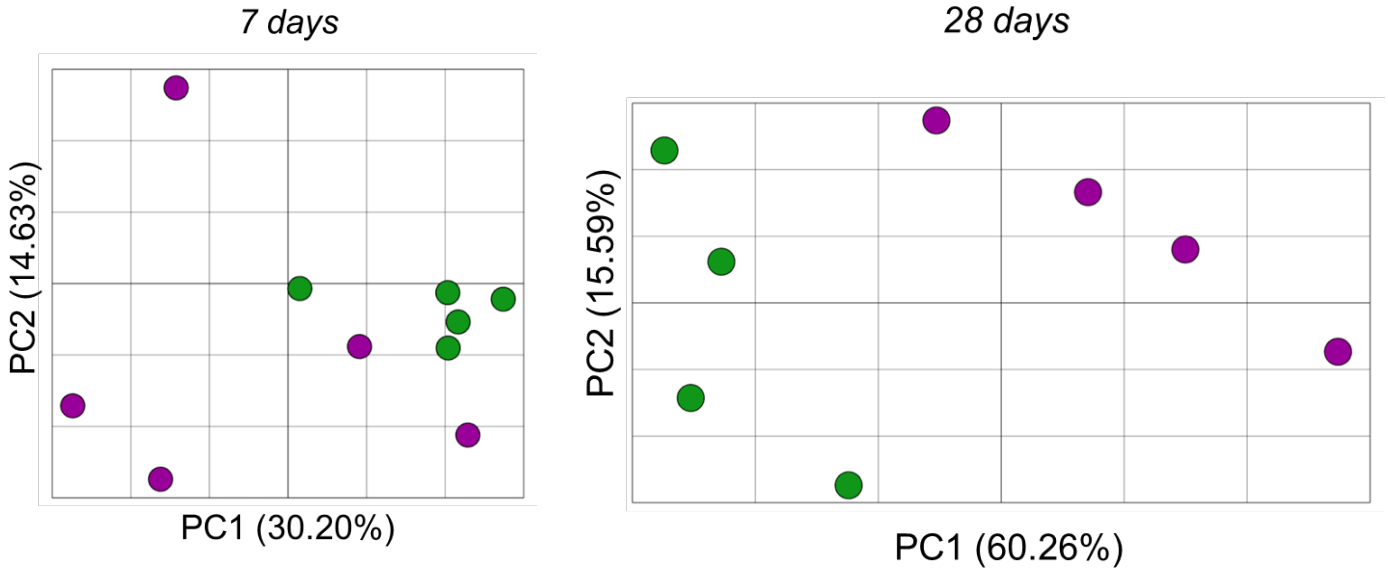
transcript profile predicts chemical potency for hepatocellular carcinoma

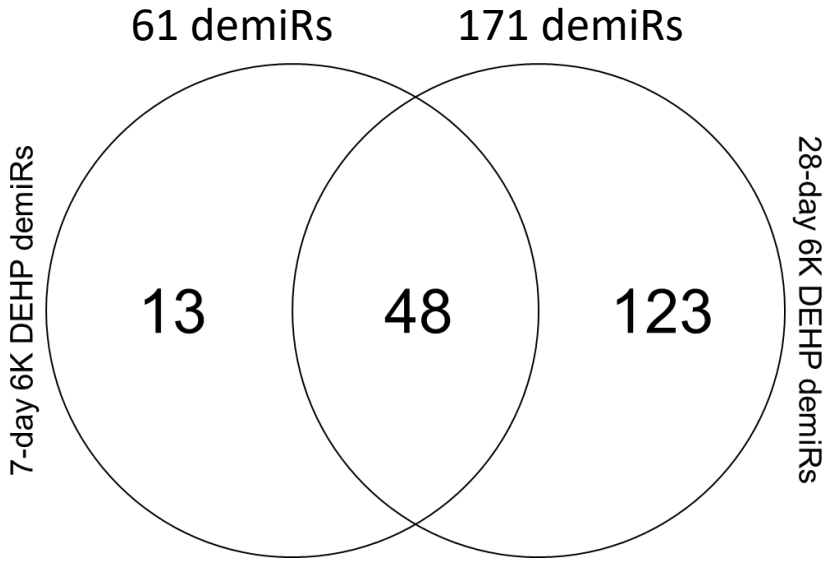
Toxicol Sci. 2016 Feb;149(2):312-25.

MicroRNA are also responsive to DEHP

Sequencing of liver RNA of 7 and 28-day DEHP treated mice

- Separation by PCA plot of liver miRNA expression
- Shared and unique miRNAs after 7 and 28 days





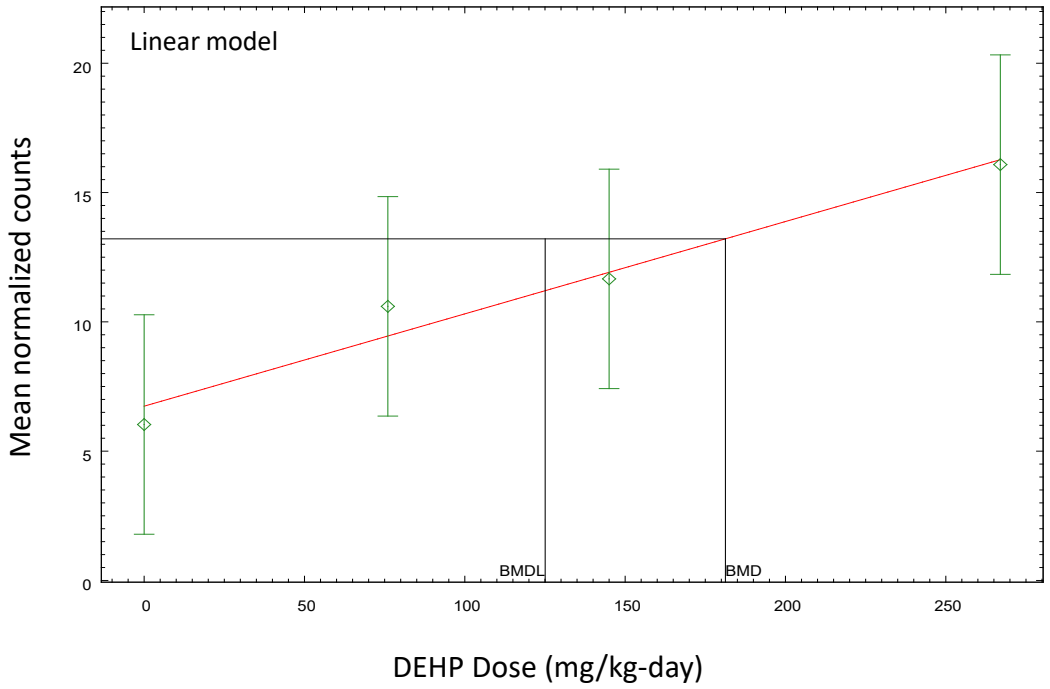
Evidence of persistent miRNA changes

Chorley et al. *Toxicol Rep*. 2020 Jun 23;7:805-815.

PPARα-linked miRNA are dose-responsive

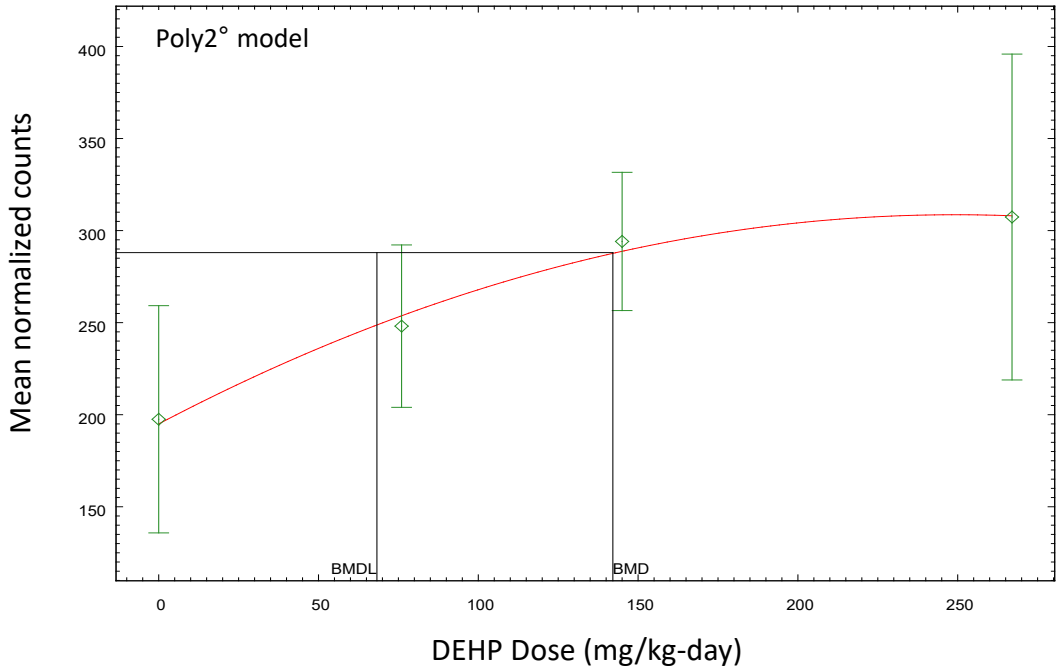
Benchmark dose (BMD) analysis of dose-responsive miRNA after 7 days

mmu-miR-182-5p



BMD_{miR} = 181mg/kg-day

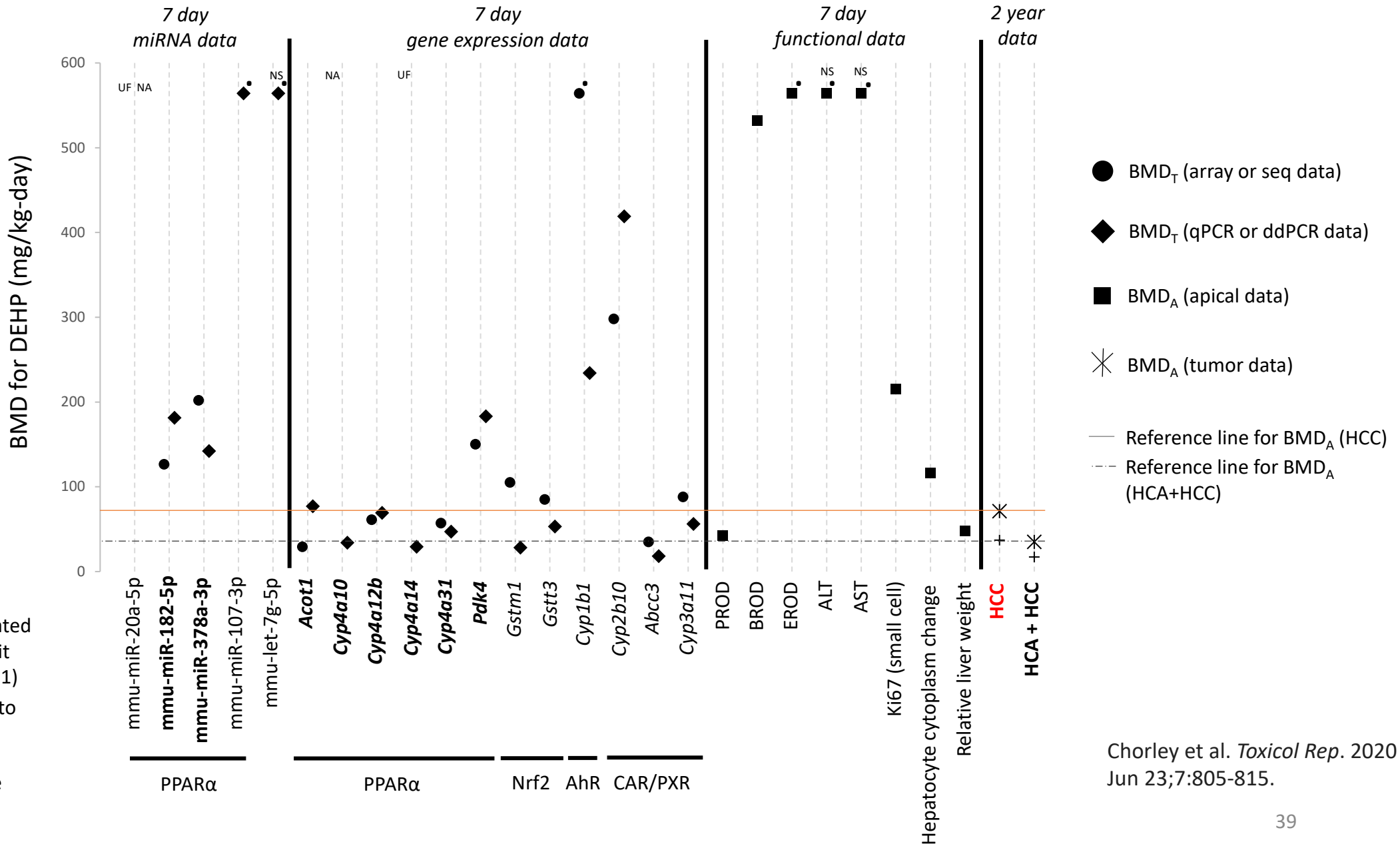
mmu-miR-378a-3p



BMD_{miR} = 142mg/kg-day

Chorley et al. *Toxicol Rep*. 2020 Jun 23;7:805-815.

microRNAs Correlate with Gene Expression and Functional Data



BMD_T (array or seq data)

BMD_T (qPCR or ddPCR data)

BMD_A (apical data)

BMD_A (tumor data)

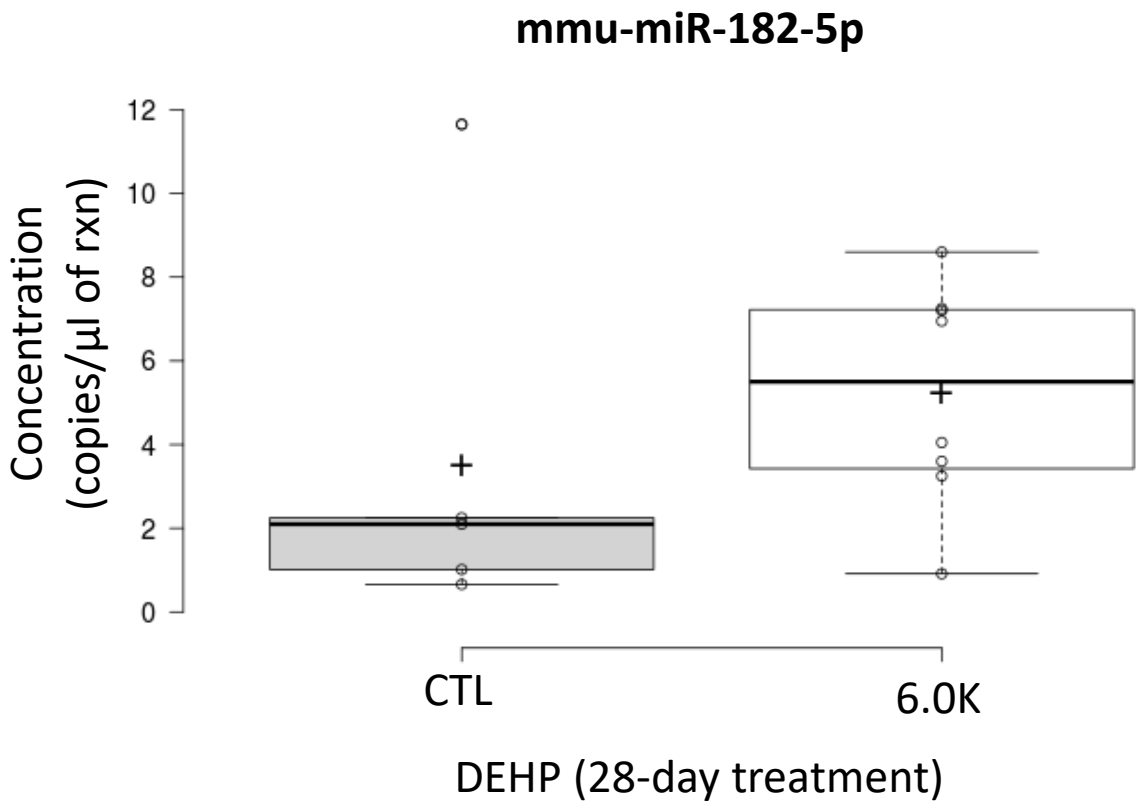
Reference line for BMD_A (HCC)

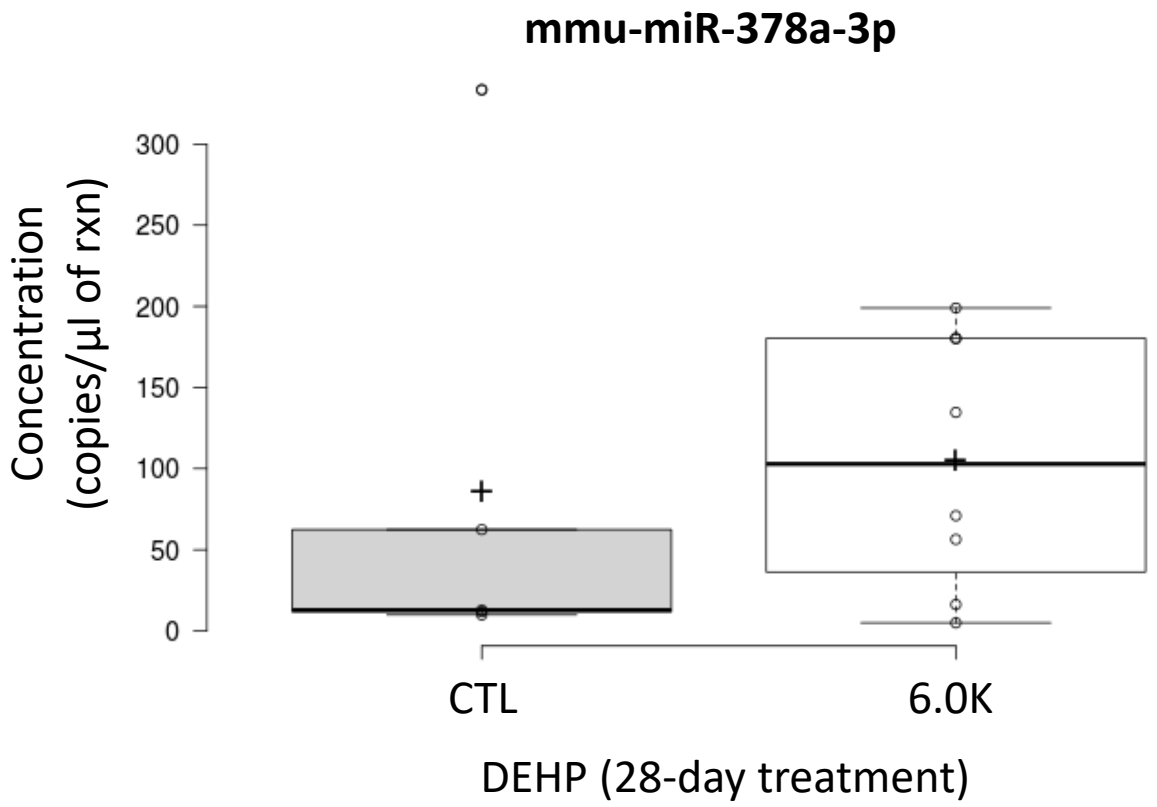
Reference line for BMD_A (HCA+HCC)

- BMD greater than than top dose tested
- UF the response was dose-related but was an Unacceptable Fit (global goodness of fit p<0.1)
- NS not significance compared to control values for any dose tested
- NA measurement not available

Chorley et al. *Toxicol Rep*. 2020 Jun 23;7:805-815.

Candidate microRNA in serum



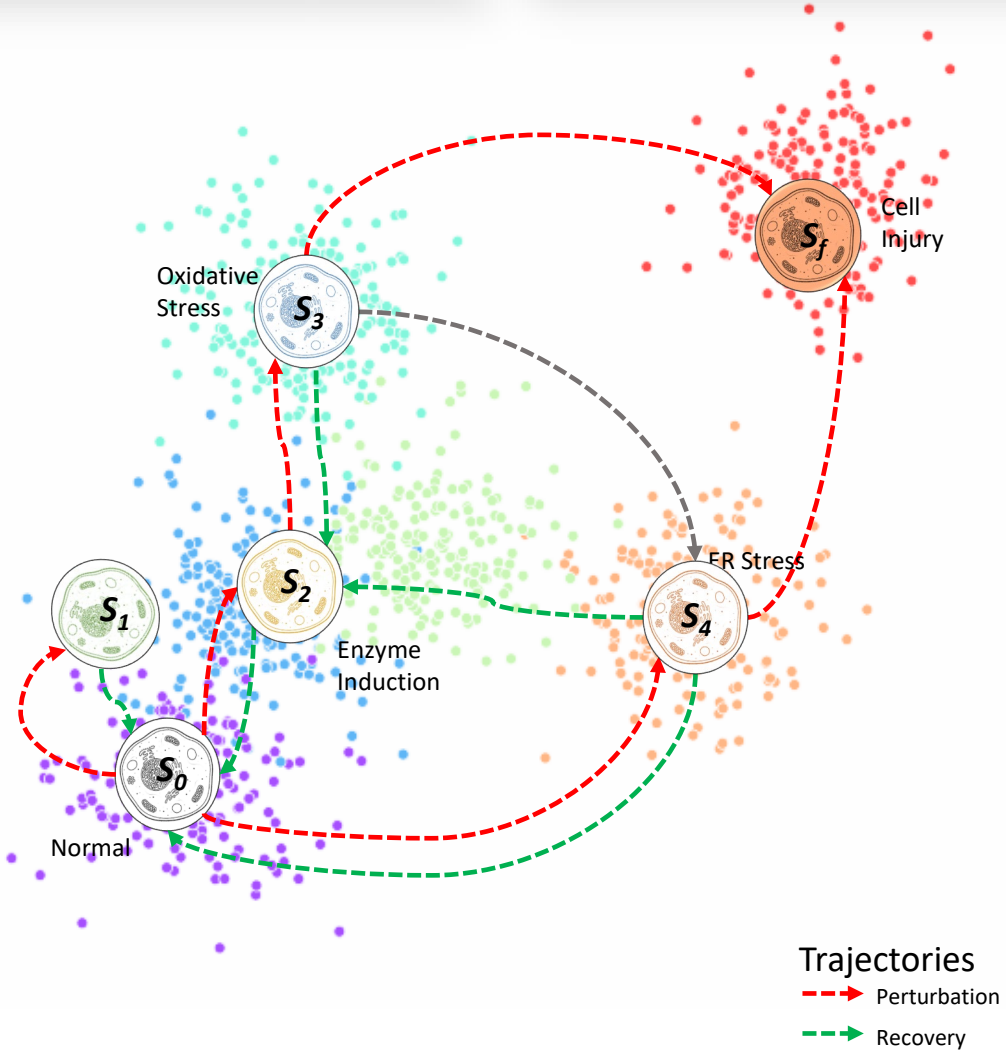


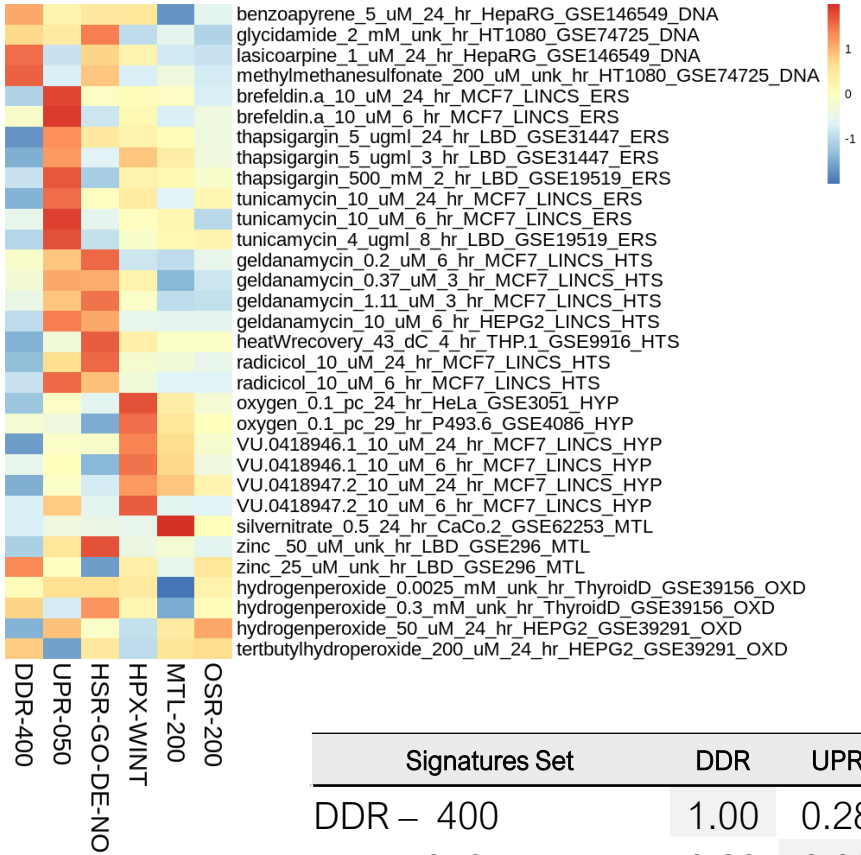
Chorley et al. *Toxicol Rep*. 2020 Jun 23;7:805-815.

Take home message: PPARα study

- In this case study, dose-responsive miRNA are linked to the known primary mechanism of action (PPARα) for DEHP-mediated mouse HCC
- Indications these miRNAs may be leaked/transferred into circulation
- *Can these miRNA patterns enhance our chemical screening efforts?*

Transcriptomic signatures *in vitro* to identify cellular stress response





Signatures Set	DDR	UPR	HSR	HPX	MTL	OSR
DDR – 400	1.00	0.28	0.47	0.27	0.60	0.68
UPR – 050	0.23	0.89	0.70	0.38	0.21	0.16
HSR – GO_DE_NO	0.72	0.34	0.97	0.17	0.34	0.50
HPX – WINT	0.28	0.57	0.47	1.00	0.20	0.20
MTL – 200	0.12	0.63	0.33	0.85	0.66	0.28
OSR – 200	0.10	0.50	0.40	0.58	0.57	0.88

Shah I, et al. Environ Health Perspect. 2016 Jul;124(7):910-9. doi: 10.1289/ehp.1409029.

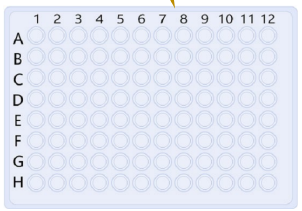
Slide courtesy of Imran Shah, Bryant Chambers, US EPA

Defining extracellular microRNAs signatures

- ***Non-destructive measurement of extracellular microRNA to define chemical mechanism-of-action***

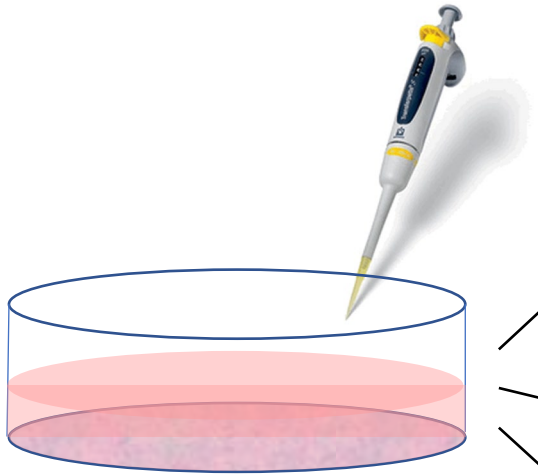
Reference chemicals for hepatotoxicant MoA

24-48 hr exposure



24 hr sample

48 hr sample



HepaRG
(human hepatocytes)

microRNA released in media

cytotoxicity and brightfield

cellular microRNA and mRNA (HTTr)

- Identify candidate miRNA measured in HepaRG media
- Optimize media volume for measurement
- Distinguish active versus passive release of miRNA into media (toxicity vs. cellular response)
- Link to gene expression networks and link cellular microRNA
- Establish extracellular microRNA patterns linked to chemical MoA

Exposure | **Sampling** | **Measurements** | **Aims**

Defining microRNAs signatures of MoA

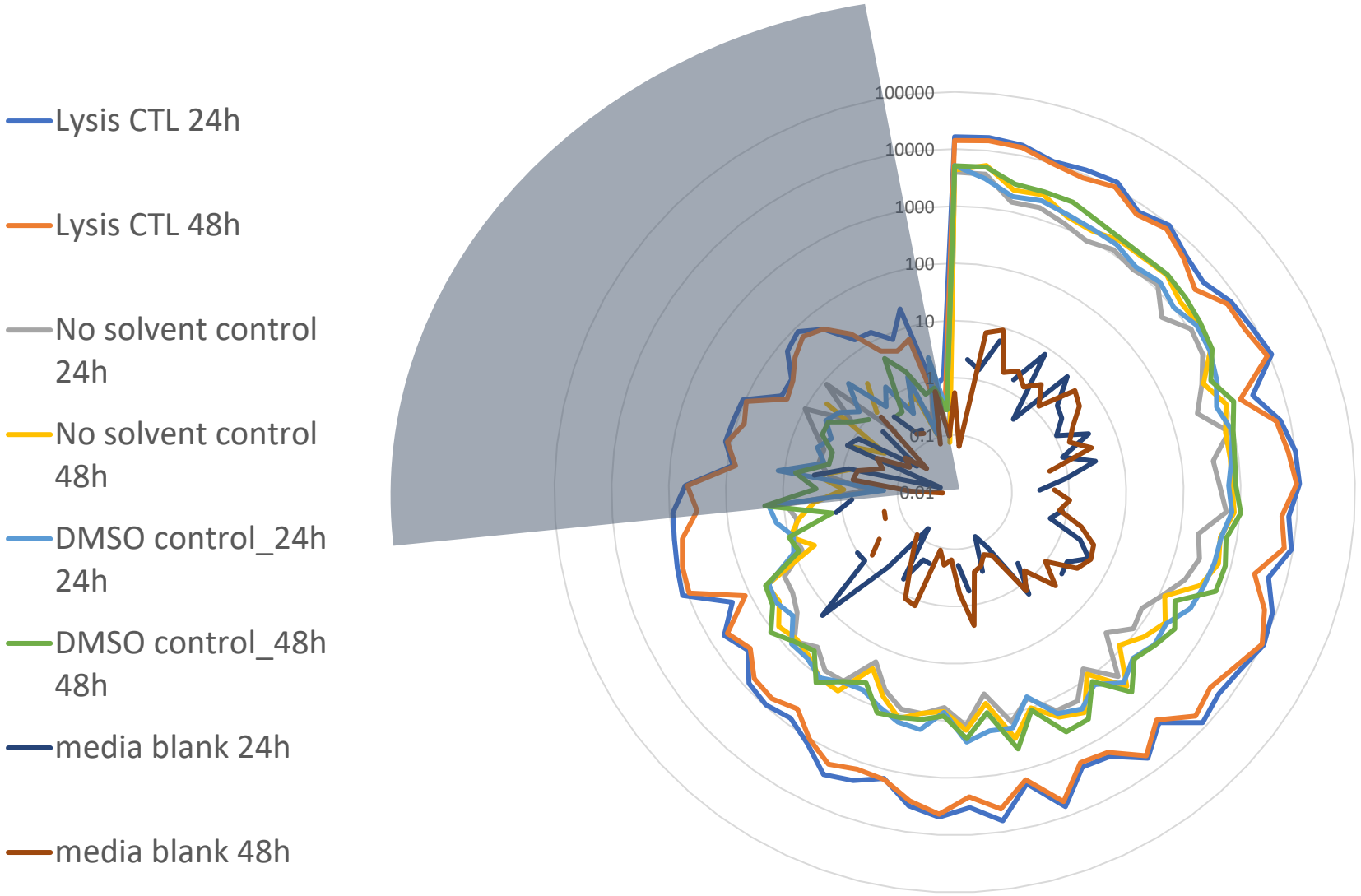
Phase I Chemicals

Benzo[a]pyrene – 10, 1, 0.25 uM	Aryl hydrocarbon receptor (AHR) agonist
Pirinixic acid (WY-14643) – 30, 3, 0.3 uM	Peroxisome proliferator-activated receptor (PPAR) α agonist
Menadione – 30, 15, 7.5 uM	Aldehyde oxidase-1 (AOX1) agonist
Ketoconazole – 10, 1, 0.1 uM	Cytochrome P450 3A4 (CYP3A4) antagonist
Retinoic acid – 10, 1, 0.1 uM	Retinoic acid receptor alpha (RAR-α) agonist
Chenodeoxycholic acid – 200, 100, 50 uM	Farnesoid X receptor (FXR) agonist
Trichostatin A – 3, 0.3, 0.03 uM	Histone deacetylase inhibitors (HDACi)
Rifampicin – 100, 50, 25 uM	Pregnane X receptor (PXR) agonist
Troglitazone – 100, 50, 5 uM	Peroxisome proliferator-activated receptor (PPAR) γ agonist
Atorvastatin – 10, 1, 0.1 uM	3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR) inhibitor

Small RNA sequencing: candidate miRNA identification

- 181 total miRNAs measured in media in small RNA-seq results
- 65 chosen for miRNA Fireplex panel
- Candidates measured at 24h and 48h post exposure

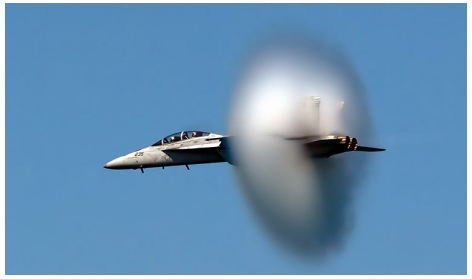
Phase I Fireplex data: The ceiling and the floor of the assay

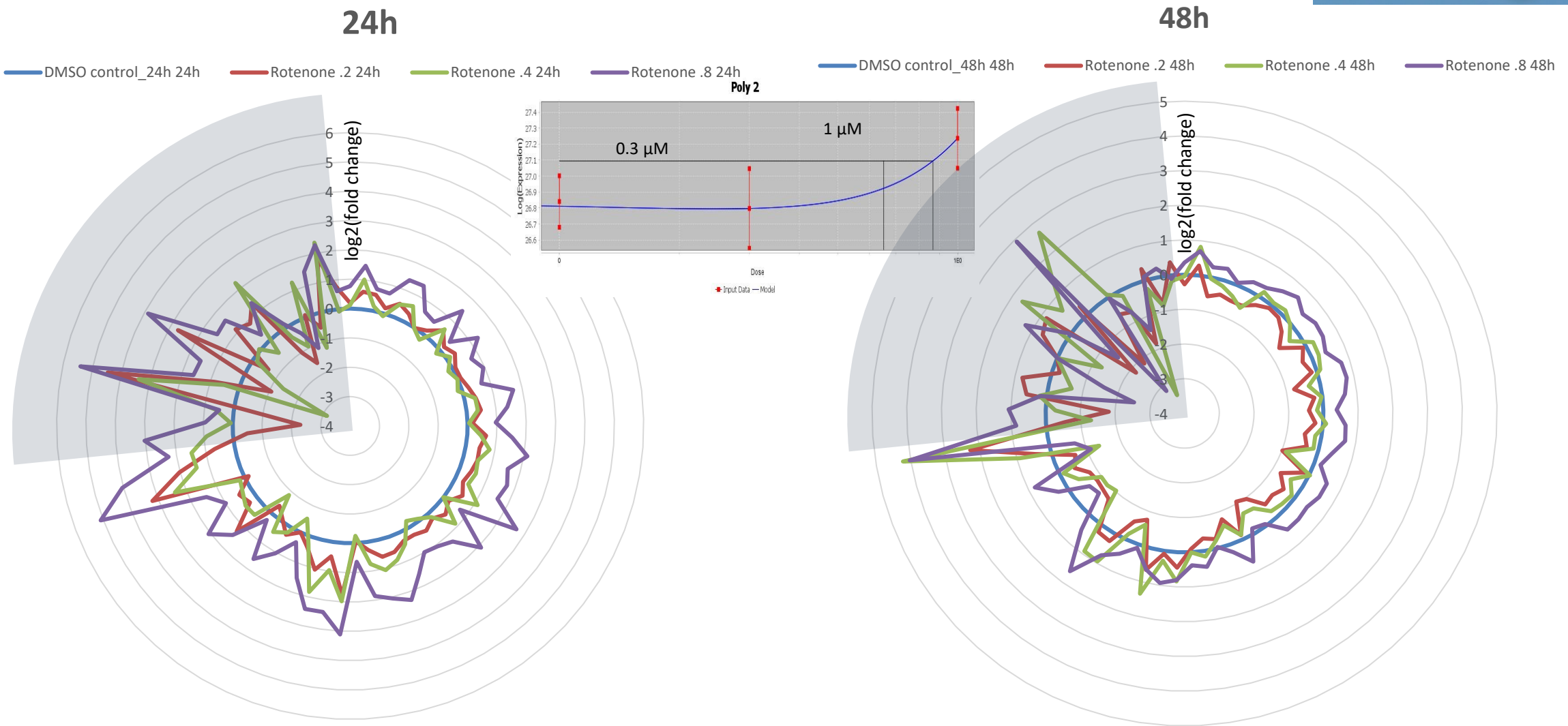


Unpublished results, please do not cite

Phase I Fireplex data

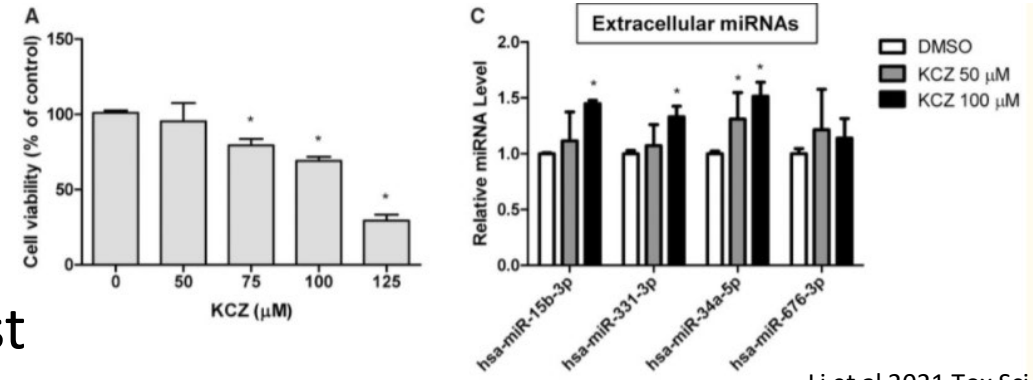
Rotenone controls; “shockwave” toxicity indicator





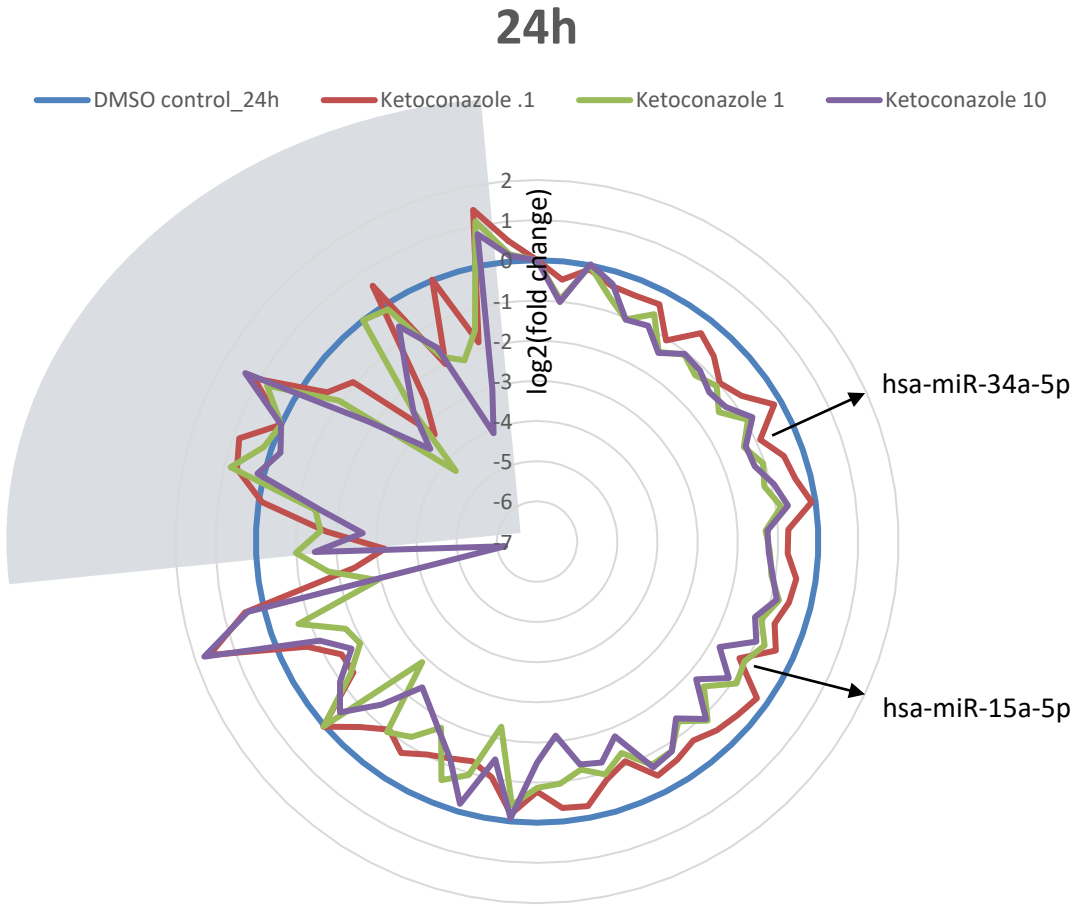
Unpublished results, please do not cite

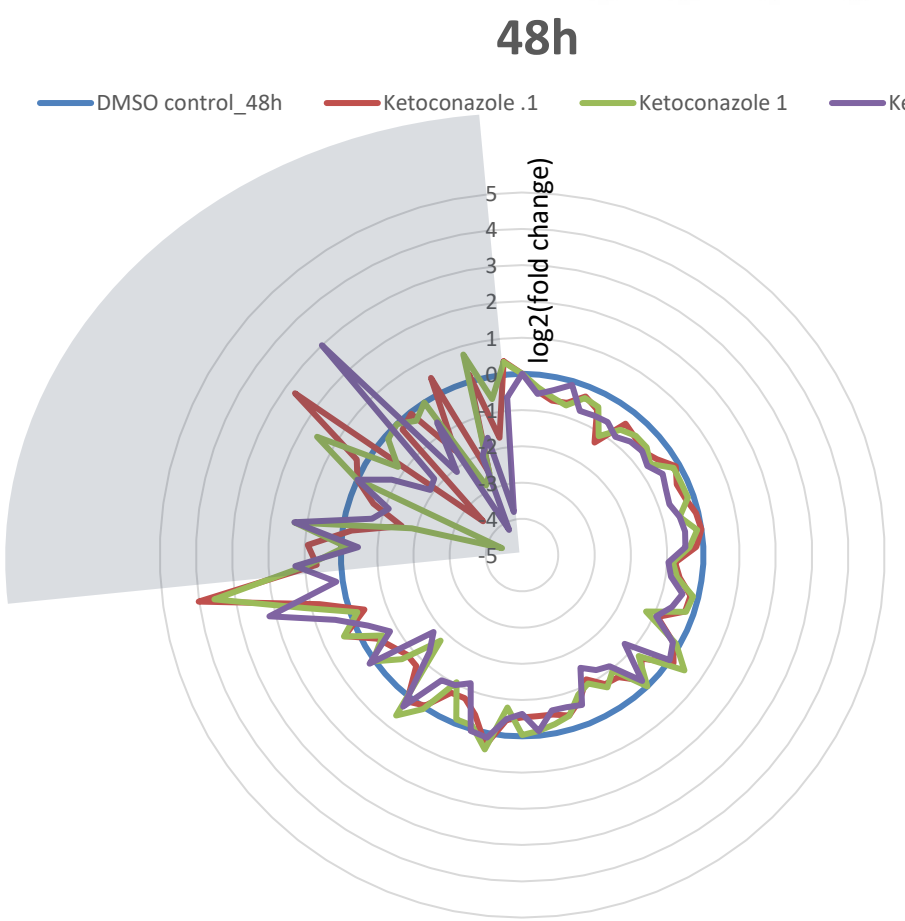
Phase I Fireplex data
"Black hole"; Suppressed signal



Li et al 2021 Tox Sci

CYP3A4 antagonist

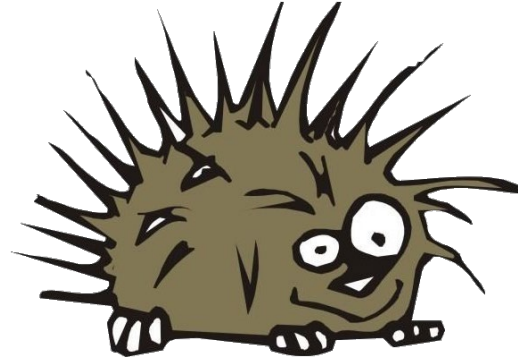




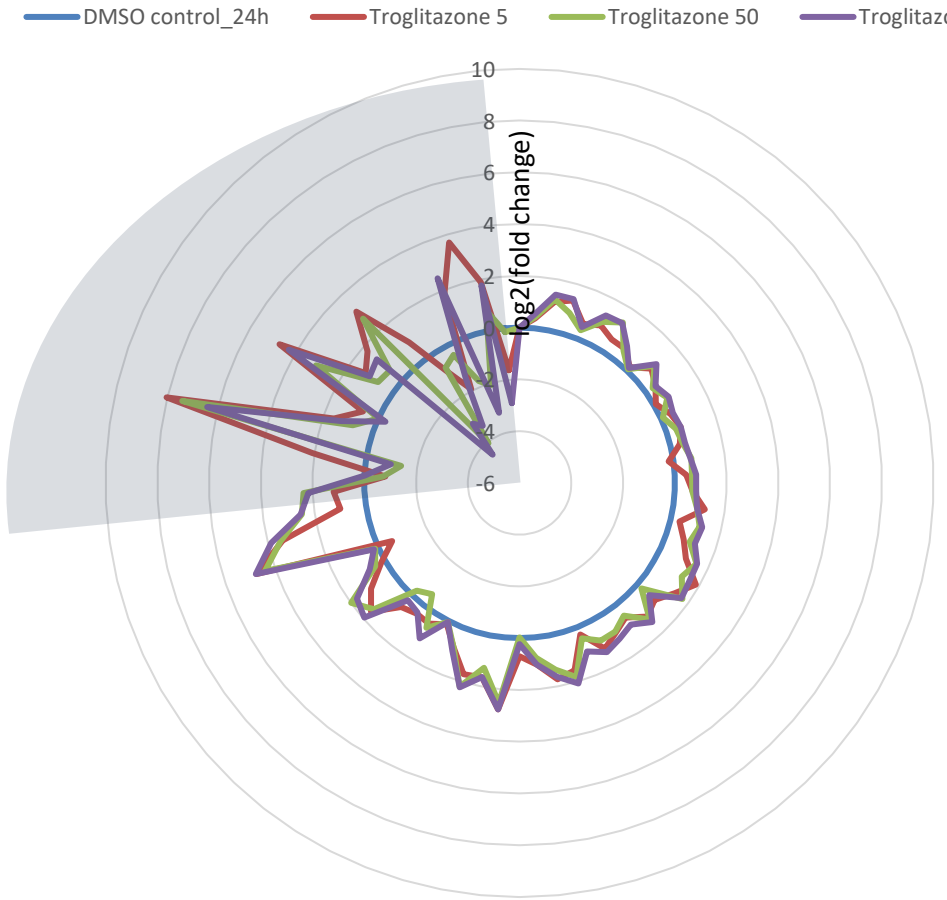
Unpublished results, please do not cite

Phase I Fireplex data

"Porcupines"; Potential signatures of MoA

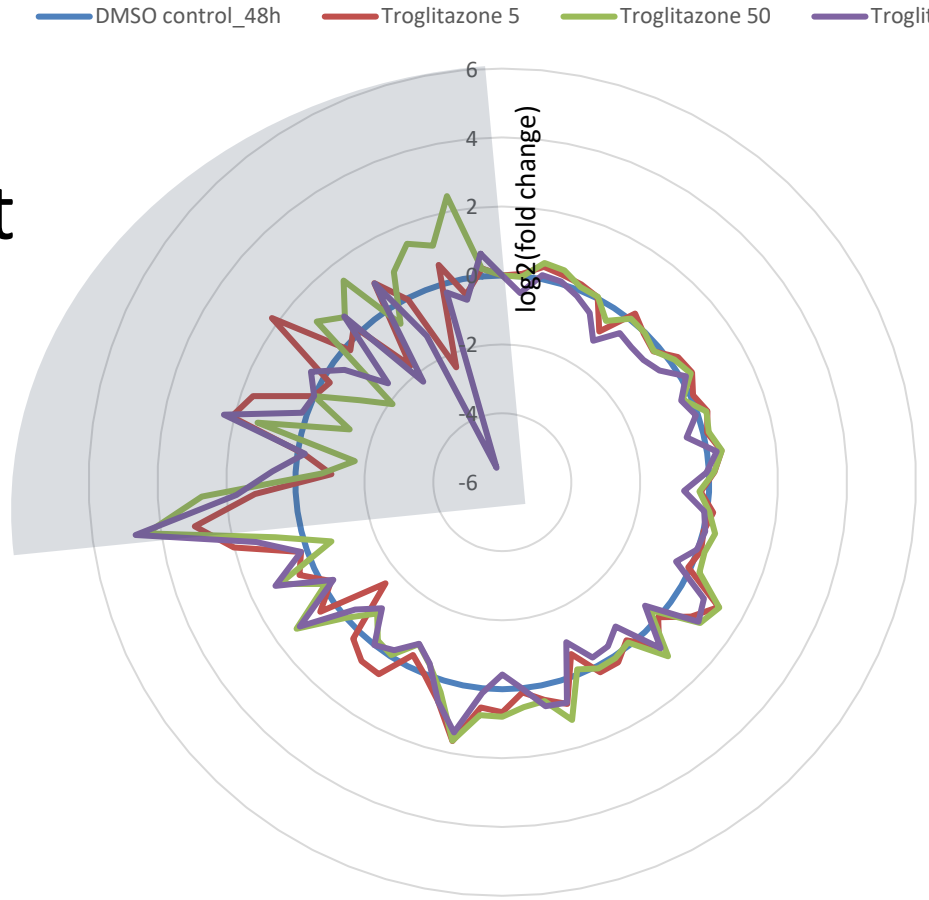


24h



PPARγ agonist

48h



Unpublished results, please do not cite

Phase II Chemicals – Can we replicate signatures?

Omeprazole	Aryl hydrocarbon receptor (AHR) agonist	30, 10, 3, 1, 0.3, 0.1, 0.03 uM
3,3'-diindolylmethane	Aryl hydrocarbon receptor (AHR) agonist	100, 30, 10, 3, 1, 0.3, 0.1 uM
Isovanillin	Aldehyde oxidase-1 (AOX1) agonist	100, 30, 10, 3, 1, 0.3, 0.1 uM
Hydralazine	Aldehyde oxidase-1 (AOX1) agonist	100, 30, 10, 3, 1, 0.3, 0.1 uM
Amiodarone	Cytochrome P450 3A4 (CYP3A4) antagonist	100, 30, 10, 3, 1, 0.3, 0.1 uM
Itraconazole	Cytochrome P450 3A4 (CYP3A4) antagonist	30, 10, 3, 1, 0.3, 0.1, 0.03 uM
GW4064	Farnesoid X receptor (FXR) agonist	10, 3, 1, 0.3, 0.1, 0.03, 0.01 uM
Obeticholic acid	Farnesoid X receptor (FXR) agonist	30, 10, 3, 1, 0.3, 0.1, 0.03 uM
Suberohydroxamic acid	Histone deacetylase inhibitors (HDACi)	30, 10, 3, 1, 0.3, 0.1, 0.03 uM
Vorinostat	Histone deacetylase inhibitors (HDACi)	30, 10, 3, 1, 0.3, 0.1, 0.03 uM
Lovastatin	3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR) inhibitor	10, 3, 1, 0.3, 0.1, 0.03, 0.01 uM
Simvastatin	3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR) inhibitor	10, 3, 1, 0.3, 0.1, 0.03, 0.01 uM
Acetaminophen	Peroxisome proliferator-activated receptor (PPAR) α agonist	100, 30, 10, 3, 1, 0.3, 0.1 uM
MEHP	Peroxisome proliferator-activated receptor (PPAR) α agonist	100, 30, 10, 3, 1, 0.3, 0.1 uM
Rosiglitazone	Peroxisome proliferator-activated receptor (PPAR) γ agonist	100, 30, 10, 3, 1, 0.3, 0.1 uM
Pioglitazone	Peroxisome proliferator-activated receptor (PPAR) γ agonist	100, 30, 10, 3, 1, 0.3, 0.1 uM
AM580	Retinoic acid receptor alpha (RAR-α) agonist	10, 3, 1, 0.3, 0.1, 0.03, 0.01 uM
Arotinoid acid	Retinoic acid receptor alpha (RAR-α) agonist	10, 3, 1, 0.3, 0.1, 0.03, 0.01 uM
Tunicamycin	Unfolded protein response (UPR)	100, 30, 10, 3, 1, 0.3, 0.1 uM
Brefeldin A	Unfolded protein response (UPR)	100, 30, 10, 3, 1, 0.3, 0.1 uM
Pyridaben	Unfolded protein response (UPR)/Hypoxia (HPX) response	30, 10, 3, 1, 0.3, 0.1, 0.03 uM
1,10-Phenanthroline	Hypoxia (HPX) response	100, 30, 10, 3, 1, 0.3, 0.1 uM
Quercetin	Hypoxia (HPX) response	100, 30, 10, 3, 1, 0.3, 0.1 uM
Chlorothalonil	Heat shock response (HSR)	100, 30, 10, 3, 1, 0.3, 0.1 uM
Cadmium Chloride	Heat shock response (HSR)	100, 30, 10, 3, 1, 0.3, 0.1 uM
Piperine	Oxidative stress response (OSR)	100, 30, 10, 3, 1, 0.3, 0.1 uM
Tert-butylhydroquinone	Oxidative stress response (OSR)	100, 30, 10, 3, 1, 0.3, 0.1 uM
1,4-Naphthoquinone	Oxidative stress response (OSR) /Hypoxia (HPX) response	100, 30, 10, 3, 1, 0.3, 0.1 uM
Etoposide	DNA damage response (DDR)	30, 10, 3, 1, 0.3, 0.1, 0.03 uM
5-Fluorouracil	DNA damage response (DDR)	100, 30, 10, 3, 1, 0.3, 0.1 uM

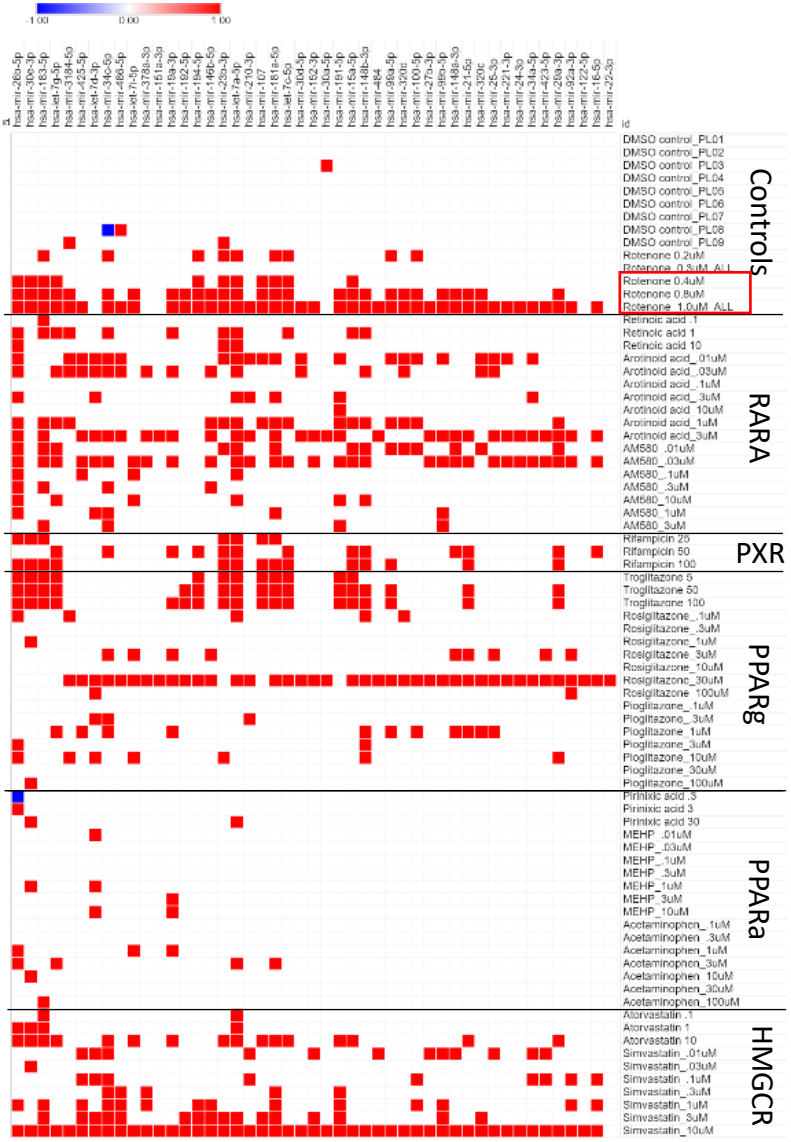
Chemical mechanism-of-action

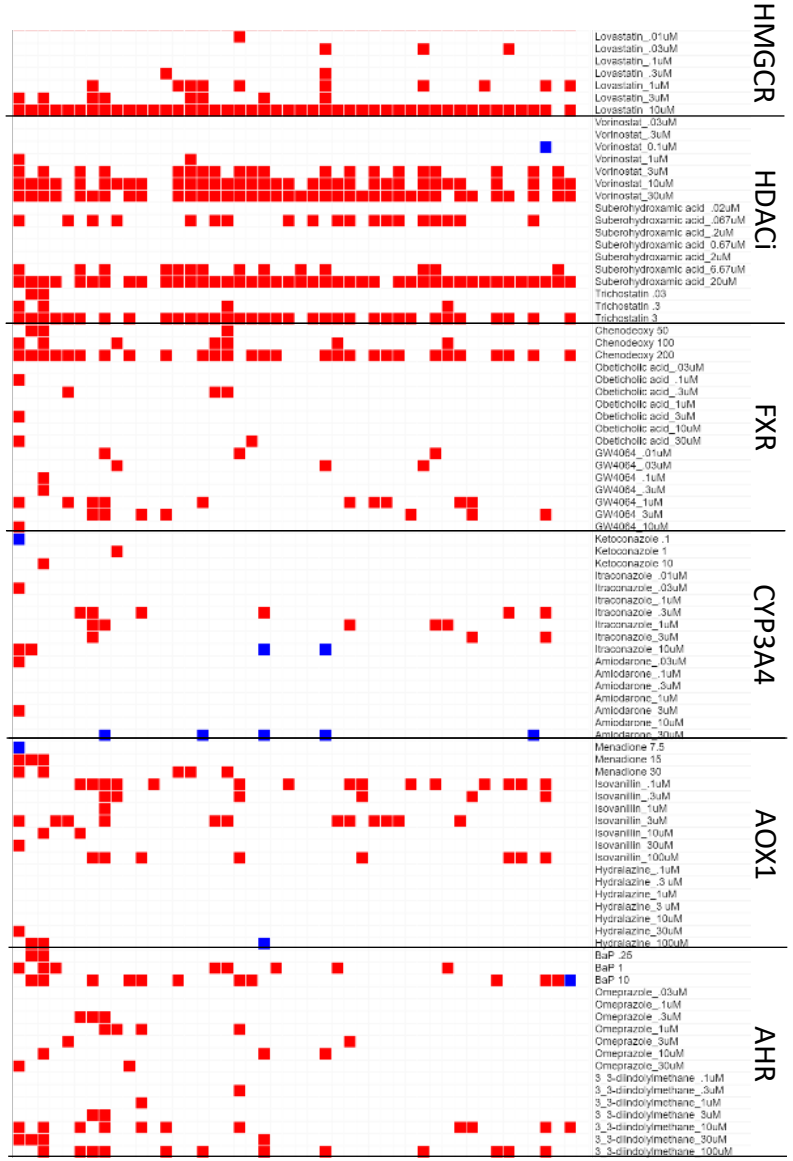
Cellular stress response

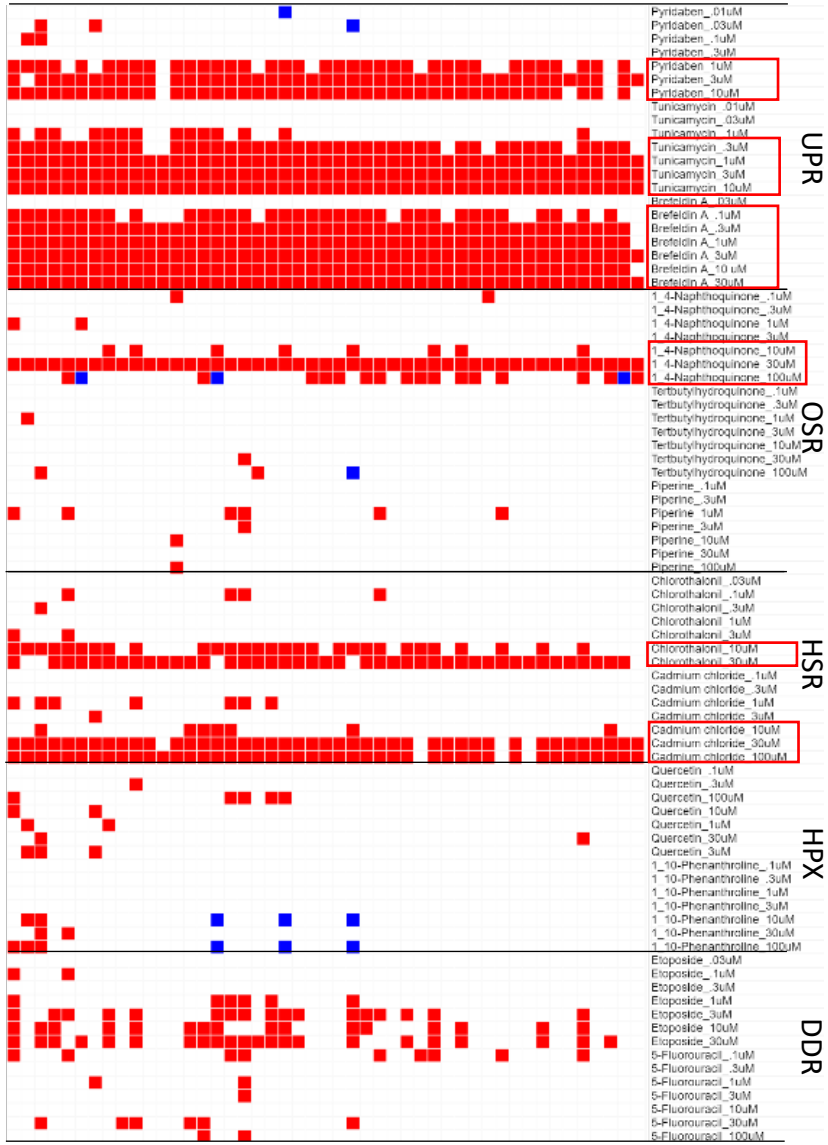
Baseline Median Absolute Deviation (BMAD) threshold calls

under 3XBMAD

over 3XBMAD

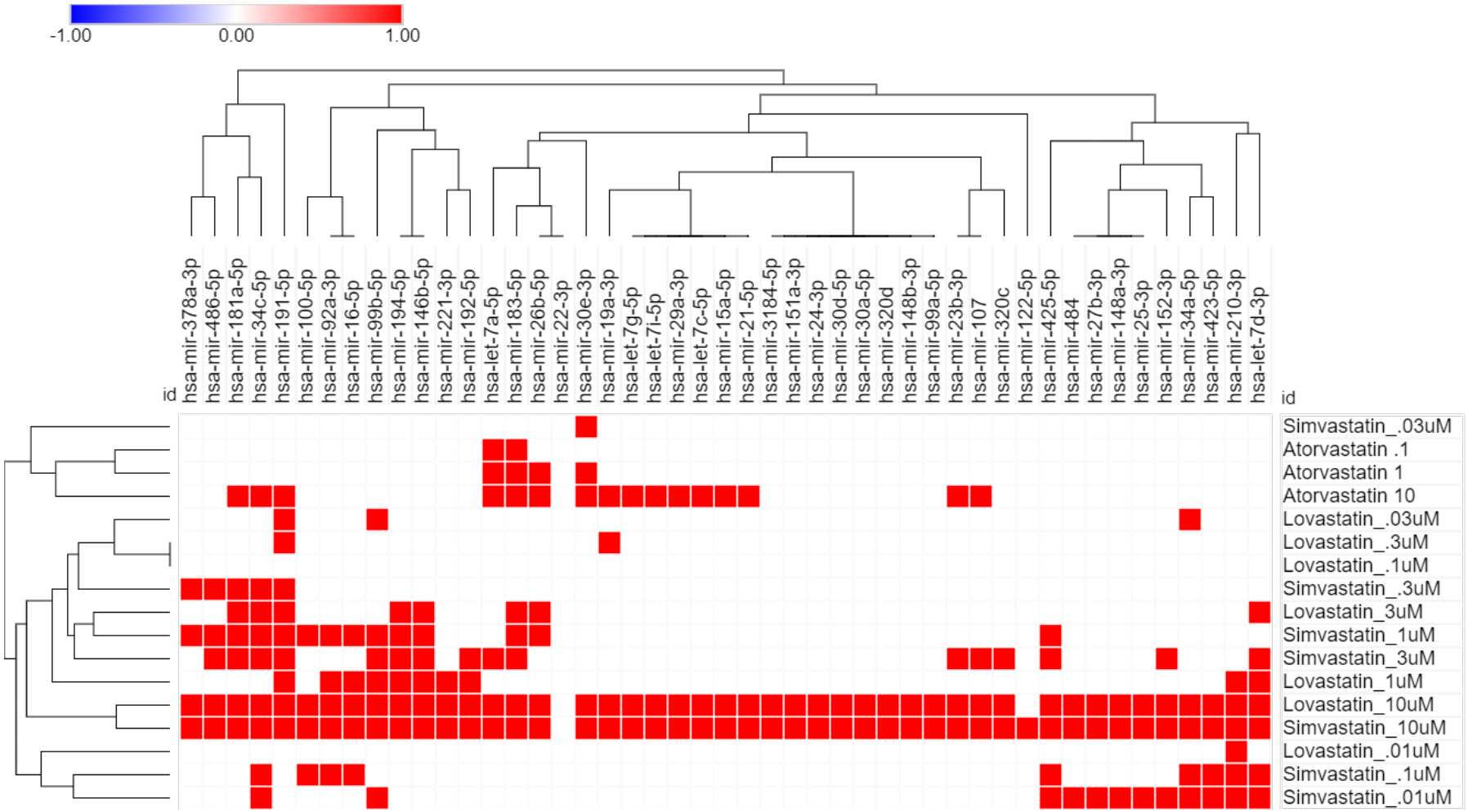






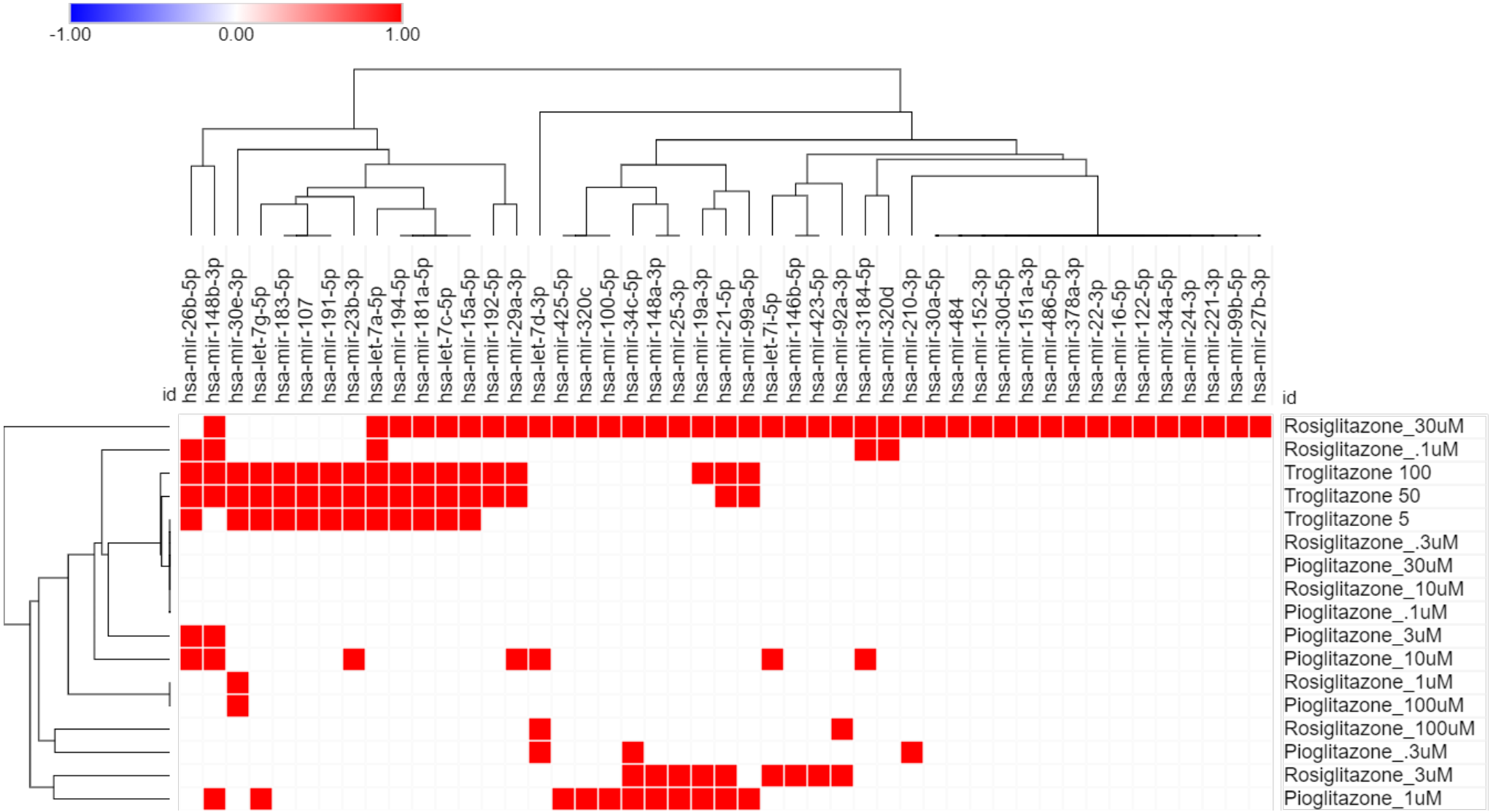
Unpublished results, please do not cite

BMAD threshold calls: Statins (HMGCR inhibitors)



Unpublished results, please do not cite

BMAD threshold calls: PPARg agonist



Unpublished results, please do not cite

Summary: HepaRG media study

- Established extracellular microRNA patterns linked to chemical mechanism-of-action
  - Cellular toxicity due to chemical exposure is correlating highly with the “shockwave” toxicity pattern
  - However, some signatures seen with non-toxic doses. Does this link to a specific MoA? Does it link with more apical cellular effect?

- Will link to gene expression networks and cellular microRNA alterations
  - HTTr data and small RNA sequencing are being performed for cell lystates
  - We will leverage this data and in silico prediction algorithms to identify correlations between miRNAs and gene expression regulation (node identification)

- Distinguish active versus passive release of miRNA into media (cellular response vs toxicity)

Conclusions

- **Overall, the evidence in these studies suggest microRNAs may serve as useful biomarkers for chemical screening and hazard identification in multiple toxicological contexts**

- In human populations, miRNAs in blood correlated with disease markers and exposure

- In short-term mouse studies of exposure, miRNAs linked to primary mechanism-of-action dose-dependently responded

- *In vitro*, non-destructive measurements of miRNA in media are indicative of mechanism-of-action

- Future studies will strengthen mechanistic relationship of miRNA alteration and cellular response

Acknowledgements: Anniston Study

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Virunya S. Bhat

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Pathfinder Innovation Projects

Pathfinder Innovation Projects challenge EPA scientists to answer the question, "Wouldn't it be amazing if we could ... ?"

US EPA

Gail Nelson

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Imran Shah

Bryant Chambers

Abcam

Mike Tackett

Thank you and any questions?