Toxicity Assessment of Treated Produced Water

- Whole effluent toxicity on aquatic organisms
- Zebrafish long-term exposure
- Human cell lines exposure
- Mice acute and chronic study
- Toxicological evaluation using mosquitoes

Yanyan Zhang

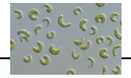
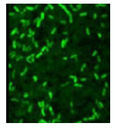
Civil and Environmental Engineering

John Xu

Biological Science

Disclaimer: The New Mexico Produced Water Research Consortium at New Mexico State University is conducting independent, science-based research to evaluate potential environmental and health effects related to the reuse of treated produced water for fit-for-purpose applications. This work is designed to generate objective data under controlled conditions, without promoting or opposing any specific reuse practices. Our goal is to provide clear, reliable information that supports informed decision-making around the safe and responsible use of produced water in New Mexico and beyond.

Whole Effluent Toxicity Assays Used

Organism	Species	Method	Type	Endpoint	Exposure time	Trophic level
Fish 	*Pimephales promelas* (Fathead	EPA 2000.0	Acute	Mortality	48 h	2nd-level consumer
		EPA	Chronic	Survival and Growth	7 days	
	Danio rerio (zebrafish)	OECD 236	Acute	Mortality	96 h	
Inv 	*Ceriodaphnia dubia*	EPA 2002.0	Acute	Mortality	48 h	1st-level consumer
		EPA 1002.0	Chronic	Survival and Reproduction	7 days	
Algae/p  t	*Raphidocelis subcapitata*	EPA 1003.0	Chronic	Growth	96 h	Producer
Bacteria  	*Vibrio fischeri*	ISO 11348:200	Acute	Bioluminescence Inhibition	15 min	Destroyer

NOEC: No observed effect concentration; **LOEC:** Lowest observed effect concentration;
IC_{25}: concentration causing 25% inhibition; **LC_{50}**: concentration causing 50% lethality;

WET Summary - Thermal Processes for Permian Basin PW

Organisms for WET assays	Scientific Name
Bacteria	*V. fischeri*
Algae	*R. subcapitata*
Water Flea	*C. dubia*
Zebrafish	*D. rerio*

Thermal #1

Raw PW

	NOEC (%)	LOEC (%)	IC_{25} or LC_{50} (%)	Effect at 100%
V. fischeri	<6.25	6.25	5.71	100
R.	<6.25	6.25	6.25	99.89
C. dubia	<6.25	6.25	1.69	100
D. rerio	<6.25	6.25	1.69	100

Thermal Distillate

	NOEC	LOEC (%)	IC_{25} or LC_{50}	Effect at 100%
V. fischeri	<6.25	6.25	8.66	100
R.	25	50	38.79	84.21
C. dubia	50	100	63.73	100
D. rerio	6.25	12.5	19.17	100

Post-treated Effluent (GAC+zeolite)

	NOEC	LOEC (%)	IC_{25} or LC_{50}	Effect at 100%
V. fischeri	100	>100	>100	3.29
R.	100	>100	>100	-21.06
C. dubia	100	>100	>100	0
D. rerio	100	>100	>100	0

Thermal #2

Thermal Distillate

	NOEC (%)	LOEC (%)	IC_{25} or LC_{50}	Effect at 100%
V. fischeri	3.125	6.25	40.36	42.37
R.	12.5	25	34.89	49.48
C. dubia	25	50	31.86	100
D. rerio	6.25	12.5	16.68	100

Post-treated effluent (GAC+zeolite)

	NOEC (%)	LOEC (%)	IC_{25} or LC_{50}	Effect at 100%
V. fischeri	3.125	6.25	>100	19.30
R.	100	>100	>100	8.42
C. dubia	100	>100	>100	5.0
D. rerio	100	>100	>100	8.11

NOEC: No observed effect concentration; **LOEC:** Lowest observed effect concentration;
IC_{25}: concentration causing 25% inhibition; **LC_{50}**: concentration causing 50% lethality;

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WET Summary - Thermal Processes for Permian Basin PW

Thermal #3

Post-treated effluent (Thermal + industry proprietary posttreatment)

20.3 mg/L methanol detected

		NOEC (%)	LOEC (%)	IC_{25} or LC_{50}	Effect at 100%
Algae	*R. subcapitata*	100	>100	>100	-21.15
Daphnia	*D. pulex*	50	100	>100	45
	C. dubia (2 d survival)	50	100	59.5	100
	C. dubia (reproduction)	50	100	59.26	100
Fish	*P. promelas* (4 d	50	100	>100	40
	P. promelas (7 d	50	100	70.71	100
	P. promelas (7 d	50	100	59.5	100

NOEC: No observed effect concentration; **LOEC:** Lowest observed effect concentration;
IC_{25}: concentration causing 25% inhibition; **LC_{50}**: concentration causing 50% lethality;

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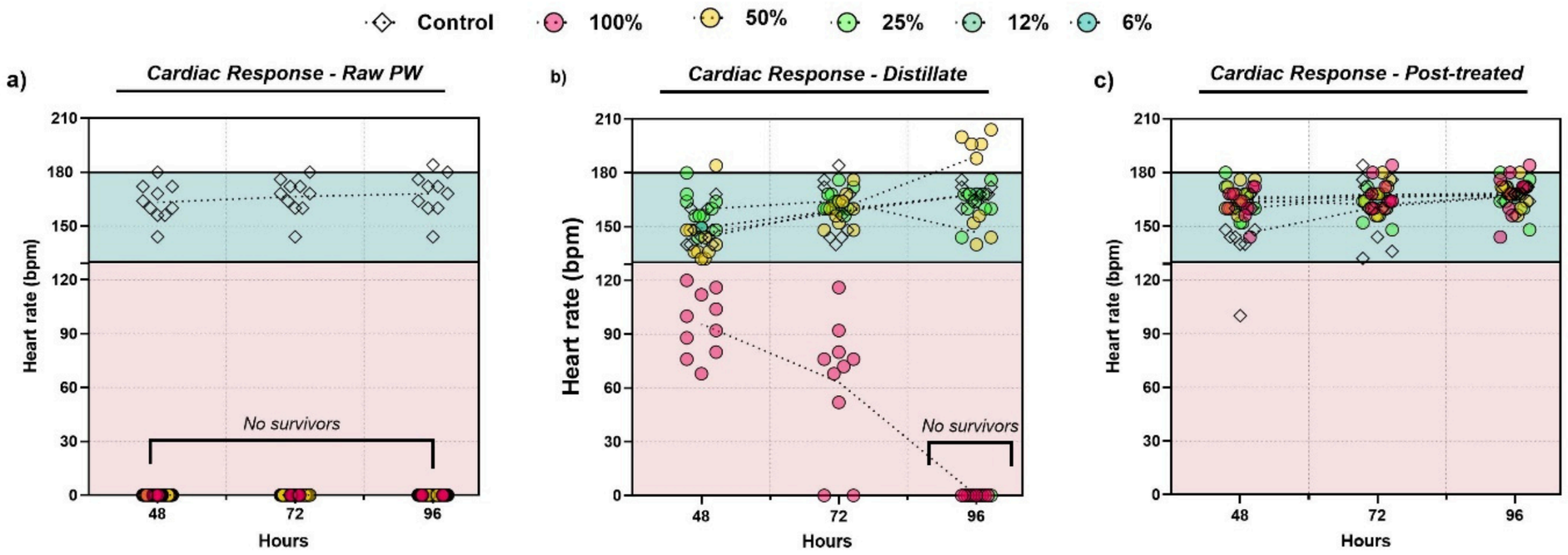
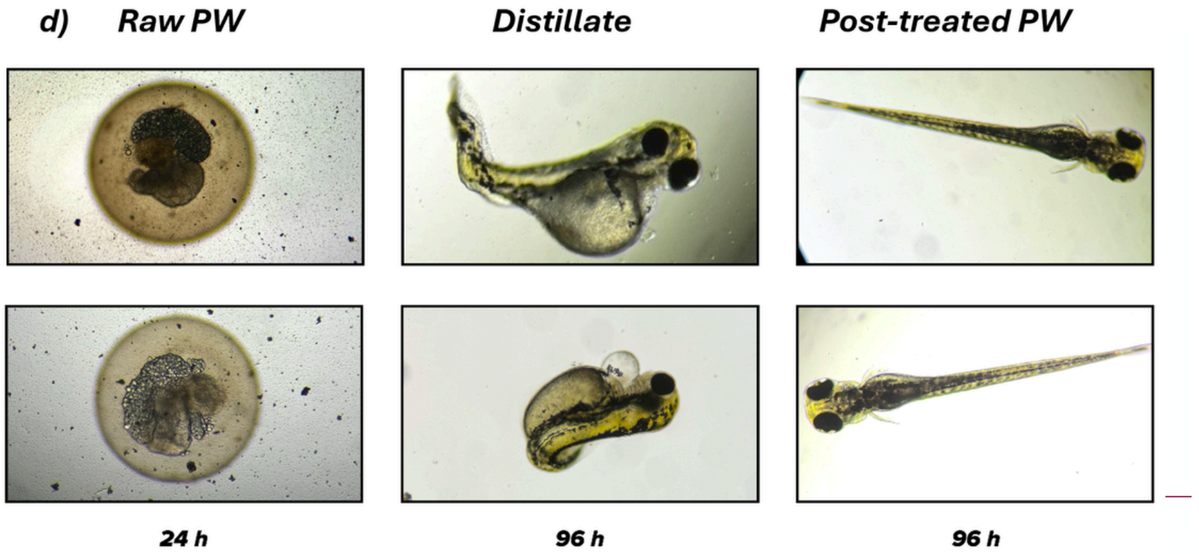
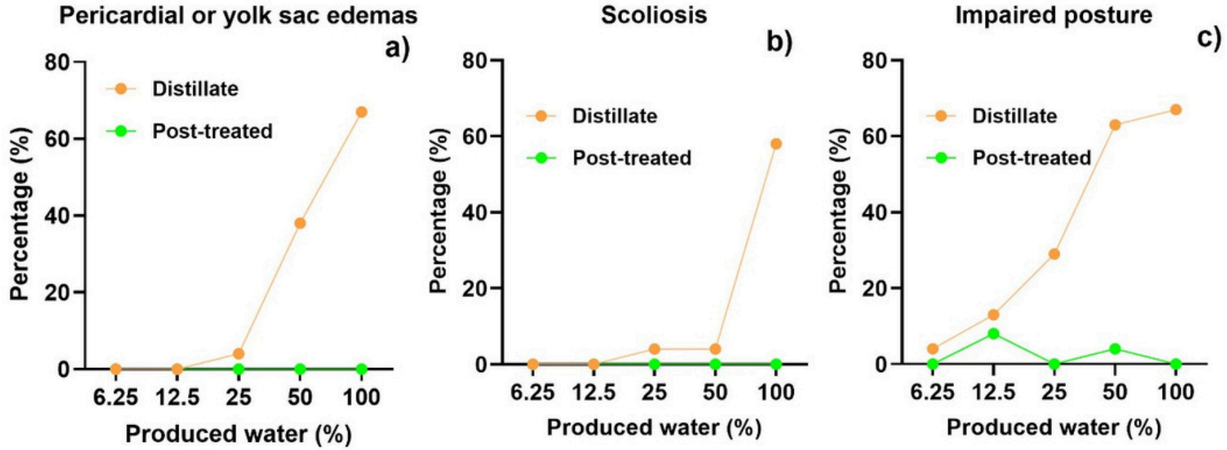
Thermal Process #1: Cardiac Response and microscope observation

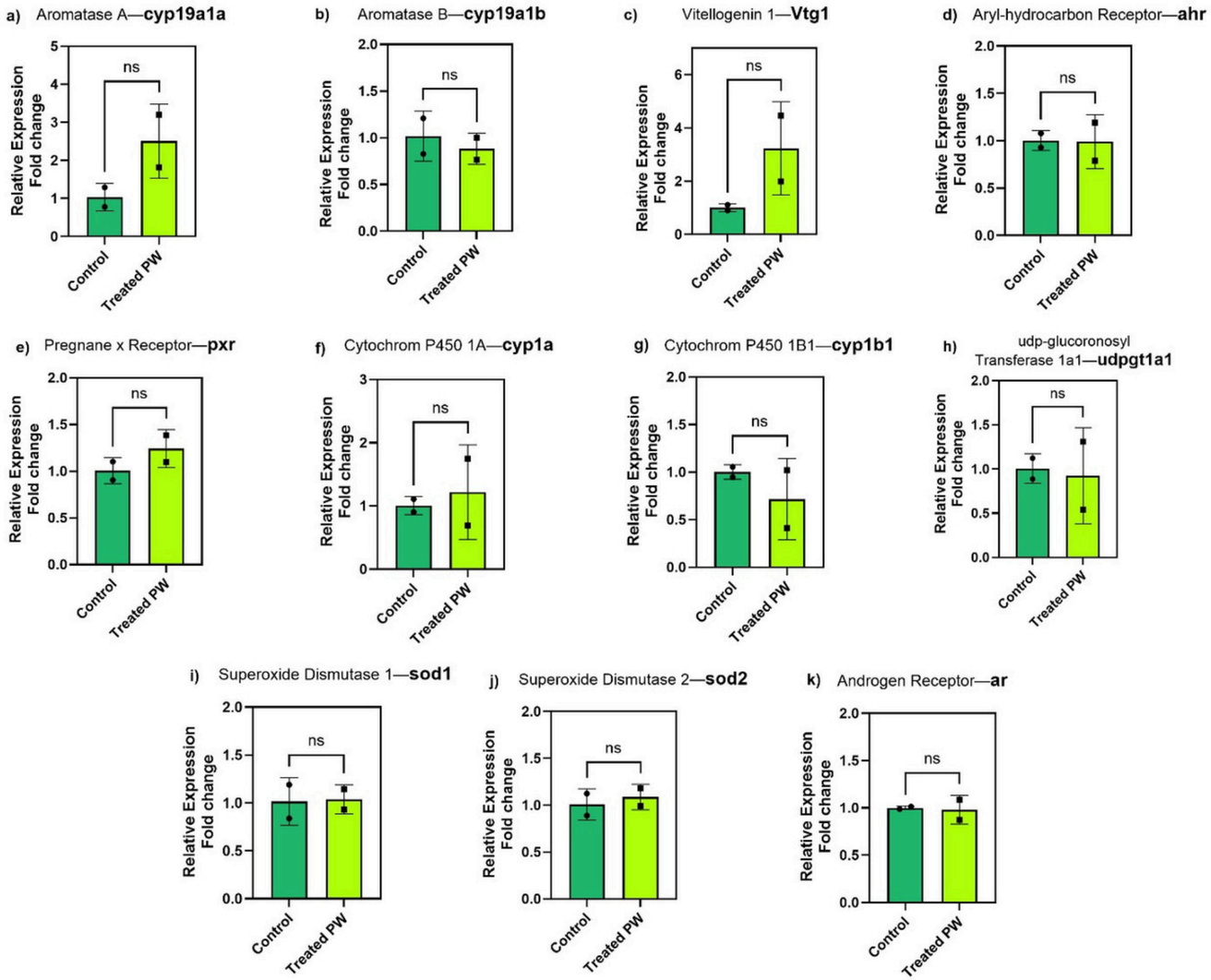






Thermal Process #1: Gene Expression in Zebrafish Embryos after 7 days Exposure

Gene Expression related to biotransformation, oxidative stress, and endocrine function



- No significant difference was observed between the control and post-treated PW groups

WET Summary - Membrane Processes for Permian Basin PW

RO permeate

Membrane #1

		NOEC (%)	LOEC (%)	IC_{25} or LC_{50} (%)	Effect at 100%
R. subcapitata	*4 d growth*	100	>100	>100	-44.6
C. dubia	2 d survival	100	>100	>100	5
	6 d survival	100	>100	>100	0
	reproduction	100	>100	>100	14.5
P. promelas	4 d survival	100	>100	>100	5
	7 d survival	100	>100	>100	2.6
	7 d growth	100	>100	>100	-13.1

RO permeate+post-treatment

Membrane

		NOEC (%)	LOEC (%)	IC_{25} or LC_{50} (%)	Effect at 100%
R. subcapitata	*4 d growth*	50	100	>100	11.7
C. dubia	2 d survival	100	>100	>100	0
	6 d survival	100	>100	>100	11.1
	reproduction	100	>100	>100	-1.6
P. promelas	4 d survival	100	>100	>100	5.1
	7 d survival	100	>100	>100	2.5
	7 d growth	100	>100	>100	1.9

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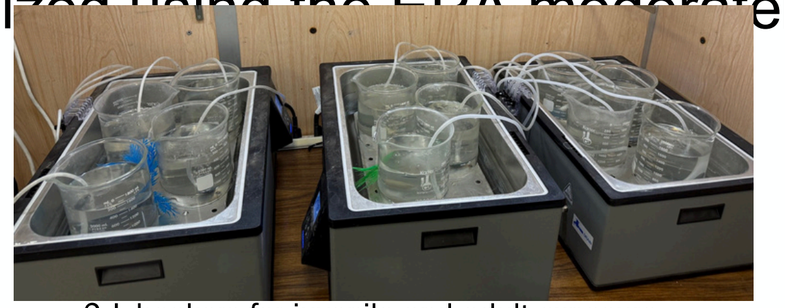
Zebrafish Long-term Exposure

	Embryo	Juvenile	Adult
Age	2 hpf (hours post-	2-month-old	8-month-old
Exposure time	7 days	98 days	28 days
Organism number per	72	10	20 (10 male and 10

- Water samples:
  - Control: City tap water after aquarium water filtration system (RO+IX)
  - tPW: Membrane process final products (TDS= 230 mg/L, TOC =0.72 mg/L, ammonia-N < 0.05 mg/L)
  - rPW: 400 times diluted raw PW (TDS = 450 mg/L, TOC =0.14 mg/L, ammonia-N = 1.45 mg/L)
  - All the water samples are mineralized using the EPA moderately hard water recipe and a

24-well plate for embryo exposure



2-L beakers for juvenile and adult exposure

Static-renewal (water change every 2 days)

Survival and Growth

Survival

1 fish died at Day 29

Juvenile Fish- Growth

$$r_2 = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} \times 100$$

Initial organism number:	**20 (10 per sex)**	**10**	**72**
Exposure time:	**28 days**	**98 days**	**7 days**

Antioxidant Enzyme Activities

Embryos

Superoxide dismutase

Catalase

Juveniles

Superoxide dismutase

Catalase

Adults

Superoxide dismutase

Male

Female

Catalase

Male

Female

Gene Expression for Xenobiotic Response

Cyp1a1: cytochrome P450 1A; *ahr*: aryl hydrocarbon receptor; *pxr*: pregnane x receptor

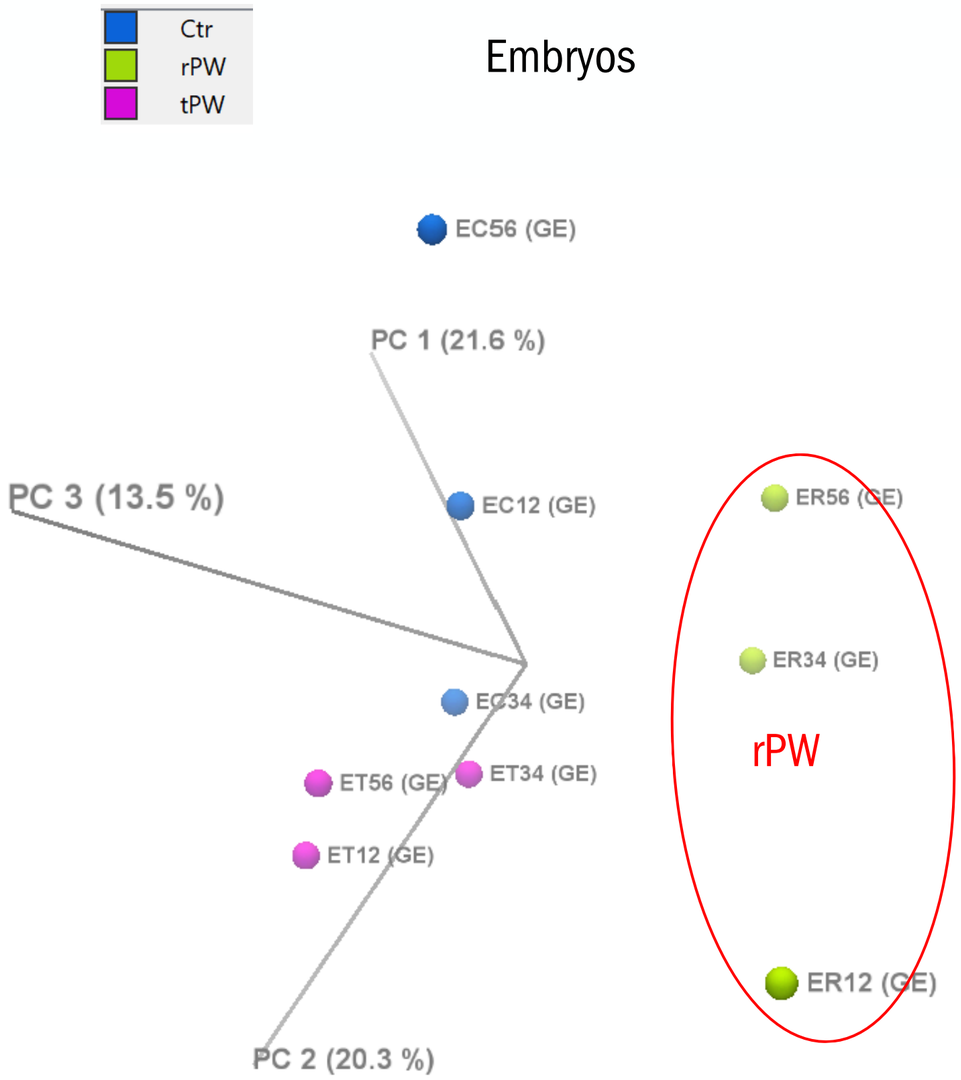
Embryos

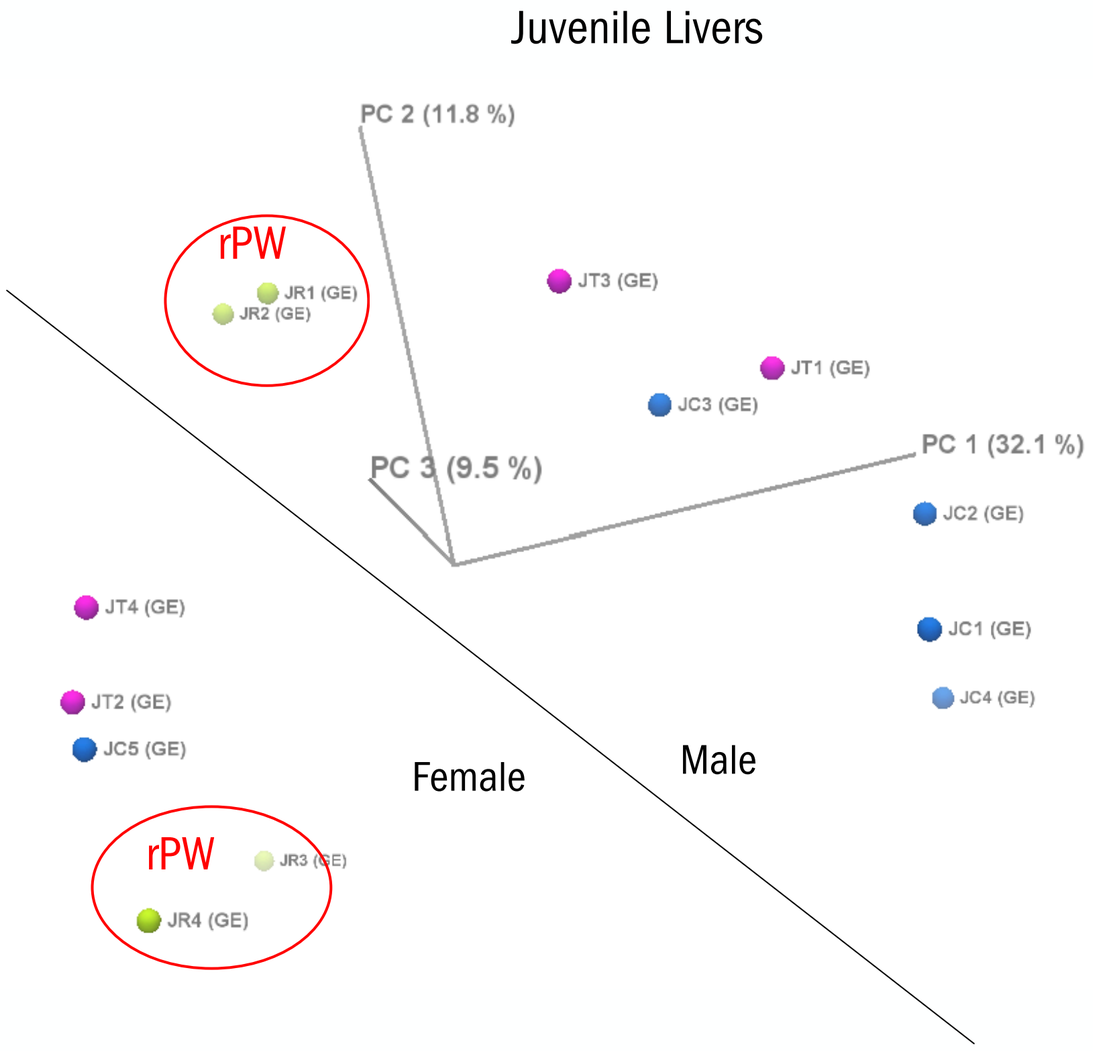
Juveniles

Male Adults

Female Adults

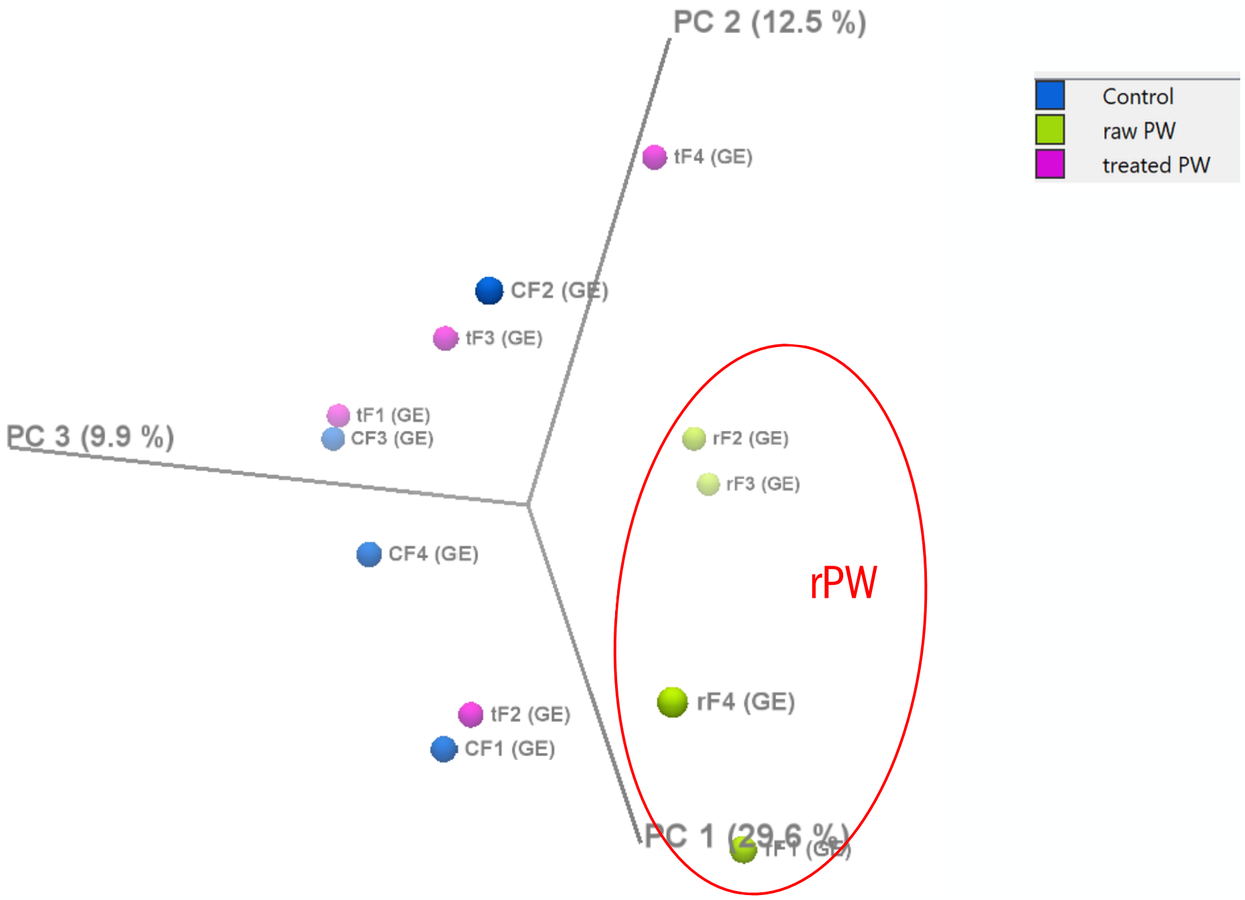
RNA-Seq PCA Analyses



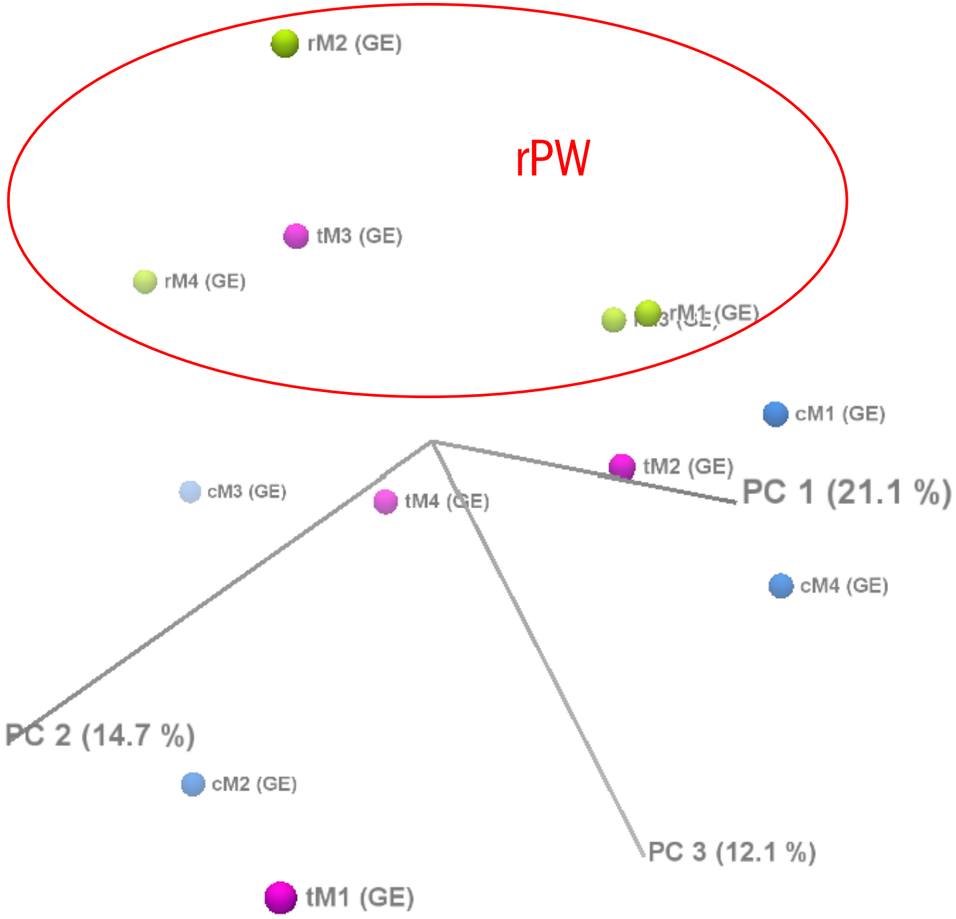


RNA-Seq PCA Analyses

Female adult livers

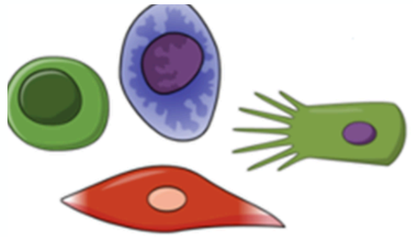


Male adult livers



Human Cell Toxicity Study

- Human cell lines:
  - MCF-7: breast cancer cells
  - HEK293: embryonic kidney cells
  - HepG2: liver cancer cells
  - A549: lung cancer cells
  - Caco-2: colon cancer cells



- Exposure time: 24 hours

- Endpoints:
  - Cell viability (ATP, enzyme activities, cell membrane damage), apoptosis, oxidative stress, activation of the aryl hydrocarbon receptor, estrogenic activities

Human Cell Line Summary-Thermal Processes for Permian Basin PW

Thermal #1

		NOEC (%)	LOEC (%)	IC_{25} (%)
Distillate	*MCF-7*	100	>100	>100
	HEK293	100	>100	>100
	Caco-2	100	>100	>100
Post-treated	*MCF-7*	100	>100	>100
	HEK293	100	>100	>100
	Caco-2	100	>100	>100

Thermal #2

		NOEC (%)	LOEC (%)	IC_{25} (%)
Distillate	*MCF-7*	100	>100	>100
	HEK293	100	>100	>100
	Caco-2	100	>100	>100
Post-treated	*MCF-7*	100	>100	>100
	HEK293	100	>100	>100
	Caco-2	100	>100	>100

Thermal #3

		NOEC (%)	LOEC (%)	IC_{25} (%)
Distillate	*MCF-7*	100	>100	>100
	HEK293	100	>100	>100
	HepG2	100	>100	>100
	A549	100	>100	>100
Post-treated	*MCF-7*	100	>100	>100
	HEK293	100	>100	>100
	HepG2	100	>100	>100
	A549	100	>100	>100

Human Cell Line Summary -Membrane Processes for Permian Basin PW

Membrane #1

		NOEC (%)	LOEC (%)	IC_{25} (%)
RO permeate (final product)	MCF-7	100	>100	>100
	HEK293	100	>100	>100
	HepG2	100	>100	>100
	A549	100	>100	>100

Membrane #2

		NOEC (%)	LOEC (%)	IC_{25} (%)
RO permeate	*MCF-7*	100	>100	>100
	HEK293	100	>100	>100
	HepG2	100	>100	>100
	A549	100	>100	>100
Final product	*MCF-7*	100	>100	>100
	HEK293	100	>100	>100
	HepG2	100	>100	>100
	A549	100	>100	>100

Sublethal Effect on Human Cells after Exposure

Activation of Aryl Hydrocarbon Receptor

- CYP1A1/CYP1B1 upregulation by gene expression
- Increased Ethoxyresorufin-O-deethylase (EROD) activity,
- Induced luminescence using commercially available luciferase reporter cells

Cell type	Process	Raw PW	Distillate	Post-treated
HepG2, MCF-7 or HEK293	Thermal #1	Yes	Yes	No
	Thermal #2	Yes	Yes	No
	Thermal #3	Yes	Yes	Yes

20.3 mg/L methanol detected in thermal #3 post-treated effluent

Cell type	Process	Raw PW	Permeate	Post-treated
HepG2, MCF-7 or	Membrane #1	Yes	Yes	NA*
	Membrane #2	Yes	Yes	No

Estrogenic activity (MCF-7 cells)

- CYP19A1 upregulation by gene expression
- Increased estrogenicity by E-screen Assay

	Raw PW	Distillate	Post-treated
Thermal #1	Yes	Yes	No
Thermal #2	Yes	Yes	No
Thermal #2	Yes	Yes	No

	Raw PW	RO Permeate	Final Products
Membrane #1	Yes	No	NA*
Membrane #2	Yes	No	No

*Post-treatment with GAC adsorption was conducted. The analysis is ongoing.

Summary

- Thermal/Membrane process followed by appropriate post-treatment effectively reduced the concentration of PW constituents at levels that do not exert toxic effects on the tested aquatic organisms.
- Treated PW did not impact the survival of embryos, juveniles, and adult fish in the long term. It did not induce oxidative stress, activate aryl hydrocarbon receptors, or cause a significant change in the transcriptomic response

- Thermal distillate and membrane permeate had no impact on human cell viability; The thermal distillate and membrane permeate still have contaminants that activate the aryl hydrocarbon receptor, highlighting the importance of post-treatment. The integrated processes can also remove compounds

Mouse Toxicity Tests

- **Mouse strain:** outbred strain, natural genetic background (no specific genotypes)
- **Water types**
  - Control water (Ctr):
    - City tap water after home countertop water filtration system (prefilter+activated carbon+RO+post carbon+UV)
  - Salt control water (Salt Ctr):
    - 7 g/L NaCl solution
  - Raw Produced Water (rPW):
    - 20 x dilution (5% rPW) for acute;
      - **TDS= 9000 mg/L, TOC= 2.86 mg/L, NH_3-N= 26.1 mg/L**
    - 400 x dilution (0.25% rPW) for subchronic exposure
      - **TDS= 450 mg/L, TOC= 0.14 mg/L, NH_3-N= 1.45 mg/L**
  - Two treated Produced Water (tPW):
    - tPW1: Membrane Process
      TDS= 288 mg/L, TOC= 0.77 mg/L, NH_3-N = 29.5 mg/L
    - tPW2: Membrane + post-treatment + remineralization
      TDS= 231 mg/L, TOC= 0.72 mg/L, NH_3-N < 0.05 mg/L

Experimental schem

Acute

28-day exposure

10-day exposure

Ctr	tPW1	tPW2	5% rPW	Salt (7g/L NaCl)
F, n = 5	F, n = 5	F, n = 5	F, n = 2	F, n = 2
M, n = 5	M, n = 5	M, n = 5	M, n = 2	M, n = 2

Subchronic

100-day exposure

Ctr	0.25% rPW	tPW1	tPW2
F, n=10	F, n=10	F, n=10	F, n=10
M, n = 10	M, n = 10	M, n = 10	M, n = 10

Liver/Body Weight Ratio

	Female	Male
Acute study		Salt ctr water caused liver enlargement
		Treated PW showed no effect
Subchronic study		0.25% rPW resulted in female liver enlargement in long term exposure

Mice Study Summary- Weight gain, Hematology, and Biochemistry

Acute Study (28 days)

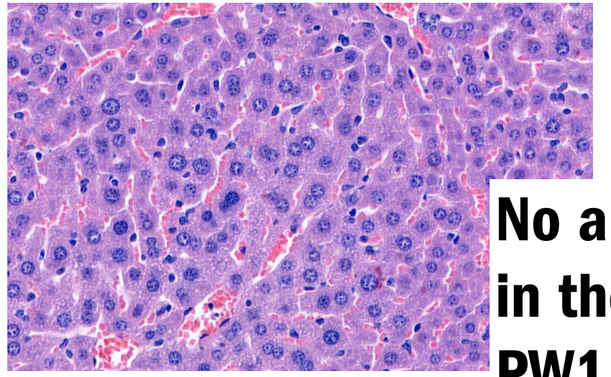
	tPW1		tPW2		5% rPW		Salt control	
	Female	Male	Female	Male	Female	Male	Female	Male
Weight gain	No change	No change	No change	No change	No change	No change	No change	No change
Hematology	No change	No change	No change	No change	No change	No change	Low Mean Corpuscular	No change
Blood Biochemistry	Low globulin	High sodium	No change	No change	High	High Alkaline	High	High Alkaline

Sub-chronic Study (100 days)

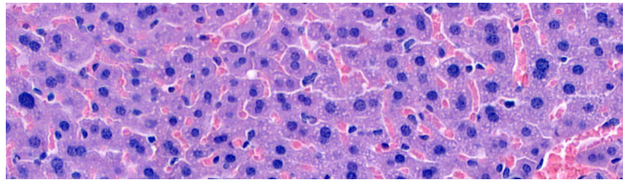
	tPW1		tPW2		0.25% rPW	
	Female	Male	Female	Male	Female	Male
Weight gain	No change	No change	No change	No change	No change	No change
Hematology	No change	Low platelet count/volume/	No change	No change	Low red blood cell count, hemoglobin, hematocrit	Low hemoglobin, hematocrit, and mean corpuscular
Blood Biochemistry	No change	Low globulin	No change	No change	Low albumin, calcium, sodium, total protein	Low albumin, calcium, sodium, total protein, globulin

Histology images

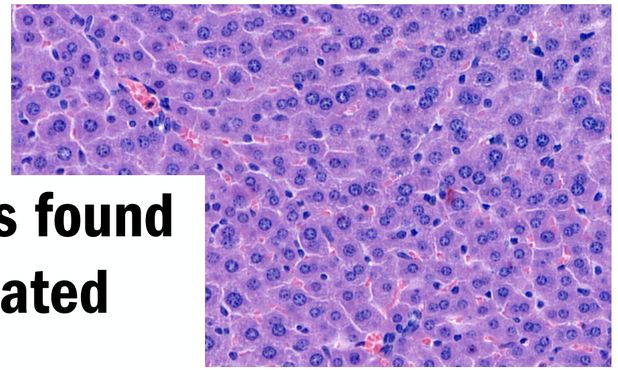
Control-female (X40)



tPW 1-female (X40)

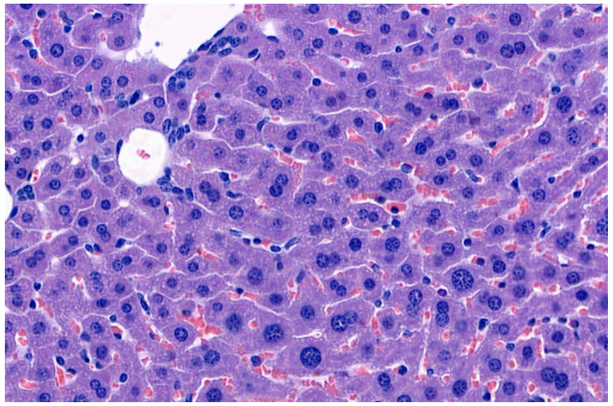


tPW 2-female (X40)

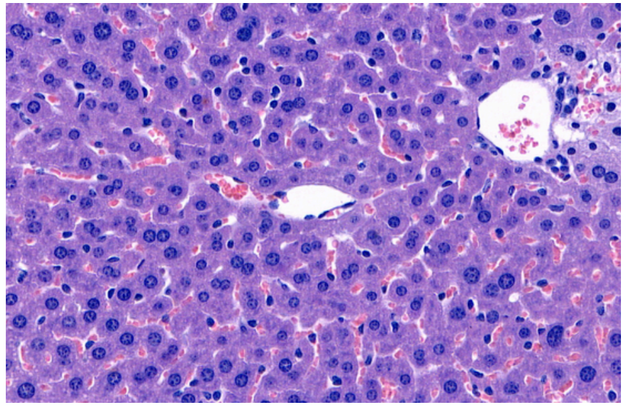


No abnormal cell structure was found in the livers exposed to the treated PW1 and PW2.

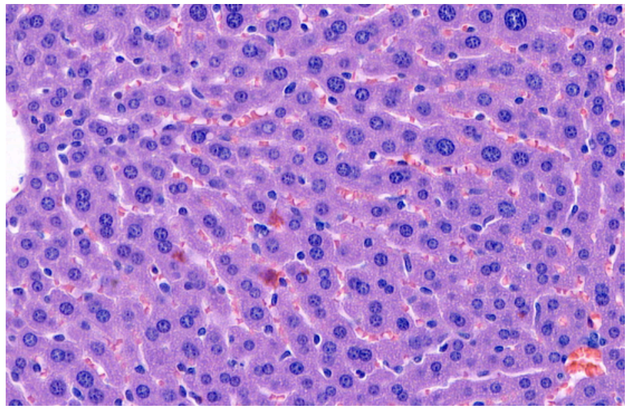
Control-male (X40)



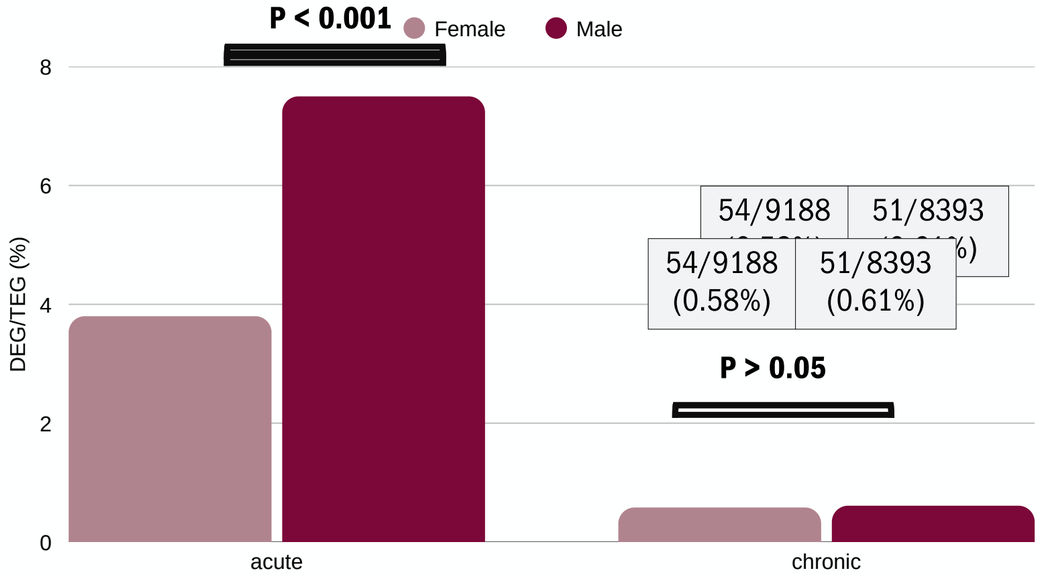
tPW 1-male (X40)



tPW 2-male (X40)

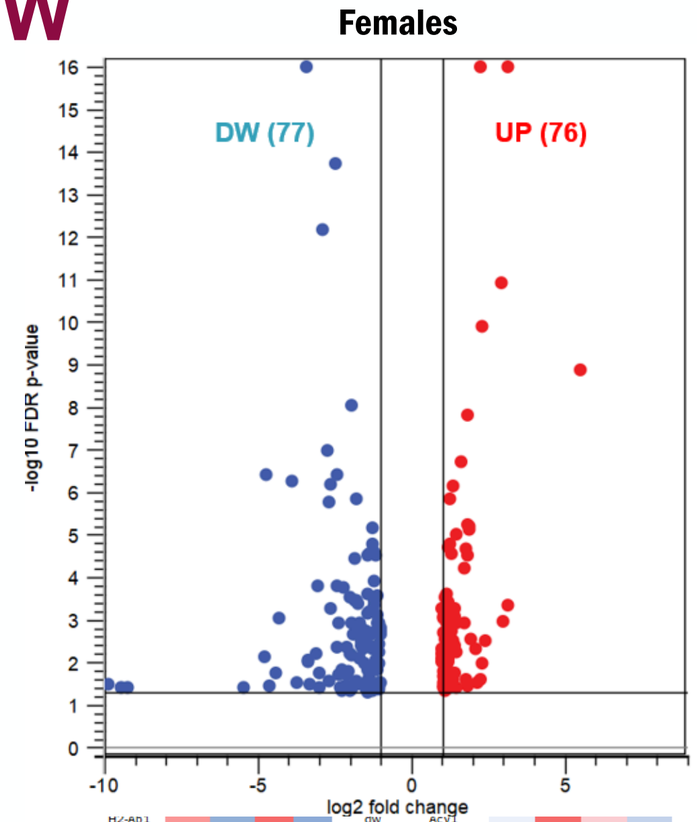


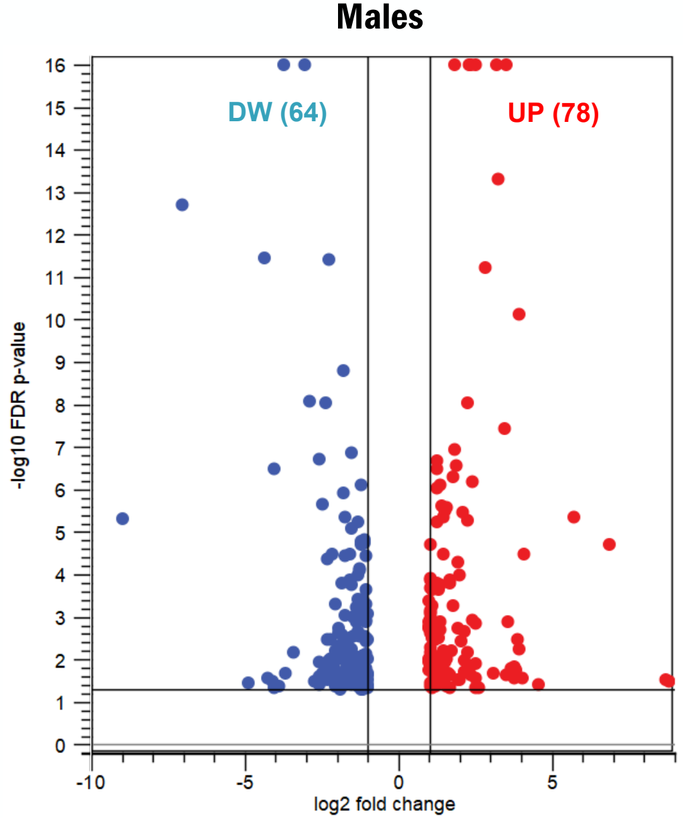
Transcriptomic analysis

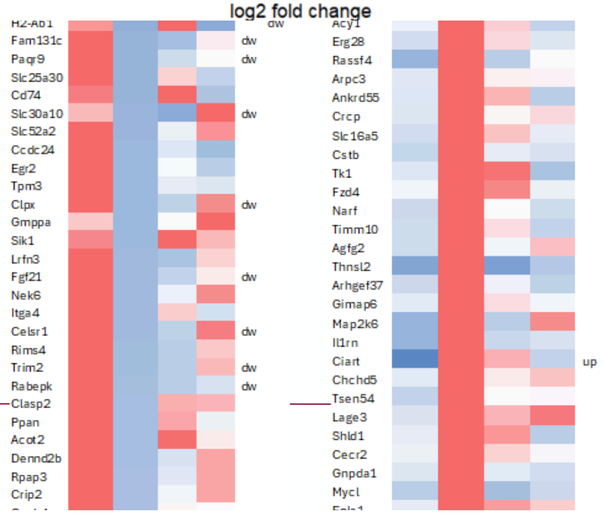


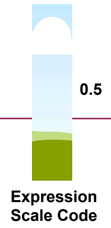
- **Sex dimorphic responses: males have more genes responsive to 0.5% rPW upon acute exposure**
- **Many fewer genes are responsive to 0.25% of rPW upon chronic exposure.**
- **Dilution significantly reduced the toxic effects.**

Treatments reversed most DEGs induced by rPW









DEGs in display

TPM ≥ 10
FDR p<0.05
|>| 2 fold

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Functional outcome based on the annotation of DEGs

→ **Metabolic stress**

→ **Xenobiotic detoxification activation**

→ **Lipid and cholesterol metabolic remodeling**

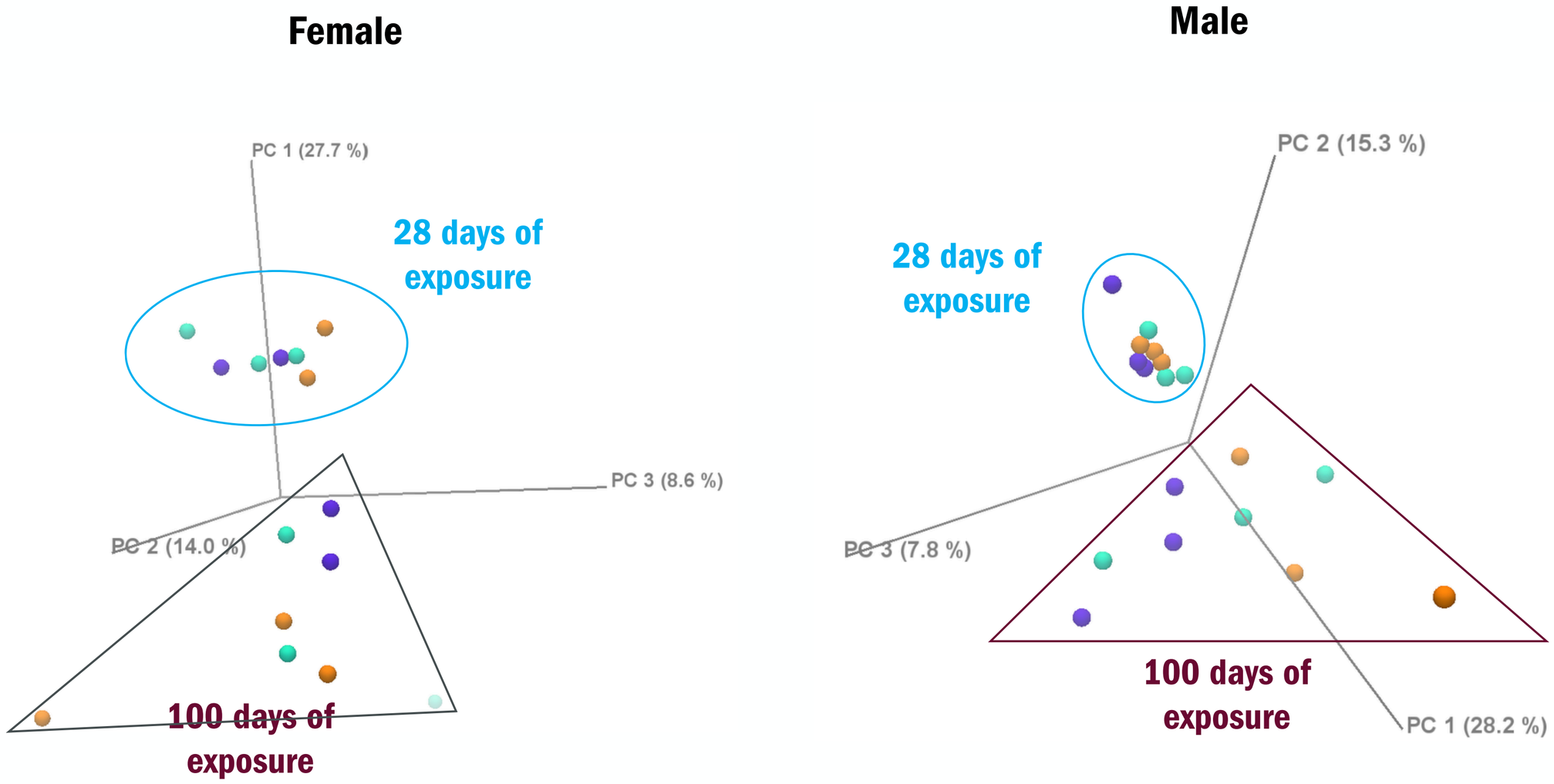
→ **Oxidative and inflammatory imbalance**

→ **Suppressed immune and endocrine signaling**

→ **Early hepatotoxicity or adaptive liver remodeling**

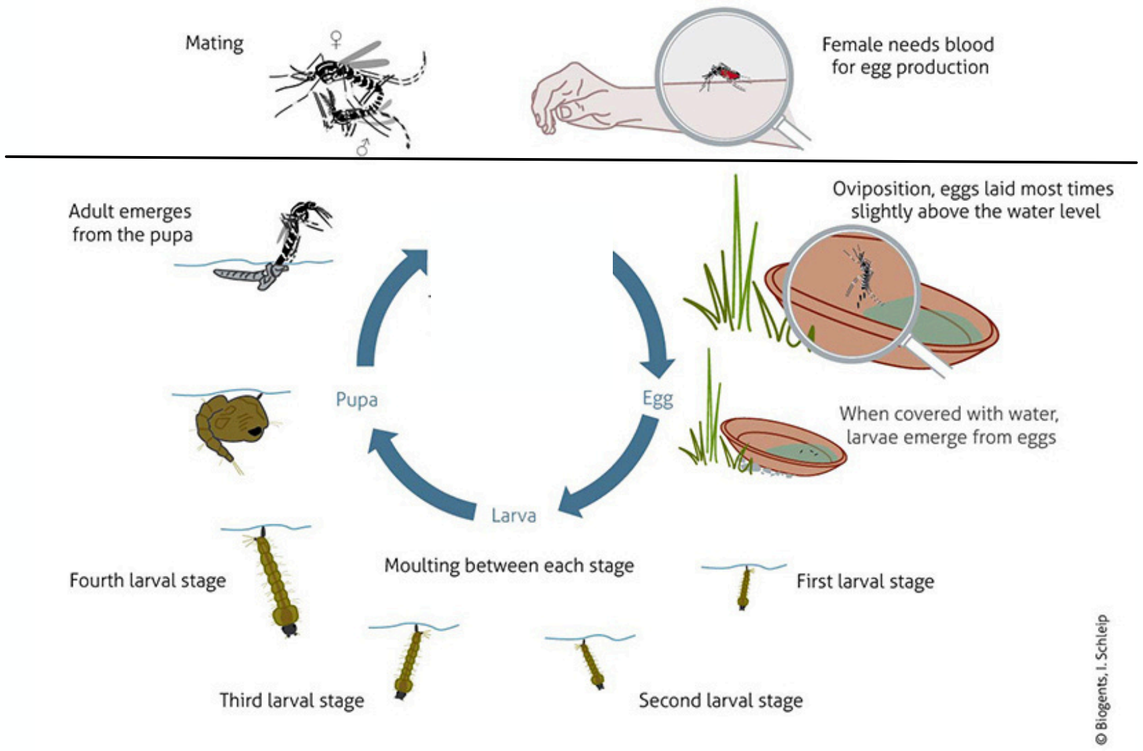
This is consistent with exposure to complex mixtures of hydrocarbons, surfactants, metals, and endocrine-active compounds commonly found in produced water.

Ctr and tPWs have similar transcriptomic profiles



Toxicological Evaluation Using Mosquitoes as a Model

Terrestrial adult stage (months)



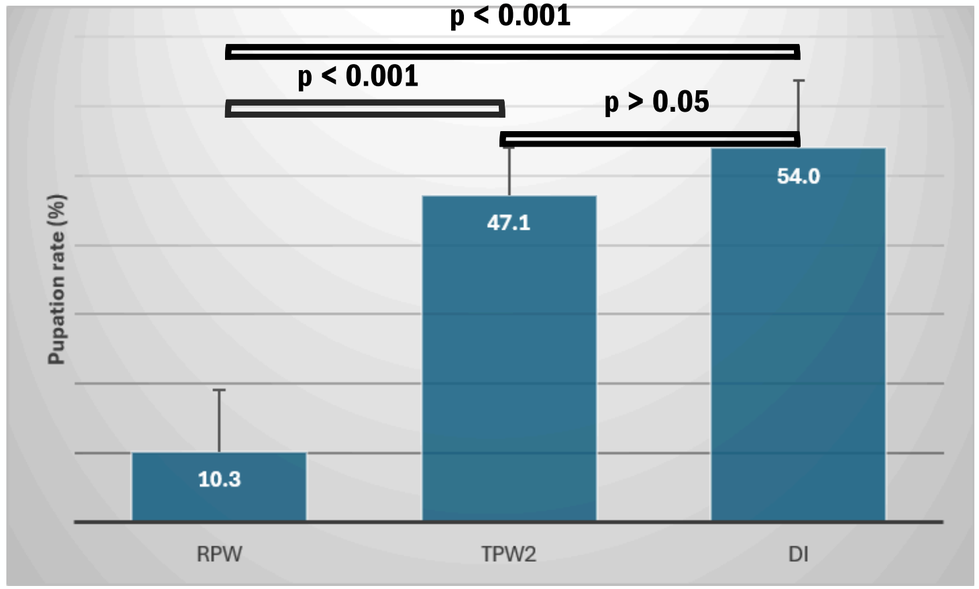
Aquatic larval stage
(10-12 days)

This obligate biphasic life history allows for an integrated bioassay to **quantify aquatic toxicity via**

- **pupation success**
- **reproductive in adults**

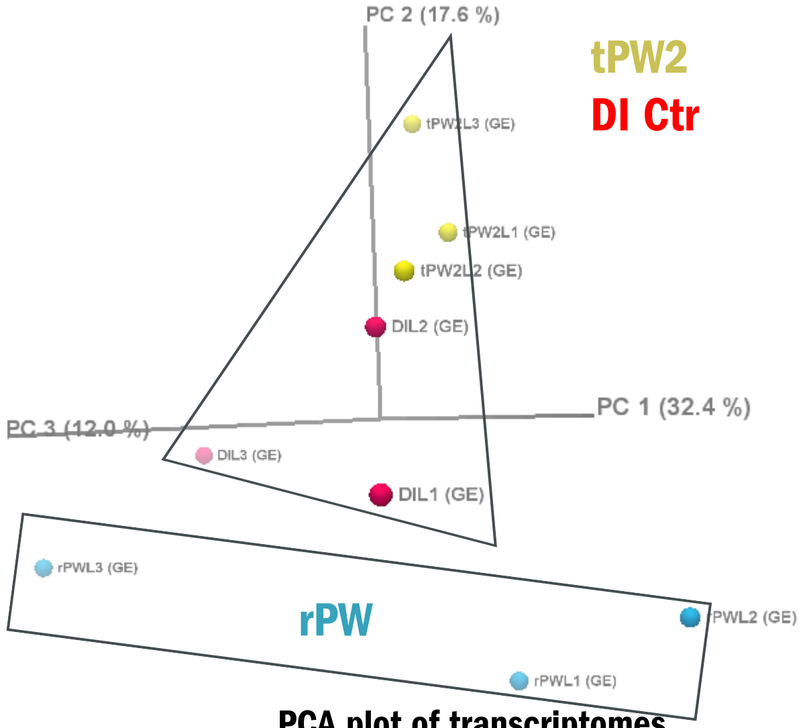
Pupation Rate and Larval Transcriptome Response

The 0.25% of rPW significantly reduced pupation success. tPW2 and Ctr water show the same pupation rate



Pupation success (R=3)

The 0.25% of rPW induced a larval transcriptomic pattern distinct from that of the tPW2 and Ctr water.



PCA plot of transcriptomes

Summary

- 5% rPW exposure caused changes in a few measures in blood biochemical assays and strongly induced liver transcriptomic responses in the 10-day-exposure setting.
- 0.25% rPW caused abnormal red blood cells and platelet indicators and reduced the calcium, sodium, albumin, total protein, and globulin concentration after 100-day exposure. It also caused a mild change in the liver transcriptome.
- There is no significant difference between the mice fed with control water and treated PW after polishing (tPW2)
  - No difference in weight gain, live/weight ratio, blood hematology, and biochemistry indicators.
  - Liver histology indicates no liver cell morphology difference between the tPWs and Ctr.
- Treated produced water, desalination (tPW1) and desalination +purification (tPW2) have similar transcriptomic profiles.
- The mosquito model can be adopted to assess the ecological impact on a broad range of invertebrate organisms.

NM STATE NMPWRC New Mexico Produced Water Research Consortium

Toxicity Summary for Permian Basin Treated PW

Non-toxic Toxic A: AhR activation E: Induced estrogenicity

		Thermal Process #1		Thermal Process #2		Thermal Process #3		Membrane Process #1		Membrane Process #2	
		Distillate	Pos-	Distillate	Pos-	Distillate	Pos-	Desalinate	RO	RO	Post-
WET assays	*V. fischeri*										
	R.										
	C. dubia										
	D. rerio										
	P. promelas										
Human Cell lines (cell viability/Ah	*HEK293*										
	Caco-2										
	HepG2						A		A		
	A549										
Zebrafish	*Embryo*										
Mice	*Acute*										
	Chronic										
	Chronic										

Published Papers on Toxicity Study

Thermal Process #1 (Chemical and WET assays)

Journal of Hazardous Materials 511 (2026) 142274

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Advancing produced water quality via integrated mechanical vapor recompression: Chemical and toxicological characterization, hazard identification and mitigation

Yeinner Tarazona [a], Joseph Alexander [b], Mike Hightower [c], Himali M.K. Delanka-Pedige [a,d], Huiyao Wang [a], Pei Xu [a], Yanyan Zhang [a,*]

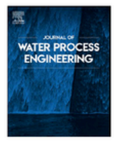
Thermal Process #2 (Chemical and WET assays)

Journal of Water Process Engineering 67 (2024) 106146

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe



Treatment of produced water from the Permian Basin: Chemical and toxicological characterization of the effluent from a pilot-scale low-temperature distillation system

Journal of Hazardous Materials 478 (2024) 135549

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Benchmarking produced water treatment strategies for non-toxic effluents: Integrating thermal distillation with granular activated carbon and zeolite post-treatment

Yeinner Tarazona [a], Haoyu B. Wang [b], Mike Hightower [c], Pei Xu [a], Yanyan Zhang [a,*]

[a] Department of Civil Engineering, New Mexico State University, Las Cruces, NM 88003, USA
[b] The University of Washington, Seattle, WA 98195, USA
[c] New Mexico Produced Water Consortium, New Mexico State University, Las Cruces, NM 88003, USA

Thermal Process #1 and #2 (Human cell cytotoxicity)

Ecotoxicology and Environmental Safety 302 (2025) 118726

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Comprehensive cytotoxicity assessment of treated produced water from thermal distillation using human cell lines

Senuri Wijekoon [a], Yeinner Tarazona [a], Mike Hightower [b], Huiyao Wang [a], Pei Xu [a], Yanyan Zhang [a,*]

[a] Department of Civil Engineering, New Mexico State University, Las Cruces, NM 88003, USA
[b] New Mexico Produced Water Consortium, New Mexico State University, Las Cruces, NM 88003, USA

Membrane Process #1 (Chemical, WET assay, and Human cell cytotoxicity)

Separation and Purification Technology 394 (2026) 137642

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Pilot-scale evaluation of hypersaline produced water treatment and reuse via freeze desalination and seawater reverse osmosis: Organic removal, aquatic and human cell line toxicity studies

E.M.N. Thiloka Edirisooriya [a], Adrianne A. Lopez [b], Danielle Smith [b], Punhasa S. Senanayake [a], P.S. Weerasinghe [a], Yanyan Zhang [a], Himali M.K. Delanka-Pedige [a,c], Pei Xu [a,*], Huiyao Wang [a,*]

[a] Department of Civil and Environmental Engineering, New Mexico State University, Las Cruces, NM 88003, United States
[b] Texas Pacific Water Resources, 15 Smith Rd Suite 2012, Midland, TX 79705, United States
[c] Department of Civil Engineering, Southern Illinois University, Carbondale, IL 62901, United States