The Effect of Developmental Exposure to Hydraulic Fracturing Chemicals on Adipocyte Size

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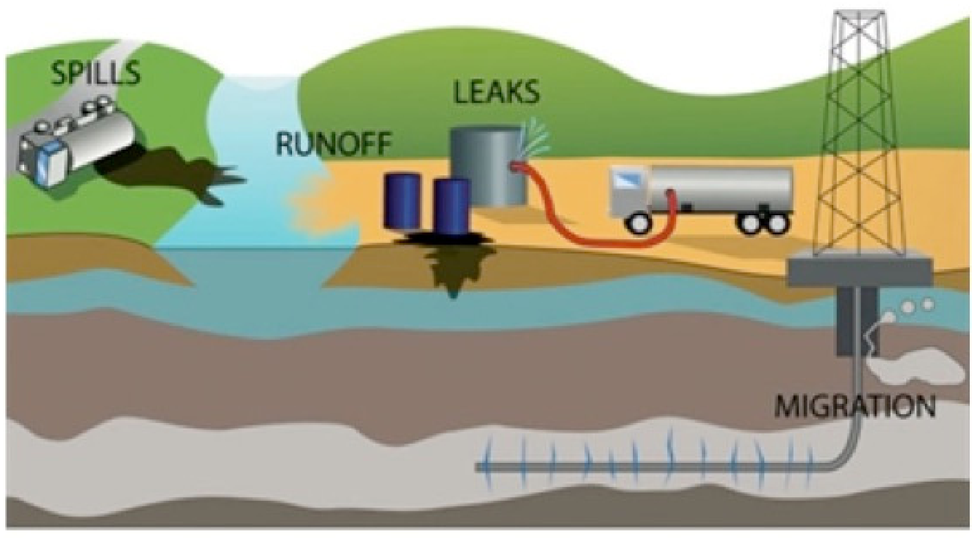
Introduction

- Hydraulic fracturing (HF) is the process of extracting natural gas and oil from rock using high-pressure injections of a mix of water and chemicals.
- The practice of HF is on the rise in many states across the country.
- The HF process is exempt from sections of six federal regulatory acts, notably the Safe Drinking Water Act and Clean Water Act, leaving oversight of the process to individual state governments.
- Over 1,000 chemicals are used in the extraction process, some of which are known endocrine disruptors, neurotoxins, and carcinogens.
- Endocrine disrupting chemicals (EDCs) have been shown to alter the function of hormones involved in the normal differentiation of adipocytes.
- Exposure to EDCs early in development may increase adipocyte size, thus predisposing an exposed individual to obesity and metabolic insufficiencies later in life.

Hypothesis

- **Many known endocrine disrupting chemicals, some of which are used in hydraulic fracturing, have the ability to disrupt the normal differentiation of adipocytes.**
- **Developmental exposure to a mixture of hydraulic fracturing chemicals between gestational day one and postnatal day 21 will result in increased adipocyte size as measured in gonadal fat pads of mouse pups.**

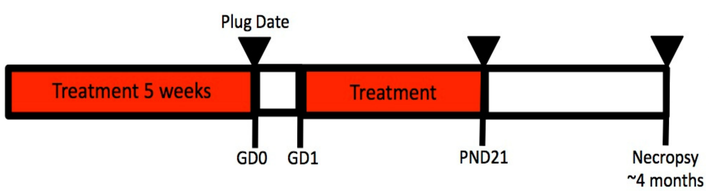
Potential Sources of Water Contamination



(Ritha & Rahm, Framework for Addressing Water Resource Impacts from Shale Gas Drilling (2010))

Figure 1: Graphic representation of possible routes of contamination for surface and ground water surrounding hydraulic fracturing. This contaminated water may eventually enter drinking water near drilling sites.

Exposure Window



- **Vehicle Control**: 0.2% ethanol
- **Mix 1.5**= 1.5 µg/kg/day
- **Mix 15**=15 µg/kg/day
- **Mix 150**=150 µg/kg/day
- **Mix 1500**=1500 µg/kg/day
- **Flutamide**=166.67 µg/kg/day
  - Positive control anti androgen

Materials and Methods

Chemicals: C57BL/6J mice were time-mated and pregnant females were administered a mixture of 23 commonly used HF chemicals (Figure 2), at each of four concentrations, flutamide, or an ethanol vehicle via drinking water from gestational day one to postnatal day 21.

Histology and Image Capture: Right gonadal fat pads from male pups of treated dams were removed, fixed in a 10% neutral buffered formalin solution, and dehydrated as standard before embedding in paraffin wax. Sections (5 µm) were cut and mounted on positively-charged glass slides and hematoxylin and eosin (H&E) staining was performed as standard. Bright-field images were captured at 10x optical magnification with a Olympus XM10 color camera microscope from Terzic Instruments.

Quantification of Fat and Lean Mass: Fat and lean masses were measured via EchoMRI—using an EchoMRI 4in1/1100 system.

Adipocyte Area Analysis: MetaMorph Microscopy Automation and Image Analysis Software was used to measure the area of 50 adipocytes per visual field. The absolute pixel area of each object was calculated and converted to µm^2. [Objects that were too small to classify as cells (<240 µm^2) were excluded as artifacts.] Microsoft Excel was used to calculate the average of all 50 measured adipocytes per animal.

HF Chemical Information

1,2,4-trimethylbenzene
2-(2-methoxyethoxy) ethanol
2-ethylhexanol
Acrylamide
Benzene
Bronopol
Cumene
Diethanolamine
Dimethyl formamide
Ethoxylated nonylphenol
Ethoxylated octylphenol
Ethylbenzene
Ethylene glycol
Ethylene glycol butyl ether
Methyl-4-isothiazolin
Napthalene
Phenol
Propylene glycol
Sodium tetraborate decahydrate
Styrene
Toluene
Triethylene glycol
Xylenes

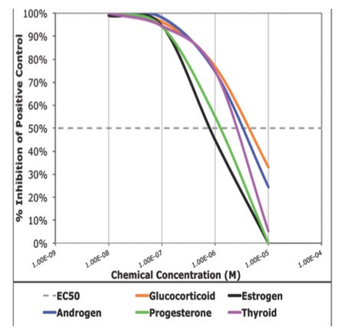


Figure 2: (A) Simulated HF fluid with a mixture of 23 common chemicals. **(B)** Antagonist activity of HF mixture in nuclear receptor reporter gene assays in human endometrial Ishikawa cancer cells for five nuclear receptors.

Adipocyte Histology

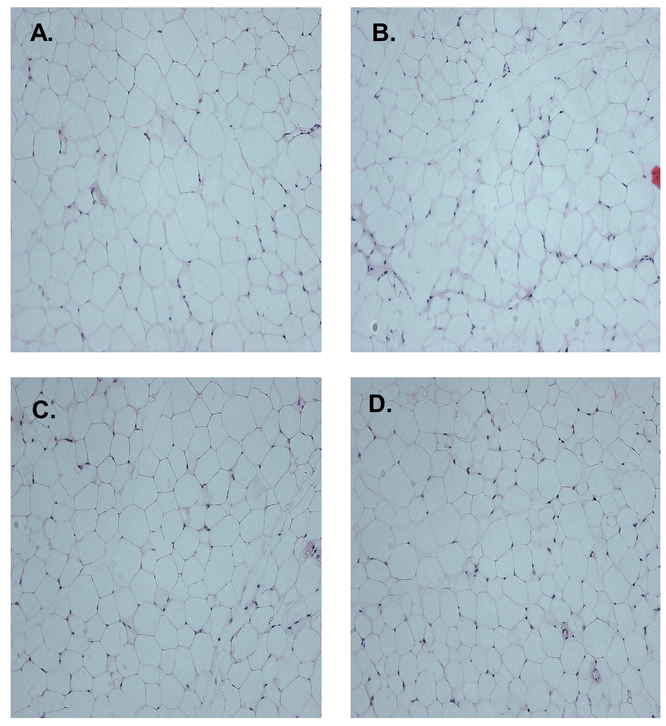
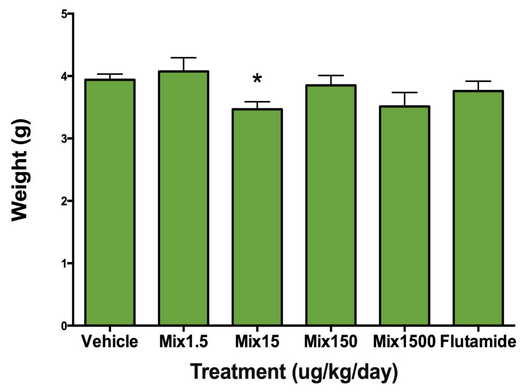
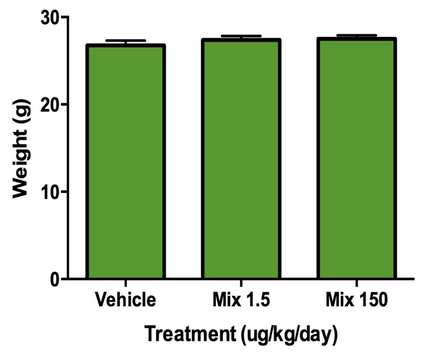


Figure 3: Image captures of adipocytes measured in gonadal fat pads. **(A)** Ethanol Vehicle. **(B)** Treatment Mix 15. **(C)** Treatment Mix 1500. **(D)** Positive Control Treatment of Flutamide.

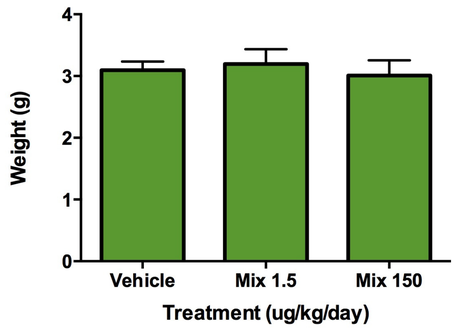
PND7 Body Weight



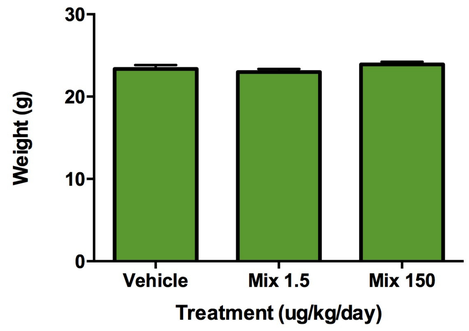
Necropsy Weight



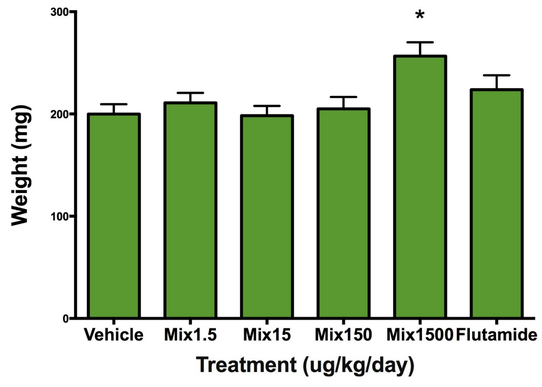
Fat Mass



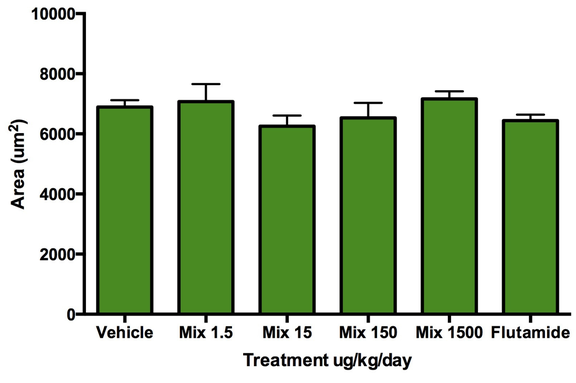
Lean Mass



Gonadal Fat Pad Weight



Average Adipocyte Area



Results and Conclusions

1. Male pups of dams treated with 1500 ug/kg/day of HF chemicals had heavier right gonadal fat pads than other treatment groups.
2. The average area of adipocytes did not vary significantly between treatment groups, though the trend follows that of gonadal fat pad weight..
3. HF chemicals did not influence average adipocyte area of male C57BL/6J pups exposed between GD 1 to PND 21.

Future Directions

1. Perform further statistical analysis considering various covariates such as necropsy location, necropsy date, and pups per litter.
2. Apply same experimental methods using a different mouse model, such as the CD1 strain.
3. Quantify immune cells in gonadal fat pads between treatment groups.