

Metabolic Disrupting Potential of Unconventional Oil and Gas Operation Chemicals and Mixtures



Duke
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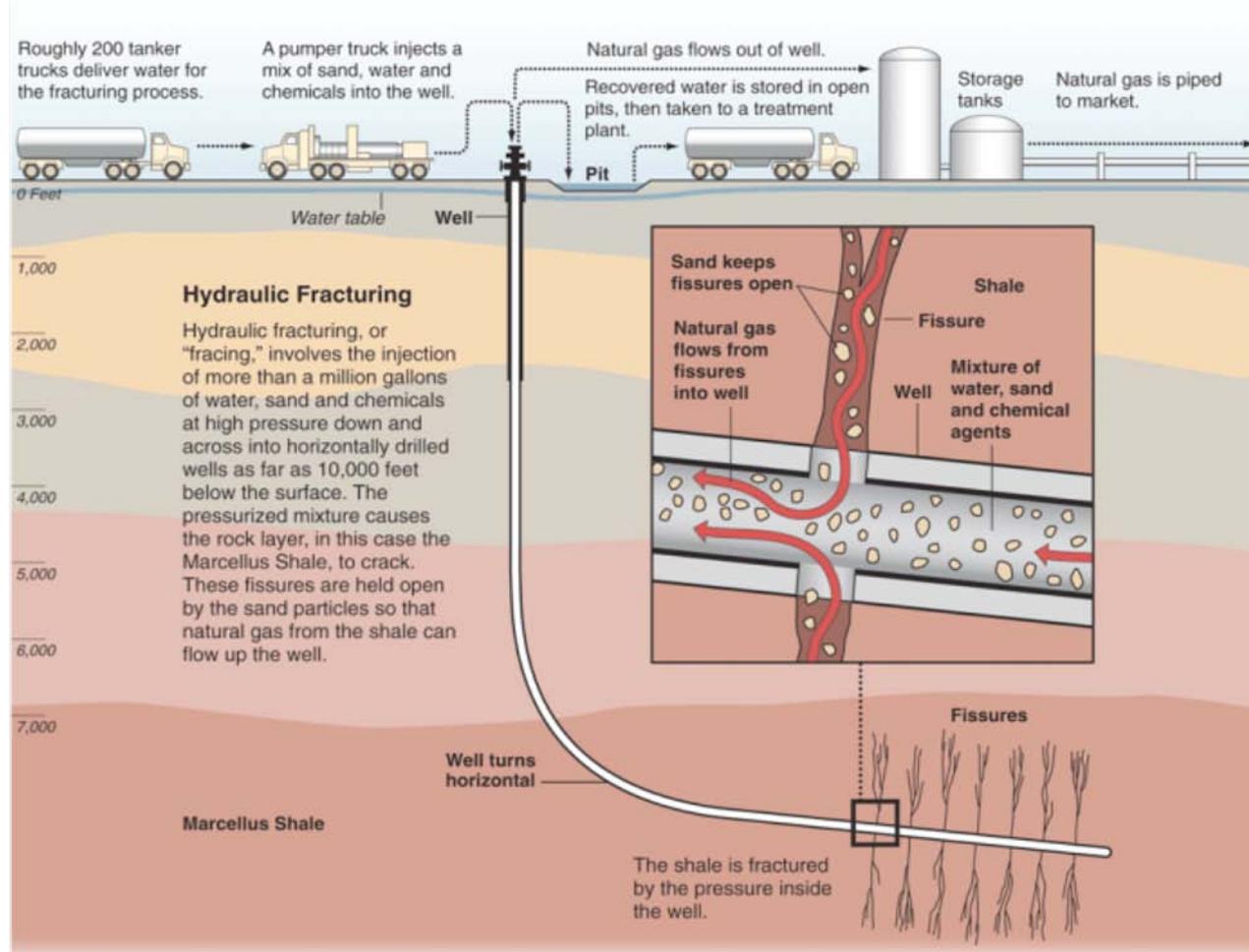
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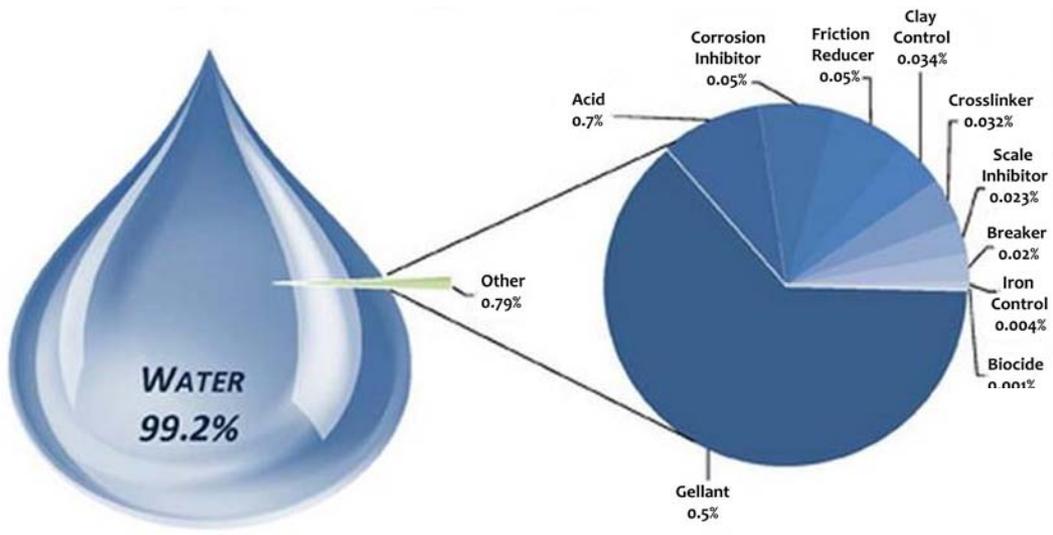
Outline

- Hydraulic fracturing/unconventional oil and gas (UOG) operations
- Endocrine disrupting chemicals (EDCs) and hormones
- Adipogenesis (fat cell development)
- Metabolic disruption potential of UOG chemicals
- Implications, future directions

The Hydraulic Fracturing Process

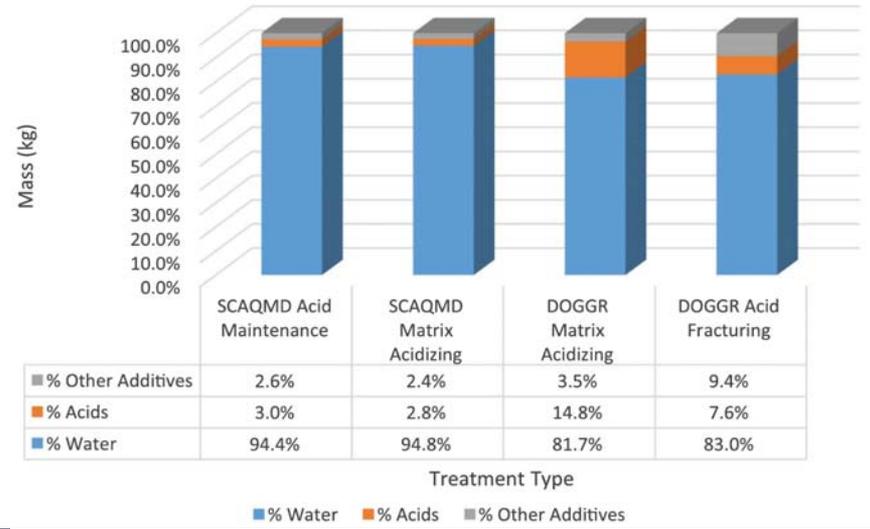


Fracturing Fluid Composition

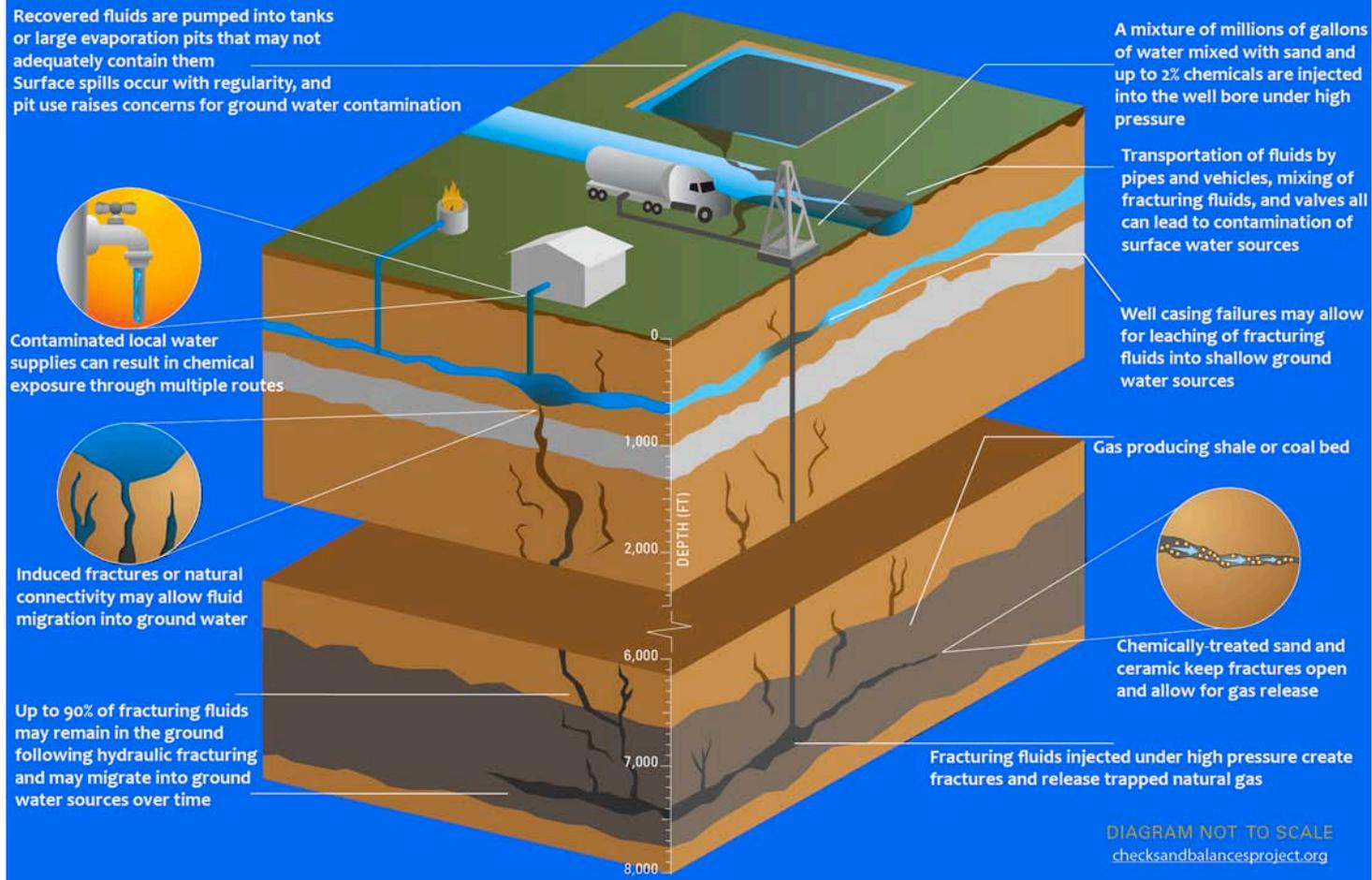


<http://fracfocus.org>

Acidizing Fluid Composition



Potential Routes of Water Contamination



Oil and Gas Development Spills

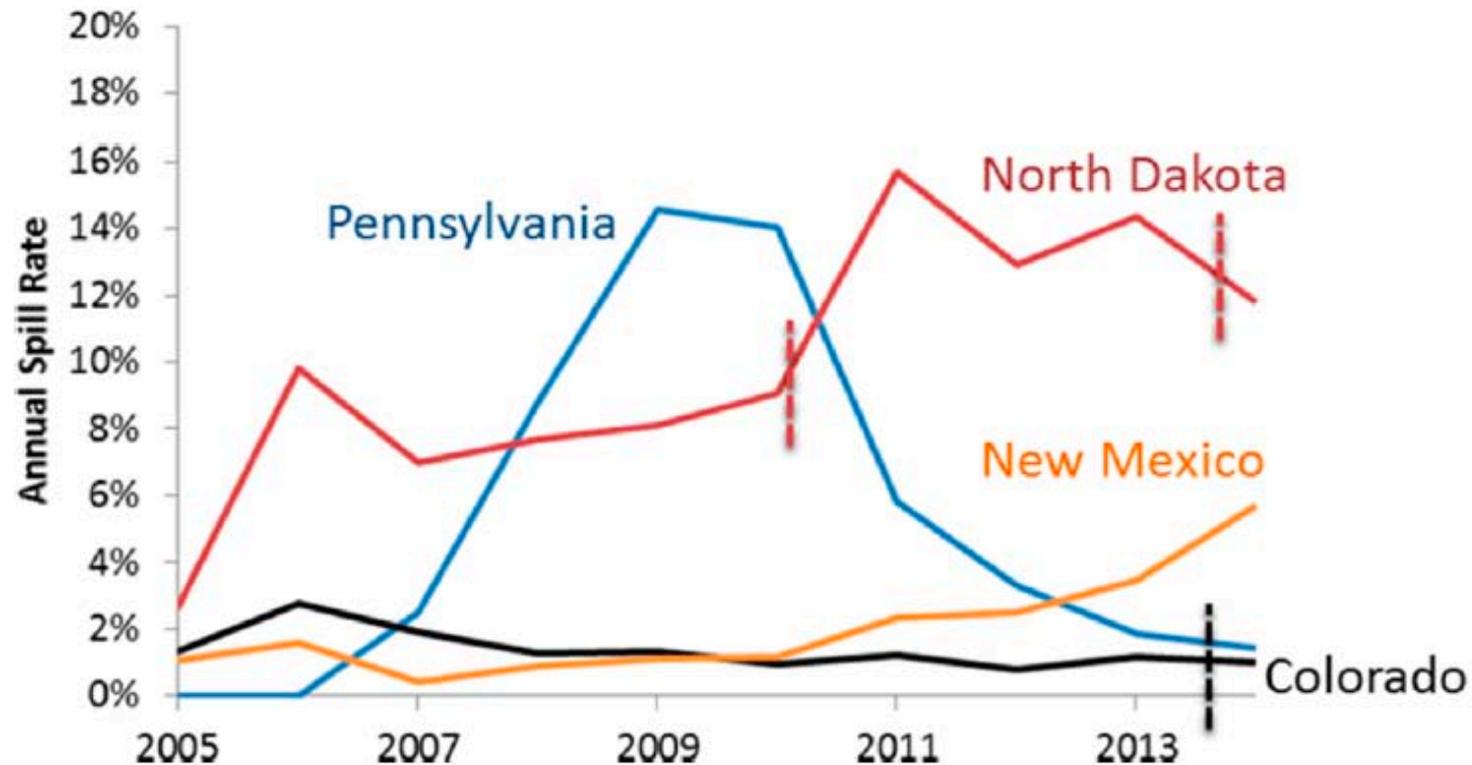
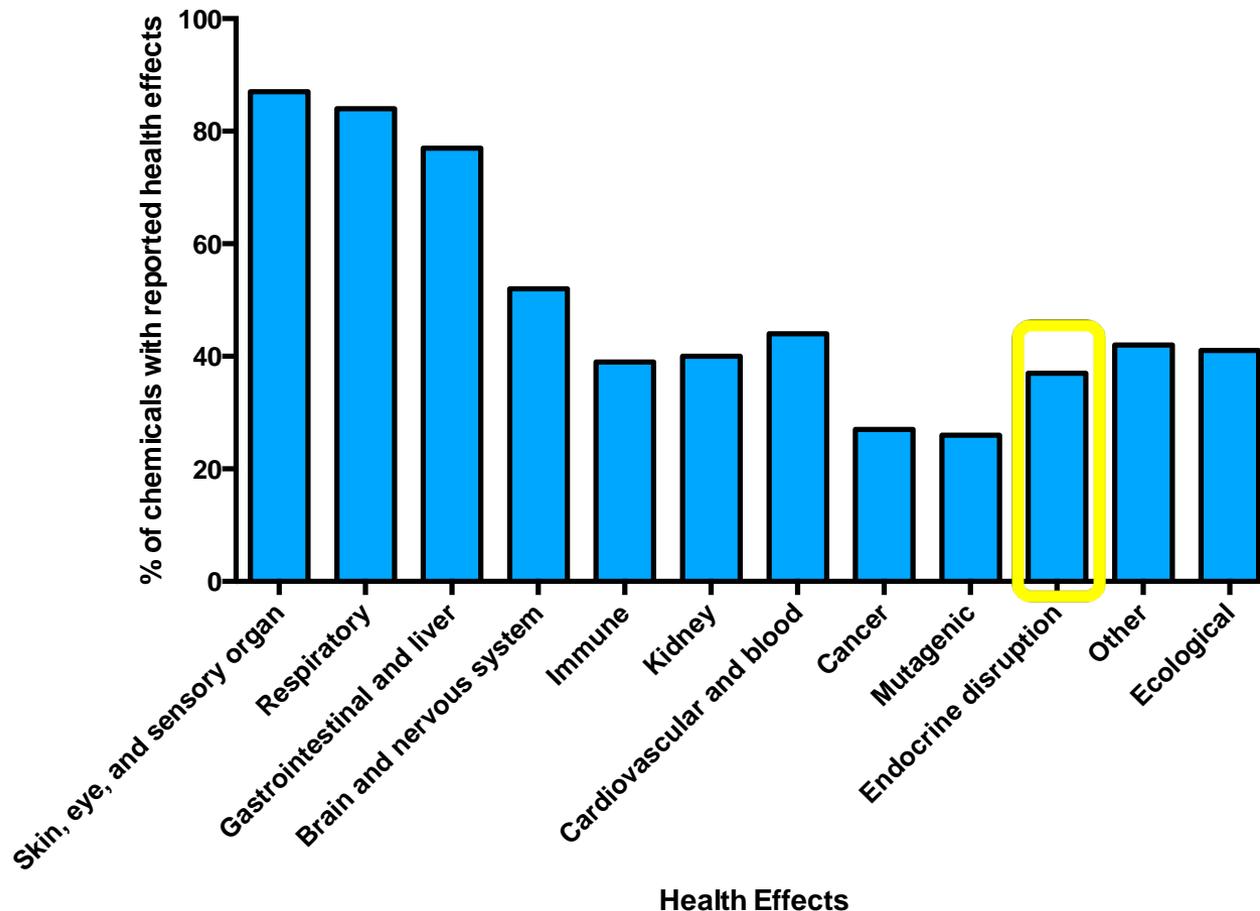


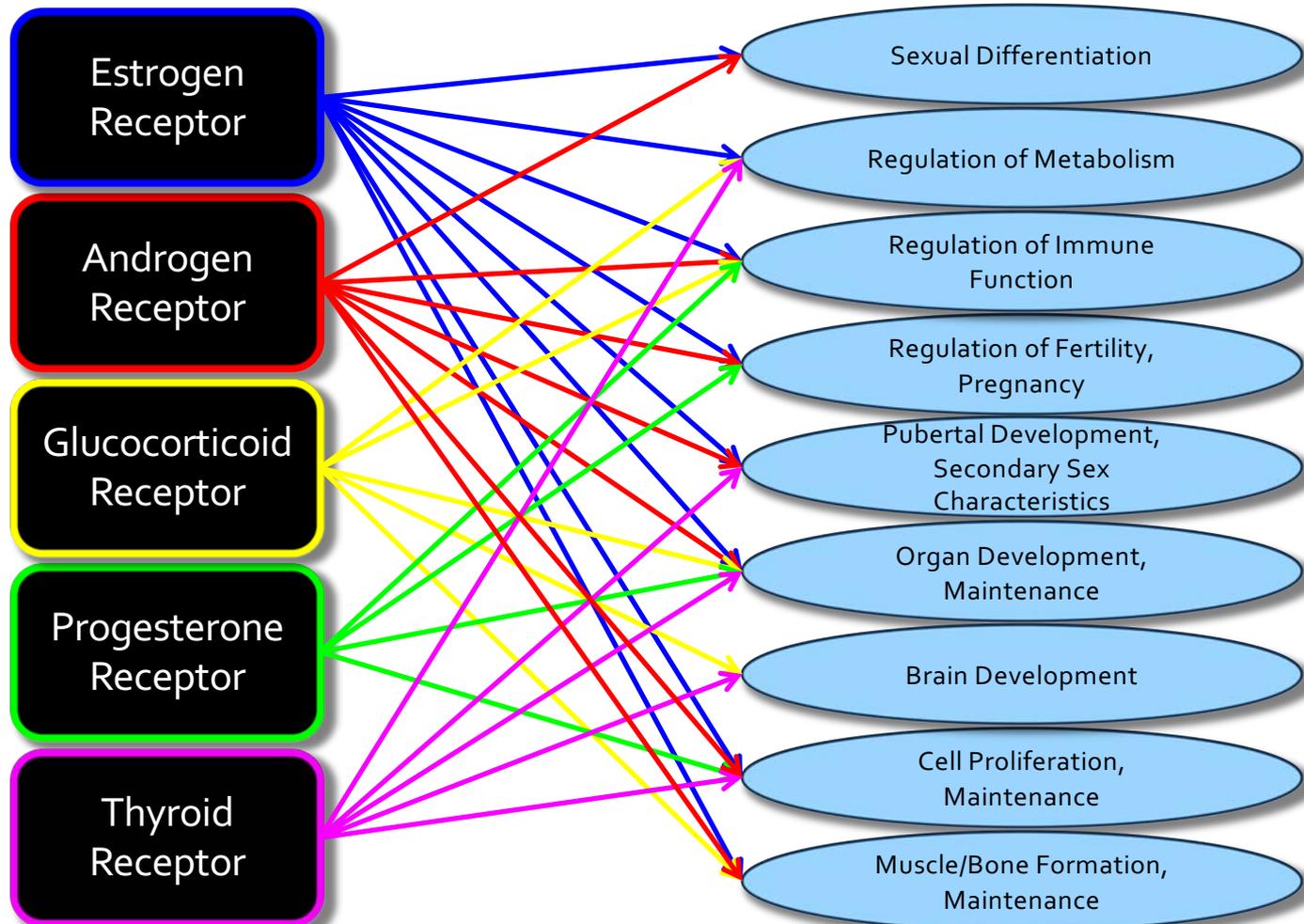
Figure 3. Annual spill rate. Dashed vertical lines represent changes in reporting requirements.

Unconventional Oil and Gas Operations as Source of Endocrine Disruptors



Theo Colborn

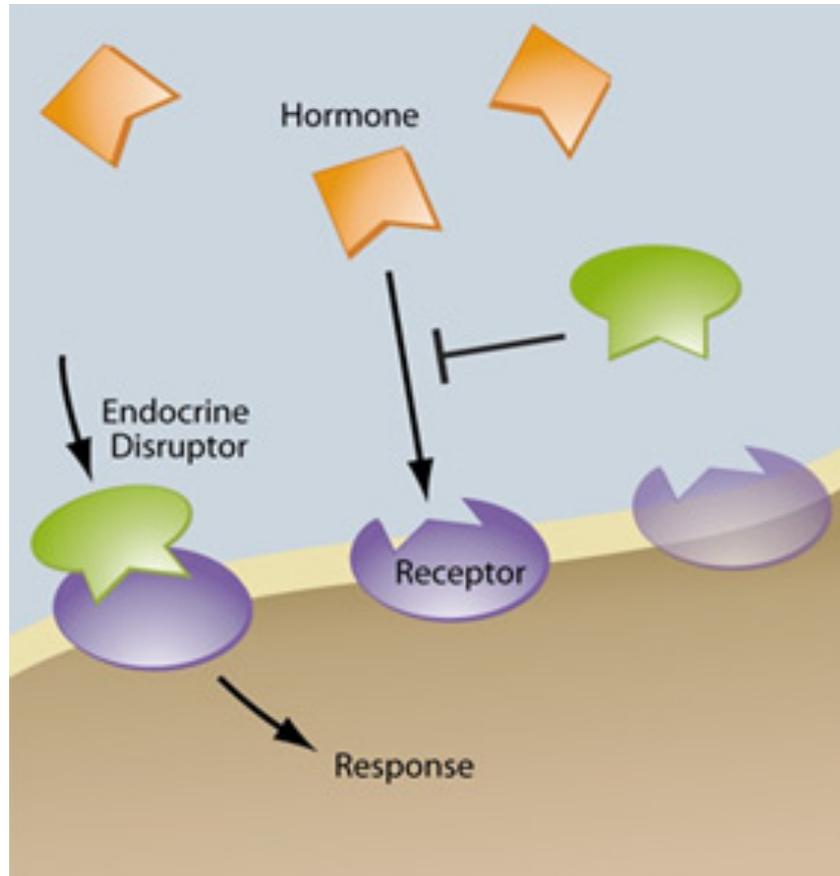
Normal Hormonal Function



Hormone/EDC Action

1) Direct Interactions

Receptor
Agonists
Antagonists

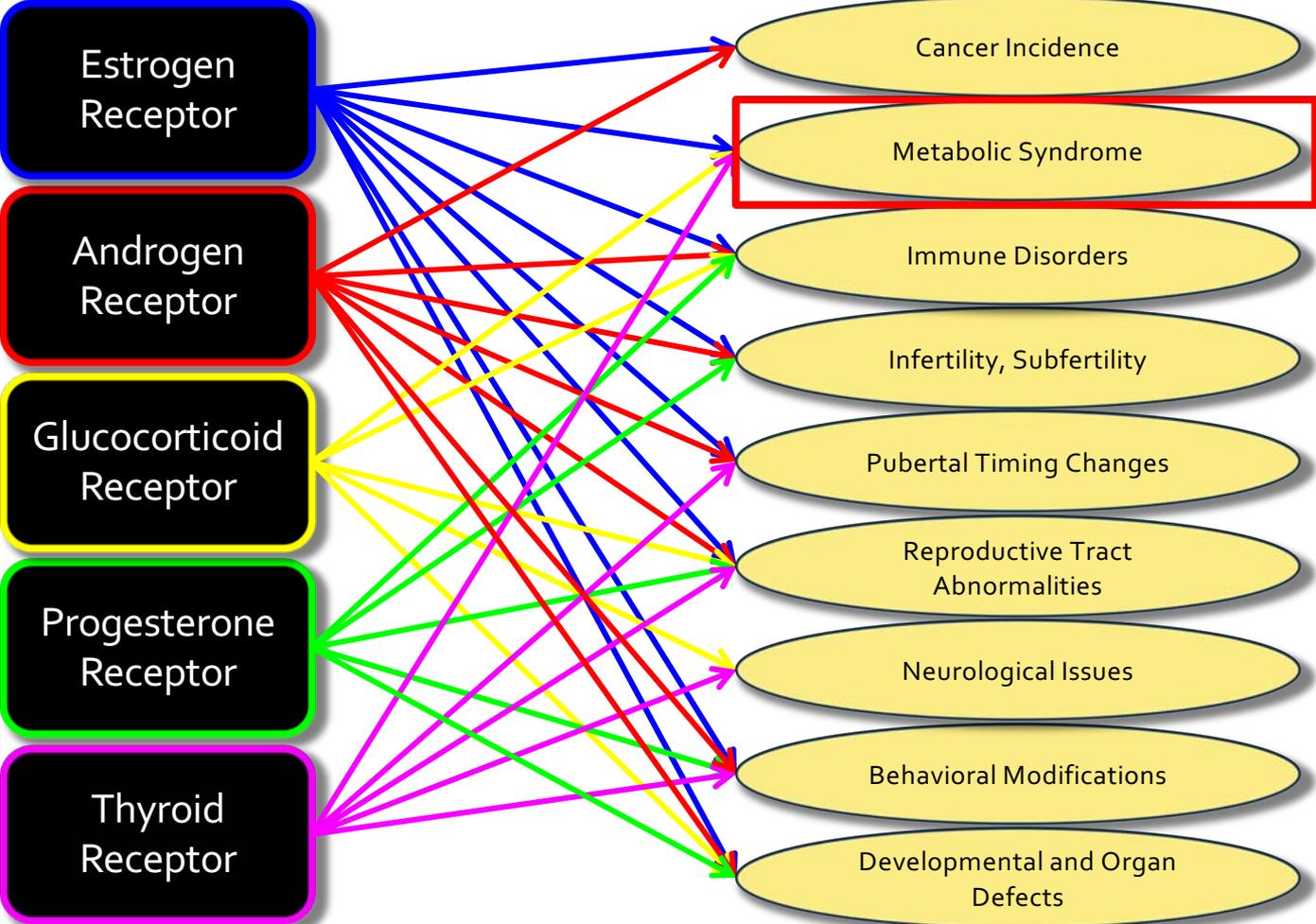


2) Indirect Interactions

Receptor Expression
Hormone Levels
Receptor Response

An endocrine disruptor is “a chemical or mixture of chemicals in the environment that interferes with any aspect of hormone action.” – The Endocrine Society, 2012.

Disruption of Hormone Receptors and Adverse Health Outcomes



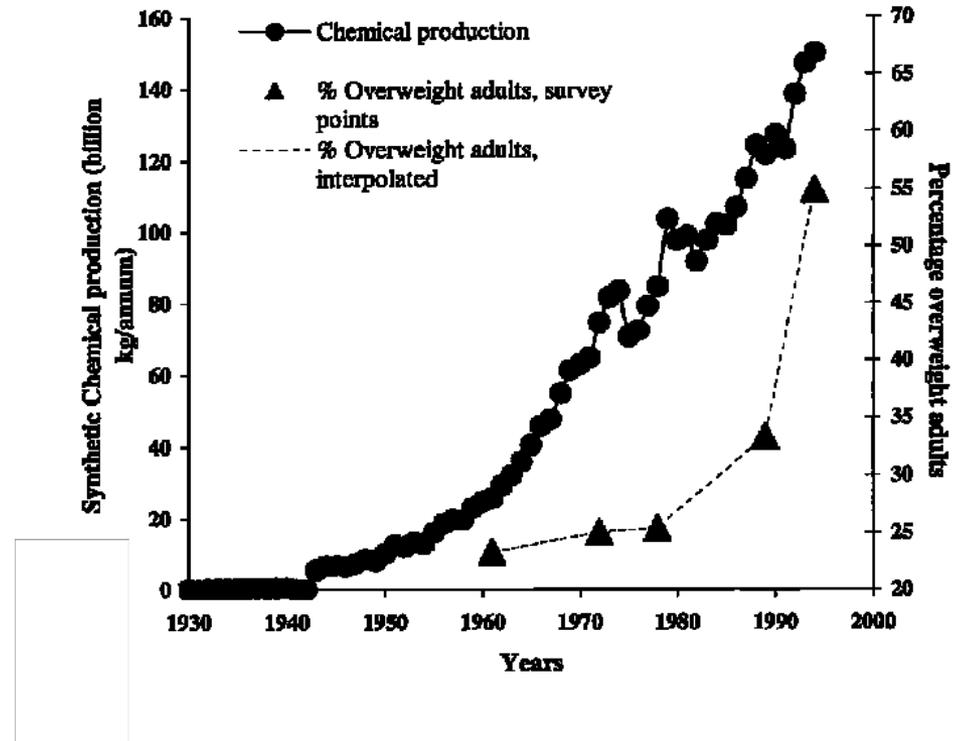
Altered Health Following Gestational Exposure to UOG Chemical Mixture

Endpoint	Males	Females
Body weight	↑	↑
Heart weight	↑	↑
Heart collagen deposition	↑	↑
Testis weight	↑	N/A
Sperm count/quality	↓	N/A
Serum testosterone	↑	N/A
Time to puberty	-	-
Litter size/sex ratio	-	-
Pituitary hormones	N/A	↓
Ovary/uterine weights	N/A	↓ ↑
Ovarian follicle development	N/A	↑
Mammary development	N/A	↑ *
Immune function/challenge response	-	↓

Potential Role of Chemicals in Increasing Obesity Rates in Humans

- + First posited in 2002, despite decades of experimental evidence.
- + Challenges caloric intake, activity, genetics as sufficient factors to explain magnitude/speed of observed trend.
- + Summarizes wealth of animal evidence on antibiotics, PCBs, plastics, pharmaceuticals, pesticides, organophosphates, heavy metals, etc.

DO CHEMICAL TOXINS CAUSE OBESITY?



Adipocyte Differentiation Process

Adipocyte commitment

Mesenchymal stem cell



Other pathways:
Myoblasts
Osteoblasts
Chondroblasts

Adipocyte differentiation

Adipoblast

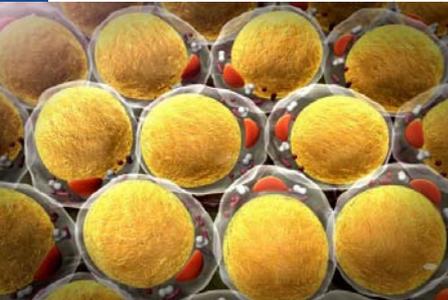
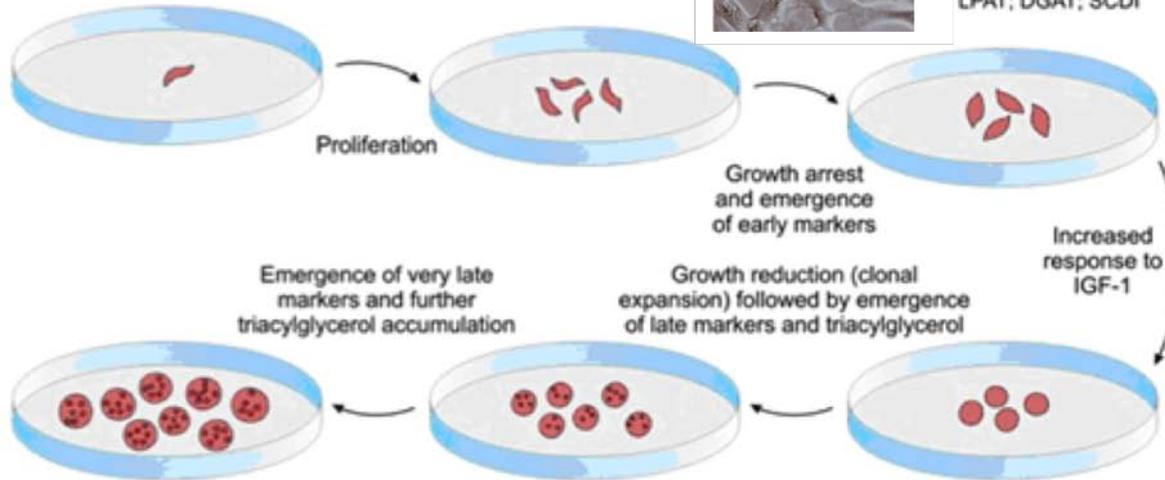
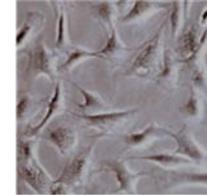
Pref-1

Preadipose cell
A2COL6/pOb24
LPLF A transport
PPARδ

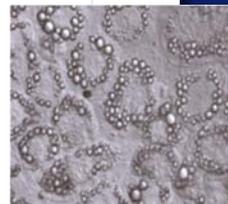
Preadipose cell
C/EBP β/δ
IGF-1
PRAR γ2

Immature adipose cell

C/EBP γ; GLUT4;
β2 AR; β3 AR; ACC
FAS; ME; ATP-citrate
lyase; GPDH; HSL;
LBP; perilipin; apoE;
low Km PDE; GPAT;
LPAT; DGAT; SCDI



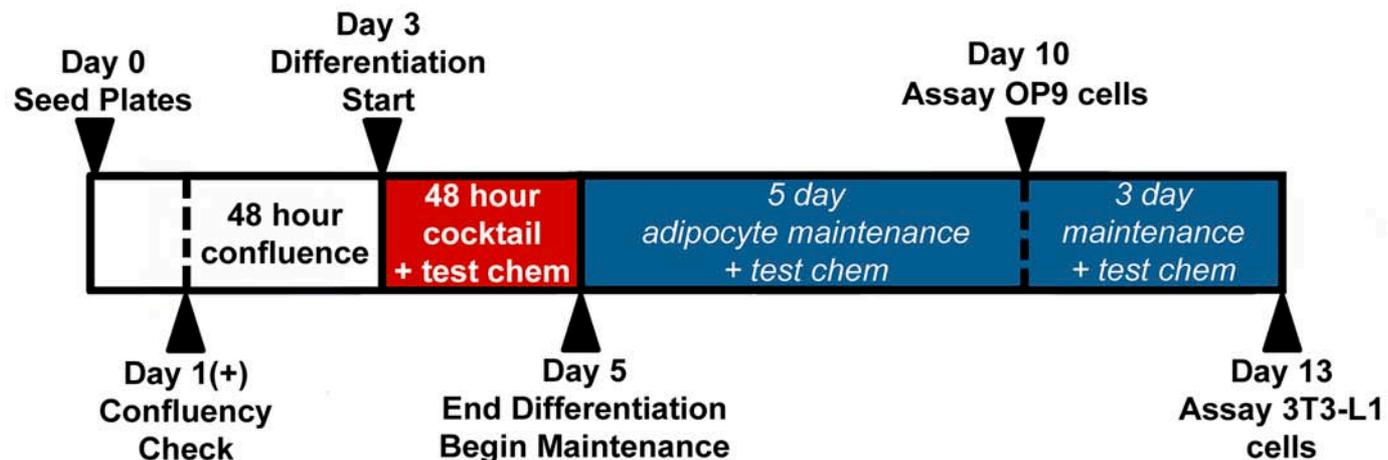
Resemble mature white adipose cell



Resemble brown/developing white adipose cell

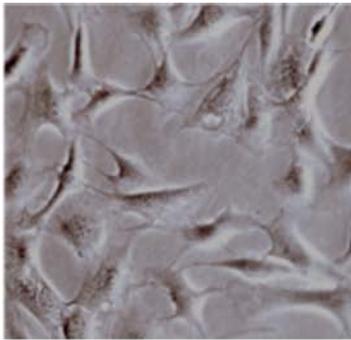
3T3-L1 Pre-adipocyte Adipogenesis Assay

- + Swiss albino mouse embryonic fibroblast cell line – committed pre-adipocytes
- + Extensively used over decades to evaluate adipogenesis
 - + Mechanisms of adipocyte differentiation well understood
 - + This assay, particularly coupled with PPAR γ reporter gene assays, has proven a reliable *in vitro* model for metabolic disruption *in vivo*.

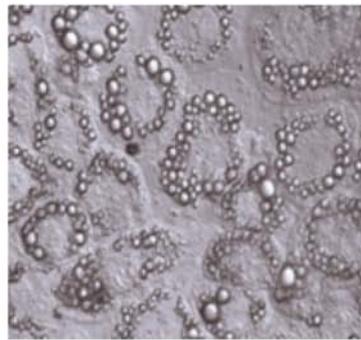


Adipogenesis Assay Measures

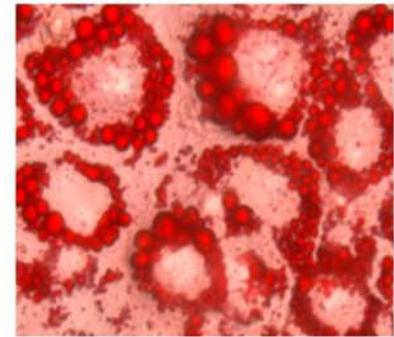
- Triglyceride accumulation
 - AdipoRed - hydrophilic fluorescent dye (Nile Red)
 - Partitions into lipid droplets in the cells, fluoresces



(A)



(B)



(C)

- Cell proliferation/cytotoxicity
 - NucBlue DNA dye (Hoechst 33342)
 - Partitions into nuclei and fluoresces upon binding DNA



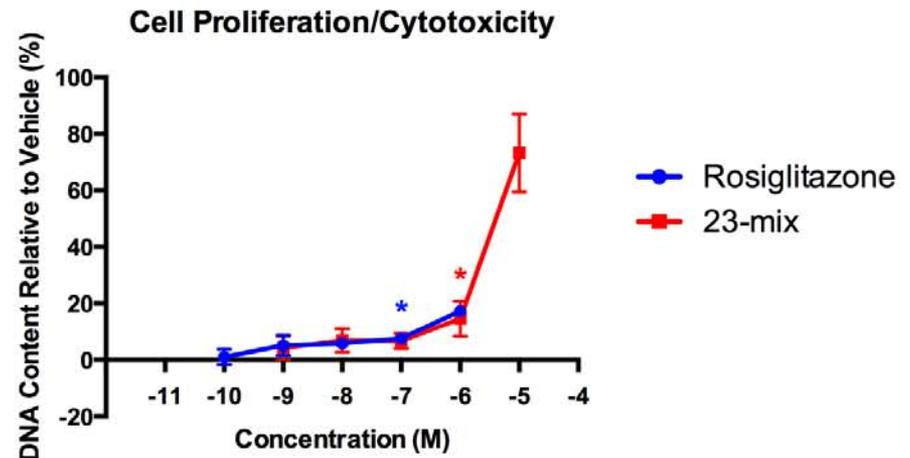
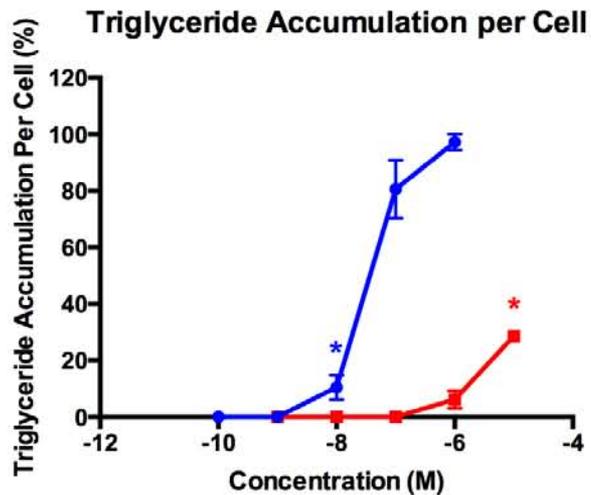
Study Design

- + Hypothesis: UOG-related chemicals will promote adipogenesis via PPAR γ -independent mechanisms.
- + Selected 8 UOG-impacted water sample extracts
 - Four from WV (wastewater injection well site; 1 reference, 1 control, 2 downstream)
 - Four from CO (near UOG well/spills; 1 control, 3 putative impacted samples)
- + Tested two UOG wastewater samples (CO)
- + Tested one mixture of 23 commonly-used UOG chemicals

Chemical Name

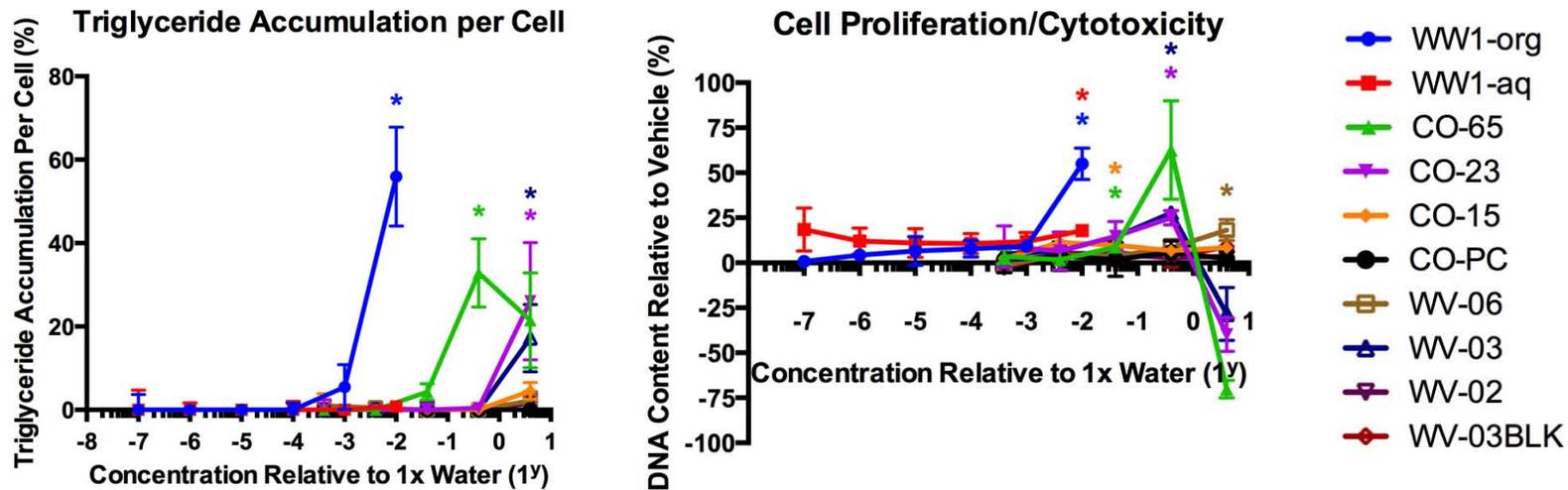
1,2,4-trimethylbenzene
 2-(2-methoxyethoxy) ethanol
 2-ethylhexanol
 Acrylamide
 Benzene
 Bronopol
 Cumene (Isopropylbenzene)
 Diethanolamine
 Dimethylformamide
 Ethoxylated nonylphenol (6)
 Ethoxylated octylphenol (4)
 Ethylbenzene
 Ethylene glycol
 Ethylene glycol monobutyl ether (2-BE)
 Methyl-4-isothiazolin
 Naphthalene
 Phenol
 Propylene glycol
 Sodium tetraborate decahydrate
 Styrene
 Toluene
 Triethylene glycol
 Xylenes (isomer mix)

Commonly-Used UOG Chemical Mixture Promotes Adipogenesis in 3T3-L1 Cells



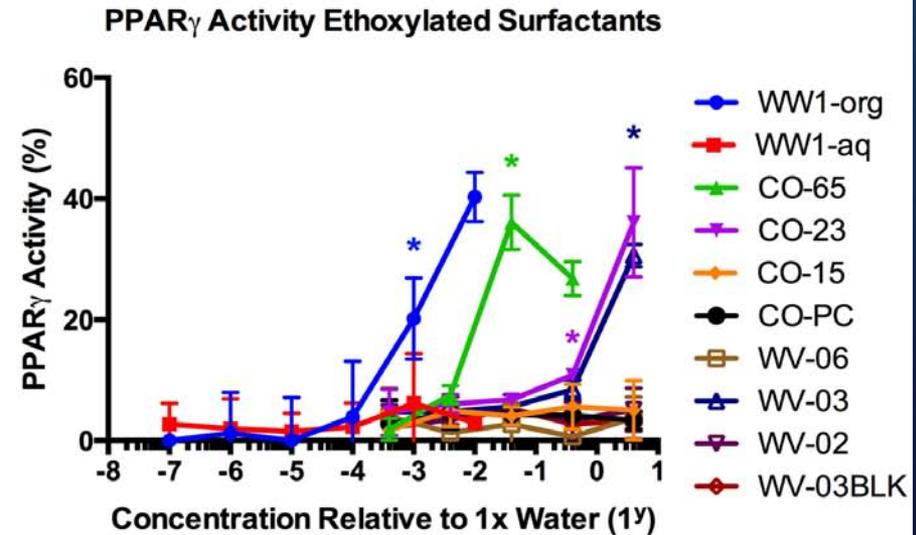
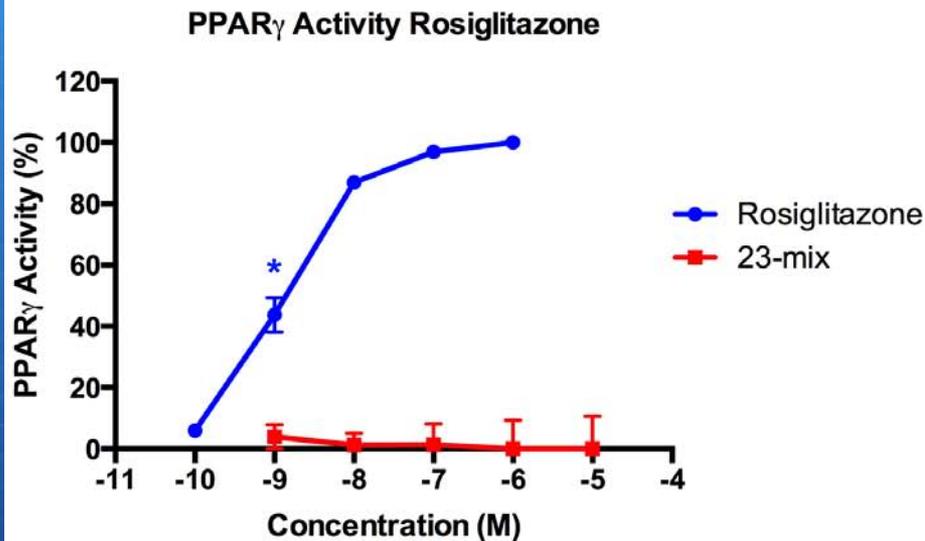
- 23-mix lab mixture exhibits significant adipogenic activity: triglyceride accumulation relative to rosiglitazone positive control, and pre-adipocyte proliferation relative to differentiated vehicle control.

UOG Wastewater-Impacted Water Extracts Promote Adipogenesis at Low Concentrations



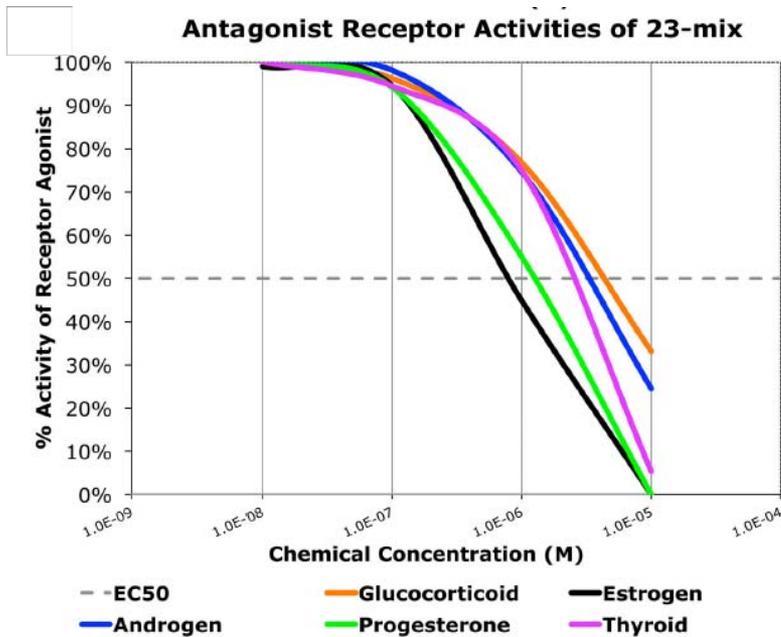
- Wastewater and wastewater-impacted water samples promote adipogenesis at diluted concentrations:
 - WW1: at 0.01x water concentration (1:100 dilution)
 - CO-65, 23, 15, WW-03 at 0.4x and 4x water concentration (SPE extracts) stimulated triglyceride accumulation
 - CO-65, 23, 15, WW-06, 03 at concentrations as low as 0.04x stimulated pre-adipocyte proliferation

UOG Mixture Promotes Adipogenesis via PPAR γ -Independent Mechanism

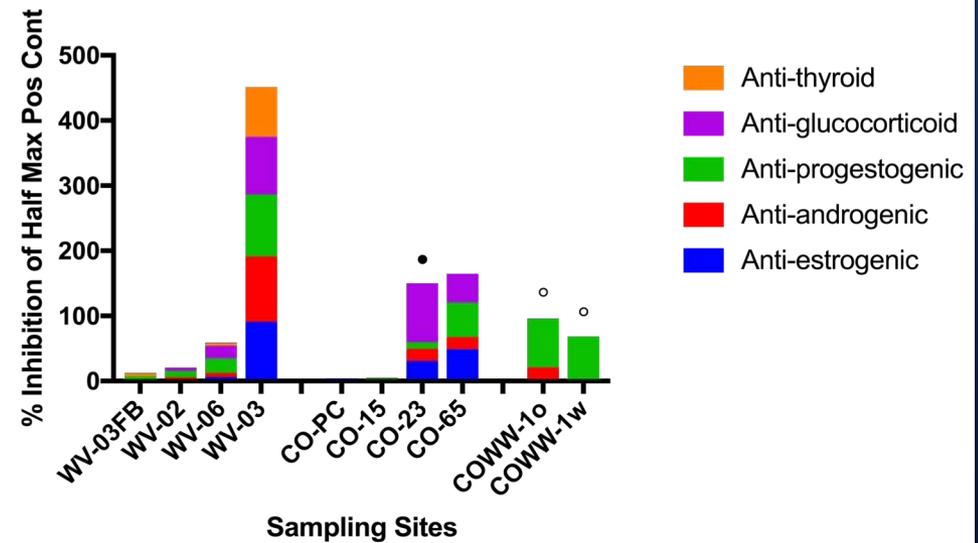


- 23-mix, WW1, CO-65, 23, WV-03 stimulate PPAR γ activity as well as adipogenesis.
- CO-15, WV-06 may be adipogenic via other mechanisms.

Nuclear Receptor Inhibition by Unconventional Oil/Gas Chemicals



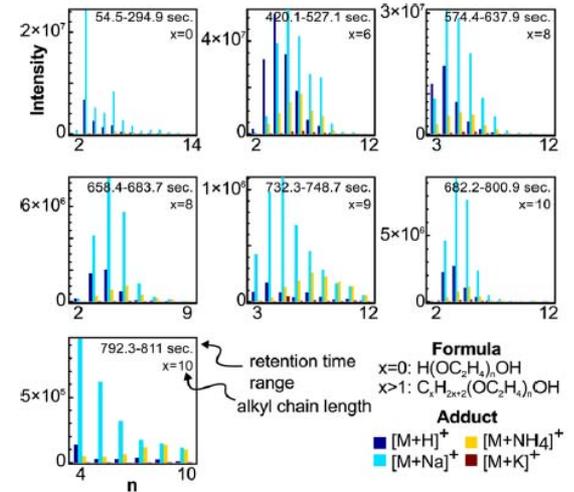
Antagonist Receptor Activity of Water Extracts



4x water concentration except: • 0.4x ◦ 0.01x (un-extracted water)

Presence of Alcohol Ethoxylates in Unconventional Oil and Gas Wastewater

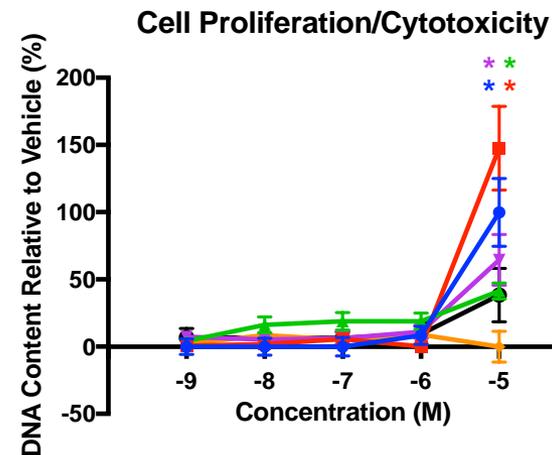
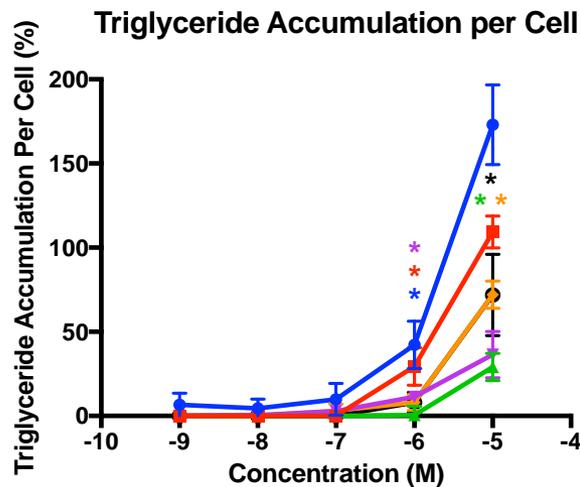
- + Suite of studies has measured alcohol and alkylphenol ethoxylates in fracking wastewater samples.
- + High-production volume chemicals
 - + >13 million metric tons, 2008
 - + >\$33 billion global revenues, 2014
- + Used widely in laundry detergents, hard-surface cleaners, paints, cosmetics, agriculture.
- + Common environmental contaminants
- + A concurrent project in our lab evaluated a series of these ethoxylated surfactants for adipogenic activity.



Getzinger et al 2015

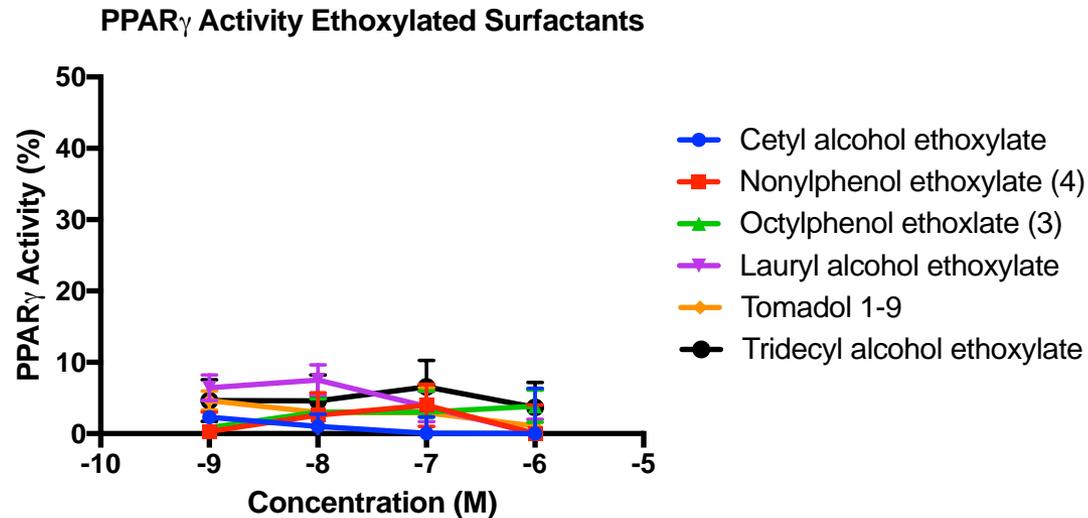


Various Alkylphenol and Alcohol Ethoxylates Induce Adipogenesis



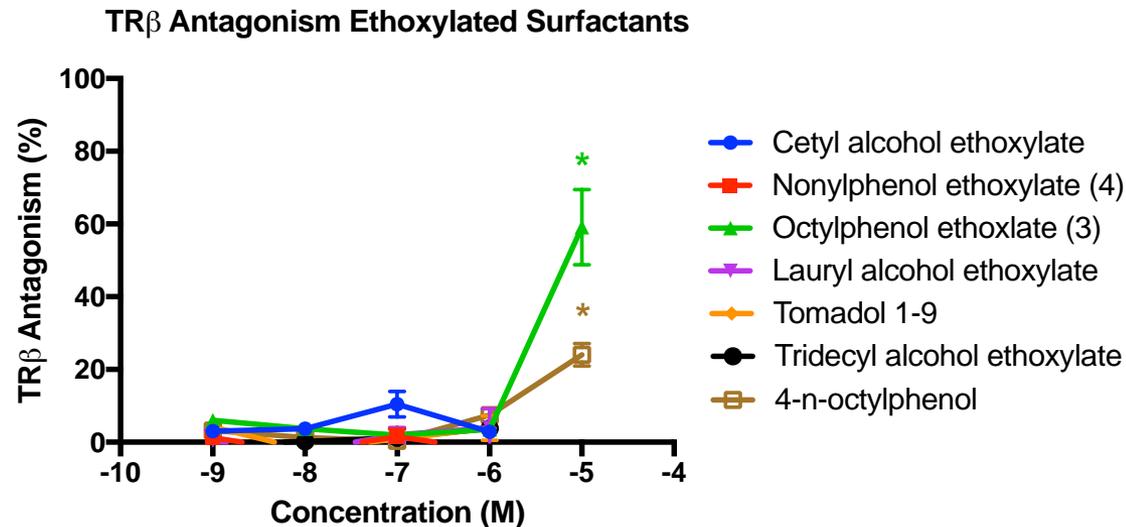
- Six ethoxylated surfactants (alkyl lengths 11-16) all induced triglyceride accumulation in 3T3-L1 cells.
 - Cetyl alcohol and NPEO induced greater maximal accumulation than the rosiglitazone control.
- 4/6 surfactants induced pre-adipocyte proliferation.

Ethoxylated Surfactants Do Not Promote Adipogenesis Via PPAR γ Activation



- Neither various alkyl length surfactants nor various ethoxylate chain length nonylphenols exhibited any activation.
- Confirmed in cell-free co-activator recruitment assay.

Ethoxylated Surfactants May Promote Adipogenesis Via TR β Antagonism



- Several ethoxylated surfactants exhibited significant TR β antagonism, independent of inhibited cell viability.
- Further work (cell-free) needed to confirm mechanism & evaluate other potential pathways.

Overall Take-Homes

- + Unconventional oil/gas production chemicals and mixtures may be able to disrupt metabolic health.
 - + High metabolic disruption potential in surface and groundwater from regions with oil and gas wastewater influence.
 - + Significant induction of fat cell development at very diluted concentrations of impacted water samples
- + Causative chemicals and molecular mechanisms promoting these effects have yet to be determined.
 - + PPAR γ activation and TR β antagonism appear to be likely mechanisms promoting at least some of the effects
 - + Polyethoxylated surfactants may be responsible for at least a portion of the adipogenic activity observed in environmental samples herein
- + Exposure to these chemicals may result in adverse health effects in humans and/or animals living nearby, particularly during critical windows.

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