Unconventional Oil and Gas Extraction and Endocrine Disruptors: Potential Implications for Human and Animal Health

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The Endocrine Disruption Exchange
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Outline

- Hormones and Endocrine Disrupting Chemicals (EDCs)
- Hydraulic Fracturing
- EDCs Associated with an Oil and Gas Wastewater Injection Operation
- Gestational Exposure and Health Effects in Male and Female Mice
Normal Hormonal Function

- Estrogen Receptor
- Androgen Receptor
- Glucocorticoid Receptor
- Progesterone Receptor
- Thyroid Receptor

- Sexual Differentiation
- Regulation of Metabolism
- Regulation of Immune Function
- Regulation of Fertility, Pregnancy
- Pubertal Development, Secondary Sex Characteristics
- Organ Development, Maintenance
- Brain Development
- Cell Proliferation, Maintenance
- Muscle/Bone Formation, Maintenance
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

- Estrogen Receptor
- Androgen Receptor
- Glucocorticoid Receptor
- Progesterone Receptor
- Thyroid Receptor

- Cancer Incidence
- Metabolic Syndrome
- Immune Disorders
- Infertility, Subfertility
- Pubertal Timing Changes
- Reproductive Tract Abnormalities
- Neurological Issues
- Behavioral Modifications
- Developmental and Organ Defects
The Hydraulic Fracturing Process

Roughly 200 tanker trucks deliver water for the fracturing process. A pump truck injects a mix of sand, water and chemicals into the well. Natural gas flows out of well. Recovered water is stored in open pits, then taken to a treatment plant. Natural gas is piped to market.

Hydraulic Fracturing

Hydraulic fracturing, or “fracking,” involves the injection of more than a million gallons of water, sand and chemicals at high pressure down and across into horizontally drilled wells as far as 10,000 feet below the surface. The pressurized mixture causes the rock layer, in this case the Marcellus Shale, to crack. These fissures are held open by the sand particles so that natural gas from the shale can flow up the well.

The shale is fractured by the pressure inside the well.

Graphic by Al Granberg
US Oil Production Boom and Bust

http://oilprice.com/
Fracturing Fluid Composition

http://fracfocus.org

Abdullah et al 2016, Tox Environ Chem
Potential Routes of Water Contamination

- Recovered fluids are pumped into tanks or large evaporation pits that may not adequately contain them. Surface spills occur with regularity, and pit use raises concerns for groundwater contamination.
- Contaminated local water supplies can result in chemical exposure through multiple routes.
- Induced fractures or natural connectivity may allow fluid migration into groundwater.
- Up to 90% of fracturing fluids may remain in the ground following hydraulic fracturing and may migrate into groundwater sources over time.
- A mixture of millions of gallons of water mixed with sand and up to 35 chemicals are injected into the wellbore under high pressure.
- Transportation of fluids by pipes and vehicles, mixing of fracturing fluids, and valves all can lead to contamination of surface water sources.
- Well casing failures may allow for leaching of fracturing fluids into shallow groundwater sources.
- Gas-producing shale or coal bed.
- Chemically-treated sand and ceramic keep fractures open and allow for gas release.
- Fracturing fluids injected under high pressure create fractures and release trapped natural gas.

Diagram not to scale. [checksandbalancesproject.org]
EDCs Used in Unconventional Oil and Gas Operations

- Skin, eye, and sensory organ
- Respiratory
- Gastrointestinal and liver
- Immune
- Brain and nervous system
- Cardiovascular and blood
- Kidney
- Cancer
- Mutagenic
- Endocrine disruption
- Other
- Ecological

% of chemicals with reported health effects

Colborn et al 2011, Hum Ecol Risk Assess
Previous Work in our Lab

- Detected greater estrogenic, anti-estrogenic and anti-androgenic activities in surface and groundwater near drilling-dense sites with history of fracking fluid spills (Kassotis et al. 2014).

- Reported that 23 of 24 commonly-used hydraulic fracturing chemicals act as agonists and/or antagonists for five nuclear receptors (Kassotis et al. 2015).

- Have begun to report chemicals and concentrations of oil/gas production chemicals in wastewater with Dr. Chung-Ho Lin (Kassotis et al. 2015).

- Reported adverse health effects in male C57 mice exposed prenatally to likely environmentally relevant concentrations of a fracking chemical mixture (Kassotis et al. 2015).
Reporter Gene Assay System
Reporter Gene Bioassay Activities

Receptor Activity of Positive and Negative Controls

- Estrogen Agonist
- Estrogen Antagonist
- Thyroid Agonist
- Thyroid Antagonist
- Progesterone Agonist
- Progesterone Antagonist
- Androgen Agonist
- Androgen Antagonist
- Glucocorticoid Agonist
- Glucocorticoid Antagonist

Percent Activity vs. Concentration, Molar

Kassotis et al 2016a, STOTEN
West Virginia Wastewater Disposal Well

Gradient of Potential Oil/Gas Contamination

Kassotis et al 2016a, STOTEN
Combined Surface Water Antagonist Activities
WV Injection Well Site

Gradient of Potential Oil/Gas Contamination

% Activity

Reference

WV-2  WV-4  WV-3FB Sample  WV-6  WV-7  WV-3

Kassotis et al 2016a, STOTEN
Combined Surface Water Agonist Activities
WV Injection Well Site

Gradient of Potential Oil/Gas Contamination

Reference

WV-2  WV-4  WV-3FB  WV-6  WV-7  WV-3
Sample IDs

% Activity

Kassotis et al 2016a, STOTEN
Elevated antagonist activities present in surface water downstream from oil and gas wastewater disposal operation.

Geochemical and organic chemical analyses (Akob et al. and Orem et al.) demonstrate unconventional oil and gas wastewater influence on stream quality.

Antagonist equivalent concentrations at levels known to result in adverse health effects in aquatic organisms.
Growing Understanding of Adverse Human and Animal Health Outcomes

**General adverse health**
- Increased reported health symptoms in humans (Rabinowitz et al. 2014) and dogs (Slizovskiy et al. 2015)
  - Increased inpatient hospital utilization rates (Jemielita et al. 2015)
- Respiratory, GI, immune, reproductive, other issues for humans, companion and food animals, wildlife, etc. (Bamberger & Oswald)
  - Symptom abatement for families, animals that left drilling areas (Bamberger & Oswald 2015)

**Reproductive/developmental effects**
- Increased rate of congenital heart defects (McKenzie et al. 2014)
- Increased rates of preterm birth, high risk pregnancies (Casey et al. 2015)
- Increased rates of low birth weight and SGA babies with greater density (Stacy et al. 2015)
Gestational Exposure in C57BL/6J Mice

- Concentrations in drinking water (each of 23 chemicals; 23-mix):
  - 0.01, 0.1, 1.0, 10 mg/L or
  - 3, 30, 300, 3,000 μg/kg/day
  - in 0.2% ethanol vehicle (~500 mg/kg/day),
  - & 50 mg/kg/day flutamide (AR antagonist) control

Kassotis et al 2015, Endocrinology
## Oil & Gas Operation Chemicals in 23-mix

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>CAS #</th>
<th>Oil and Gas Operation Use</th>
</tr>
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<tbody>
<tr>
<td>1,2,4-trimethylbenzene</td>
<td>95-63-6</td>
<td>Surfactant</td>
</tr>
<tr>
<td>2-(2-methoxyethoxy) ethanol</td>
<td>111-77-3</td>
<td>Biocide, Surfactant</td>
</tr>
<tr>
<td>2-ethylhexanol</td>
<td>104-76-7</td>
<td>Defoamer, Breaker</td>
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<tr>
<td>Acrylamide</td>
<td>79-06-1</td>
<td>Scale Control, Friction Reducer</td>
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<tr>
<td>Benzene</td>
<td>71-43-2</td>
<td>Paraffin Inhibitor, Surfactant</td>
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<tr>
<td>Bronopol</td>
<td>52-51-7</td>
<td>Biocide</td>
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<tr>
<td>Cumene (Isopropylbenzene)</td>
<td>98-82-8</td>
<td>Paraffin Inhibitor</td>
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<tr>
<td>Diethanolamine</td>
<td>111-42-2</td>
<td>Friction Reducer, Corrosion Inhibitor</td>
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<tr>
<td>Dimethylformamide</td>
<td>68-12-2</td>
<td>Corrosion Inhibitor</td>
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<tr>
<td>Ethoxylated nonylphenol</td>
<td>9016-45-9</td>
<td>Surfactant, Corrosion Inhibitor</td>
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<td>Ethoxylated octylphenol</td>
<td>9036-19-5</td>
<td>Surfactant, Corrosion Inhibitor</td>
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<tr>
<td>Ethylenebenzene</td>
<td>100-41-4</td>
<td>Non-emulsifier, paraffin inhibitor</td>
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<tr>
<td>Ethylene glycol</td>
<td>107-21-1</td>
<td>Crosslinker, Friction reducer</td>
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<tr>
<td>Ethylene glycol monobutyl ether (2-BE)</td>
<td>111-76-2</td>
<td>Surfactant, Foamer</td>
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<tr>
<td>Methyl-4-isothiazolin</td>
<td>2682-20-4</td>
<td>Biocide</td>
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<tr>
<td>Naphthalene</td>
<td>91-20-3</td>
<td>Surfactant, Acid Inhibitor</td>
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<tr>
<td>Phenol</td>
<td>108-95-2</td>
<td>Resin-coating for proppants</td>
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<td>Propylene glycol</td>
<td>57-55-6</td>
<td>Gellant, Breaker</td>
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<td>Sodium tetraborate decahydrate</td>
<td>1303-96-4</td>
<td>Crosslinker</td>
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<td>Styrene</td>
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<td>Proppant</td>
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<td>Toluene</td>
<td>108-88-3</td>
<td>Non-emulsifier, paraffin inhibitor</td>
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<td>Triethylene glycol</td>
<td>112-27-6</td>
<td>Biocide, Dehydration</td>
</tr>
<tr>
<td>Xylenes</td>
<td>1330-20-7</td>
<td>Non-emulsifier, Breaker</td>
</tr>
</tbody>
</table>
Altered Body and Organ Weights in Developmentally Exposed Mice

Ground water directly below surface spills, *Gross et al*  
Kassotis et al 2015, *Endocrinology*

Kassotis et al 2016b, in prep
Adverse Reproductive Health Outcomes in Developmentally Exposed Male Mice

- **PND21 Testes Weight**
- **PND85 Sperm Count**
- **PND85 Testosterone (ng/mL)**

Kassotis et al 2015, Endocrinology
Disrupted Heart Development in Developmentally Exposed Female Mice

Kassotis et al 2016b, in prep
Suppressed Pituitary Hormones in Developmentally Exposed Female Mice

Kassotis et al. 2016b, in prep
Disrupted Folliculogenesis in Developmentally Exposed Female Mice

Kassotis et al 2016b, in prep
Overall Take-Homes

- Some chemicals used in and/or produced by oil and natural gas operations can act as nuclear receptor agonists and antagonists.

- Humans and animals are likely exposed to these chemicals via multiple routes in drilling-dense areas
  - Drinking water, inhalation, and dermal absorption.

- Injection sites may represent another route through which oil and gas operations may influence EDC contributions to surface/groundwater.

- Gestational exposure to a mixture of oil and gas operation chemicals at likely environmentally-relevant concentrations resulted in adverse health outcomes in C57 mice.
  - Increased body weights, reduced sperm counts in males.
  - Increased body weights, suppressed pituitary hormones, altered folliculogenesis in females.
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