Endocrine disrupting activity associated with unconventional oil and natural gas operations

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Endocrine Disrupting Chemicals (EDC)

“A chemical, or mixture of chemicals, that interferes with any aspect of hormone action.”
EDCs are found in many products.
EDCs disrupt hormone receptors
Steroid Receptors Are Ligand activated Transcription Factors

- Estradiol
- Testosterone
- Progesterone
- Cortisol
- Aldosterone
Why is endocrine disruption important to human health and disease?

- Hormones and EDCs can act at low concentrations
- Human exposure can be within the range of bioactivity
- Developmental exposure can alter adult health & disease

<table>
<thead>
<tr>
<th>Parts Per</th>
<th>trillion</th>
<th>billion</th>
<th>million</th>
<th>thousand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>1 (pg/ml)</td>
<td>10 (ng/ml)</td>
<td>1 (µg/ml)</td>
<td>1 (mg/ml)</td>
</tr>
<tr>
<td>BPA</td>
<td>1 (pg/ml)</td>
<td>10 (ng/ml)</td>
<td>1 (µg/ml)</td>
<td>1 (mg/ml)</td>
</tr>
</tbody>
</table>
Hormones are essential for normal health and development.
Fetal and early life exposure to EDCs is associated with adult disease

- Breast Cancer
- Bone Health
- Endometriosis
- Infertility
- Obesity
- Reduced Sperm
- Heart Disease
- Diabetes
- Hypertension
- Testicular Cancer

Pollutants → Nutrition → Maternal Health and Disease
Unconventional oil and natural gas extraction is a potential source of endocrine disrupting chemicals
Unconventional oil & gas (UOG) extraction: Hydraulic Fracturing + Horizontal Drilling

Hydraulic Fracturing

Hydraulic fracturing, or “fracing,” 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.
Fracturing Fluid Composition

0.5% Chemicals

- Acid 0.7%
- Other 0.79%
- Corrosion Inhibitor 0.05%
- Friction Reducer 0.05%
- Clay Control 0.034%
- Crosslinker 0.032%
- Scale Inhibitor 0.023%
- Breaker 0.02%
- Iron Control 0.004%
- Biocide 0.001%

WATER 99.2%

Millions of gallons of water
Thousands of gallons of chemicals

Source: FracFocus data August 2012
Millions of gallons of wastewater

- “Flowback” returns immediately to surface
- “Produced water” returns over the life of the well and contains fracking chemicals, liberated salts and radioisotopes from deep underground
- Wastewater spills and leaks
- Direct disposal into surface water
- Well casing failures
- Underground migration
Hypothesis: Surface and ground water at natural gas drilling spill sites contain more endocrine disrupting activity than reference sites.

Garfield County

Red = Active wells, Orange = permitted wells, Yellow = Abandoned wells
Solid Phase Extraction & Reporter Gene Assays
Ground Water Activity By Site

% Activity

- Anti-androgenic
- Anti-estrogenic
- Estrogenic

Reference

Kassotis, et al 2014
Surface Water Activity By Site

Kassotis, et al 2014
Disposal Facility in West Virginia
Hormone Blocking Activity Associated with Injection/Disposal Well

- Blank Background
- Anti-thyroid
- Anti-progestogenic
- Anti-estrogenic
Our hypothesis: Chemicals used in hydraulic fracturing will disrupt hormone receptors.
Hydraulic Fracturing and EDCs

- Tested 24 chemicals
- Five nuclear receptors
- Measured receptor activation
- Measured receptor inhibition
<table>
<thead>
<tr>
<th>Fracking Chemicals We Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,4-trimethylbenzene</td>
</tr>
<tr>
<td>Ethylbenzene</td>
</tr>
<tr>
<td>2-(2-methoxyethoxy) ethanol</td>
</tr>
<tr>
<td>Ethylene glycol</td>
</tr>
<tr>
<td>2-ethylhexanol</td>
</tr>
<tr>
<td>Ethylene glycol butyl ether</td>
</tr>
<tr>
<td>Acrylamide</td>
</tr>
<tr>
<td>Methyl-4-isothiazolin</td>
</tr>
<tr>
<td>Benzene</td>
</tr>
<tr>
<td>Naphthalene</td>
</tr>
<tr>
<td>Bisphenol A</td>
</tr>
<tr>
<td>Phenol</td>
</tr>
<tr>
<td>Bronopol</td>
</tr>
<tr>
<td>Propylene glycol</td>
</tr>
<tr>
<td>Cumene</td>
</tr>
<tr>
<td>Sodium tetraborate decahydrate</td>
</tr>
<tr>
<td>Diethanolamine</td>
</tr>
<tr>
<td>Styrene</td>
</tr>
<tr>
<td>Dimethyl formamide</td>
</tr>
<tr>
<td>Toluene</td>
</tr>
<tr>
<td>Ethoxylated nonylphenol</td>
</tr>
<tr>
<td>Triethylene glycol</td>
</tr>
<tr>
<td>Ethoxylated octylphenol</td>
</tr>
<tr>
<td>Xylenes</td>
</tr>
</tbody>
</table>
Estrogen Receptor Inhibition

% Inhibition of Receptor Activity

- Ethylene glycol butyl ether
- Diethanolamine
- Boronol
- Styrene
- Ethyl-1-hexanol
- Ethylene glycol
- Methyl-4-isothiazolidine
- Dimethylformamide
- Cumene
- Xylenes
- Phenol
- Benzene glycol
- Toluene
- Ethylbenzene
- Acrylamide
- Bisphenol A
- 23-mix
- 24-mix
- 9-mix

% Inhibition of Receptor Activity

- 0%
- 10%
- 20%
- 30%
- 40%
- 50%
- 60%
- 70%
- 80%
- 90%
- 100%
Androgen Receptor Inhibition

% Inhibition of Receptor Activity

Bisphenol A
Ethylene glycol
Diethanolamine
Ethylene glycol butyl ester
2,2'-[2,2'-biphenyl]-4,4'-diol
Methyl-1-ethoxyhexanol
Cumene
Naphthalene
Benzene
Ethylbenzene
Dimethylphenoxyamine
Sodium tetraborate
Triethylene glycol
Phenol
Xylenes
Acrylamide
Toluene
Bronopol
Propylene glycol
Styrene
24-mix
9-mix
Progesterone Receptor Inhibition

% Inhibition of Receptor Activity

- Bisphenol A
- Ethoxylated phenol
- Ethoxylated octylphenol
- 2-Ethyl-1-hexanol
- Xylenes
- Naphthalene
- Cumene
- Bromopol
- Ethylene glycol
- Diethanolamine
- Dimethylformamide
- Phenol
- 1,2,4-Trimethylbenzene
- 2,4-D
- Acrylamide
- Benzene
- Ethylbenzene
- Ethylene glycol butyl
- Methyl-4-isothiazolin
- Propylene glycol
- Sodium tetraborate
- Styrene
- Toluene
- Triethylene glycol
Glucocorticoid Receptor Inhibition

% Inhibition of Receptor Activity

- Bisphenol A
- Bronopol
- Naphthalene
- 2-Ethyl-1-hexanol
- Ethoxylated octyphenol
- Methyl 4-isothiazolin
- 1,2,4-Trimethylbenzene
- Styrene
- Acrylamide
- Benzene
- Cumene
- Diethanolamine
- Ethylbenzene
- Ethylene glycol butyl
- Phenol
- Propylene glycol
- Sodium tetraborate
- Toluene
- Triethylene glycol
- Xylenes
Thyroid Receptor Inhibition

% Inhibition of Receptor Activity

Bisphenol A
Naphthalene
Ethylene glycol
Ethoxylated octylphenol
Styrene
1,2,4-Trimethylbenzene
2-Ethyl-1-hexanol
2,4-DBP
Acrylamide
Benzene
Cumene
Diethanolamine
Methyl-4-isothiazolinone
Propylene glycol
Sodium tetraborate
Toluene
Triethylene glycol
Xylenes
# EDC Activity of 24 Fracking Chemicals

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Activation</th>
<th>Inhibition</th>
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<tbody>
<tr>
<td>Estrogen</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Androgen</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Progesterone</td>
<td>1</td>
<td>12</td>
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<tr>
<td>Glucocorticoid</td>
<td>0</td>
<td>10</td>
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<tr>
<td>Thyroid</td>
<td>2</td>
<td>7</td>
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</table>
Inhibitory Activity of a Mixture of 23 UOG Chemicals

![Graph showing the inhibitory activity of various chemicals](image_url)
Developmental exposure to a mixture of 23 UOG chemicals via drinking water

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Mix 1</th>
<th>Mix 2</th>
<th>Mix 3</th>
<th>Mix 4</th>
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<tbody>
<tr>
<td>Prenatal</td>
<td>0</td>
<td>3000 µg/kg</td>
<td>300 µg/kg</td>
<td>30 µg/kg</td>
<td>3 µg/kg</td>
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<tr>
<td>Birth</td>
<td>0</td>
<td>300 µg/kg</td>
<td>30 µg/kg</td>
<td>3 µg/kg</td>
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<tr>
<td>Treatment</td>
<td>11</td>
<td>21</td>
<td>70</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2014 Colorado Trip: 48 Sites Sampled
Identify chemicals in water responsible for EDC activity

- Bioassay samples
- Bioassay Guided Fractionation
- Identify unknowns
- Directed Analysis

- Estrogen
- Androgen
- Glucocorticoid
- Progesterone
- Thyroid
<table>
<thead>
<tr>
<th>Chemical</th>
<th>Chemical</th>
</tr>
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<tbody>
<tr>
<td>1,2,4-trimethylbenzene</td>
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<td>Xylenes</td>
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Conclusions

1. Hormones and EDCs work at low concentrations
2. EDCs are associated with disease in people
3. Of 24 fracking chemicals tested, 23 are EDCs
4. All five hormone receptors tested were disrupted by fracking chemicals
5. Surface and ground water from hydraulic fracturing “preventable discharge” sites had greater endocrine disrupting activity than control sites
Research Team

Kassotis  Davis  Nagel  McElroy

Lin  Tillit  Vengosh  Balise

Funding: The Passport Foundation, University of Missouri, EPA STAR Fellowship to Kassotis
<table>
<thead>
<tr>
<th>Site #</th>
<th># of Samples</th>
<th>Wells within 1 mile</th>
<th>Incident</th>
<th>Year</th>
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<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>43</td>
<td>Natural gas upwelling</td>
<td>2008</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>78</td>
<td>Discharge into stream</td>
<td>2009</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>69</td>
<td>Fuel or produced water spill</td>
<td>2008</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>136</td>
<td>Produced water tank leak</td>
<td>2004</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>95</td>
<td>Produced water line leak</td>
<td>2010</td>
</tr>
</tbody>
</table>
Steroid Receptor Reporter Gene assay

Testosterone [M]
# Analytical Measurement of Selected Chemicals in Produced Water

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>CAS #</th>
<th>PW1 Aqu</th>
<th>PW1 Org</th>
<th>PW2</th>
<th>PW3</th>
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</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>91-20-3</td>
<td>3.5</td>
<td>264.5</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Styrene</td>
<td>100-42-5</td>
<td>&lt;LOD</td>
<td>52.0</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Benzene</td>
<td>71-43-2</td>
<td>0.9</td>
<td>331.5</td>
<td>1.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>100-41-4</td>
<td>7.3</td>
<td>1099.0</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Cumene</td>
<td>98-82-8</td>
<td>4.2</td>
<td>128.9</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>2-ethylhexanol</td>
<td>104-76-7</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>trimethylbenzene</td>
<td>95-63-6</td>
<td>78.5</td>
<td>5873.0</td>
<td>0.3</td>
<td>2.7</td>
</tr>
<tr>
<td>Toluene</td>
<td>108-88-3</td>
<td>27.3</td>
<td>1410.0</td>
<td>4.8</td>
<td>11.8</td>
</tr>
<tr>
<td>m-xylene</td>
<td>108-38-3</td>
<td>&lt;LOD</td>
<td>885.5</td>
<td>1.6</td>
<td>8.2</td>
</tr>
<tr>
<td>p-xylene</td>
<td>106-42-3</td>
<td>40.1</td>
<td>1172.0</td>
<td>(m+p)</td>
<td>(m+p)</td>
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<td>o-xylene</td>
<td>95-47-6</td>
<td>15.0</td>
<td>396.4</td>
<td>0.9</td>
<td>2.7</td>
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<tr>
<td>2-butoxyethanol</td>
<td>111-76-2</td>
<td></td>
<td></td>
<td>77.5</td>
<td></td>
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