



**Endocrine-Disrupting Chemicals and Oil and
Natural Gas Operations: Potential Environmental
Contamination and Recommendations to Assess
Complex Environmental Mixtures**

**Christopher D. Kassotis, Donald E. Tillitt, Chung-Ho Lin,
Jane A. McElroy, and Susan C. Nagel**

<http://dx.doi.org/10.1289/ehp.1409535>

Received: 27 November 2014

Accepted: 22 July 2015

Advance Publication: 27 August 2015

This article will be available in a 508-conformant form upon final publication. If you require a 508-conformant version before then, please contact ehp508@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

Endocrine-Disrupting Chemicals and Oil and Natural Gas Operations: Potential Environmental Contamination and Recommendations to Assess Complex Environmental Mixtures

Christopher D. Kassotis,¹ Donald E. Tillitt,² Chung-Ho Lin,³ Jane A. McElroy,⁴ and Susan C. Nagel¹

¹Department of Obstetrics, Gynecology and Women's Health, University of Missouri, Columbia, Missouri, USA; ²U.S. Geological Survey, Columbia Environmental Research Center, Columbia, Missouri, USA; ³Department of Forestry, School of Natural Resources, University of Missouri, Columbia, Missouri, USA; ⁴Department of Family and Community Medicine, University of Missouri, Columbia, Missouri, USA

Address correspondence to Susan C. Nagel, University of Missouri, Obstetrics, Gynecology and Women's Health, M659 Medical Sciences Building, 1 Hospital Drive, University of Missouri-Columbia, Columbia, MO 65211 USA. Telephone: 573-884-3028. E-mail: nagels@health.missouri.edu

Short running title: Assessing EDC mixtures in oil and gas operations

Acknowledgments: We wholeheartedly thank Drs. Carol Kwiatkowski and John J. Bromfield for their comments on the development of this manuscript.

Writing supported by STAR Fellowship Assistance Agreement no. FP-91747101 awarded by the U.S. Environmental Protection Agency (CDK). The views expressed are those of the authors and of the US Geological Survey; however, they are not necessarily the views of the US

Environmental Protection Agency. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

Competing financial interests: The authors declare no competing financial interests.

Abstract

Background: Hydraulic fracturing technologies, developed over the last 65 years, have only recently been combined with horizontal drilling to unlock oil and gas reserves previously deemed inaccessible. While these technologies have dramatically increased domestic oil and natural gas production, they have also raised concerns for the potential contamination of local water supplies with the approximately 1,000 chemicals used throughout the process, including many known or suspected endocrine-disrupting chemicals.

Objectives: We discuss the need for an endocrine component to health assessments for drilling-dense regions in the context of hormonal and anti-hormonal activities for chemicals used.

Methods: We discuss the literature on 1) surface and ground water contamination by oil and gas extraction operations, and 2) potential human exposure, particularly in context of the total hormonal and anti-hormonal activities present in surface and ground water from natural and anthropogenic sources, with initial analytical results and critical knowledge gaps discussed.

Discussion: In light of the potential for environmental release of oil and gas chemicals that can disrupt hormone receptor systems, we recommend methods for assessing complex hormonally active environmental mixtures.

Conclusions: We describe a need for an endocrine-centric component for overall health assessments and provide supporting information that using this may help explain reported adverse health trends as well as help develop recommendations for environmental impact assessments and monitoring programs.

Introduction

A novel source of human and animal exposure to endocrine-disrupting chemicals (EDCs) is through their use in oil and gas drilling operations. EDCs are exogenous compounds that have the ability to disrupt development and normal hormone action through directly interacting with hormone receptors as agonists/antagonists or indirectly, *e.g.*, by altering endogenous hormone concentrations, delivery to receptors, modulation of endogenous hormone responses, enzyme activities, or other mechanisms (Bergman et al. 2013; Diamanti-Kandarakis et al. 2009; Zoeller et al. 2015). Importantly, oil and gas operation chemicals have been shown to act through both direct and indirect mechanisms (Andric et al. 2006; Kassotis et al. 2014; Knag et al. 2013; Thomas and Budiantara 1995). EDCs can exhibit effects at extremely low, environmentally relevant concentrations, particularly during sensitive windows when exposure can alter normal development and result in adverse health outcomes during adulthood (Vandenberg et al. 2012; Vandenberg 2014; vom Saal et al. 2007; Welshons et al. 2003). While chemicals used in and produced by oil and gas operations include EDCs, carcinogens, radioactive compounds, and other toxicants, herein we will focus on the unique issues posed by their endocrine-disrupting activities.

In hydraulic fracturing, millions of gallons of water, tens of thousands of gallons chemicals, and suspended solids are injected into the ground under high pressure. Hydraulic fracturing serves to fracture the shale or coal bed layer and release trapped natural gas or oil, allowing for increased well production. While hydraulic fracturing technologies have been developed over the last 65 years, they have only recently been combined with horizontal drilling to unlock vast new oil and gas reserves around the globe previously deemed either inaccessible or unprofitable (Waxman et al. 2011; Wiseman 2008). Chemicals are added throughout the entire production process

(including drilling, fracturing, and through closure) for a number of reasons (Table 1; Deutch et al. 2011; Riedl et al. 2013; Waxman et al. 2011). In total, approximately 1,000 chemicals are known to be used throughout the process (EPA 2015; Waxman et al. 2011).

Following the initial injection into the well to generate fractures, a portion of the injected volume returns to the surface immediately and is termed “flow-back”. The remaining fluids either permeate into the shale or coal bed formation and/or return to the surface over the life of the producing well, termed “produced water”. Both types of wastewater can contain fracturing fluids, naturally occurring salts, radioactive materials, heavy metals, and other chemicals from the shale formation such as polycyclic aromatic hydrocarbons, alkenes, alkanes, and other volatile and semi-volatile organic compounds (Deutch et al. 2011; Fontenot et al. 2013; Harkness et al. 2015; Harvey et al. 1984; Maule et al. 2013; Warner et al. 2012). Wastewater is disposed of via injection wells, open evaporation pits, landfills, treatment plants, through on-site burial, being spread over road or fields, and/or is treated and reused in future hydraulic fracturing operations (Deutch et al. 2011; Gilmore et al. 2014; Lee et al. 2011; Wiseman 2008). Treatment of wastewater for reuse or disposal varies via geological region due to differing chemical compositions, and may include biological treatment, filtration or aeration steps, and/or reverse-osmosis separation (Lester et al. 2015).

Potential Routes of Exposure to Oil and Natural Gas Operation Chemicals

Water. Oil and natural gas operations can lead to the contamination of surface and ground water, both sources of drinking water (reviewed in Brantley et al. 2014; Burton et al. 2014; Vengosh et al. 2014). Contamination can occur through spills of chemicals during transport to and from the fracturing site, the drilling and fracturing processes, improper treatment and disposal of

wastewater, failure of well casings, and from structural failure in abandoned wells (Ingraffea et al. 2014; Kell 2011; Mauter et al. 2014; Rozell and Reaven 2012).

In 2013, spills were reported at 1% of Colorado wells (550/51,000 active wells) and it has been estimated that 50% of surface spills contaminate ground water based on data from Weld County, CO (Gross et al. 2013). An analysis of permitted Pennsylvania wells suggests a similar total spill rate of 2% (103/5,580 active wells; Souther et al. 2014). Although all 24 states with active shale reservoirs report spills, reporting limits and information required vary widely, and only five states require maintenance of public records for spills and violations (Soraghan 2014; Souther et al. 2014). Given the limited mandatory reporting, it is likely that the magnitude of oil and gas operations' impact on water quality is underestimated (Soraghan 2014; Souther et al. 2014). For example, an analysis in Pennsylvania found that industry had reported 59% of documented spills (Souther et al. 2014).

Wastewater is commonly sent to wastewater treatment plants in many regions (Gilmore et al. 2014) that are not able to remove many of the anthropogenic or naturally occurring compounds present in wastewater from shale operations (Braga et al. 2005; Campbell et al. 2006; Westerhoff et al. 2005). This can result in the discharge of these compounds into surface water following treatment (Ferrar et al. 2013b; Harkness et al. 2015; Warner et al. 2013, 2014). Transportation of chemicals for drilling and fracturing to well pads and wastewater away from well pads poses risks for contamination (Burton et al. 2014). Spills and leaks occur during transportation through wastewater pipelines and during transfer to trucks at well pads and during vehicular transport to disposal facilities (Gilmore et al. 2014).

Ground water contamination associated with oil and gas operations has also been reported (Fontenot et al. 2013; Jackson et al. 2013; Osborn et al. 2011; Vengosh et al. 2014). This contamination can occur through migration of chemicals from the surface or underground. An investigation of wastewater pits and impoundments in the Marcellus Shale region reported lack of maintenance of containment and transport systems, with spills impacting ground water largely due to equipment failures and corrosion of pipes and tanks (Ziemkiewicz et al. 2014). Surface spills of fracturing fluids can also contaminate ground water, and elevated BTEX (benzene, toluene, ethylbenzene, and xylenes) concentrations have been reported in ground water near surface spills (Gross et al. 2013; Ziemkiewicz et al. 2014). A recent EPA report conclusively linked hydraulic fracturing to drinking water contamination at some wells within five of six retrospective study regions, with no existing baseline testing for the sixth region (EPA 2015). Underground migration potential is also a concern. Heavy metal concentrations have been shown to increase in drinking water with proximity to natural gas wells (Fontenot et al. 2013), and thermogenic (shale-origin) gas concentrations in drinking water from close proximity to natural gas wells have been reported to be higher than water from more distant sources (Jackson et al. 2013; Li and Carlson 2014; Osborn et al. 2011). Recent work suggests this may be due primarily to faulty well casings (Darrah et al. 2014).

Air. Oil and natural gas production processes also contribute contaminants to the air, creating another potential route of exposure for humans and animals (Colborn et al. 2014; Helmig et al. 2014; Macey et al. 2014; Moore et al. 2014). Potential sources of inhalation exposure for these chemicals include: evaporation from surface spills and evaporation pits, flaring at the surface, surface transfers, and during processing (Colborn et al. 2014; Trimble 2012). High-level releases of chemicals are episodic (Brown et al. 2014, 2015). Elevated volatile organic compounds

(VOCs) such as BTEX, alkenes, alkanes, aromatic compounds, and aldehydes are reported during drilling, production, and completion from nearby wells (Colborn et al. 2014; McKenzie et al. 2012; Roy et al. 2014; Steinzor et al. 2013), in some cases exceeding levels observed in heavily polluted inner cities (Helmig et al. 2014).

Endocrine-disrupting Chemicals and Oil and Gas Operations

EDC Activity of Chemicals Used in Oil and Natural Gas Operations. Our lab has tested the estrogen and androgen receptor activities of twelve chemicals commonly used in oil and gas operations using a luminescence-based reporter gene bioassay in human cancer cells. We measured stimulation of receptors (agonist) or inhibition of positive control-induced expression (antagonist). We found one estrogen receptor agonist, eleven estrogen receptor antagonists, and ten androgen receptor antagonists, with several chemicals exhibiting multiple receptor activities (Kassotis et al. 2014).

A 2011 analysis reported approximately 120 known or suspected EDCs out of 353 oil and gas operation chemicals with Chemical Abstract Service (CAS) numbers (Colborn et al. 2011). Importantly, only half of the known oil and gas operation chemicals had CAS numbers at that time, greatly limiting the health assessment for other chemicals used (Waxman et al. 2011). Still other chemicals remain proprietary information (Shonkoff et al. 2014; Wiseman 2011). For example, recent work found that 67%, 37%, and 18% of assessed wells were fractured with ≥ 1 , 5 or 10 proprietary chemicals, respectively (Souther et al. 2014).

EDC Activity in Water Near Oil and Natural Gas Operations. We have assessed the estrogen and androgen receptor activities of water samples collected from five sites in a drilling-dense region of Garfield County, Colorado that had experienced industry related spills or preventable

discharges relative to surface and ground water collected immediately outside of the drilling-dense region (Kassotis et al. 2014). Analysis of these samples revealed that surface and ground water from Garfield County spill sites contained significantly elevated estrogen agonist, estrogen antagonist, and androgen antagonist activities relative to reference sites (Kassotis et al. 2014). Independent analytical water testing at these sites identified chemicals that others or we have shown to exhibit these same agonist and antagonist activities (discussed in Kassotis et al. 2014). Other researchers have reported estrogen agonist and androgen antagonist activities associated with oil sands and oil production wastewaters (He et al. 2011; Thomas et al. 2004, 2009; Tollefsen et al. 2007).

Concentration of Oil and Natural Gas Operation Chemicals in Water. Hydraulic fracturing wastewater is reported to contain hundreds of organic chemicals (polyethylene glycols, ethoxylated surfactants, BTEX compounds, biocides, polycyclic aromatic hydrocarbons, aromatic amines, and more), with total dissolved organ carbon as high as 5.5 g/L and many individual compounds present at >500 mg/L and up to g/L concentrations (Kahrilas et al. 2015; Maguire-Boyle and Barron 2014; Orem et al. 2014; Thurman et al. 2014). A recent report analyzed publically available data on FracFocus, an industry disclosure website (<http://www.fracfocus.org/>), and reported benzene at up to 4.1%, and naphthalene and ethylbenzene up to 0.45% of total fracturing fluid volume, resulting in mg/L concentrations for these and other chemicals (Schaeffer and Bernhardt 2014).

Surface spills have been found to contaminate ground water with oil and gas operation chemicals (Gross et al. 2013). Groundwater at surface spill sites contained 1.4, 2.2, 0.2, and 2.6 mg/L benzene, toluene, ethylbenzene, and xylene, respectively, and these concentrations decreased over time and distance from the spill sites (Gross et al. 2013). Sampling of ground water in

Pavillion, WY by the US EPA, in a region where no specific accident or spill had occurred, revealed concentrations of BTEX, naphthalene, ethylene glycols, and other oil and gas chemicals at concentrations ranging from 0.01-8 mg/L (DiGiulio et al. 2011). As some of these chemicals have been shown to disrupt multiple hormone receptors *in vitro* at concentrations in the µg/L range (Kassotis et al. 2014), these ground water samples contain concentrations within the bioactive range in our reporter gene assays. To date there are few comprehensive analyses of oil and gas operation-derived chemicals in drinking water samples.

Potential Endocrine-Related Health Effects of Oil and Gas Operation Chemicals

Oil and Gas Operation Chemicals and Health Effects. Evidence of potential harm from exposure to hazardous chemicals, pollutants, and emissions used in oil and natural gas operations has been reported. These have most frequently been case series involving natural experiments using quasi-experimental design, involving domestic animals and wild life (Bamberger and Oswald 2012). Researchers, have also begun to document in reports and white papers the content and quantities of hazardous chemicals, pollutants, and emissions associated with these operations (Eastern Research Group 2011; Ethridge 2010; Steinzor et al. 2013; Witter et al. 2008). Concurrent with these environmental testing projects, surveys of local residents were also obtained with reports suggesting living in close proximity to oil and gas operations may have the potential to affect human and environmental health (Ferrar et al. 2013a; Rabinowitz et al. 2015; Steinzor et al. 2013; Subra 2009, 2010). Currently a limited number of epidemiology studies have been conducted to explore the association with health effects and exposure to oil and gas operation chemicals as described herein and reviewed in (Webb et al. 2014; Werner et al. 2015).

The biological plausibility of health effects associated with exposure to hazardous chemicals, pollutants, and emissions used in oil and natural gas operations has also been explored. Many of

these chemicals have documented adverse health effects in humans, are designated priority pollutants by the EPA, and/or are known or suspected EDCs (Colborn et al. 2011; Waxman et al. 2011). For example, exposure to naphthalene, a constituent of crude oil and a chemical used for hydraulic fracturing processes by industry (Waxman et al. 2011) and reported in air and water near operations (Colborn et al. 2014; DiGiulio et al. 2011; Wolf Eagle Environmental 2009), can result in altered steroid hormone levels, increased reproductive abnormalities, and impaired sexual maturation in animal models or *in vitro* (Hansen et al. 2008; Pollino et al. 2009; Thomas and Budiantara 1995; Tintos et al. 2006), though generally at greater concentrations than those reported near these sites.

Occupational Exposures. As with all environmental exposures, those who work around or with hazardous chemicals face significantly higher exposure risk compared to the general population. For the oil and natural gas extraction industry, the National Institute of Occupational Health and Safety (NIOSH) has published two studies; one about work crew exposures to respirable crystalline silica and the other about work crew exposures to VOCs (Esswein et al. 2013, 2014). In both cases, these pilot data indicated that some workers' exposures exceeded NIOSH and/or ACGIH safe levels (reported therein) for crystalline silica, flammable hydrocarbon emissions and benzene, respectively.

Reproductive Effects. Exposure to VOCs including but not limited to benzene, toluene, ethylbenzene, toluene, and formaldehyde, all chemicals used in and produced by oil and natural gas operations (Colborn et al. 2011; Waxman et al. 2011), are associated with reproductive health effects in both humans and animals. These effects include impaired fertility and fecundity via reduced semen quality and impaired menstrual cycles as well as increased risk of miscarriage, stillbirth, preterm birth, and birth defects, reviewed in Webb et al. 2014. Other

adverse endocrine health effects due to exposure to single chemicals used in and produced by oil and gas operations have been assembled and are available at:

<http://endocrinedisruption.org/chemicals-in-natural-gas-operations/chemicals>.

Adverse Pregnancy Outcomes. Using spatial analysis, McKenzie et al evaluated the likelihood of adverse pregnancy outcomes in a cohort of 12,842 live births for mothers living within 10 miles of drilling well operations compared to those with no drilling wells within 10 miles. A significant increased risk for congenital heart defects (adjusted odds ratio [AOR]: 1.3; 95% CI, 1.2-1.5) and neural tube defects (AOR: 2.0; 95% CI, 1.0-3.9) but no association for oral clefts (AOR: 0.82; 95% CI: 0.55-1.2) was observed. In contrast, low birth weight has mixed results using a similar study design (McKenzie et al. 2014; Stacy et al. 2015). In two case-control studies, maternal or paternal occupational exposure to glycol ethers (hormonally active chemicals used in fracturing fluids; EPA 2015; Kassotis et al. 2014; Waxman et al. 2011) and other chemicals (pesticides, polychlorinated compounds, phthalates, bisphenol A, alkylphenolic compounds, heavy metals and miscellaneous agents), during pregnancy has been associated with congenital malformations (Cordier et al. 1997).

Cancer. In a health impact assessment, McKenzie et al used spatial modeling based on residence proximity ($\leq 1/2$ mile versus $>1/2$ mile) to oil and gas operations in Colorado and found an elevated cumulative cancer risk for people living near drilling wells (10 in a million versus 6 in a million; McKenzie et al. 2012). Two studies calculated standardized incidence ratios. One was a cancer cluster analysis for several cancers in a drilling-dense Texas town compared to state rates using 3-years of cancer incidence data. Mokry et al reported a statistically significant elevated rate for breast cancer (SIR: 1.3; 95% CI, 1.1-1.5; Mokry 2010). The other study compared Pennsylvania counties before and after launching of drilling operations. Fryzek et al, found a

slight increased rate of one cancer, central nervous system tumors (SIR: 1.13; 95% CI, 1.02-1.25) after unconventional drilling operations began in Northeast Pennsylvania (Fryzek et al. 2013).

Limitations and Data Gaps. Limitations of the above-mentioned studies are the lack of direct exposure assessment as well as information on residential mobility of study participants. To date, no longitudinal study has enrolled a cohort of residents in a community that has an active oil and natural gas extraction industry so that biomarkers can be obtained in a timely manner. Known and suspected risk factors need to be collected to fully model the exposure risk. The critical route/timing of exposure and hazardous chemicals associated with oil and natural gas operations has yet to be established. Drilling wells release different amounts of air pollutants at different stages of the development and production processes (Brown et al. 2014; Colborn et al. 2014; Helmig et al. 2014; McKenzie et al. 2012), and residents, including pregnant women, may be exposed to these pollutants throughout the process or only during specific stages. Drinking water exposure may show considerable heterogeneity due to the hydrogeology of underground water flow associated with natural and man-made chemicals released, and limited data are available on contamination of drinking water in areas that have oil and natural gas operations.

Recommendations

The endocrine system is designed to respond to extremely low concentrations of hormones, making it uniquely equipped to assess exposure to low levels of exogenous hormonally active contaminants. While toxicological studies often assess adverse outcomes from higher exposure scenarios more relevant to occupational exposure, endocrinological studies can assess outcomes from lower level exposure that may be more relevant to humans living near oil and natural gas operations. Combining existing *in vivo* EDC studies with knowledge of the hormone receptor

activity profile of chemicals used in oil and natural gas operations, we can identify adverse health outcomes for epidemiological assessment in areas where humans and animals are exposed to these chemicals. Subsequently, we can then use a modified Bradford-Hill approach to assess causality between environmental exposures and adverse health outcomes, as suggested previously (Zoeller et al. 2015). The risks related to potential exposure and adverse outcomes in humans and wildlife populations have not been afforded complete evaluations due in part to exemptions from parts of six key federal regulatory acts that traditionally act to safeguard US water sources, including the Safe Drinking Water Act and Clean Water Act (Deutch et al. 2011; Clean Water Act 1972; Safe Drinking Water Act 1974).

Based on the hypothesis that exposure to oil and natural gas chemicals may contribute to negative health outcomes, we offer the following recommendations to evaluate the risks posed to humans and wildlife: 1) integrate endocrine-centric endpoints into human health assessments in areas of unconventional drilling operations; 2) perform biomonitoring studies for chemicals and their metabolites in people; 3) develop an effect-directed screening approach to assess endocrine-related effects of mixtures; 4) perform controlled laboratory animal studies of exposure to complex mixtures of oil and natural gas chemicals to assess adverse health outcomes; and 5) perform in vitro bioassays to assess receptor-interactions with complex mixtures.

1) Endocrine health assessments. We suggest an endocrine-centric component to overall human and environmental health assessments. An endocrine-centric health component would assume additivity of chemicals, a concept that has been shown to be reasonable for chemicals acting through similar mechanisms of action (Payne et al. 2000; Rajapakse et al. 2002; Silva et al. 2002). This approach would assess common adverse endocrine endpoints shown to result from disruption of specific hormone receptors alone and in combination, including 1) reproductive

(infertility, subfertility, reduced sperm counts, miscarriage, preterm birth, birth weight, puberty), 2) developmental (cryptorchidism, hypospadias, NTD, congenital heart defects), and 3) cancer, particularly hormone-responsive types such as testicular, breast, prostate, and brain (reviewed in Bergman et al. 2013; Diamanti-Kandarakis et al. 2009; Vandenberg et al. 2012; Zoeller et al. 2012).

2) Measurement of chemicals in humans and wildlife (biomonitoring). One of the major limitations in human risk assessment of oil and natural gas operations is the paucity of chemical exposure information, considering the number of chemicals used and proprietary disclosure rules. Most research has thus far focused on airborne emissions (reviewed in Moore et al. 2014) and water contamination (reviewed in Rozell and Reaven 2012; Vengosh et al. 2014). While epidemiological studies have begun to assess adverse health outcomes near drilling operations (McKenzie et al. 2014), to our knowledge no researchers have yet published data on concentrations of oil and gas operation chemicals in humans or wildlife.

Chemical characterization is required to determine most likely biomonitoring candidates. Recent work has detailed analytical approaches for characterization of the various classes of compounds present in hydraulic fracturing wastewater (Ferrer and Thurman 2015). We suggest that oil and gas wastewater should be used to determine chemicals present that can result in the observed agonist and/or antagonist responses. Initial identification should occur via reverse matching to known compound lists such as the National Institute of Standards and Technology (NIST) Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library. These compounds can be further reverse matched to known oil and gas operation chemicals (EPA 2015; Colborn et al. 2011, 2014; Waxman et al. 2011). As this step may miss proprietary compounds not currently reported by industry, it should be used as a supplement to reverse-matching databases. These

compounds can then be confirmed with authentic standards. These chemicals can be further tested in bioassays to determine receptor activities and likely presence and contribution to activities in water. These data can then guide the development of analytical methods for target compounds and their metabolites as biomonitoring candidates in humans living near extraction operations.

3) Using effects-directed analysis to identify chemicals responsible for EDC activity. Analytical identification of the hormonally active chemicals present in both water and air must be performed to better characterize source, exposure, and to assess risk. Whenever possible, analysis of complex environmental samples should be performed using an effect-directed analysis approach (Burgess et al. 2013; Liscio et al. 2014; Rostkowski et al. 2011) coupled with a response-balance approach (Cargouet et al. 2004; Schriks et al. 2010; Sun et al. 2008).

This effect-directed/response-balance approach should target the most hormonally active samples from drilling regions (as well as reference sites to eliminate background activity/chemicals) for chemical fractionation and testing. These procedures should include orthogonal separations and screening of the resulting fractions in bioassays to refine and isolate bioactive chemicals. Refined fractions can then be analyzed using mass spectrometry (MS) tools described below and reported recently (Ferrer and Thurman 2015) to help identify chemicals responsible for observed activities. Once candidate chemicals are identified, authentic standards may be used to confirm MS identification and bioactivity in bioassays. This method has been used successfully to identify novel bioactive compounds and represents the best approach for characterizing EDCs most responsible for observed activities (Liscio et al. 2014; Rostkowski et al. 2011). Lastly, biological activity can be coupled with chemicals concentrations from

environmental monitoring to determine relative contributions to observed receptor activities, as described by others (Cargouet et al. 2004; Schriks et al. 2010; Sun et al. 2008).

4) *EDC-centric laboratory animal health assessments.* Laboratory animal models can and should be used to test for causal relationships between exposure and negative health outcomes that might be expected in drilling-dense regions. Humans and wildlife living in these regions are likely exposed during different developmental windows and known critical periods such as prenatal, perinatal, childhood, and puberty should be targeted. Studies of adult exposure should also be performed to assess occupational exposure and chronic exposure at environmentally relevant levels encountered by nearby residents. We further recommend that route of exposure remain as relevant as possible. Likely exposure to chemicals may occur through oral, dermal, and/or inhalation routes, and parameters such as volatility and partition coefficients will help determine which exposure routes are of the highest concern for individual chemicals. Route of administration is crucial to understanding health effects, as varying routes of exposure can result in very different bioavailability of EDCs, as described recently for bisphenol A (Gayrard et al. 2013; Hormann et al. 2014; vom Saal and Welshons 2014). Adverse health outcomes that should be targeted are described above in both the Human Health section as well as Recommendation 1 and are known to result from exposure to EDCs (reviewed in Bergman et al. 2013; Diamanti-Kandarakis et al. 2009; Vandenberg et al. 2012; Zoeller et al. 2012); many protocols have been described for the evaluation of these endpoints (EPA 2009a, b, c; Diamanti-Kandarakis et al. 2009; Schug et al. 2013; Zoeller et al. 2012). These data can provide important information for further refining human epidemiological studies as well as studies on pets and wildlife populations, which have recently been shown to be impacted via endocrine health concerns

(Bamberger and Oswald 2012, 2014, 2015; Grant et al. 2015; Papoulias and Velasco 2013; Slizovskiy et al. 2015).

5) *Bioassays for complex mixtures*. With approximately 1,000 chemicals used in and produced by oil and gas operations (EPA 2015), there is a critical need for methods to assess EDC activity of these complex mixtures. Methods of assessing activity and potential health risks of mixtures that can appropriately address interplay between receptor systems are limited. Observed outcomes *in vivo* can often be the result of disruption of several hormone receptor systems by either single chemicals or mixtures. Statistical modeling (Orton et al. 2012), *in vitro* and *in vivo* assays (Silva et al. 2002), quantitative structure analysis (Nishihara et al. 2000), gene expression (Richter et al. 2014), and other tools have been used to assess a number of laboratory-defined mixtures that interact with single hormone receptors.

Modeling complex mixtures has the ability to greatly reduce the number of independent tests. For example, Bertin et al. used a neural networking model to assess mixture toxicity, achieving a predictive model with approximately 10% of actual interactions tested (Bertin et al. 2013). However, despite clear successes with less complicated mixtures, analysis of more complicated mixtures appears to be beyond current capabilities (Kortenkamp et al. 2014; Orton et al. 2012), due to insufficient knowledge of: inter-receptor interactions and indirect chemical-receptor interactions (Kortenkamp et al. 2014). Another concern involves indirect interactions between chemicals and receptors. For example, interaction with the aryl hydrocarbon receptor can result in the activation of cytochrome P450 enzymes, well understood to alter endogenous and exogenous chemical metabolism and therefore exposure (Anzenbacher and Anzenbacherova 2001; Markowitz et al. 2003). Inactive chemicals can be metabolized into active metabolites, resulting in mixtures of inactive chemicals that can act as agonists or antagonists in mixtures

only (Gauger et al. 2007). Better characterization of these interactions will provide a clearer understanding of the utility and limitations models provide towards assessing *in vivo* outcomes.

As all combinations of chemicals can never be tested *in vitro* and/or *in vivo*, we recommend guided *in vitro* and *in vivo* research that focuses on receptor interactions. We suggest reporter gene assays be used for *in vitro* testing due to low cost, ease of use, reliability, high sensitivity, and ease of adapting for multiple receptor systems (Naylor 1999; Rajapakse et al. 2002; Silva et al. 2002; Soto et al. 2006). Similar assays including the yeast receptor screens (YES, YAS, etc.) tend to be less robust and less sensitive though are less susceptible to toxicity, while cell proliferation assays (E-SCREEN, A-SCREEN, etc.) can be equally sensitive and unlike reporter gene assays are able to measure non-genomic effects through cell-surface receptors, though are generally less applicable for diverse receptor testing (Leusch et al. 2010). Current high-throughput assay options such as Tox21 or ToxCast are of great use as diverse first-pass screens for individual compounds, though it is unclear if they will be helpful in the assessment of complex mixtures (Filer et al. 2014; Tice et al. 2013). Rather than the single-receptor tests utilized by these systems, assessing chemicals and mixture of chemicals in controlled multiple-receptor systems is critical to better understand and account for receptor interplay.

Improvement of the utility of *in vitro* assay systems should take place in several steps. First, receptor interaction can be assessed through testing positive controls both in the presence and absence of other receptors. Ideally, this should be done across several cell lines to identify chemical impingement on receptor interactions and tissue-specific comodulators. Once multiple receptor experiments are carried out with single chemicals, introducing simple mixtures with clearly defined receptor activity profiles can be used to determine how simultaneous interactions with several receptors can modulate responses. Further work should be coupled with *in vivo*

experiments to understand these interactions in a whole animal model and to confirm *in vitro* multiple-receptor results.

Potential Implications

Recent analysis of EDC exposures and potential contributions to adverse endocrine health outcomes, such as obesity, cancers (particularly hormone-dependent), reproduction/infertility, metabolic diseases, and developmental abnormalities, has been estimated to account for 1.8% to 40% of societal health care costs (Hunt and Ferguson 2014; Olsson 2014; Trasande 2014). More recently, a suite of studies estimated the potential health care costs for the European Union (EU) due to: neurobehavioral deficits and disorders (>150 billion euros; Bellanger et al. 2015), obesity and diabetes (>18 billion euros; Legler et al. 2015), and male reproductive disorders and diseases (>15 billion euros; Hauser et al. 2015). Altogether, the median cost to the EU for EDCs with the highest probability of causation was estimated at 157 billion euros per year (Trasande et al. 2015). While exposure to oil and gas operation chemicals individually would likely result in only a fraction of these costs, increasing exposure to additional hormonally active chemicals is cause for concern given the additive nature of many of these receptor systems. As such, there are potentially large financial implications of exposure to EDCs from their use in oil and gas operations.

Conclusions

We provide a series of recommendations herein that will allow scientifically defensible, accurate assessments of the potential endocrine-related risks from chemical exposure associated with oil and natural gas operations. We present these recommendations in light of the growing information regarding chemical concentrations in the environment as well as adverse health outcomes reported both in humans and wildlife. We suggest that these approaches will lead to

better information for resource management decisions and ultimately protect and improve human health.

References

- Andric NL, Kostic TS, Zoric SN, Stanic BD, Andric SA, Kovacevic RZ. 2006. Effect of a PCB-based transformer oil on testicular steroidogenesis and xenobiotic-metabolizing enzymes. *Reprod Toxicol* 22:102-110.
- Anzenbacher P, Anzenbacherova E. 2001. Cytochromes p450 and metabolism of xenobiotics. *Cellular and Molecular Life Sciences* 58:737-747.
- Bamberger M, Oswald RE. 2012. Impacts of gas drilling on human and animal health. *New Solutions* 22:51-77.
- Bamberger M, Oswald RE. 2014. Unconventional oil and gas extraction and animal health. *Environmental Science: Processes & Impacts* 16:1860-1865.
- Bamberger M, Oswald RE. 2015. Long-term impacts of unconventional drilling operations on human and animal health. *Journal of Environmental Science and Health Part A, Toxic/Hazardous Substances & Environmental Engineering* 50:447-459.
- Bellanger M, Demeneix B, Grandjean P, Zoeller RT, Trasande L. 2015. Neurobehavioral deficits, diseases and associated costs of exposure to endocrine disrupting chemicals in the European Union. *The Journal of Clinical Endocrinology and Metabolism* 100:1256-1266.
- Bergman A, Heindel JJ, Jobling S, Kidd KA, Zoeller RT. 2013. State of the science of endocrine disrupting chemicals 2012. World Health Organization. Available at: <http://www.who.int/ceh/publications/endocrine/en/>
- Bertin MJ, Moeller P, Guillette LJ, Jr., Chapman RW. 2013. Using machine learning tools to model complex toxic interactions with limited sampling regimes. *Environmental Science & Technology* 47:2728-2736.
- Braga O, Smythe GA, Schafer AI, Feitz AJ. 2005. Steroid estrogens in primary and tertiary wastewater treatment plants. *Water Science and Technology* 52:273-278.
- Brantley SL, Yoxtheimer D, Arjmand S, Grieve P, Vidic R, Pollak J, et al. 2014. Water resource impacts during unconventional shale gas development: The Pennsylvania experience. *The International Journal of Coal Geology* 126:140-156.
- Brown D, Weinberger B, Lewis C, Bonaparte H. 2014. Understanding exposure from natural gas drilling puts current air standards to the test. *Reviews on Environmental Health* 29:277-292.

- Brown DR, Lewis C, Weinberger BI. 2015. Human exposure to unconventional natural gas development: A public health demonstration of periodic high exposure to chemical mixtures in ambient air. *Journal of Environmental Science and Health Part A, Toxic/Hazardous Substances & Environmental Engineering* 50:460-472.
- Burgess RM, Ho KT, Brack W, Lamoree M. 2013. Effects-directed analysis (EDA) and toxicity identification evaluation (TIE): Complementary but different approaches for diagnosing causes of environmental toxicity. *Environmental Toxicology and Chemistry* 32:1935-1945.
- Burton GA, Jr., Basu N, Ellis BR, Kapo KE, Entrekin S, Nadelhoffer K. 2014. Hydraulic "fracking": Are surface water impacts an ecological concern? *Environmental Toxicology and Chemistry* 33:1679-1689.
- Campbell C, Borglin S, Green F, Grayson A, Wozel E, Stringfellow W. 2006. Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: A review. *Chemosphere* 65:1265-1280.
- Cargouet M, Perdiz D, Mouatassim-Souali A, Tamisier-Karolak S, Levi Y. 2004. Assessment of river contamination by estrogenic compounds in Paris area (France). *The Science of the Total Environment* 324:55-66.
- Clean Water Act of 1972. 1972. Public Law 92-500.
- Colborn T, Kwiatkowski C, Schultz K, Bachran M. 2011. Natural gas operations from a public health perspective. *International Journal of Human and Ecological Risk Assessment* 17:1039-1056.
- Colborn T, Schultz K, Herrick L, Kwiatkowski C. 2014. An exploratory study of air quality near natural gas operations. *Human and Ecological Risk Assessment* 20:86-105.
- Cordier S, Bergeret A, Goujard J, Ha MC, Ayme S, Bianchi F, et al. 1997. Congenital malformation and maternal occupational exposure to glycol ethers. Occupational exposure and congenital malformations working group. *Epidemiology* 8:355-363.
- Darrah TH, Vengosh A, Jackson RB, Warner NR, Poreda RJ. 2014. Noble gases identify the mechanisms of fugitive gas contamination in drinking-water wells overlying the Marcellus and Barnett Shales. *PNAS* 111:14076-14081.

- Deutch J, Holditch S, Krupp F, McGinty K, Tierney S, Yergin D, et al. 2011. The Secretary of the Energy Board Shale Gas Production Subcommittee Ninety-Day Report. Available at: <http://energy.gov/downloads/90-day-second-report-shale-gas-production-secretary-energy-advisory-board>
- Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, et al. 2009. Endocrine-disrupting chemicals: An endocrine society scientific statement. *Endocrine reviews* 30:293-342.
- DiGiulio DC, Wilkin RT, Miller C, Oberley G. 2011. Investigation of ground water contamination near pavillion, wyoming. EPA 600/R-00/000.
- Eastern Research Group and Sage Environmental Consuling. 2011. City of Fort Worth natural gas air quality study. Available at: http://fortworthtexas.gov/uploadedFiles/Gas_Wells/AirQualityStudy_final.pdf
- Environmental Protection Agency. 2009a. Test method for assessment of amphibian metamorphosis (frog). EPA 740-C-09-002. Available at: <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0576-0002>
- Environmental Protection Agency. 2009b. Test method for fish short-term reproduction assay. EPA 740-C-09-007. Available at: <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0576-0007>
- Environmental Protection Agency. 2009c. Test method for assessment of pubertal development and thyroid function in intact juvenile/peripubertal male rats. EPA 740-C-09-012. Available at: <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0576-0010>
- Environmental Protection Agency. 2015. Assessment of the potential impacts of hydraulic fracturing for oil and gas on drinking water resources. EPA/600/R-15/047a. Available at: <http://cfpub.epa.gov/ncea/hfstudy/recordisplay.cfm?deid=244651>
- Esswein EJ, Breitenstein M, Snawder J, Kiefer M, Sieber WK. 2013. Occupational exposures to respirable crystalline silica during hydraulic fracturing. *Journal of Occupational and Environmental Hygiene* 10:347-356.
- Esswein EJ, Snawder J, King B, Breitenstein M, Alexander-Scott M, Kiefer M. 2014. Evaluation of some potential chemical exposure risks during flowback operations in unconventional oil and gas extraction: Preliminary results. *Journal of Occupational and Environmental Hygiene* 11:D174-184.

- Ethridge S. 2010. Texas Commission on Environmental Quality Interoffice Memorandum. (Vickery MR, ed). Available at:
http://www.tceq.state.tx.us/assets/public/implementation/barnett_shale/2010.01.27-healthEffects-BarnettShale.pdf.
- Ferrar KJ, Kriesky J, Christen CL, Marshall LP, Malone SL, Sharma RK, et al. 2013a. Assessment and longitudinal analysis of health impacts and stressors perceived to result from unconventional shale gas development in the Marcellus Shale region. *International Journal of Occupational and Environmental Health* 19:104-112.
- Ferrar KJ, Michanowicz DR, Christen CL, Mulcahy N, Malone SL, Sharma RK. 2013b. Assessment of effluent contaminants from three facilities discharging Marcellus Shale wastewater to surface waters in Pennsylvania. *Environmental Science & Technology* 47:3472-3481.
- Ferrer I, Thurman EM. 2015. Chemical constituents and analytical approaches for hydraulic fracturing waters. *Trends in Environmental Analytical Chemistry* 5:18-25.
- Filer D, Patisaul HB, Schug T, Reif DM, Thayer KA. 2014. Test driving Toxcast: Endocrine profiling for 1858 chemicals included in Phase II. *Current Opinion in Pharmacology* 19:145-152.
- Fontenot BE, Hunt LR, Hildenbrand ZL, Carlton Jr DD, Oka H, Walton JL, et al. 2013. An evaluation of water quality in private drinking water wells near natural gas extraction sites in the Barnett Shale formation. *Environmental Science & Technology* 47:10032-10040.
- Fryzek J, Pastula S, Jiang X, Garabrant DH. 2013. Childhood cancer incidence in Pennsylvania counties in relation to living in counties with hydraulic fracturing sites. *Journal of Occupational and Environmental Medicine* 55:796-801.
- Gauger KJ, Giera S, Sharlin DS, Bansal R, Iannacone E, Zoeller RT. 2007. Polychlorinated biphenyls 105 and 118 form thyroid hormone receptor agonists after cytochrome p4501a1 activation in rat pituitary GH3 cells. *Environmental Health Perspectives* 115:1623-1630.
- Gayraud V, Lacroix MZ, Collet SH, Viguie C, Bousquet-Melou A, Toutain PL, et al. 2013. High bioavailability of bisphenol A from sublingual exposure. *Environmental Health Perspectives* 121:951-956.
- Gilmore KR, Hupp RL, Glathar J. 2014. Transport of hydraulic fracturing water and wastes in the Susquehanna River Basin, Pennsylvania. *Journal of Environmental Engineering* 140:10.

- Grant CJ, Weimer AB, Marks NK, Perow ES, Oster JM, Brubaker KM, et al. 2015. Marcellus and mercury: Assessing potential impacts of unconventional natural gas extraction on aquatic ecosystems in Northwestern Pennsylvania. *Journal of Environmental Science and Health Part A, Toxic/Hazardous Substances & Environmental Engineering* 50:482-500.
- Gross SA, Avens HJ, Banducci AM, Sahmel J, Panko JM, Tvermoes BE. 2013. Analysis of BTEX groundwater concentrations from surface spills associated with hydraulic fracturing operations. *J Air Waste Manag Assoc* 63:424-432.
- Hansen BH, Altin D, Vang SH, Nordtug T, Olsen AJ. 2008. Effects of naphthalene on gene transcription in calanus finmarchicus (crustacea: Copepoda). *Aquat Toxicol* 86:157-165.
- Harkness JS, Dwyer GS, Warner NR, Parker KM, Mitch WA, Vengosh A. 2015. Iodide, bromide, and ammonium in hydraulic fracturing and oil and gas wastewaters: Environmental implications. *Environmental Science & Technology* 49:1955-1963.
- Harvey TG, Matheson TW, Pratt KC. 1984. Chemical class separation of organics in shale oil by thin-layer chromatography. *Analytical Chemistry* 56:1277-1281.
- Hauser R, Skakkebaek NE, Hass U, Toppari J, Juul A, Andersson AM, et al. 2015. Male reproductive disorders, diseases, and costs of exposure to endocrine-disrupting chemicals in the European Union. *The Journal of Clinical Endocrinology and Metabolism* 100:1267-1277.
- He Y, Wiseman SB, Hecker M, Zhang X, Wang N, Perez LA, et al. 2011. Effect of ozonation on the estrogenicity and androgenicity of oil sands process-affected water. *Environmental Science & Technology* 45:6268-6274.
- Helmig D, Thompson C, Evans J, Park JH. 2014. Highly elevated atmospheric levels of volatile organic compounds in the Uintah Basin, Utah. *Environmental Science & Technology* 48:4707-4715.
- Hormann AM, Vom Saal FS, Nagel SC, Stahlhut RW, Moyer CL, Ellersieck MR, et al. 2014. Holding thermal receipt paper and eating food after using hand sanitizer results in high serum bioactive and urine total levels of bisphenol A (BPA). *PloS ONE* 9:1-12.
- Hunt A, Ferguson J. 2014. Health costs in the European Union - how much is related to EDCs? Health and Environment Alliance (HEAL). Available at: <http://www.env-health.org/news/latest-news/article/health-costs-in-the-eu-how-much-is>

- Ingraffea AR, Wells MT, Santoro RL, Shonkoff SB. 2014. Assessment and risk analysis of casing and cement impairment in oil and gas wells in Pennsylvania, 2000-2012. *PNAS* 111:10955-10960.
- Jackson RB, Vengosh A, Darrah TH, Warner NR, Down A, Poreda RJ, et al. 2013. Increased stray gas abundance in a subset of drinking water wells near Marcellus Shale gas extraction. *PNAS* 110:11250-11255.
- Kahrilas GA, Blotevogel J, Stewart P, Borch T. 2015. Biocides in hydraulic fracturing fluids: A critical review of their usage, mobility, degradation, and toxicity. *Environmental Science & Technology* 49:16-32.
- Kassotis CD, Tillitt DE, Davis JW, Hormann AM, Nagel SC. 2014. Estrogen and androgen receptor activities of hydraulic fracturing chemicals and surface and ground water in a drilling-dense region. *Endocrinology* 155:897-907.
- Kell S. 2011. State oil and gas agency groundwater investigations and their role in advancing regulatory reforms. (A Two-State Review: Ohio and Texas). Groundwater Protection Council. Available at:
https://fracfocus.org/sites/default/files/publications/state_oil_gas_agency_groundwater_investigations_optimized.pdf
- Knag AC, Verhaegen S, Ropstad E, Mayer I, Meier S. 2013. Effects of polar oil related hydrocarbons on steroidogenesis in vitro in H295R cells. *Chemosphere* 92:106-115.
- Kortenkamp A, Scholze M, Ermler S. 2014. Mind the gap: Can we explain declining male reproductive health with known antiandrogens? *Reproduction* 147:515-527.
- Lee DS, Herman JD, Elsworth D, Kim HT, Lee HS. 2011. A critical evaluation of unconventional gas recovery from the Marcellus Shale, Northeastern United States. *KSCE Journal of Civil Engineering* 15:679-687.
- Legler J, Fletcher T, Govarts E, Porta M, Blumberg B, Heindel JJ, et al. 2015. Obesity, diabetes, and associated costs of exposure to endocrine-disrupting chemicals in the European Union. *The Journal of Clinical Endocrinology and Metabolism* 100:1278-1288.
- Lester Y, Ferrer I, Thurman EM, Sitterley KA, Korak JA, Aiken G, et al. 2015. Characterization of hydraulic fracturing flowback water in Colorado: Implications for water treatment. *The Science of the Total Environment* 512-513C:637-644.

- Leusch FD, de Jager C, Levi Y, Lim R, Puijker L, Sacher F, et al. 2010. Comparison of five in vitro bioassays to measure estrogenic activity in environmental waters. *Environmental Science & Technology* 44:3853-3860.
- Li H, Carlson KH. 2014. Distribution and origin of groundwater methane in the Wattenberg oil and gas field of Northern Colorado. *Environmental Science & Technology* 48:1484-1491.
- Liscio C, Abdul-Sada A, Al-Salhi R, Ramsey MH, Hill EM. 2014. Methodology for profiling anti-androgen mixtures in river water using multiple passive samplers and bioassay-directed analyses. *Water Research* 57C:258-269.
- Macey GP, Breech R, Chernaik M, Cox C, Larson D, Thomas D, et al. 2014. Air concentrations of volatile compounds near oil and gas production: A community-based exploratory study. *Environmental Health* 13:82.
- Maguire-Boyle SJ, Barron AR. 2014. Organic compounds in produced waters from shale gas wells. *Environmental Science Processes & impacts* 16:2237-2248.
- Markowitz JS, Donovan JL, DeVane CL, Taylor RM, Ruan Y, Wang JS, et al. 2003. Effect of St John's Wort on drug metabolism by induction of cytochrome p450 3a4 enzyme. *JAMA* 290:1500-1504.
- Maule AL, Makey CM, Benson EB, Burrows IJ, Scammell MK. 2013. Disclosure of hydraulic fracturing fluid chemical additives: Analysis of regulations. *New Solutions* 23:167-187.
- Mauter MS, Alvarez PJ, Burton A, Cafaro DC, Chen W, Gregory KB, et al. 2014. Regional variation in water-related impacts of shale gas development and implications for emerging international plays. *Environmental Science & Technology* 45:8298-8306.
- McKenzie LM, Witter RZ, Newman LS, Adgate JL. 2012. Human health risk assessment of air emissions from development of unconventional natural gas resources. *The Science of the Total Environment* 424:79-87.
- McKenzie LM, Guo R, Witter RZ, Savitz DA, Newman LS, Adgate JL. 2014. Birth outcomes and maternal residential proximity to natural gas development in rural Colorado. *Environmental Health Perspectives* 122:412-417.
- Mokry BJ. 2010. Summary of investigation into the occurrence of cancer, zip codes 75022 and 75028, Flower Mound, Denton County, Texas, 1998-2007, 2007-2009. Department of State Health Services Texas Cancer Registry. Available at: https://www.dshs.state.tx.us/epitox/consults/flower_mound32010.doc

- Moore CW, Zielinska B, Petron G, Jackson RB. 2014. Air impacts of increased natural gas acquisition, processing, and use: A critical review. *Environmental Science & Technology* 48:8349-8359.
- Naylor LH. 1999. Reporter gene technology: The future looks bright. *Biochemical Pharmacology* 58:749-757.
- Nishihara T, Nishikawa J-i, Kanayama T, Dakeyama F, Saito K, Imagawa M, et al. 2000. Estrogenic activities of 517 chemicals by yeast two-hybrid assay. *Journal of Health Science* 46:282-298.
- Olsson I-M. 2014. The cost of inaction - a socioeconomic analysis of costs linked to effects of endocrine disrupting substances on male reproductive health. Nordic Council of Ministers. Available at: <http://norden.diva-portal.org/smash/get/diva2:763442/FULLTEXT04.pdf>
- Orem W, Tatu C, Varonka M, Lerch H, Bates A, Engle M, et al. 2014. Organic substances in produced and formation water from unconventional natural gas extraction in coal and shale. *International Journal of Coal Geology* 126:20-31.
- Orton F, Rosivatz E, Scholze M, Kortenkamp A. 2012. Competitive androgen receptor antagonism as a factor determining the predictability of cumulative antiandrogenic effects of widely used pesticides. *Environmental Health Perspectives* 120:1578-1584.
- Osborn SG, Vengosh A, Warner NR, Jackson RB. 2011. Methane contamination of drinking water accompanying gas-well drilling and hydraulic fracturing. *PNAS* 108:5.
- Papoulias DM, Velasco AL. 2013. Histopathological analysis of fish from Acorn Fork Creek, Kentucky, exposed to hydraulic fracturing fluid releases. *Southeastern Naturalist* 12:92-111.
- Payne J, Rajapakse N, Wilkins M, Kortenkamp A. 2000. Prediction and assessment of the effects of mixtures of four xenoestrogens. *Environmental Health Perspectives* 108:983-987.
- Pollino CA, Georgiades E, Holdway DA. 2009. Physiological changes in reproductively active rainbowfish (*melanotaenia fluviatilis*) following exposure to naphthalene. *Ecotoxicology and Environmental Safety* 72:1265-1270.
- Rabinowitz PM, Slizovskiy IB, Lamers V, Trufan SJ, Holford TR, Dziura JD, et al. 2015. Proximity to natural gas wells and reported health status: Results of a household survey in Washington County, Pennsylvania. *Environmental Health Perspectives* 123:21-26.

- Rajapakse N, Silva E, Kortenkamp A. 2002. Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. *Environmental Health Perspectives* 110:917-921.
- Richter CA, Martyniuk CJ, Annis ML, Brumbaugh WG, Chasar LC, Denslow ND, et al. 2014. Methylmercury-induced changes in gene transcription associated with neuroendocrine disruption in largemouth bass (*micropterus salmoides*). *General and Comparative Endocrinology* 203:215-224.
- Riedl J, Rotter S, Faetsch S, Schmitt-Jansen M, Altenburger R. 2013. Proposal for applying a component-based mixture approach for ecotoxicological assessment of fracturing fluids. *Environ Earth Sci* 70:3907-3920.
- Rostkowski P, Horwood J, Shears JA, Lange A, Oladapo FO, Besselink HT, et al. 2011. Bioassay-directed identification of novel antiandrogenic compounds in bile of fish exposed to wastewater effluents. *Environmental Science & Technology* 45:10660-10667.
- Roy AA, Adams PJ, Robinson AL. 2014. Air pollutant emissions from the development, production, and processing of marcellus shale natural gas. *Journal of the Air & Waste Management Association* 64:19-37.
- Rozell DJ, Reaven SJ. 2012. Water pollution risk associated with natural gas extraction from the Marcellus Shale. *Risk Analysis* 32:1382-1393.
- Safe Drinking Water Act of 1974. 1974. Public Law 93-523.
- Schaeffer E, Bernhardt C. 2014. Fracking's toxic loophole. Environmental Integrity Project. Available at: <http://environmentalintegrity.org/wp-content/uploads/FRACKINGS-TOXIC-LOOPHOLE.pdf>
- Schriks M, Van Leerdam JA, Van Der Linden SC, Van Der Burg B, Van Wezel AP, and, De Voogt P. 2010. High-resolution mass spectrometric identification and quantification of glucocorticoid compounds in various wastewaters in the Netherlands. *Environmental Science & Technology* 44:4766-4774.
- Schug TT, Abagyan R, Blumberg B, Collins TJ, Crews D, DeFur PL, et al. 2013. Designing endocrine disruption out of the next generation of chemicals. *Green Chemistry* 15:181-198.
- Shonkoff SB, Hays J, Finkel ML. 2014. Environmental public health dimensions of shale and tight gas development. *Environmental Health Perspectives* 122:787-795.

- Silva E, Rajapakse N, Kortenkamp A. 2002. Something from "nothing"--eight weak estrogenic chemicals combined at concentrations below noecs produce significant mixture effects. *Environmental Science & Technology* 36:1751-1756.
- Slizovskiy IB, Conti LA, Trufan SJ, Reif JS, Lamers VT, Stowe MH, et al. 2015. Reported health conditions in animals residing near natural gas wells in Southwestern Pennsylvania. *Journal of Environmental Science and Health Part A, Toxic/Hazardous Substances & Environmental Engineering* 50:473-481.
- Soraghan M. 2014. Oil and gas: Spills up 17 percent in U.S. in 2013. *Environment & Energy Publishing*. Available at: <http://www.eenews.net/stories/1059999364>
- Soto AM, Maffini MV, Schaeberle CM, Sonnenschein C. 2006. Strengths and weaknesses of in vitro assays for estrogenic and androgenic activity. *Best Practice & Research Clinical Endocrinology & Metabolism* 20:15-33.
- Souther S, Tingley MW, Popescu VD, Hayman DT, Ryan ME, Graves TA, et al. 2014. Biotic impacts of energy development from shale: Research priorities and knowledge gaps. *Frontiers in Ecology and the Environment* 12:330-338.
- Stacy SL, Brink LL, Larkin JC, Sadovsky Y, Goldstein BD, Pitt BR, et al. 2015. Perinatal outcomes and unconventional natural gas operations in Southwest Pennsylvania. *PloS ONE* 10:e0126425.
- Steinzor N, Subra W, Sumi L. 2013. Investigating links between shale gas development and health impacts through a community survey project in Pennsylvania. *New Solutions* 23:55-83.
- Subra W. 2009. Health survey results of current and former residents of Dish/Clark, Texas resident. Earthworks' Oil & Gas Accountability Project. Available at: https://www.earthworksaction.org/files/publications/DishTXHealthSurvey_FINAL_hi.pdf
- Subra W. 2010. Community health survey results: Pavillion, Wyoming. Earthworks' Oil & Gas Accountability Project. Available at: <https://www.earthworksaction.org/files/publications/PavillionFINALhealthSurvey-201008.pdf>
- Sun Q, Deng S, Huang J, Shen G, Yu G. 2008. Contributors to estrogenic activity in wastewater from a large wastewater treatment plant in Beijing, China. *Environmental Toxicology and Pharmacology* 25:20-26.

- Thomas KV, Balaam J, Hurst MR, Thain JE. 2004. Identification of in vitro estrogen and androgen receptor agonists in north sea offshore produced water discharges. *Environmental Toxicology and Chemistry* 23:1156-1163.
- Thomas KV, Langford K, Petersen K, Smith AJ, Tollefsen KE. 2009. Effect-directed identification of naphthenic acids as important in vitro xeno-estrogens and anti-androgens in north sea offshore produced water discharges. *Environmental Science & Technology* 43:8066-8071.
- Thomas P, Budiantara L. 1995. Reproductive life history stages sensitive to oil and naphthalene in atlantic croaker. *Marine Environmental Research* 39:147-150.
- Thurman EM, Ferrer I, Blotevogel J, Borch T. 2014. Analysis of hydraulic fracturing flowback and produced waters using accurate mass: Identification of ethoxylated surfactants. *Analytical Chemistry* 86:9653-9661.
- Tice RR, Austin CP, Kavlock RJ, Bucher JR. 2013. Improving the human hazard characterization of chemicals: A Tox21 update. *Environmental Health Perspectives* 121:756-765.
- Tintos A, Gesto M, Alvarez R, Miguez JM, Soengas JL. 2006. Interactive effects of naphthalene treatment and the onset of vitellogenesis on energy metabolism in liver and gonad, and plasma steroid hormones of rainbow trout *oncorhynchus mykiss*. *Comparative Biochemistry and Physiology Toxicology & Pharmacology* 144:155-165.
- Tollefsen KE, Harman C, Smith A, Thomas KV. 2007. Estrogen receptor (ER) agonists and androgen receptor (AR) antagonists in effluents from Norwegian North Sea oil production platforms. *Marine Pollution Bulletin* 54:277-283.
- Trasande L. 2014. Further limiting bisphenol A in food uses could provide health and economic benefits. *Health Affairs* 33:316-323.
- Trasande L, Zoeller RT, Hass U, Kortenkamp A, Grandjean P, Myers JP, et al. 2015. Estimating burden and disease costs of exposure to endocrine-disrupting chemicals in the European Union. *The Journal of Clinical Endocrinology and Metabolism* 100:1245-1255.
- Trimble DC. 2012. Unconventional oil and gas development - key environmental and public health requirements. U.S. Government Accountability Office. GAO-12-874. Available at: <http://www.gao.gov/products/GAO-12-874>

- Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Jr., Lee DH, et al. 2012. Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. *Endocrine Reviews* 33:378-455.
- Vandenberg LN. 2014. Non-monotonic dose responses in studies of endocrine disrupting chemicals: Bisphenol A as a case study. *Dose-Response* 12:259-276.
- Vengosh A, Jackson R, Warner N, Darrah TH, Kondash A. 2014. A critical review of the risks to water resources from unconventional shale gas development and hydraulic fracturing in the United States. *Environmental Science & Technology* 48:8334-8348.
- vom Saal FS, Akingbemi BT, Belcher SM, Birnbaum LS, Crain DA, Eriksen M, et al. 2007. Chapel hill bisphenol A expert panel consensus statement: Integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. *Reprod Toxicol* 24:131-138.
- vom Saal FS, Welshons WV. 2014. Evidence that bisphenol A (BPA) can be accurately measured without contamination in human serum and urine, and that BPA causes numerous hazards from multiple routes of exposure. *Molecular and Cellular Endocrinology* 398:101-113.
- Warner NR, Jackson RB, Darrah TH, Osborn SG, Down A, Zhao K, et al. 2012. Geochemical evidence for possible natural migration of Marcellus formation brine to shallow aquifers in Pennsylvania. *PNAS* 109:11961-11966.
- Warner NR, Christie CA, Jackson RB, Vengosh A. 2013. Impacts of shale gas wastewater disposal on water quality in Western Pennsylvania. *Environmental Science & Technology* 47:11849-11857.
- Warner NR, Darrah TH, Jackson RB, Millot R, Kloppmann W, Vengosh A. 2014. New tracers identify hydraulic fracturing fluids and accidental releases from oil and gas operations. *Environmental Science & Technology* 48:12552-12560.
- Waxman HA, Markey EJ, DeGette D. 2011. Chemicals used in hydraulic fracturing. U.S. House of Representatives. Available at:
<http://democrats.energycommerce.house.gov/sites/default/files/documents/Hydraulic-Fracturing-Chemicals-2011-4-18.pdf>

- Webb E, Bushkin-Bedient S, Cheng A, Kassotis CD, Balise V, Nagel SC. 2014. Developmental and reproductive effects of chemicals associated with unconventional oil and natural gas operations. *Reviews on Environmental Health* 29:307-318.
- Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS. 2003. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environmental Health Perspectives* 111:994-1006.
- Werner AK, Vink S, Watt K, Jagals P. 2015. Environmental health impacts of unconventional natural gas development: A review of the current strength of evidence. *The Science of the Total Environment* 505C:1127-1141.
- Westerhoff P, Yoon Y, Snyder S, Wert E. 2005. Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. *Environmental Science & Technology* 39:6649-6663.
- Wiseman HJ. 2008. Untested waters: The rise of hydraulic fracturing in oil and gas production and the need to revisit regulation. *Fordham Environmental Law Review* 20:115-169.
- Wiseman HJ. 2011. Trade secrets, disclosure, and dissent in a fracturing energy revolution. *Columbia Law Review* 111:1-13.
- Witter R, Stinson K, Sackett H, Putter S, Kinney G, Teitelbaum D, et al. 2008. Potential exposure-related human health effects of oil and gas development: A literature review (2003-2008). University of Colorado School of Public Health. Available at: http://docs.nrdc.org/health/hea_08091702.asp
- Wolf Eagle Environmental. 2009. Town of Dish, Texas ambient air monitoring analysis - final report. Available at: http://townofdish.com/objects/DISH_-_final_report_revised.pdf
- Ziemkiewicz PF, Quaranta JD, Darnell A, Wise R. 2014. Exposure pathways related to shale gas development and procedures for reducing environmental and public risk. *Journal of Natural Gas Science and Engineering* 16:77-84.
- Zoeller RT, Brown TR, Doan LL, Gore AC, Skakkebaek NE, Soto AM, et al. 2012. Endocrine-disrupting chemicals and public health protection: A statement of principles from the Endocrine Society. *Endocrinology* 153:4097-4110.
- Zoeller RT, Bergman A, Becher G, Bjerregaard P, Bornman R, Brandt I, et al. 2015. A path forward in the debate over health impacts of endocrine disrupting chemicals. *Environmental Health* 14:118.

Table 1. Functional categories of hydraulic fracturing chemicals (adapted from Colborn et al. 2011)

Chemical Categories	Technical Hydraulic Fracturing Use	Example Compounds
Acids	To achieve greater injection ability or penetration and later to dissolve minerals and clays to reduce clogging, allowing gas to flow to the surface.	Hydrochloric acid
Biocides	To prevent bacteria that can erode pipes and fittings and break down gellants that serve to ensure that fluid viscosity and proppant transport are maintained.	1-methyl-4-isothiazolin-3-one, bronopol, glutaraldehyde
Breakers	To allow the breakdown of gellants used to carry the proppant, added near the end of the hydraulic fracturing sequence to enhance flowback.	Ammonium persulfate, magnesium peroxide
Clay stabilizers	To create a fluid barrier to prevent mobilization of clays, which can plug fractures.	Tetramethyl ammonium chloride, sodium chloride
Corrosion inhibitors	To reduce the potential for rusting in pipes and casings.	Ethoxylated octylphenol and nonylphenol, isopropanol
Crosslinkers	To thicken fluids, often with metallic salts, in order to increase viscosity and proppant transport.	Ethylene glycol, sodium tetraborate decahydrate, petroleum distillate
Defoamers	To reduce foaming after it is no longer needed in order to lower surface tension and allow trapped gas to escape.	2-ethylhexanol, oleic acid, oxalic acid
Foamers	To increase carrying capacity while transporting proppants and decreasing the overall volume of fluid needed.	2-butoxyethanol, diethylene glycol

Friction reducers	To make water slick and minimize the friction created under high pressure and to increase the rate and efficiency of moving the hydraulic fracturing fluid.	Acrylamide, ethylene glycol, petroleum distillate, methanol
Gellants	To increase viscosity and suspend sand during proppant transport.	Propylene glycol, guar gum, ethylene glycol
pH control	To maintain the pH at various stages using buffers to ensure maximum effectiveness of various additives.	Sodium hydroxide, acetic acid
Proppants	To hold fissures open, allowing gas to flow out of the cracked formation, usually composed of sand and occasionally glass or ceramic beads.	Styrene, crystalline silica, ceramic, graphite
Scale inhibitors	To prevent build up of mineral scale that can block fluid and gas passage through the pipes.	Acrylamide, sodium polycarboxylate
Surfactants	To decrease liquid surface tension and improve fluid passage through pipes in either direction.	Naphthalene, 1,2,4-trimethylbenzene, ethanol, methanol, 2-butoxyethanol

Categories and uses for commonly applied chemicals throughout the hydraulic fracturing process with specific examples provided for each class. Adapted with permission from Colborn et al. 2011: Natural Gas Operations from a Public Health Perspective.