Endocrine Disrupting Activity of Hydraulic Fracturing Chemicals and *in vivo* Adverse Health Outcomes.

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There has been a rapid rise in the use of hydraulic fracturing to produce natural gas and oil. Over 750 chemicals are used in this process and many are known toxicants, carcinogens, and/or endocrine disrupting chemicals (EDCs). Spills of wastewater associated with this process are common and can contaminate surface and ground water. We have previously found an association between hydraulic fracturing spills and endocrine disrupting activity in surface and ground water. Water samples collected from sites with documented natural gas drilling contamination exhibited the highest levels of activity, samples collected from the Colorado River had intermediate levels of activity, and reference sites in areas away from natural gas drilling exhibited the lowest levels.

We previously found that eleven of twelve chemicals used in hydraulic fracturing exhibited significant anti-estrogenic activity and nine exhibited significant anti-androgenic activity. Based on this small subset of chemicals and the vast number of chemicals used, we hypothesized that a wider analysis of chemicals would reveal other hormonally active chemicals. Initial work focused exclusively on the estrogen and androgen receptor, while this study extends the analysis to include agonist and antagonist activities of the estrogen, androgen, progesterone, glucocorticoid, and thyroid receptors for 24 individual chemicals used in the fracturing process. To date, we have identified 21, 21, 10, 10, and 7 chemicals that exhibit antagonist activities for the estrogen, androgen, progesterone, glucocorticoid, and thyroid receptors, respectively. Twenty-three of 24 chemicals tested exhibited disruption of one or more receptor systems.

Previous work has reported additivity of EDCs with the same mechanism of action. With hundreds of chemicals used, it is essential to begin to assess *in vitro* and *in vivo* effects of complex mixtures of the EDCs used throughout the natural gas drilling process. Chemical mixtures will be made at equimolar concentrations for 1) all 24 EDCs analyzed, 2) 9 EDCs that we have analytically measured in hydraulic fracturing wastewater, and at equipotent concentrations for 3) chemicals interacting with each of the five receptors individually. These mixtures will all be tested in an *in vitro* system, and the smaller mixture of analytically identified EDCs will also be tested in an *in vivo* model. Briefly, timing of puberty and other estrogen, androgen, and thyroid-related endpoints will be assessed in mice following peripubertal exposure to four concentrations of the mixture, provided via drinking water. Overall, we have shown that many chemicals used in hydraulic fracturing are EDCs. Completion of the *in vivo* studies will substantially increase our knowledge associated with exposure to complex mixtures of EDCs used in hydraulic fracturing and increase our understanding of the potential health risks.