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Title:
Integrated assessment of runoff from concentrated animal feeding operations: Analytical approaches, in vitro bioassays, and in vivo fish exposures

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Abstract:
While the trend toward using concentrated animal feeding operations (CAFOs) has resulted in increased efficiency in food production, this has prompted concern regarding the impact these operations have on the environment. For example, animal waste from CAFOs can contain natural and synthetic androgens and/or estrogens which can pollute surrounding waterways. To assess the potential effects on aquatic animals of exposure to mixtures of these endocrine-active chemicals, a suite of instrumental chemical analyses, in vitro bioassays, and a short-term in vivo fish test were used to assess surface stream water from six basins receiving livestock CAFO input during runoff conditions. These basins included cattle or poultry livestock, and the sites were selected for their homogenous livestock type. In these studies, we measured concentrations of select endocrine-active chemicals using GC/MS/MS and determined estrogenic and androgenic activities using the T47D-Kbluc and MDA-kb2 in vitro bioassays, respectively. We also conducted 48 h static-renewal exposures with sexually mature male and female fathead minnows (*Pimephales promelas*) exposed to four surface water dilutions (0%, 25%, 50%, and 100% surface water), or 10 ng 17α-ethynylestradiol/L and 50 ng 17β-trenbolone/L as positive controls. Targeted gene expression and gonadal ex vivo testosterone (T) and 17β-estradiol (E2) production were measured in the fish. The T47D-Kbluc assay indicated that there was some estrogenic activity associated with all of the water samples, with the two sites eliciting the greatest response also having the highest levels of measured estrone and 17β-estradiol. The MDA-kb2 assay detected slight androgenic activity in only one of the six surface water samples. Corresponding in vivo exposures demonstrated that exposure to surface water from the livestock

basins had no significant dose-dependent effect on ex vivo T or E2 production in fathead minnows, with the exception of increased male T production at one site. Significant up-regulation of hepatic vitellogenin mRNA expression in male fish (a response indicative of estrogenicity) was observed using water from one site. Overall, our study provides insights as to the utility of an integrated approach with analytical measurements, in vitro bioassays, and in vivo tests for assessing the presence and possible effects of complex mixtures of endocrine active chemicals.

Keywords: Endocrine disruptors, Surface water, Adverse outcome pathway, Biomarkers

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