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### **Nature of the Problem**

My research objectives center on the protection of our water resources; specifically, limiting chemical and nutrient transport from agricultural operations. My motivation has evolved toward a proactive approach to mitigating pollution after previous experience concentrated on monitoring and assessment of water quality and community health in aquatic ecosystems.

Improper animal waste management continues to pose a threat to water quality in the United States. A transition towards concentrated animal feeding operations (CAFOs) has magnified problems related with handling animal by-products. Inherent in manure disposal are the associated risks of surface and ground water contamination. Although the focus in this arena has centered on nutrient management, it is becoming increasingly apparent that hormonal compounds found in manure present a human health risk and imperil wildlife communities. The EPA Office of Research and Development has addressed the risks of endocrine disrupting chemicals and the Office of Science Coordination and Policy currently lists endocrine disruptor screening as a priority setting (<http://www.epa.gov/oscpmont/oscpendo/index.htm>).

The potential impacts of hormonal compounds on human health and wildlife communities are extensive. Evidence suggests estrogenic compounds found in animal waste induce breast cancer (Dickson 1986), while reduced sperm counts and increased incidents of human testicular cancer have been linked to environmental exposure (Carlsen et al. 1995). Natural estrogen compounds have also been documented to cause sex reversals in fish (Nakamura 1984), premature heifer development (Shore et al. 1988), and reproductive disorders in wildlife (Kramer et al. 1998).

Land application of manures presents an environmental loading of estrogens that may result in regional surface or ground water concentrations significantly above ambient levels. Elevated concentrations of excreted steroidal compounds have been recognized in livestock waste (Mathur and Common 1969) for some time, but environmental impacts continued unrecognized. Recent concerns over hormonal exposure warrant further research on the fate and transport of these chemicals following land application of animal wastes.

Despite numerous physiological investigations, literature on the fate and transport of steroidal compounds in environmental systems is limited. Research has begun to address this issue from a monitoring standpoint, but essentially no examination of specific chemical behavior (degradation, sorption, etc.) exists. Preliminary findings suggest transport mechanisms differ between hormonal compounds (Shore 1995). Nichols et al. (1997, 1998) found 17 $\beta$ -estradiol migrates off-site when broiler litter was applied at agronomic rates. However, the monitoring aspect of these studies did not permit

evaluation of degradation, sorption, or leaching on hormone concentration. Furthermore, the methodology presented used enzyme-linked assay kits that only determine the presence of 17 $\beta$ -estradiol as an indicator compound.

Monitoring 17 $\beta$ -estradiol in broiler litter disregards both other hormones (i.e., metabolites) and other animal waste management systems. Furthermore, different soil types receiving animal waste should be investigated due to matrix influence on chemical mobility. By increasing the analytical rigor of chemical fate and transport using new and emerging techniques, a better understanding of the hormonal behavior will be achieved. Using an approach that progresses beyond rapid assessment methods for 17 $\beta$ -estradiol will provide more accurate and precise determination of estrogenic risk to human and wildlife populations.

My proposed research systematically examines the specific chemical behavior of hormonal compounds. Current research on estrogens has been limited to edge-of-field concentrations following broiler litter application to fescue plots (*Festuca arundinacea*). Restricting investigation of hormonal compounds to broiler litter disregards other animal waste management systems. My research will focus on liquid handling systems used by certain CAFOs, including layer and dairy operations. The long term goal of my research will provide an understanding of the chemical behavior of hormones in the environment so that scientists and livestock/poultry facility operators can limit the transport of these compounds to waters of concern. The research proposed here will begin to investigate the behavior of 17 $\beta$ -estradiol and other estrogenic compounds inherent to waste streams. The Pesticide Fate Research Laboratory at Texas A&M University has performed numerous studies investigating herbicide behavior, chemicals that greatly resemble hormonal compounds in structures and will hence require similar methodologies. Furthermore, an influx of CAFOs has magnified problems related with handling animal by-products in Texas. This combination of facilities, expertise and proximity to areas at risk presents an ideal situation to study these chemicals.

## **Research Objectives**

The first goal of the project will identify the primary metabolites that form during degradation of lagoon effluent. Lagoon samples will be analyzed using GC-MS to determine the total estrogenic concentration and identify the corresponding compounds. This method improves upon the use of enzyme-linked assay kits that have been utilized by previous studies to determine the presence of 17 $\beta$ -estradiol as an indicator compound. Despite the benefits of rapid assessment, there are a number of drawbacks associated with this technique. First, cross contamination with other chemicals can occur, failing to yield precise compound concentrations. Second, no research has addressed the potential estrogenic metabolites that may persist after degradation of the parent compound. Sumpter and Jobling (1995) found mixtures of estrogenic compounds resulted in substantially greater feminization of fish populations compared to individual compound analysis. GC-MS analysis eliminates the shortcomings of assay tests. This approach also provides base line concentrations not explored in previous publications.

The project will then focus on degradation rates of 17 $\beta$ -estradiol by using commercially prepared spikes and raw and soil applied dairy lagoon effluent. Soil applied effluent will be applied at representative agronomic land disposal rates to soils representative of Texas dairy operations. Samples will be placed under simulated sunlight lamps representing field conditions and temperature will be maintained at room temperature. Analysis following the procedure outlined above will be conducted at appropriate time intervals so that data can be fitted to a decaying exponential function ( $y = ae^{(-kt)}$ ) and the decay constants can be calculated using curve-fitting software.

The principal metabolites will then be used to determine adsorption characteristics in the soils used to determine degradation rates. Raw excreta and lagoon effluent will be applied to soils and standard methods will be used (e.g. indirect batch-suspension measurement procedure) to determine isotherms for the key metabolites. In conjunction with the jar adsorption experiments, leaching chambers will be used to determine the leaching properties of the key metabolites. Large columns provide a unique setup that will more greatly represent field conditions due to their large size compared to standard column tests. Agronomic disposal rates of lagoon effluent will be added to the column and representative rainfall rates will be simulated. Analysis of estrogenic compounds at various depths in the columns will be conducted to determine effective leaching of 17 $\beta$ -estradiol and metabolites.

The final experimental portion of this research program will use a small watershed approach in a test plot fitted with lysimeters. Leaching and runoff samples will be analyzed to determine the transport mechanisms associated with each species. This field experiment brings together the degradation, adsorption, and primary movement characteristics determined in the laboratory in a real-world situation.

The outlined strategy will then allow for incorporation of the knowledge gained in the laboratory and field experiments to develop a model. This end product will provide a tool that can be used by agency and academic personnel in developing best management practices (BMPs) that promote the retention of steroidal compounds originating from agricultural operations. The expected results will add to the current knowledge of estrogenic behavior in the environment, bridge the gap in understanding their potential fate and transport and allow for the protection of Texas waters.

### **Intended Career Path Statement**

I have concentrated both my studies and work experience in mitigating water pollution. My interests continue to center around water quality, but subsequent to matriculation my attention has evolved to include additional agricultural issues. I am fascinated by the evolving paradigm of sustainable land management practices that will help restore and maintain ecological integrity to water bodies in agricultural areas by limiting pollutants at their source. After graduate training I hope to start an academic career in which I can work toward improved water quality by investigating better management practices. Being a professor in a comprehensive agricultural program at a land-grant university is an ideal way to conduct research while passing along knowledge to both students and agricultural

producers.