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TRANSPORT AND FATE OF THE ENDOCRINE DISRUPTING HORMONE, TRENBOLONE ACETATE, IN VEGETATED SYSTEMS

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ABSTRACT: Harmful concentrations of endocrine disrupting chemicals (EDCs), such as steroid hormones, have been detected in water bodies nationwide. One source of hormone contaminants is runoff from animal waste applications. It is estimated that 95% of beef cattle in the United States receive steroidal hormone therapy to increase production. By far, the most widely used implants contain a combination of a synthetic androgen, TBA, combined with the natural estrogenic steroid, E2. Several key transport characteristics have been documented for E2. However, many of the same values have not been developed for TBA. Because of the relative lack of information for TBA, the major aims of this study are to document the transport and fate of TBA in natural systems and to assess pollution prevention measures for TBA once it is present in the environment.

This project will consist of a three-phase study conducted at scales ranging from the lab to the field. The lab and mesocosm scale work will generate data that will be helpful in better understanding the physical, biological and chemical processes affecting transport and fate of TBA. The sorption rates, equilibrium data, and partitioning coefficients developed in the lab studies will be used in the mesocosm studies to help determine plant assisted degradation. The combination of the lab and mesocosm studies will lead to the field scale determination of the potential of vegetated buffer systems, a commonly used best management practice for livestock operations, to serve as a pollution prevention measure for TBA in runoff from cattle feedlots.

The successful completion of this research will provide baseline data leading to improved scientific understanding of the transport and fate of TBA in the environment. Research on the effectiveness of these pollution prevention vegetated systems will significantly add to the scientific basis for environmental management decisions regarding EDCs. With greater understanding of EDCs in the environment, the great risk to animal and ecosystem health can be reduced.
KEY TERMS: trenbolone acetate (TBA), endocrine disrupting chemical, vegetated buffer system, runoff, pollution prevention

Harmful concentrations of endocrine disrupting chemicals, such as steroid hormones, have been documented in the United State's surface water and groundwater (Peterson et al., 2000; Ying et al., 2002). These chemicals' frequency of detection combined with their serious health effects at low levels is a major cause for concern (Kolpin et al., 2002). To decrease the exposure risk to animals as well as humans, it is necessary to develop pollution prevention measures to reduce contamination of water bodies with this type of "emerging contaminant." The long-term goal of this project is to investigate the transport and fate of the steroid trenbolone acetate (TBA) from implanted beef cattle and to assess the potential for pollution prevention through the use of vegetated buffer systems.

It is estimated that 95% of American beef cattle receive steroidal hormone therapy to increase production. The most widely used beef implants contain a combination of a synthetic androgen, TBA, and the natural estrogenic steroid, E2. (Dr. E. Minton, KSU, personal communication, 2006). Because these hormones are emerging contaminants, there is little environmental fate research for either compound; however, there is far less literature available for TBA compared to E2. It is thought that due to the hydrophobicity of TBA and its metabolites, sorption may occur (Schiffer et al., 2001). In the field, both TBA metabolites have been detected in runoff from manure mounds (Schiffer et al., 2001). Moreover, detectable levels of the androgenic hormone have been found in the soil up to nearly two months after solid waste land applications (Schiffer et al., 2001). It is hypothesized that, like E2, TBA would have a relatively high K_{ow} value, indicating that plant uptake would not occur (Trapp and McFarlane, 1995; Briggs et al., 1982). Therefore, microbial degradation of the hormones within the rhizosphere, rather than plant uptake, will play the largest role in removal.

This study will quantify the sorption/desorption of TBA at the bench scale, characterize plant uptake and degradation of TBA under controlled mesocosm conditions, and evaluate vegetated buffer effectiveness in reduction of TBA at two field sites. It is hypothesized that the use of vegetated buffer systems can aid in the removal of this hormone in runoff destined for surface water bodies. Vegetated buffer systems (VBSs) (i.e. vegetated filter strips, riparian corridors) are

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commonly used best management practices (BMPs) for livestock operations. The underlying concept for these BMPs is that vegetation is used to intercept runoff and effectively reduce runoff volume and remove non-point source pollutants (Koelsch et al., 2006; USDA, 1999).

For this research, animal waste samples containing TBA will be taken either directly from steers at the Beef Cattle Research Center or from the KSU animal waste lagoon. Aqueous TBA will be quantified either directly using reverse phase HPLC and fluorescence detection, or after liquid-liquid extraction with ethyl acetate followed by concentration by solid-phase extraction in C18 and silica gel cartridges (Naoki et al., 2000; MacNeil et al., 2003). Enzyme Linked ImmunoSorbent Assays (ELISAs) will be used as a quality check for all TBA metabolites (RIDASCREEN® Trenbolon; R-Biopharm). Analysis of variance (ANOVA) (α=0.05) and Least Significant Difference statistical procedures will be used to determine differences between the treatment means.

Phase I: Microcosm scale research will consist of a lab scale study of the transport and fate of TBA. These laboratory scale phase distribution experiments for TBA metabolites will yield accurate sorption rate and equilibrium data that can be used to predict transport of the synthetic hormone via surface runoff. Batch sorption experiments will be conducted in centrifuge tube batch reactors containing a representative agricultural silt loam soil. This soil will be contacted with TBA solutions at aqueous concentrations ranging over three orders of magnitude and will be analyzed with the methods described above.

Phase II: Mesocosm scale research will include the study of plant-assisted degradation of TBA. Plant growth chambers will be used to house 36 half-gallon containers of silt loam soil planted with grasses and treated with animal waste (2 grass varieties plus unvegetated control x 3 waste treatments x 4 replications). The three waste treatments in this randomized design will consist of: (1) a "control" waste from animals that were not implanted, (2) waste from animals given TBA, and (3) control waste from the non-treated animals mixed with synthetic TBA. Brome grass (*Bromus* L.), a cool season grass, and switch grass (*Panicum virgatum* L.), a native warm season prairie grass, will be the two grass varieties under investigation as both grasses are commonly used in vegetated BMPs.

Phase III: Macrocosm scale, field scale, research will be be conducted to look at the fate of TBA in the natural environment as well as the potential of VBSs as a possible pollution prevention measure. In order to replicate the grasses used in Phase II of the project, two sites will be used. The first site, located near Manhattan, Kansas on Fort Riley military base, has existing stands of native prairie grasses (mixture of switch grass, big bluestem, little bluestem, and Indian grass). The other site, located on the KSU Animal Science Farm, also in Manhattan, KS has several experimental brome grassed waterways. Manure from TBA implanted steers will be spread at predetermined rates on the field plots, which will be arranged in a randomized, block design. A rainfall simulator will be used to apply 1.3-5.1 cm/hr rainfall events over the growing season at each location. Data from the other phases will be used to estimate TBA metabolite degradation and sorption rates. Runoff at the outlet of each plot will be diverted to a tipping bucket gage, and the time of each tip will be recorded using a digital counter and data logger, allowing determination of the runoff hydrograph for each experimental unit separately. The combination of hydrograph and flow-weighted samples will allow mass outflows. Samples will be analyzed for TBA metabolites, total suspended sediments, total nitrogen, total phosphorus, and COD.

The successful completion of this research will provide baseline data leading to improved scientific understanding of the transport and fate of one endocrine disrupting chemical (EDC), TBA, in the environment. Research on the pollution prevention potential of vegetated buffer systems will significantly add to the scientific basis for environmental management decisions regarding EDCs. With greater understanding of EDCs in the environment, such as this work will provide, the great risk to animal and ecosystem health can be reduced.

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