U.S. EPA Workshop on Fate and Effects of Hormones in Waste From Concentrated Animal Feeding Operations

U.S. Environmental Protection Agency
Region 5
Chicago, IL

August 20–22, 2007

Draft Executive Summary

OVERVIEW

The U.S. Environmental Protection Agency (EPA) Workshop on Fate and Effects of Hormones in Waste From Concentrated Animal Feeding Operations was held August 20–22, 2007, in Chicago, Illinois. The workshop was sponsored by the National Center for Environmental Research (NCER), which is part of EPA's Office of Research and Development (ORD). Scientists from academic, government, and industry sectors assembled to discuss research addressing hormones in concentrated animal feeding operation (CAFO)-derived wastes and their impact on human and environmental health. Emphasis was placed on fitting waste-derived hormones into the context of all CAFO waste contaminants. The meeting provided an opportunity for grantees in the EPA-funded Science to Achieve Results (STAR) Program to present their research early in the grant period and to identify and interact with the greater community of CAFO researchers. Collaborations were encouraged with stakeholders from EPA, federal, state, and local agencies. Several of the projects were awarded as cooperative agreements with EPA, so this meeting allowed for investigators to meet to discuss the projects in detail. The meeting also provided a chance to review related research being conducted in ORD laboratories. Approximately 80 individuals attended the meeting.

Welcome and Introductory Remarks
Susan Laessig, EPA/ORD/NCER

In the past, research involving CAFOs focused on nutrient, bacterial, and antibiotic contaminants in discharges. The potential impact of hormones in CAFO waste is an emerging topic of concern. Consequently, EPA has awarded seven new grants and cooperative agreements to investigators proposing to study the environmental transport, fate, and ecological effects of natural and synthetic steroid hormones that accompany discharges and the disposal of animal wastes from CAFOs. The results of this research will contribute to improving estimates of the occurrence and risks of steroid hormones associated with animal waste, and developing new or improved animal waste handling systems and risk management options for steroid hormones in animal waste. The STAR grantees will conduct research toward defining the scope of the problem and incorporating hormone fate and effects into a contextual framework with other CAFO-derived environmental stressors. This emerging research area is complex and multidisciplinary, and collaborations are important for creating relevant and useful datasets. The funded projects span CAFOs in different geographic regions and employing different waste management practices. Proposed research utilizes biological, chemical, and computational methods in both laboratory and field settings.

CAFO Rule and Future Research Needs
Roberta Parry, EPA/Office of Water

The regulatory history of CAFOs began with the Clean Water Act (1977). The 2003 CAFO rule describes animal feeding operations (AFOs) as sites where animals are confined for 45 days or more in a 12-month period. CAFOs are those AFOs that supersede designated size thresholds of animals per unit area; contain a stream through the confinement area or a manmade conveyance to surface water; or otherwise are denoted a significant contributor of pollutants by authorities who conduct an onsite visit. It is estimated that 5 percent of all AFOs are CAFOs; however, CAFOs contribute 60 percent of all AFO manure. CAFOs that discharge waste to the environment are required to obtain permits and annually document their practices of animal confinement, raw material and manure storage, and application of fertilizer to land. CAFO operators also must follow Nutrient Management Plans (NMPs), which are developed on a CAFO-specific basis and regulate many aspects of the operation. For large CAFOs, effluent guidelines are added to the permit. These include rigorous stormwater discharge requirements with the primary goal of minimizing transport of nitrogen and phosphorus to surface water. It is expected that other pollutants—hormones, metals, pesticides, and antibiotics—will in turn be controlled by the NMPs. In general, the federal requirements for CAFOs set the minimum standards, and individual states are encouraged to exceed these guidelines in their NMPs and permitting approaches. Permits are beneficial to CAFOs because they provide a shield from litigation if waste discharge occurs under the terms of the permit. EPA estimates that 44 percent of CAFOs are currently permitted and expects NMPs to be implemented fully by 2009. Notably, the first CAFO rule did not provide information on the regulation of hormones. Background documents for the rule noted that hormones were present in manure and were linked to endocrine disruption, but minimal data existed on how manure handling technologies and management practices affect hormone dynamics. In addition, a pivotal court ruling (Waterkeeper Alliance, et al., versus EPA)—involving the 2003 requirement that CAFOs with the "potential to discharge" obtain permits—has led to a revised CAFO proposal in recent months. The updated language states that any CAFOs that discharge waste or "propose to discharge" waste must apply for a permit, but if the discharge is exclusively agricultural stormwater, a permit is not required. Research initiatives involving pollutant sources, environmental impacts, effectiveness of management practices, cost controls, and feasibility of implementation all have the potential to impact policy. Participants were directed to the EPA CAFO Web Site (http://cfpub.epa.gov/npdes/home.cfm?program_id=7) and the CAFO Effluent Guidelines Web Site (http://www.epa.gov/waterscience/guide/cafo/) for further information.

Discussion

A participant asked about the types of records that would need to be filed by CAFOs producing only agricultural stormwater discharges. Ms. Parry responded that the individual CAFO's NMP must be followed. If the operator does not provide appropriate documentation and uphold technical standards, he or she may be charged.

Dr. Steve Hutchins (EPA) asked how many states require NMP documentation. Ms. Parry responded that there are 23 states thus far that have required NMPs; most have fulfilled this requirement. Another participant asked if there was a Web site with more information about the NMP-bearing states. A participant responded that there is a matrix database of this information. It can be accessed through the National Association of State Departments of Agriculture (http://www.cnmpwatch.com). Ms. Parry referred participants to the National Agriculture Compliance Assistance Center (Ag Compliance Center) for information about environmental requirements that affect the agricultural community. The Ag Center was created by the U.S. Environmental Protection Agency (EPA) with the support of the U.S. Department of Agriculture (USDA) and it can be accessed from the EPA Agriculture homepage (http://www.epa.gov/agriculture/).

Ms. Suzanne Stevenson (EPA) commented that she would like to know the details of the EPA/U.S. Department of Agriculture (USDA) joint strategy. Ms. Parry explained that the USDA National Research Conservation Service (NRCS) has conservation districts and state conservationists. Each state has an individual program and is encouraged to prepare comprehensive NMPs. Ms. Stevenson asked about EPA's involvement in this process, and Ms. Parry explained that EPA facilitates the state-run projects and assists in meetings.

A participant asked whether EPA is evaluating seasonal variation in hormones from a wildlife source to establish a baseline, "no discharge" case. Ms. Parry answered that such research is being funded through the STAR Grant Program. Dr. Laessig added that collaborative projects through ORD also are addressing this issue.

A participant asked about the changes that occur when a CAFO implements the Clean Water Act. Ms. Parry replied that the CAFO may need to modify its storage plan and prepare an NMP. In addition, the nutrient balance would be assessed to ensure that nitrogen and phosphorus discharge was minimized.

A participant via telephone asked Ms. Parry to expound upon current practices of nutrient trading for CAFOs. For instance, if a poultry operation gives litter to a farm as fertilizer, who is responsible if runoff occurs? Mr. Hank Zygmunt stated that the state provides checks and balances and tracks litter transport. However, there is no federal responsibility to keep track of litter transport from one CAFO to another. The source CAFO only needs to document the amount of waste that was shipped—not how it was applied at the receptor CAFO. A participant added that this is not normally an issue on the Eastern Shore, where poultry operations are generally not CAFOs. Ms. Parry stated that EPA did not want to discourage operators from outsourcing manure to other facilities in need of nutrient supplements.

Dr. John Classen (North Carolina State University) asked for a clarification of the "no discharge" guidelines. Ms. Parry responded that in 2003, operations could only meet the no discharge rule if they could store waste through a hypothetical 100-year, 24-hour storm. In the current proposal, that language is removed, and operators would have to prove no discharge on a case-by-case basis. Mr. Scott Piggott (Michigan Farm Bureau) asked Ms. Parry to define "proposed discharge." She replied that it is better not to overly deconstruct the language of the proposal and emphasized that most farmers do not purposely discharge waste.

Mr. Allan Stokes (National Pork Board) mentioned that Publicly Owned Treatment Works (POTWs) have developed technologies to deal with the potential for discharge. He noted that these methods may be transferred to the agricultural sector. Dr. Steve Hutchins affirmed that research was ongoing in Ohio to determine the crossover benefit of conventional wastewater treatment in removing hormones from CAFO waste.

SESSION I: STAKEHOLDER INVOLVEMENT IN CAFO ENVIRONMENTAL ISSUES

Session Moderator: **Steve Jann, Deputy Branch Chief, NPDES Programs Branch**

EPA Regional CAFO Waste Issues
Matthew Gluckman, EPA, Region 5 CAFO Coordinator

At the national level, 500 million tons of manure are created annually in the United States—three times the wet weight of human sanitary sewage. Ninety percent of this manure is applied to crops or forage land. The states in EPA Region 5 are top producers of corn, milk, eggs, meat, and other agricultural products. Most of the livestock in this region are confined within AFOs and CAFOs. In these operations, rainfall and snowmelt runoff are the primary routes of manure pollutants entering the surface water. Manure from AFOs has contaminated wells, killed fish, and contributed to chronic nutrient and pathogen

problems, making this a topic of particular concern. In response to these widespread water quality problems, many states in Region 5 have instituted rigorous permitting of all large CAFOs. This entails a full evaluation of animal confinement procedures and manure, litter, wastewater, and raw material storage areas (production areas) as well as the land to which manure, litter, or processed wastewater is applied as fertilizer (land application area). Unpermitted discharges from production areas are illegal. Similarly, discharges from land application areas are prohibited unless such discharges are the effect of precipitation. Research priorities for AFOs include monitoring the phosphorus index (established by USDA) as a predictor of phosphorus transport from land application sites and minimizing the risk of runoff from frozen and snow-covered ground. In addition, research is needed to determine whether species-specific effects of hormones partitioning to the solid or liquid phases of land-applied manure can impact the hormone concentration in runoff. Aside from the above research priorities for hormones, research toward evaluating and improving the practices of competing out nutrients from waste discharge also would be useful.

Discussion

Dr. Hutchins commented that there were 52 cases of manure runoff in Wisconsin in 2006; these cases appeared to be from solid waste application. Dr. Jim Lauderdale (Lauderdale Enterprises, Inc.) stated that 100 percent of the Wisconsin CAFOs are registered, but state authority only extends across large CAFOs. Small and medium facilities may have been the sources of these runoff events.

Dr. Classen requested information on CAFOs in Illinois. He commented that in the past, Illinois provided an annual summary of its inspections. Dr. Rich Breckenridge (Illinois Environmental Protection Agency) commented that current summaries only document by CAFO size and the animal species contained there. He directed Dr. Classen to the Illinois Environmental Protection Agency Web Site (http://www.epa.state.il.us) for agricultural information and inspection summaries. Mr. Jerry Foster (National Pork Board/Cargill Pork) questioned why Illinois continues to struggle with waste issues despite the rigors of their permitting program. Dr. Breckenridge responded that the 1997 Management Act identified more than 1,000 animal unit facilities in Illinois; these would need to be permitted with the Illinois Department of Agriculture.

Mr. Zygmunt commented that scientists in EPA Region 3 (MD, VA, DE, PA, WV, and DC) should communicate with ORD regional liaisons during the early phases of their research. He also mentioned the opportunity to turn a grant into a cooperative agreement. Such agreements allow EPA to bring additional resources to the project and recruit additional partners from federal, academic, and industrial sectors to assist with project implementation.

Environmental Assessment for Veterinary Pharmaceuticals
Charles Eirkson, U.S. Food and Drug Administration, Center for Veterinary Medicine

The U.S. Food and Drug Administration (FDA) is the primary federal agency that reviews and approves pharmaceuticals and personal care products. The FDA Center for Veterinary Medicine (CVM) regulates actions related to veterinary pharmaceuticals and feed additives as articulated in the Federal Food, Drug, and Cosmetic Act. Drug regulations cover hormones for use in animals, mainly cattle. The National Environmental Policy Act (NEPA, 1969) is the basic national charter for the protection of the environment. It requires that federal agencies consider the environmental impacts of their regulations and stresses the importance of accurate scientific analyses, expert comment, and public scrutiny. The FDA implements NEPA through the Council on Environmental Quality. The review spans administration, excretion, and disposal of pharmaceuticals. Through this legislation, the FDA may review environmental assessments (EAs) and categorical exclusions (CEs) and determine the appropriate actions. The EA is a concise, objective, and well-balanced public document that describes a pharmaceutical's use, analysis, risk characterization, potential mitigations, and disposal. EAs include laboratory studies on chemistry, fate, and effects of drugs and additives on invertebrates, fish, and plants. Measurement endpoints are the

same as used for pesticides—mortality, immobilization, reproduction, growth, and biogeochemical cycling. The assessment provides sufficient evidence and analysis and finds either "no significant impact" or issues an "environmental impact statement (EIS)." CEs are classes of actions that individually or cumulatively do not significantly affect the quality of the human environment are usually excluded from the requirement to prepare an EA. However, if information indicates that a normally excluded specific action may significantly affect the human environment, categorical exclusion may be denied. Numerous activities are ongoing with the Office of Science and Technology, the U.S. Geological Survey (USGS) Toxics Program, and EPA field offices. FDA is working to improve its predictive power for environmental exposure levels and is sharing its analytical methods with USGS and the Office of Water. FDA plans to scrutinize hormonally active products more closely and encourage people to adopt conservation practices. Data are needed on the background levels of natural hormones, other estrogenic compounds, minimal effects, estrogen degradation dynamics, and the effectiveness of buffer zones. Information also is needed on predicted and actual levels of pharmaceuticals.

Dr. Eirkson directed participants to the Environmental Impact Assessment for Veterinary Medicinal Products; Phase I Web Site (http://www.fda.gov/cvm/guidance/guide89.pdf) and the Phase II Web Site (http://www.fda.gov/cvm/guidance/guide166.pdf). Guidelines at Phase I are less than 1 ppb if the drug is introduced into water and less than 100 ppb if introduced onto land. The risk quotient is assessed in Phase II through a method of determining the predicted environmental concentration (PEC) versus the predicted no-effect concentration (PNEC) to determine the risk quotient.

Discussion

Ms. Nancy Shappell (USDA) asked whether it is known how many cattle are implanted with zeranol, a synthetic estrogen used as a growth promoter. Dr. Eirkson replied that most cattle used in meat production are steers that undergo hormonal implants. Dr. Hutchins added that *Environmental Science & Technology* published a review article on implants for beef cattle (Renner R. Do cattle growth hormones pose an environmental risk? *Environmental Science & Technology* 2002;36(9):194A-197A; see also Willingham EJ. Trenbolone and other cattle growth promoters: need for a new risk-assessment framework. *Environmental Practice* 2006;8(1):58-65). In the article, it was estimated that 90 percent of steers continue to be implanted; this leads to leaner beef and lower hormonal outputs than bulls without implants. Moreover, implanted steers are less aggressive. Dr. Lauderdale agreed that implants are beneficial to producers, the meat market, and the environment.

Overview of U.S. Department of Agriculture (USDA) Research on Hormones
Mary Ann Rozum, USDA, Cooperative State, Research, Education and Extension Service

The USDA Cooperative State Research, Education, and Extension Service (CSREES) funds research addressing such topics as hormone discharge from animal production facilities and subsequent transport of hormones to the environment. Dr. Rozum highlighted preliminary findings of these projects, which include the following: (1) The USDA Agricultural Research Service (ARS) found that composting is an effective method to reduce, but not eliminate, the introduction of estradiol and testosterone into the environment; (2) Tennessee researchers comparing dairy and swine wastes concluded that swine farrowing facilities pose the greatest threat as environmental estrogen pollutants; and (3) Purdue University studies determined that androgen and trenbolone and the antibiotics tylosin, monension, and lasalocid are highly sorbed to soils and have a relatively short half-life, so that concentrations in nearby water bodies are likely to be small. She added that Maryland scientists found that substantial quantities of poultry litter-derived estradiol can be transported to surface waters via runoff and persist for weeks to months at environmentally relevant concentrations. This is significant because fields under no-till management practices can lose up to 10 times more estradiol than fields employing conventional tillage.

Cliff Rice, USDA, ARS

Researchers at the ARS in the pharmaceutical and personal care product (PPCP) sector currently are involved in the development of a document to assess human and ecological risk from pharmaceuticals in the environment. This document is spearheaded by the Pharmaceuticals in the Environment (PiE) workgroup and will be available by December 2007. In addition, USDA and ARS are working together to develop and evaluate methods to control CAFO-mediated releases of endocrine disrupting chemicals (EDCs) to the environment. Their objective is to protect public and environmental health while still promoting the safe use of manure as fertilizer. Ongoing research includes the measurement of EDCs in complex mixtures, management practices and technologies to degrade EDCs and/or prevent their discharge, predictors of EDC fate and transport, and documentation of the above methods. Current options for controlling EDCs on farms consist of composting, lagoon storage and wetlands, and manure piling. Composting is associated with a decrease in testosterone and 17ß-estradiol over time. Similarly, passage through lagoons and wetlands decreases estrogenic activity significantly. Other ongoing research at ARS targets the fate of dairy lagoon hormone wastes, leaching to ground water, and poultry litter runoff. ARS also conducts research on the environmental impact of other hormone-active chemicals, such as alkylphenols.

Discussion

Dr. David Sedlak (University of California, Berkeley) asked about intercomparisons of data among laboratories. He commented that past agency intercomparisons in the United Kingdom lacked consistency. Dr. Rice responded that ARS is active with the European Union; the organizations compare wastewater treatment methods and have found comparable results. Dr. Sedlak recommended that ARS confirm that its data on steroid hormones are repeatable internally and outsource to other laboratories for intercomparisons to verify reproducibility. Dr. Rice responded that ARS searches actively for competent laboratories with the time and energy to test their methods. He added that they are looking specifically for a laboratory to reanalyze estradiol data generated by ARS. Dr. Hutchins added that STAR grant collaborations are being founded, particularly for comparisons of estrogen data.

A participant commented that all hormones are not equal. For instance, they may decompose differently in wetlands. She asked whether a distinction could be made between removal of wastes from the CAFO and transfer through wetlands or lagoons. In these instances, the phosphorus/nitrogen-to-hormone conversion may no longer exist. Dr. Rice agreed with the comment and reiterated that the bioactive portion must be determined in these cases.

U.S. Geological Survey (USGS) Research on Emerging Water-Quality Issues Related to CAFOs: Research Themes, Capabilities, and Opportunities for Collaboration
Sheridan Haack, USGS, Toxic Substances Hydrology Program

The USGS was established to: (1) provide the nation with reliable, unbiased information to describe and understand the earth; (2) minimize loss of life and property from natural disasters; (3) manage water, biological, energy, and mineral resources; and (4) enhance and protect the quality of life. Notably, the USGS mission has no regulatory component. Instead, it emphasizes science, health, and resource management. The USGS Toxic Substances Hydrology Program has developed the Emerging Contaminants in the Environment Project, which disseminates information about the risk of contaminants to regulators, resource managers, industry, and the public. Since 1998, more than 135 reports have been published under this project. Scientists at USGS have developed sensitive methods for the detection and characterization of hormones, pharmaceuticals, and personal care products in water, soil, sediments, waste, and tissue. Members of USGS specialize in chemistry, hydrology, and biology. These scientists monitor sources and pathways, environmental occurrence, chemical transport and fate, and exposure impacts. An ongoing study linking environmental contamination and ecological effects is the assessment of EDCs in relation to the feminization of smallmouth bass in the Potomac River Basin. Researchers are evaluating the different types of EDCs to which these fish populations are exposed. Work with relevance to CAFOs includes the evaluation of chemical dissemination, persistence, and effects on diverse biota

from waste lagoon leaching and land application runoff. The USGS is collaborating with Dr. Seth Kullman (North Carolina State University) under an EPA STAR Grant to understand hormone distribution and fate in a farrowing swine CAFO. Additional collaborations are welcomed, particularly ones that: (1) address the source-to-receptor concept; (2) are interdisciplinary in scope; (3) develop information for resource management and decisionmaking; and (4) improve the understanding of environmental fate, transport, and effects.

Sarah Gerould, USGS, Contaminant Biology Program

The Contaminant Biology Program at USGS includes program goals in toxicology, chemistry, contaminated habitats, and multiple stressors. CAFO-derived contamination is an important research topic within the contaminated habitats category. Researchers employ bioconcentration methods that mimic organismal exposure to lipophilic and hydrophilic waterborne chemicals to conduct time-integrated field sampling. Other analytical methods include *in vitro* and *in vivo* approaches in a number of model organisms to understand endocrine disruption and intersex characteristics. A current research thrust is determining the level at which testosterone masculinizes fish embryos, and similarly, the level of estrogen that feminizes fish.

Question and Answer Session

Ms. Shappell commented that aquatic systems in the West are effluent-dominated. She asked for evidence in the Chesapeake Bay of direct effects from steroid discharge where freshwater systems are much more dilute. Dr. Dan Fisher (University of Maryland) responded that this would be discussed on Day 2 of the meeting. Much of his research involves freshwater systems, and although he observes large dilution effects in these systems, there also is a great deal of runoff, particularly in the spring.

A participant commented on the CAFO Report's comparison between the 500 million tons of waste generated by CAFOs and the three-fold lesser magnitude of waste generated by humans. He was concerned because although there may be three times as much waste from CAFOs, it does not necessarily translate to three times the nutrients or hormones. A participant confirmed that the statistic originated in the 2003 CAFO Report and was a comparison to human wet weight sanitary sewage. A participant commented that he was concerned that the statistic would be used by people who did not understand its significance. Another participant added that the statistic is not as misleading as some might think because manure has three times more estradiol than human waste.

A participant commented that in the Midwest, many communities use lagoon technology. This should be considered in current research.

Dr. Laessig thanked participants for attending and offering useful comments. She adjourned discussions for Day 1 at 4:35 p.m.

SESSION II: CURRENT CAFO RESEARCH AT EPA

Session Moderator: **Gary Ankley, EPA/ORD/ National Health and Environmental Effects Research Laboratory (NHEERL)**

Session Introduction: Endocrine Disruptor Research Program and CAFOs
Elaine Francis, EPA, National Program Director for Endocrine Disruptors

The risk of exposure to environmental agents that interfere with the endocrine system is a cause for global concern. The Endocrine Disrupting Chemicals (EDCs) Research Program was instituted by EPA to better understand and manage these risks. Since its inception in 1994, the EDC Program has published requests for applications (RFAs) to foster extramural research through the STAR Grant Program and has sponsored scientific workshops covering both broad and targeted topics. Currently, the EDC extramural program's portfolio includes 55 grants. Throughout its history, the EDC extramural program has encouraged collaborations among STAR grantees and EPA scientists. Research topics were broad initially and have become defined more precisely over time. Recently, more awards are made as cooperative agreements instead of grants. This provides a greater opportunity of sharing data and leveraging resources. The Multi-Year Plans (MYPs) serve as blueprints for research expectations over the next 5 to

10 years in each EPA program. MYPs also specify which center or organization will be responsible for each research topic, when research products are planned to emerge, and how these research products will advance the overall mission of the Agency. Within the EDC Program, the Long Term Goals (LTGs) include understanding the effects, exposure assessment, and management of EDCs; determining the health impacts of EDCs, including those derived from CAFOs; and supporting EPA's screening and testing program. Ongoing work in the third LTG has led to a remarkable array of *in vitro* and *in vivo* assays. The EDC Program is in the process of updating its MYP in conjunction with a Board of Scientific Counselors (BOSC) program review. Dr. Francis expressed interest in collaborations that lead to more effective communication of results. Current communications efforts include Web site development and publishing a document that synthesizes intramural and extramural research results.

Cell-based Assays for Monitoring EDC Activity in Complex Environmental Samples, Including CAFO Discharges
Vickie Wilson, EPA/ORD/NHEERL

Steroid hormones enter cells by diffusion and bind to receptors in the nucleus. Ligand-receptor pairs dimerize with each other and bind to DNA at hormone response elements to initiate gene transcription. Research toward detecting EDC activity in complex environmental samples has taken advantage of this process to assay for the presence and binding affinity of hormone-active agents (competitive binding assays) or to characterize the binding agent as an agonist or antagonist (transcriptional activation assays). Cells *in vitro* are engineered to express androgen or estrogen receptors and luciferase and, therefore, can respond to hormone exposure in a predictable way by expressing luciferase, which emits light. Exposure of fathead minnows to paper mill effluents (PMEs) is observed to have a masculinizing effect on female fathead minnows suggesting that androgens are present in the effluents. These observations were further supported *in vitro* using androgen-responsive cells. Each assay developed in NHEERL's Reproductive Toxicology Division has unique benefits and challenges. For instance, sterilizing samples without inadvertently affecting the hormonal milieu can be particularly difficult; *in vitro* treatments with effluents expose cells to all of the contaminants in the effluents, which make it a challenge to pinpoint the specific hormone effects; exposure responses are concentration-dependent, and care must be taken to assay cells in the linear response range rather than the maximum response range, which can confound results or have lethal effects. Despite the struggles, the toolbox of assays generated in this research project may be useful for collaborations throughout the field of hormone fate and transport.

Discussion

Mr. Foster asked about analysis of samples downstream of POTWs. Dr. Wilson responded that work to detect the effects of water treatment is ongoing. Specifically, researchers are using bioactivity and chemical assays before and after treatment of effluents with POTW technologies.

Dr. Lauderdale commended Dr. Wilson on the astounding array of assays developed at NHEERL. He asked whether it was correct to infer that false positives were impossible by this approach. Dr. Wilson responded that it would be very rare, but a false positive is possible. False negatives are more common and can occur, for instance, when the treatment concentration is cytotoxic. The best confirmation is replication of experimental results with different assays. Dr. Lauderdale asked if a standard protocol exists to rule out false positives or false negatives. Dr. Wilson replied that there are enough built-in controls and internal replicates to effectively rule out false results.

Dr. Sedlak expressed concern with a slide displaying bioactivity in a cell culture assay detected at a nanomolar level. He stated that data from other assays (e.g., yeast screens) report activity levels orders of magnitude lower. Dr. Wilson explained that the assay she described would detect anything estrogenic, rather than specific estrogen species. She also noted that the assay is more sensitive than yeast screens and may detect activity that is not biologically relevant. A participant added that the particular treatment plant

used to produce those data was distinct from other plants in its high percentage of pulp effluent; that also may have contributed to the heightened levels measured.

Assessing Ground and Surface Water Impacts from Hormones in CAFOs
Steve Hutchins, EPA/ORD/ National Risk Management Research Laboratory (NRMRL)

Scientists at NRMRL develop risk management strategies to assess and minimize EDC exposure. Research efforts at the Ground Water and Ecosystems Restoration Division (GWERD) focus on sources of hormones such as CAFOs, wastewater, and drinking water treatment facilities. Ground water sources provide 40 percent of the nation's drinking water and 36 percent of the nation's irrigation water. Moreover, it serves as an important conduit, accounting for approximately 40 percent of the annual stream flow. Potential ground water stressors include nutrients, metals, pathogens, antibiotics, and EDCs, the last of which include both natural and synthetic hormones. Generally, ground water is monitored for contamination by lagoons, but not usually for contamination by land-applied fertilizer. However, CAFO waste may be applied to land after lagoon-only treatment and contaminants may still be present. To address this, GWERD developed a method to analyze low levels of natural estrogens (estrone, estriol, and ß-estradiol) in ground water and swine lagoon effluent. The method involves solid phase extraction, derivatization, and gas chromatography tandem mass spectrometry (GC/MS/MS) and allows for sensitive detection of free estrogens. A modified analysis using liquid chromatography tandem mass spectrometry (LC/MS/MS) directly quantifies conjugated estrogens; these species also can be measured by indirect enzyme hydrolysis and GC/MS/MS. Estrogen conjugates are secreted naturally by animals and are inactive; however, deconjugation to bioactive forms is possible and it is important to measure the total population of free and conjugated forms. Estrogen conjugates accounted for at least one-third of the total estrogens in the swine lagoons under study. At one swine lagoon site in Canton, Ohio, natural estrogens were detected in lagoons but not in ground water. At a different lagoon site that had been inoperable since 1999, high concentrations of estrogens were found in ground water adjacent to an unlined lagoon. Researchers at GWERD plan to continue monitoring estrogenic contamination at this site. Future studies include monitoring lagoons from swine, poultry, dairy, and beef operations. In addition, GC/MS/MS methods to detect androgens are in development.

Discussion

Dr. Kullman asked whether liquids and solids are distinguished in NRMRL's lagoon work. Dr. Hutchins agreed that there is a great deal of colloidal matter in these samples. Although their procedure involves filtering and centrifuging, complete removal of solids is not achievable. Dr. Hutchins added that this is consistent with the solution applied to land.

Dr. Ann Wilkie (University of Florida) commented that applied CAFO wastes are not necessarily mobile and may not enter water sources. She added that it would be useful to know about the facilities themselves, the type of year, the ambient temperature, and the microbial environment. Dr. Hutchins answered that the data are intended as a snapshot, rather than a set of results that could represent all lagoons. These studies were a first step toward assessing the risk of using lagoons for land application.

Mr. Kevin Elder (Ohio Department of Agriculture) commented that what is called a "lagoon" might be something else. Dr. Hutchins replied that all the lagoons studied were anaerobic, final lagoons.

A participant asked if CAFO facilities were characterized by the degree to which the operator complied to their NMP. Dr. Hutchins noted that the inoperable facility mentioned in his presentation had no NMP. GWERD is progressing toward studies of extant sites with well-designed NMPs to determine whether EDCs continue to be a problem.

Overview of the EPA, ORD, CAFO Research Project
Gary Ankley, EPA/ORD/NHEERL

Endocrine disruptor effects emerged as a major issue in the early 1990s, when biologists from the United Kingdom discovered fish in wastewater treatment plant (WWTP) outputs with external male features and female gonads. These observations led to field studies in which fish exposed to effluents developed female sexual characteristics. Subsequent work indicated that natural and synthetic steroids in effluents are the likely causative factor and confirmed that the problem spans Europe, Asia, and North America. CAFOs consist of many animals confined to a small area, and like WWTPs, CAFO wastes contain a variety of natural and synthetic hormones. Because CAFOs are often adjacent to bodies of water and undergo minimal treatment, the potential threats posed to nearby biota must be assessed. EPA, under the Clean Water Act, strives to prevent and control water pollution from CAFOs; however, very little is known about the fate, transport, and impact of exposure to the natural and synthetic hormones from CAFO discharge. A team of scientists from EPA's exposure, effects, and risk management laboratories are collaborating to develop methods and characterize endocrine-active compounds present in CAFO discharge; identify genomic biomarkers of exposure in aquatic species; evaluate fate, transport, and metabolism of CAFO-derived EDCs; assess ecological impacts; evaluate the capability of existing risk management technologies to reduce exposure to EDCs; and characterize the magnitude and extent of impact of CAFO-derived hormones. Work in this emerging field has determined that trenbolone, a synthetic androgen used as a growth promoter in beef CAFOs, is present in feedlot effluents and is associated with changes in sexually dimorphic characteristics in resident fathead minnows. In the laboratory, female fathead minnows exposed consistently to pure trenbolone undergo masculinization, displaying male secondary sex characteristics and produce an increased number of eggs. Subsequent experiments using cell-based assays on runoff from beef CAFOs detected consistent androgenic activity. Follow-up studies are ongoing and will involve the further application of *in vitro* analytical techniques, laboratory and field studies, identification of biomarkers, and assessment of existing risk management strategies to effectively control estrogen- and androgen-active compounds in the environment. Modified regulations of veterinary pharmaceuticals (e.g., trenbolone) and a better understanding of the impacts of pesticides and antibiotics in CAFO wastes are possible outcomes from these studies, but a basic understanding of the impact of these compounds in the environment is crucial for developing regulations and is the goal of this collaborative project.

Discussion

Dr. Peter Van Veld (Virginia Institute of Marine Science) asked whether the progeny of trenbolone-exposed female fish exhibit an altered sex ratio. Dr. Ankley explained that the trenbolone concentration capable of affecting intersex characteristics inhibits spawning.

A participant asked whether the feedlot data were taken from discharged wastes or treated wastes that were later land-applied. Dr. Jim Lazorchak (EPA) responded that the samples had discharged through a tile drainage system. In addition, there was a composting area behind the facility from which "aged" androgens may have leaked. Dr. Ankley stated that the current data is intriguing, but preliminary, and collaborations with STAR grantees will be beneficial toward understanding this problem from a large-scale, multiple-operation perspective.

Dr. James Minton (Kansas State University) asked about the association between timing of trenbolone implants and detection of androgens in runoff from beef CAFOs. Dr. Ankley replied that such an experiment might be difficult because facilities often stagger their hormone implant schedule. However, it would be interesting to investigate whether spikes in androgen output are associated with implantation of cattle confined to distinct areas of a given CAFO. This would contribute to the understanding of the fate and transport of hormones in waste from CAFOs.

A participant asked Dr. Ankley to comment on the different species of trenbolone he expects to find (a-trenbolone versus ß-trenbolone). Dr. Ankley responded that he would expect the ratio to be constant but insufficient data exist on the topic to speculate further.

Dr. Lauderdale asked about Dr. Ankley's predictions of population-level effects. He mentioned that a-trenbolone has been used in Nebraska for 15 years. Dr. Ankley responded that preliminary results suggest reproductive sensitivity in fish populations but more monitoring data are needed.

SESSION III: NEW EPA STAR AWARDS ON FATE AND EFFECTS OF HORMONES IN CAFO WASTE

Session Moderator: **Susan Laessig, EPA/ORD/NCER**

Fate of Hormones in Tile-Drained Fields and Impact to Aquatic Organisms Under Different Animal Waste Land-Application Practices
Linda Lee, Purdue University

In the Midwest, agricultural soils are fertile but poorly drained. Tile networks are used to improve drainage and flush excessive soil water; without these drains, many farms would become wetlands. The drainage systems output to water sources, but the dynamics of fertilizers in receiving waters are unknown. In addition, the potential impact of hormones and other compounds on existing aquatic life is not understood. It is hypothesized that tile drains will discharge the greatest manure-borne hormone loads in the first rain event following land application. In addition, researchers expect that hormones will degrade more readily when applied to soils than when stored in lagoons or manure pits. Moreover, the synthetic and natural hormones released from animal wastes may affect irreversible gonadal changes in exposed fish under certain conditions. Work for this cooperative agreement will be conducted at Purdue University's Animal Science Research & Education Center, Indiana, an operation with several species of distinctly housed livestock. Cropland is tile drained at this site, and various waste application practices are used (e.g., effluent irrigation, subsurface injection, and solid broadcasting). Current results suggest hormone concentrations below a level of concern in plots where swine manure was subsurface injected. At this site, the type, timing, location, and amount of waste applied as fertilizer is well documented. Nitrogen needs of different crops dictate waste application nature and magnitude. The operation uses a sequential lagoon system for waste treatment, and beef, dairy, and swine and poultry lagoons effluents are isolated from each other during treatment. Samples of land-applied manure and post-lagoon effluents will be obtained for hormone monitoring. Researchers will assay for natural sex steroids, natural and synthetic steroids used to promote growth, and phytoestrogens. Early-life stage fathead minnows will be exposed under controlled laboratory conditions to representative compounds from each group of chemicals. Reproductive capacity and sexual differentiation will be measured as *in vivo* endpoints. Fathead minnows also will be exposed to discharge via a flow-through ditch apparatus constructed on the CAFO site. Sex ratios, gonad histology, and gene expression will be analyzed. Population level effects will be predicted from population-matrix models. Future goals include the detection of other potentially active chemicals, such as herbicides and nitrates, in this experimental system.

Discussion

Dr. Laessig asked about the relevance of studying tile drainage systems and whether the site being targeted was applicable to other agricultural sites. Dr. Lee explained that tile drains are prevalent in Indiana, Illinois, Ohio, Iowa, and Minnesota, and they are increasing in number. She added that it is important to the health of Midwestern people and wildlife to focus research to that area.

A participant asked Dr. Lee to expound upon their research focus on plant estrogens. Dr. Lee replied that phytoestrogens were not detected from plants in their preliminary studies.

Ms. Shappell expressed concern with the chosen CAFO, which mixes beef and dairy waste before application to fields. She stated that the norm is to use fertilizer from a single livestock type. Dr. Lee responded that the CAFO operator does not use all of the lagoons simultaneously. She added that the research plan is to monitor the lagoons for 3 years; if it becomes evident that a mixture of manure from different species is complicating data interpretation, the plan can be modified.

Assessing Occurrence, Persistence, and Biological Effects of Hormones Released from Livestock Waste
Jocelyn Hemming, University of Wisconsin–Madison

It is not known how various animal waste handling and management strategies—such as lagoon storage and spraying of liquid manure versus deep-stacking and field application of solid manure—impact the transport, fate, exposure, and effects of steroid hormones discharged from CAFOs. In this project, several Wisconsin CAFO sites will be assessed for hormone management. The research project will partner with Discovery Farms and will focus on USGS sampling sites that are already in place and span a variety of livestock species, soil settings, and monitoring infrastructures. Researchers will measure hormones in manure and urine from representative cattle, dairy, swine, and poultry operations. Hormone transport and fate will be measured across both environmental and engineered reservoirs, tile drains, ground water, soils, waste storage facilities, and field-applied manure slurry. Solid phase extraction followed by LC/MS/MS will be used to measure steroids in samples. Bioactivity will be assessed using a suite of molecular and cellular-based assays including the colorimetric E-screen and A-screen for estrogenic and androgenic compounds, respectively. Chemical and biological assay data will be compared to determine whether specific compounds in CAFO discharges are associated with bioactivity. Fathead minnows will be exposed to a dilution series of lagoon wastes and histopathology, fecundity, secondary sex characteristics, plasma vitellogenin (Vtg) induction, and hormone fingerprints will be examined as endpoints. These will be compared with the bioassays to identify biomarkers of endocrine disruption. Additionally, if algae are present on these sites, researchers will determine whether hormonal uptake occurs in these organisms. Research is expected to shed light on the relative importance of different natural and synthetic hormones to the biological activity of CAFO wastes and effluents. The results will assist CAFO operators in the improvement of their management practices. It also will provide regulators with risk assessment tools, as biologically relevant chemicals will be identified, quantified, and ranked.

Discussion

Dr. Kullman asked whether Dr. Hemming was sure that additive effects could be assumed in the proposed *in vitro* assays; he commented that synergism might also be possible. Dr. Hemming replied that they had conducted quality assurance experiments with estrogenic mixtures and confirmed that the effects were additive. Dr. Kullman then asked whether fish can survive in lagoon effluents. Dr. Hemming agreed that ammonia in lagoon samples would be toxic. She explained that a dilution series would be used to ensure that subtoxic levels of ammonia are present but that fish are still subjected to the entire chemical milieu present in the lagoon.

Dr. Sedlak commented that exposing whole organisms to hormones also might be difficult because compounds may break down over time. He suggested using a flow-through or caged system. Dr. Terence Barry responded that the researchers would replace the lagoon effluents in fish containment areas every 2 weeks. Dr. Sedlak wondered whether that was often enough. The participant responded that in the field, the lagoon sits in the sun, and hormones may break down naturally. Dr. Lee commented that lagoons are anaerobic and contain high concentrations of ammonia; hormones would not naturally degrade there. The participant disagreed, saying that similar degradation occurs in anaerobic and aerobic conditions; this has been measured experimentally.

A question was raised regarding the relevance of these studies given that the lagoon effluent is spread on land before they runoff to water sources. If fish are exposed to lagoon water directly without a land

application/runoff step, they may be exposed to a different complement of substances. Dr. Hemming replied that in this preliminary phase, researchers will attempt to identify a dominant compound in the mixture and assess subsequently whether changes need to be made to the experimental design.

Transport/Fate/Ecological Effects of Steroids from Poultry Litter, and Evaluations of Existing/Novel Management Strategies
Daniel Fisher, University of Maryland

On the eastern shore of the United States, poultry operators have decreased the number of days to market, the feed-to-meat ratio, and the animal mortality while increasing the market weight. Overall, broiler production has increased by 2,000 percent. The Delmarva Peninsula in Maryland is the most densely concentrated producer of poultry in the United States. At this site, 1.6 billion pounds of poultry litter are generated annually, and the primary disposal method is land application of litter. Excessive land application can have severe impacts on regional surface and ground water quality; excessive nutrient impacts are most frequently cited. However, poultry litter also may contain bacterial and viral contaminants, pesticides, polycyclic aromatic hydrocarbons, antibiotics, metals, and natural steroids. (Notably, synthetic steroids are not administered to birds.) Fecal steroids are transported via runoff as a function of precipitation amount and type of tillage practice employed. Prior studies suggest that estradiol persists on the order of weeks to months in surface waters. The researchers hypothesize that such runoff will exert a direct steroidal effect on nearby aquatic organisms. Preliminary work has been conducted under controlled laboratory conditions, in field settings, and *in situ* watersheds. Free and conjugated steroids were monitored over time in the laboratory and field to determine degradation rates and pathways. Fathead minnows were exposed to poultry litter-associated contaminants (PLACs) to assess Vtg induction and gender differentiation. Preliminary results suggest a significant, dose-dependent effect from these treatments. In the proposed studies, particular attention will be paid to agricultural management tilling practices—no till will be compared with subsurface application of litter in controlled field runoff assays. A novel method of subsurface application exists in which a trench is cut, litter is applied, and the trench is closed and compacted. Preliminary results indicate hormone transport under no till was 2–10 times greater than under conventional tillage. Under the current STAR grant, the chemistry of sex steroids and their derivatives will be characterized fully at different steps in the hormone transport cycle—prior to application, after runoff, and in receiving waters. Solid phase extraction followed by LC/MS/MS will be used. Results will be compared to estradiol radioimmunoassay data collected prior to this grant award. Proposed research includes examining the ability of testosterone to mitigate estrogen exposure effects (poultry litter contains both testosterone and estradiol); the investigation of different fish and frog populations for hormone effects; and investigations into other endocrine disrupting chemicals, such as arsenic.

Discussion

A participant recommended that Dr. Fisher conduct testosterone and estradiol exposures with male and female minnows. Dr. Fisher replied that they had conducted a fathead minnow reproductive assay and found no decrease in the production of eggs in females.

The same participant asked whether an assay was available to measure vitellogenin (Vtg) protein. Dr. Van Veld responded that a universal, quantitative assay was recently published. He stated that the assay takes advantage of the high molecular weights and abundance of phosphates on the vitellogenin molecule and uses a simple polyacrylimide gel electrophoresis for separation by mass. Dr. Van Veld added that antibodies are becoming less crucial to these types of analyses because sequencing is so straightforward.

A participant confirmed that traditional Index of Biological Integrity (IBI) protocols were conducted. He asked whether additional metrics would be employed to detect the subtle reproductive effects of EDCs. Dr. Fisher explained that their study would look for intersex and Vtg induction in a variety of fish and frog species.

Dr. Marisol Sepulveda (Purdue University) asked whether there was a decreased bioavailability of steroids in sediments. She suggested using clean sediment to mimic field settings in the laboratory. Dr. Fisher noted that poultry litter was not stored in lagoons and stated that the observed changes in species diversity in the receiving waters were profound.

Fate of Hormones in Waste from Concentrated Broiler Feeding Operations
Miguel Cabrera, University of Georgia

In Georgia, poultry operations use poultry broiler litter to fertilize grass pastures. These STAR grant researchers are working to understand the variability of hormone and metabolic concentrations in broiler litter and the impact of hormones in waste. This work will complement previous studies involving transformation and transport and evaluate the effect of management to reduce the impact of hormones in waste. From a subset of broiler litter samples collected at the Agricultural and Environmental Services Laboratory of the University of Georgia, estradiol, estrone, testosterone, and metabolites will be assessed across different flock numbers, poultry grow lengths, cleaning regimens in broilers, litter treatments, and freshness of litter when it is applied to land. Researchers will monitor the effect on hormone and metabolite concentrations of storing broiler litter in stack houses across different temperatures and humidity levels. Litter will be tested at the time of storage in stack houses and at the time of removal and land application. Preliminary research indicates that testosterone decomposes while litter is stored, and decomposition is positively correlated with water content. Estradiol decomposes to a much lesser extent. The ability of soil temperature and water potential to affect decomposition of estradiol, estrone, and testosterone in pure solution or in solution mixed with litter will be determined. Radioactively labeled estradiol, estrone, or testosterone will be incubated under these conditions for 6 months and analyzed by liquid scintillation and GC/MS or LC/MS. Stock solutions of estradiol and testosterone will be combined with litter and applied to soil columns to quantify transport when soil is intact versus disturbed. Runoff induced by simulated rainfall at different times following litter application to land will be analyzed for hormone concentrations. Finally, the effect of mechanical grassland aeration on runoff concentrations of hormones and metabolites will be tested. This research will develop essential information needed to reduce hormone concentrations at specific steps from collection of poultry litter to its potential runoff into receiving waters. In particular, the research on grassland aeration may lead to risk management approaches to reduce contamination of surface runoff.

Discussion

A participant asked whether litter was applied to land and then mechanically aerated or vice versa. Dr. Cabrera responded that poultry litter contains many clumps and is difficult to spread evenly. For this reason, litter was applied and then soil was aerated to prevent litter clumps from clogging newly made trenches.

Dr. Lauderdale asked whether the researchers have a statistical model prepared for data analysis. Dr. Cabrera responded that they are in the process of finalizing their statistical model. Currently, they are determining where the greatest variability occurs so they know where to concentrate their efforts.

Dr. Wilkie asked about the texture and organic matter content of soil columns. Dr. Cabrera explained that data are taken on soil that is composed of 15–20 percent clay, 10 percent silt, and sand. The most absorptive of these is clay.

Dr. Wilkie asked how the industry trend toward using caked litter will impact the estrogen scenario. Dr. Cabrera agreed that caked litter was being used more frequently in Georgia. He summarized the data, saying that nutrient levels were similar between caked litter and regular litter. He speculated that a decrease in hormones may occur in the caked litter, because it was clumped by water whereas the regular litter was dry.

An Integrated Approach to Developing a Total Facility Estrogen Budget at a Swine Farrowing CAFO
Seth Kullman, North Carolina State University

CAFOs are increasing in number on the East Coast. These facilities generate abundant waste and have the potential for environmental impact. Previous pilot studies on the biological consequences of male exposure to estradiol in medaka breeding pairs found a decrease in fertilized eggs and a decrease in the number of mature spermatozoa in a dose-dependent manner. Remarkably, females produced fewer eggs after males were exposed to estradiol, even though females were not themselves exposed. Clearly, the effect of naturally occurring estrogens must be determined and fit into the context of other waste contaminants—pesticides, phosphates, nitrogens, and so on. This work proposes to develop an estrogen budget at a swine farrowing CAFO facility on the Neuse River, North Carolina, where testing facilities are already in place. The site houses a variety of livestock species of different growth phases and reproductive cycles. (Operators at this facility synchronize females in estrus.) The total estrogen output will be determined based on characteristics of each animal housed at the CAFO. The fate of estrogens in the three lagoons present at the facility will be examined, as lagoons are the largest reservoir of estrogens on a CAFO. Lagoon volume and rates of addition and removal of lagoon contents will be established. Researchers also will assess partitioning of estrogens between solid and liquid phases in lagoon effluent samples and will collect data on both free and conjugated estrogens. Post-lagoon spreading will be assessed through LC/MS/MS analysis and bioassays in cell culture. Estrogen transport from lagoons to adjacent first- and second-order streams and aquifers will be examined across different fertilizer application practices. At the CAFO being studied, waste is managed by open pit lagoons that are later applied to fields as fertilizer. A novel waste management initiative, entitled the Smithfield Project, may provide a promising mechanism to modify waste with low environmental impact; however, implementation will be costly and slow. In addition, the proposed research involves the development of a Bayesian model that will characterize causal relationships for a total facility estrogen budget in a probabilistic manner. The Bayesian approach will conduct an initial proof of principle analysis—the estrogen budget will be predicted from measured input parameters and modeling of estrogen dynamics in each facility. This work will help to define the elements that impact concentration, fate, and transport of estrogens in these facilities. Results from this project will assist in the prioritization of waste management practices and will help provide recommendations to minimize contributions of total estrogens to the environment.

Discussion

Dr. Cabrera asked whether data exist on minimum exposure times. Following a runoff event, hormones infiltrate the environment and the concentration in streams will increase at that point. How long must biota be exposed before effects are observed? Dr. Kullman responded that laboratory studies give endpoints that are dose- and time-dependent. His proposed research assumes a 3-day minimum exposure time, but researchers could use shorter or longer times based on higher or lower exposure concentrations, respectively.

A participant asked if the estrogen budget is affected by the application of solids, which Dr. Kullman affirmed. The same participant then asked whether the larger estrogenic activity following a rain event was due to aqueous or colloidal estrogen. Dr. Kullman answered that they centrifuge samples and conduct solid phase extraction, but still some solids persist, and they may contain conjugated steroid hormones. The participant noted that conjugates are more soluble and therefore would be transported.

A participant asked what types of samples are taken from animals. Dr. Kullman responded that both urine and fecal samples will be tested.

Transport and Transformation of Natural and Synthetic Steroid Hormones at Beef Cattle and Dairy CAFOs
David Sedlak, University of California–Berkeley

California is the largest producer of milk in the United States. In California dairy watersheds, steroid hormones are not detected in tile drainage systems. This suggests that the high concentrations of estrone observed after rain events are due to overflow from surface waters rather than ground water. Moreover, lagoons and animal feeding areas may "recharge" ground water hormone concentrations. In grazing rangelands, cows have access to water and will eat, drink, urinate, and defecate in the area. During the rainy season from December to May, rain flushes excrement directly into the surface waters without any filtration or treatment processes. Researchers hypothesize that steroids are attenuated as surface water infiltrates ground water. In addition, it is hypothesized that synthesized hormones are more persistent than endogenous hormones when other conditions are held constant. The proposed research will address these questions by assessing stormwater at a University of California at Davis research feedlot where hormone administration and waste handling procedures are rigorously controlled. Samples also will be collected at full-scale commercial beef CAFOs in California, Colorado, and Iowa, and at dairy CAFOs in California. The occurrence, fate, and transport of synthetic and endogenous steroid hormones from these sites will be assessed. Specifically, stormwater runoff and animal waste lagoons will be monitored for steroid hormone runoff via surface waters. Tile drains and animal waste lagoons will be assessed for additional steroid hormone contamination in ground waters. Samples will be quantitated for estrogens, androgens, progestins, and alkylphenolethoxylates (APEs) using GC/MS/MS and ELISA. Soil studies will be conducted to complement an overall hormone transport model. Transport modeling efforts will use HYDRUS ground water models, surface water models of rainfall and runoff, and mixtures effects models. This work will provide a better understanding of the relative importance of surface and ground water pathways for steroid hormone releases from CAFOs and may help develop policy guidelines and agricultural practices to minimize hormone release. Dr. Sedlak welcomed collaborations in analytical method development and testing, bioassay development, and applications of modeling or water quality parameters. He noted that common water quality parameters may provide insight into steroid hormone fate and transport. For instance, *E. coli* levels might be used as an indicator of direct discharge from grazing rangelands. Dr. Sedlak recommended that STAR grantees focus on biological effects of CAFOs beyond feminization or masculinization. He listed the examples of immune system dysfunction, reproductive abnormalities, and pheromonal effects, all of which may result from aquatic exposure to steroid hormones.

Discussion

Ms. Shappell asked how well the pre-extraction internal standard was recovered. Dr. Sedlak replied that the pre-extraction recovery was not quantitated.

Ms. Shappell asked why 17a-estradiol was measured in dairy cattle but not in range cattle. She asked if Dr. Sedlak would expect that hormone species in range cattle. Dr. Sedlak replied that estrone is observed most frequently, but both a and ß forms of estradiol also are observed.

Effects of Cattle Manure Handling and Management Strategies on Fate and Transport of Hormones in the Feedlot and the Field
Daniel Snow, University of Nebraska–Lincoln

There are an estimated 1.3 million livestock operations in the United States. Of these, about 20 percent are CAFOs, which generate 500 million tons of waste annually. There is a potential for natural and synthetic hormones in livestock waste to reach ground water and surface waters through surface runoff, leaching from holding tanks and lagoons, and composting facilities. Steroid hormones in cattle waste include both endogenous and exogenous compounds. Excreted levels are highly variable, depending on the chemical itself and on the sex, diet, and reproductive status of the animal. In this study, feedlots and

fertilized croplands with irrigated soil systems in Nebraska will be sampled and surveyed over a climactic gradient for the fate and transport of hormones. Experiments will include: (1) hormone quantification across different manure handling and management steps; (2) hormone quantification from runoff and erosion from different land application procedures; and (3) detection of hormones in select grass species in fertilized buffer strips. Steroids will be analyzed by LC/MS/MS using an atmospheric pressure photoionization method. Reactors will be used to characterize rates and degradation products. Vertical movement of hormones can be confirmed by analyzing soil leachate across different land application strategies. It is hypothesized that cattle treated with hormones will excrete more hormones than untreated cattle. Researchers expect endogenous and exogenous hormones and their transformation products to occur in cattle manure and persist in manure-fertilized soils and possibly in runoff; however, hormone losses in runoff will be greater when manure is surface-applied rather than incorporated into the soil and when land application occurs immediately before a rainfall event. Composting and other management procedures should increase the degradation rate of hormones compared to stockpiling. The proposed work will inform the scientific and regulatory communities about waste management practices and their influence on the fate of manure-derived hormones. These data also will help operators balance agricultural productivity and environmental protection.

Discussion

Dr. Lee asked, given the potential limits of detection, how the researchers expect to detect steroids in plants. Dr. Snow replied that the potential exists for natural steroids to exist in grasses. He suggested that the researchers might test roots for uptake. Dr. Lee commented that detection of steroids from roots could be due to adsorption of steroids on the root surface. She recommended using a radioactively labeled steroid to determine uptake.

Dr. Van Veld asked whether the researchers were experiencing any major problems with LC/MS/MS analysis. Dr. Snow replied that they are doing steroid analysis with toxicology work, but he affirmed that steroids are not simple to analyze. He added that GC and LC versions of tandem mass spectrometry both have strengths and weaknesses. Electrospray is useful as well, but if the samples are very contaminated, problems will emerge. Dr. Snow stated that, like Dr. Sedlak, he believed that GC/MS/MS is a better separation method and affords higher resolution.

Dr. Lee asked about derivitization before LC/MS/MS. Dr. Snow replied that at this point, the results are promising with derivitization methods, but he stated that a higher signal does not necessarily mean a better result.

GUIDED DISCUSSION

Question and Answer with STAR Principal Investigators

The seven STAR grantees convened for a panel discussion during which participants had time to ask questions that were not asked during the initial question and answer period following each presentation. Dr. Laessig encouraged new collaborations, noting that grants could be converted to cooperative agreements at any time. Currently, three of the seven grantees (Drs. Lee, Fisher, and Snow) are carrying out their research through a cooperative agreement.

Dr. Lauderdale commented that in each study, he saw the potential for confounding results. This was especially true for Dr. Hemming's research plan because she had proposed to use eight sites that were significantly different in livestock and production. Dr. Hemming responded that she intended to place different levels of focus on each farm and her emphasis would be on particular steroids. If it becomes necessary, she plans to design the experiments differently as the research progresses. She clarified that the

first year of the research would be composed of surveying and the subsequent years would be hypothesis testing.

A participant asked Dr. Lee whether there was previous research from Purdue University to document that there was no cross-contamination between CAFO blocks that are under study. Dr. Lee responded that the design of the blocks lent itself to isolation. It is highly unlikely that cross-contamination will occur because slurry walls are perforated from within. If it becomes necessary, she can follow up with additional control measures to confirm no cross-contamination or could identify cross-contamination by major flow changes with rain events. The same participant suggested that Dr. Lee use a nontoxic dye to visualize the runoff if it occurs. Dr. Lee thanked the participant for a useful suggestion and mentioned that such a test was done on one of the plots.

A question was raised about whether lagoons were still used regularly. The participant suggested that lagoons are decreasing in number, especially in swine operations. If the future research focuses on non-lagoon waste management systems, what will that mean for the current research projects? Dr. Kullman responded that he expected the number of lagoons in North Carolina would increase. He reiterated that new technology would be implemented slowly, with larger operations converting first. Dr. Lee asked what would result if lagoon systems were eliminated. She assumed that pits of effluent would still be stored in some way. Dr. Hutchins replied that lagoons would not be phased out in the West and Southwest; he said lagoons are implemented constantly in those regions. In the swine industry, anaerobic lagoons are used almost exclusively for treatment. Dr. Lee asked for a clarification of the strict definition of a lagoon and its comparison to a holding pond. Dr. Lara Moody (Iowa State University) answered that lagoons are anaerobic and maintain an inoculum for microbial digestion of contents. In contrast, the contents of holding ponds occasionally are pumped out entirely. A participant responded that several processes can inactivate microbes in lagoons. Dr. Lee suggested that the researchers have a set of parameters to differentiate lagoons from holding ponds. Dr. Moody replied that an agricultural engineer or someone otherwise familiar with a CAFO would want to know that the STAR project team had asked the right questions of the system. The researchers would need to provide an explanation of the treatment process. She added that the main parameters of CAFOs are hydraulic loading and organic loading. A holding pond is temporary, contents are only held for a few weeks.

A participant from EPA Region 3 reminded everyone that in large beef operations, the manure is dry. He did not see much discussion of this aspect in the seven STAR grants discussed and said it should be something to consider. Dr. Snow responded that, for the Nebraska study, the researchers would be working mainly with dry manure. A participant asked about covered facilities where animals are all indoors and there is no holding pond. In this case, a different form of dry waste results, although moisture would probably still be needed for composting. Another participant stated that these questions represent small parts of CAFO productions.

Dr. Laessig asked Dr. Sepulveda about her findings of high species diversity in streams and drainage systems. Dr. Laessig was surprised by these results, because she had thought that drainage ditches were devoid of species diversity. Dr. Sepulveda responded that eutrophication leads productivity in these ditches, but productivity did not necessarily mean the water source was healthy. Dr. Laessig asked if a comparison site is available, to which Dr. Sepulveda responded that the forest served as a control site. In forest samples, some species are different, but she was unsure how they compared. Dr. Lee added that the nomenclature of a “ditch” in that experiment was flawed, because a tremendous amount of water could be present there. In the Midwest, lots of natural systems are called ditches; this is simply an improper naming mechanism.

A participant revisited the previous discussion of lagoons being appropriate for operators in less temperate regions. He noted that bacterial degradation is different from winter to summer; therefore, estrogen would persist differently. Dr. Elder mentioned that an even loading rate must be applied to the lagoon because the lagoon conditions affect estrogen removal. Enough water must be present for the

lagoon effluents to form a slurry. Otherwise, bacteria will not grow uniformly. Dr. Lee summarized the discussion to say that that if researchers are reporting lagoon effluent concentrations, they would need to report how the lagoon is managed. Mr. Stokes agreed that the main point is that researchers define fully the facility on which they are working. In their publications, researchers should include whether they are targeting a holding pond or a lagoon, which is designed to treat its contents as well as store them. The panelists agreed, and stated that when they describe systems it will be important to acknowledge their differences. A panelist added that the efficiency with which a lagoon degrades hormones is still unknown.

A participant added that in all seven grantee presentations, each grantee stated that the study would be used for policy regulations or waste management at different facilities. He asked if the panelists expected that their sites of choice would be indicative of the entire field. Dr. Laessig replied that EPA is a regulatory agency; she cannot say how the data would be used, but could only ensure that it is scientifically rigorous and of high quality. She added that the STAR RFA was not designed to derive regulations; instead, its purpose is to foster knowledge. She emphasized that research and development (R&D) is not the same as the regulatory arm of EPA. Data collected will be used to better understand hormones in CAFOs. The data are not expected to be used for regulation, but rather to drive the focus of future research efforts.

Dr. Lee commented that, from a research perspective, balance is key. The grantees must be sure that their research is presented fairly; however, there is no sound foundation of research in this field, so it is necessary that the grantees answer questions for the public. Research teams must be analytical, and they must report their observations under the conditions in which they observe them. Dr. Lee sees the seven grant projects as a baseline to produce sound science for the emerging field.

A participant commented that in addition to STAR, the Pork Board is also doing research that has dealt with hormone issues. He asked whether it would be feasible to coordinate research so that scientists from different agencies did not ultimately create a disjointed grouping of isolated projects or a number or duplicated projects. Dr. Kullman stated that the workshop was organized for that reason.

The question was raised as to whether EPA would follow up with these grantees in the near future. Dr. Laessig agreed that they would hold a second workshop in the next 12–24 months and adjourned the meeting at 5:20 p.m.

U.S. EPA Workshop on Fate and Effects of Hormones in Waste From Concentrated Animal Feeding Operations

U.S. Environmental Protection Agency
Region 5
Chicago, IL

August 20–22, 2007

Participants List

Al Alwan
U.S. Environmental Protection Agency

Gary Ankley
U.S. Environmental Protection Agency

Terence Barry
The University of Wisconsin–Madison

Elizabeth Bayne
U.S. Environmental Protection Agency

Alok Bhandari
Kansas State University

Lindsey Blake
U.S. Environmental Protection Agency

Richard Breckenridge
Illinois Environmental Protection Agency

Miguel Cabrera
University of Georgia

Sangsook Choi
U.S. Environmental Protection Agency

John Classen
North Carolina State University

Cecilia Conway
Vreba-Hoff Dairy Development

Marcia Damato
U.S. Environmental Protection Agency

Charles Eirkson
U.S. Food and Drug Administration

Kevin Elder
Ohio Department of Agriculture

Tom Ferguson
Perdue AgriRecycle

Daniel Fisher
University of Maryland

Michael Formica
National Pork Producers Council

Jerry Foster
National Pork Board/Cargill Pork

Elaine Francis
U.S. Environmental Protection Agency

Paige Gay
University of Georgia

Sarah Gerould
U.S. Geological Survey

Matthew Gluckman
U.S. Environmental Protection Agency

Sheridan Haack
U.S. Geological Survey

Phillip Hartig
U.S. Environmental Protection Agency

Dan Heacock
Illinois Environmental Protection Agency

Curtis Hedman
Wisconsin State Laboratory of Hygiene

Jocelyn Hemming
University of Wisconsin–Madison

David Horak
U.S. Environmental Protection Agency

Lisa Huff
U.S. Environmental Protection Agency

Stephen Hutchins
U.S. Environmental Protection Agency

Abua Ikem
Lincoln University

Steve Jann
U.S. Environmental Protection Agency

Roxanne Johnson
North Dakota State University

Brian Koch
Illinois Environmental Protection Agency

Rick Koelsch
University of Nebraska

Alan Kolok
University of Nebraska at Omaha

Bala Krishnan
U.S. Environmental Protection Agency

Seth Kullman
North Carolina State University

Leila Lackey
The Johns Hopkins School of Public Health

Susan Laessig
U.S. Environmental Protection Agency

Boris Lau
Northwestern University

James Lauderdale
Lauderdale Enterprises, Inc.

David Laxton
Intervet Inc.

Jim Lazorchak
U.S. Environmental Protection Agency

Linda Lee
Purdue University

Hui Li
Michigan State University

Xunde Li
University of California–Davis

David Macarus
U.S. Environmental Protection Agency

Elizabeth Hinchey Malloy
U.S. Environmental Protection Agency

Dalma Martinovic
U.S. Environmental Protection Agency

Theogene Mbabaliye
U.S. Environmental Protection Agency

Michael Meyer
U.S. Geological Survey

James Minton
Kansas State University

Lara Moody
Iowa State University

Beth Murphy
U.S. Environmental Protection Agency

Todd Nettesheim
U.S. Environmental Protection Agency

Andy Palmeter
Fort Dodge Animal Health

Roberta Parry
U.S. Environmental Protection Agency

Craig Payne
University of Missouri

Scott Piggott
Michigan Farm Bureau

Donna Porter
U.S. Environmental Protection Agency

Sean Ramach
U.S. Environmental Protection Agency

Clifford Rice
U.S. Department of Agriculture

Michael Roberts
Michigan State University

Mary Ann Rozum
U.S. Department of Agriculture

David Scarfe
American Veterinary Medical Association

David Sedlak
University of California, Berkeley

Marisol Sepulveda
Purdue University

Nancy Shappell
U.S. Department of Agriculture

Gary Sides
Pfizer Animal Health

Rob Sip
Minnesota Department of Agriculture

Daniel Snow
University of Nebraska-Lincoln

William Spaulding
U.S. Environmental Protection Agency

Suzanne Stevenson
U.S. Environmental Protection Agency

Allan Stokes
National Pork Board

Troy Strock
U.S. Environmental Protection Agency

Monte Tucker
Vreba-Hoff Dairy Development

Barbara Vantil
U.S. Environmental Protection Agency

Peter Van Veld
Virginia Institute of Marine Science

Charles Whisnant
North Carolina State University

Ann C. Wilkie
University of Florida

Vickie Wilson
U.S. Environmental Protection Agency

Robert Wright
U.S. Department of Agriculture

Lance Yonkos
University of Maryland

Hank Zygmunt
U.S. Environmental Protection Agency

Contractor Support

Cerena Cantrell
The Scientific Consulting Group, Inc.

Jennifer Griffin
The Scientific Consulting Group, Inc.