Endocrine Disruptors in the Environment: A U.S. EPA Science To Achieve Results (STAR) Progress Review

Tampa Marriott Waterside Hotel
Tampa, FL

November 16, 2008

Final Executive Summary

OVERVIEW

The Endocrine Disruptors in the Environment: A U.S. EPA Science To Achieve Results (STAR) Progress Review was held November 16, 2008, in Tampa, Florida. 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 and government sectors assembled to discuss research addressing endocrine disruptors in the environment and their effects on human and environmental health. The meeting provided an opportunity for grantees in the EPA-funded STAR Program to present their research and interact with EPA staff and others conducting endocrine disrupting chemicals (EDCs) research. The meeting also permitted a chance to review related research being conducted in ORD laboratories. Approximately 50 individuals attended the meeting.

Welcome
Susan Laessig, EPA/ORD/NCER

Dr. Susan Laessig welcomed the participants and explained that EPA has awarded five grants to support research to develop new exposure methods to detect, measure, and track EDCs in the environment. These projects began in 2005 and are now in their third year. Seven grants have been awarded to characterize and assess the impacts of hormones in livestock waste from large animal feeding operations. These projects began in 2007 and are now in their second year. To better leverage resources and improve and advance the research, four of the projects are cooperative agreements between the STAR grantees and collaborators in EPA's laboratories.

Introduction to EPA's Endocrine Disruptors Research Program
Elaine Francis, EPA/ORD/National Program Director for the Endocrine Disruptors Research Program

Dr. Elaine Francis welcomed the participants and provided an overview of the research in EPA's EDCs Research Program. As a whole, EPA's EDCs Research Program is addressing three key long-term goals (LTGs) as identified in the Agency's Multi-year Plan (MYP) for Endocrine Disruptors. LTG 1 seeks to provide a better understanding of the science underlying the effects, exposure, assessment, and management of endocrine disruptors. LTG 2 aims to determine the extent of the impact of endocrine disruptors on humans, wildlife, and the environment. The goal of LTG 3 is to support the Agency's screening and testing program. Most of the research projects being presented at this meeting fall under LTG 2. Overall funding for the EDCs Research Program has been reduced in recent years, with STAR grant funding completely eliminated in 2005. There is much Congressional interest in the research conducted in the EDCs Research Program. There are many opportunities for improved communication and partnerships. STAR researchers are encouraged to propose/organize/participate in targeted workshops, serve as advocates for the research being conducted and its funding, keep ORD informed of the use of their research products, inform ORD before the release of major publications, provide updated

biosketches and other information as requested, and assist ORD by agreeing to present a poster at an EPA Board of Scientific Counselors (BOSC) review or at the Annual EPA Science Forum.

ASSESSING AQUATIC EXPOSURE TO ENDOCRINE DISRUPTING CHEMICALS (EDCS)

Methods to Measure Indicators of Exposure in Real-World Aquatic Environments
James Lazorchak, EPA/ORD/National Exposure Research Laboratory

Few studies have successfully tied EDC exposure and effects work in the laboratory to changes in wildlife in the field. To date, only two studies have shown population effects on wildlife from pharmaceuticals. A field study showed effects in different types of fish following exposure to ethynylestradiol (EE2), including higher vitellogenin (Vtg) gene expression in males, impaired reproduction, and population declines. In another study, fathead minnows (FM) were exposed to river water that received runoff from dairies and feedlots. Male FMs were found to be producing Vtg protein, suggesting a biologically significant exposure to estrogens. Histology at 1 month was inconclusive but suggested that the male FMs were no longer producing sperm. The next research steps include using an array of measurements to determine sources, duration, and possible contaminants and concentrations. Studies in Ohio and Indiana have shown possible androgen exposure in FMs in water downstream from concentrated animal feeding operations (CAFOs) and animal feeding operations (AFOs). Many different substances were found in these waters, making it difficult to determine the cause(s) of any changes in the fish. Future questions to be addressed include: How do we use laboratory-derived data to set protective standards given the paucity of data showing population effects? What endpoints should we use to measure EDC exposures? Do we really need to show population effects of EDCs? What are the new thresholds? Endpoints? The level of protection? How do we address the exposure soup?

Exposure Measurement Tools for Endocrine Disrupting Chemicals in Mixtures
Heiko Schoenfuss, St. Cloud University

The primary goal of this research project is to assess the hypothesis that mixtures of estrogenic compounds will have adverse effects on aquatic vertebrate reproductive fitness that can be rapidly assessed by quantifying alterations in the neuroendocrine system of fish. This will be accomplished by assessing the synergistic interactions of mixtures; assembling a broad, biologically relevant effects matrix; and developing neuroendocrine endpoints for rapid assessment. Researchers began by determining the concentration of various chemicals in effluent from two different samples of treated wastewater. Diatoms, *Daphnia magna*, and fathead minnows (FMs) were then exposed to chemicals. Controlled experiments showed that diatom health was affected by alkylphenols but not by estrogens, and that diatoms differentially bind alkylphenols. Daphnids were exposed to different mixture concentrations, but the researchers saw no effects on molting frequency, survival, production of male daphnids, or embryonic development. When the daphnids were exposed to diatoms that had been previously exposed to nonylphenol, the nonylphenol had an indirect, food-mediated effect on *Daphnia* (increased molting frequency). Predator avoidance studies showed that embryo and larvae performance was adversely affected after 12 days of exposure. Adult FMs were then exposed and compared with control males to determine the effects of exposure on vitellogenin, secondary sex characteristics, nest holding, and intersex. Estrogen effects were additive, but mixture responses at times contradicted the single exposure responses. Neuroendocrine endpoints were then analyzed before nest competition in male FMs; estrone exposure had an effect while estradiol and ethynylestradiol showed no effects. Limited effects were seen after mixture exposure. After the nest competition, the same compounds showed different effects. Thus, it is crucial to consider how the social hierarchy of the fish resulting from reproductive competition affects their response to chemicals. Validation of these studies in the field has begun.

Discussion

A participant asked Dr. Schoenfuss what endpoint was used to determine intersex. Dr. Schoenfuss replied that the endpoint used was multiple clearly definable oocytes in testicular tissue. Another participant asked if the researchers planned to expand the trophic experiments to FMs. Dr. Schoenfuss said that this was the plan for the upcoming year. Another participant asked what the concentrations of the individual estrogen compounds were in the mixture. Dr. Schoenfuss responded that there was 50 µg/L of each compound in the mixture. A participant asked if the researchers found that nonylphenol was binding to glass. Dr. Schoenfuss said that he had no definitive answer to this question. He explained that his team had measured the mucous layer surrounding the diatoms and found that at higher levels of exposure, the mucous layer was removed by alkylphenols. If the alkylphenols are removing the mucous layer, it is likely that they are binding to the remaining glass shell.

Development of Receptor- to Population-Level Analytical Tools for Assessing Endocrine Disruptor Exposure in Wastewater-Impacted Estuarine Systems
Lee Ferguson, University of South Carolina

Grand challenges for assessing EDC exposure in the aquatic environment include assessing identity, sources, and exposure levels; and determining which components of complex mixtures contribute to EDC activity. High-performance mass spectrometry (HPLC-MS/MS) is considered the gold standard for analyzing EDCs because of its high sensitivity and amenability to polar compounds, but there is a need for broadband, sensitive, mode-of-action based methods for surveying exposure to EDCs in mixtures. This research project is working to develop nuclear hormone receptor-affinity extraction techniques for isolating EDCs from complex wastewater mixtures. These methods will be applied in combination with HPLC-MS/MS for activity-directed analysis of EDCs in wastewater and estuarine receiving waters on the South Carolina coast. Sensitive vertebrate (fish) and invertebrate (copepod) EDC exposure laboratory assays will be used to link exposure measurements to biological effects. Novel biomolecular endpoints will be developed and applied to assess EDC exposure in sensitive meiobenthic invertebrates in wastewater-impacted estuarine environments. Nuclear receptor activity is implicated in many EDC modes of action and these proteins form the basis of many common EDC screening tools. Using the standard HPLC-MS/MS method to test water samples, the researchers found high levels of estrogens in certain places, but the samples also included a lot of noise. Using the effluent extract after immunosorbent cleanup showed much clearer results from HPLC-MS/MS. The researchers identified a purified, soluble estrogen receptor ligand-binding domain that is stable and competently binds to estrogens. A fluorescence polarization ligand-binding assay was used to verify that this substance maintained binding affinity for a number of different estrogens and zenoestrogens. A number of environmental contaminants were found to bind to the estrogen receptor. Testing this method in the field, estrogenic compounds were successfully separated from the mixture. Future plans include integrating the mysid shrimp ecdysone receptor/ultraspiracle heterodimer (EcR/USP) into receptor-affinity extraction methods and fluorescence-polarization EDC screening.

Discussion

A participant asked how dynamic the relationship between the ligand and the receptor was. Dr. Ferguson responded that their experiments showed that both estradiol and nonylphenol were able to successfully bind to the receptor.

INNOVATIVE METHODS FOR RAPID DETECTION

Systems Approach to Assessing Cumulative Exposure to Endocrine Disrupting Chemicals
Gerald LeBlanc, North Carolina State University

Existing measures to assess the influence of cumulative exposure to EDCs by conventional analytical chemistry or reporter gene approaches are insufficient because of the complex nature of EDC mixtures, which can elicit toxicity via a range of mechanisms. To address this limitation, the current study is examining cumulative exposure to EDCs by taking a systems approach that assesses multiple endocrine signaling circuits simultaneously. This approach will incorporate toxicokinetic and toxicodynamic actions and also will provide information on mechanisms of action (MOAs) and interactivity. The main objective is to develop an approach for evaluating exposure to EDCs based upon changes in the expression profile of endocrine-related genes in a whole organism. Expression of targeted genes will be evaluated by quantitative real-time polymerase chain reaction (PCR) to identify genes of interest, sequence genes, generate primers, sequence PCR products, and optimize all procedures. To date, 25 nuclear receptors have been identified in the *Daphnia magna*. Daphnids were exposed to a number of chemicals suspected to affect endocrine activity. Experiments have shown that differential expression of a suite of at least six genes can be used to assess exposure of daphnids to EDCs. This exposure detection system has application for assessing exposure to several classes of EDCs. The gene battery also holds promise for the assessment of exposure to chemicals that elicit toxicity via the generation of free-oxygen radicals.

Discussion

A participant asked if *Daphnia* genes change over the 3-day molt cycle. Dr. LeBlanc replied that they do change. The animals in the time course experiments were all the same age and the data were corrected for changes in the level of gene expression in the untreated animals.

Microfluidic Systems for Bioreporting, Separations, Vibrational Spectroscopy, and Microcantilever Sensing of EDCs
Michael Sepaniak, University of Tennessee

The goal of this project is to develop a fully integrated microfluidic device for the detection of EDCs. The research accomplishments to date include: optimization of protein receptor immobilization on microcantilevers (MCs), analysis for EDCs using endocrine receptor functionalized MCs, and analysis of thyroid disrupting chemicals using thyroid hormone transport protein functionalized MCs. Representative EDCs have been surveyed for surface-enhanced Ramen spectroscopy (SERS) response signatures using nanocomposite substrates, but sensitivity was not adequate. The researchers demonstrated the possibility of creating advanced substrates using a direct electron beam lithography (EBL) procedure; sensitivity was more controlled, but still not adequate. Next, nanotransfer printing and a stamp and repeat process were used to address the scalability limitation of EBL; this approach was successful. Future work will include pursuing further improvements in "hot" substrates with several novel approaches. The enhanced science and products of this research should reduce uncertainty in risk assessment/management by: improving the existing technology and attacking the problem of EDC exposure and activity monitoring with an arsenal of informative tools; clarifying and elucidating which chemicals, and in which combinations, can mimic or inhibit endocrine signaling molecules; and providing faster and more portable analytical techniques to facilitate screening and characterization of samples in the field. Future directions include functionalizing the MC arrays and developing field-deployable systems.

Discussion

A participant asked Dr. Sepaniak if this approach could differentiate between different types of estrogens. Dr. Sepaniak said that it could not.

Rapid Detection of Trace Endocrine Disrupting Chemicals in Complex Mixtures: A Full-Spectrum Deconvolution Technique With a UV-Transparent Passive Concentrator
Tohren Kibbey, University of Oklahoma

This research is aimed at developing a rapid, inexpensive screening method that can detect EDCs and other related compounds. Traditional analyses are labor-intensive and costly because they rely on extraction steps and on specialized laboratory equipment. A low-cost approach would allow more waters to be screened and these results could be used to prioritize testing using more complex and costly methods. The method being evaluated uses a UV-transparent polymer that concentrates chemicals and acts as an optical cell for detection. Preliminary partitioning results showed Dow Corning Sylgard 184 Silicone Encapsulant (PDMS) to be the most promising polymer candidate, so further tests were conducted with PDMS. Detection limits for the chemicals tested were found to depend on partition coefficients (higher K_{PW} = better detection), UV absorbance (more chemospheres typically equal better detection), and polymer thickness (thicker = better detection). Estimated detection levels were high enough for most of the chemicals with the exception of estrone and estradiol. Field testing showed no interference from higher turbidity. Dissolved organic carbon (DOC) in landfill water appeared to influence the spectrum slightly, but did not seem to interfere with detection. Future work includes measuring more basis spectra in PDMS, revisiting polymer sampler design, quantitatively assessing uncertainty and the effects of unknown compounds, exploring other deconvolution methods, exploring multi-polymer deconvolution to increase selectivity, and conducting preliminary field testing.

FATE AND EFFECTS OF HORMONES IN ANIMAL WASTE—CATTLE, SWINE, AND POULTRY

Integrated EPA Studies on EDCs in CAFOs
Vickie Wilson, EPA/ORD/National Health and Environmental Effects Research Laboratory (NHEERL)

CAFO wastes include both synthetic steroids and natural hormones and often end up in water bodies. Unlike human waste, CAFO wastes are often unregulated and receive minimal treatment. Studies have shown adverse reproductive effects in fathead minnows (FMs) exposed to trenbolone, an androgenic steroid that is commonly used in CAFOs. EPA assembled a research team to characterize occurrence and ecological impacts of estrogenic and androgenic chemicals from CAFO waste. The research approach includes developing and validating *in vitro* and analytical methods to identify and quantify compounds responsible for endocrine activity in CAFOs across the United States; assessing the ecological impacts using a combination of laboratory and field studies; identifying ecologically relevant biomarkers in aquatic species using genomic approaches; evaluating the environmental fate, transport, and metabolism of CAFO-derived EDCs in surface and groundwaters; and evaluating the capability of existing risk management technologies for CAFOs to reduce exposure to EDCs. The researchers have encountered a number of issues with effluent testing in *in vitro* assays and have devised or are working on solutions. Studies conducted to date have shown that the level of EDCs can vary greatly at different places in a single watershed; thus, a single point assessment is not sufficient. *In vitro* assays appear be good tools for bioactivity-directed analytical chemistry and for overall assessment of hormonal activity. Estrogens were identified more often than androgens. The group will continue to address other issues and will ultimately study the ecological impact of EDCs.

Discussion

A participant noted that the seven androgens in the mixture effects experiment and the dose-response curves seemed to fall into two groups and asked Dr. Wilson if she could explain this. Dr. Wilson explained that this could be because the androgens are normalized to the trenbolone on the same plate. Another participant asked if any researchers were studying atmospheric transport of EDCs. Dr. Wilson

said that her laboratory has found androgenic activity in some products of combustion. Dr. Francis commented that she knew of some nitrogen-focused research that is being done.

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

The Purdue team is working in collaboration with EPA to quantify the contribution of tile-drained agricultural fields under different manure and lagoon effluent application practices to hormone loads in aquatic systems. To do so, animal wastes were applied to two field sites using three different methods. Initial sampling proved to be much more time consuming than anticipated. A more efficient sampling method is being implemented and QA/QC of current hormone and flow data is ongoing. The group also is working to assess hormone persistence in fields under different manure and lagoon effluent practices, studying the persistence in surface soils and stream sediments, tracking average hormone concentrations in manure and lagoons 3 months after beef implanting, performing laboratory-based degradation studies quantifying the effect of temperature and moisture content on trenbolone sorption, and supporting sorption studies. Data collected to date have shown that degradation appears reversible in soil and in the tissues of unintended receptors such as fish. The group also is working to evaluate the impacts of these hormone loads (relevant levels and mixtures) on aquatic organisms. The researchers are studying fish and turtles to identify reproductive effects from EDCs. Laboratory studies have shown changes in gene expression in fathead minnow (FM) larvae exposed to both potent and weak estrogens, showing that the effects on young FMs may be different than the effects on adult FMs. Field studies showed that fish abundance decreased throughout the year, especially at the CAFO sites and higher species richness was seen at the control site. Creek chubs from the site closest to the CAFO were either immature or had very undeveloped gonads compared to the other sites. The researchers are developing quantitative PCR techniques for evaluating changes in gene expression for this species. For the turtle studies, the researchers developed a semi-quantitative vitellogenin assay; plasma samples will be used to quantify sex hormones.

Discussion

Dr. Francis noted that there were more females than males further downstream from the Box Site and wondered if this was significant. Dr. Lee responded that she did not know the reason for this. Sampling will be repeated over the next year, which will help the researchers identify trends.

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

The objectives of this project were to: quantify hormones in various stages of the manure processing pathway in cattle feedlots; determine the effects of different handling practices of cattle feedlot wastes on the stability and availability of hormones; determine the effects of different land application strategies on the fate and transport of hormones in vadose zone soils; and determine if grasses grown in conservation buffers assimilate hormones. Much of the work to date has focused on quantifying the differences in cattle waste as influenced by handling practices such as stockpiling, composting, and runoff retention basins. Two feedlot studies have been completed in an attempt to identify and quantify products from biological degradation. Additional project tasks included: determination of the effect of tillage on hormone levels in field runoff using five manure sources, quantification of the uptake of hormones in manure to selected grass species commonly used in buffer strips, and determination of the fate and transport of hormones in vadose zone soil by examining leaching. Hormones were extracted and analyzed using microwave-assisted solvent extraction, automated solid-phase extraction, and liquid chromatography in tandem with mass spectrometry. In the preliminary results from feedlot runoff samples, few samples contained detectable levels of synthetic growth promoters. However, natural steroid

hormones (an average of << 0.5 ppb of androsterone, 4-androstenedione, estrone, 17β-estradiol, and progesterone) were detected in both treatment and control pens. The amount of steroid hormones in the feedlot pen surface samples (feces) was comparable to that in the runoff samples, but no steroid hormones were detected in freshly scraped samples, and trace levels of synthetic hormone metabolites were detected in pen surface samples collected after implanting.

Discussion

A participant asked if no chemicals at all were found in the samples. Dr. Snow responded that in the first feedlot runoff samples, no synthetic hormones were detected; however, the team did find substantial levels of endogenous hormones, specifically estrogens and progesterone. Most hormones were found in the solids.

Transport and Transformation of Natural and Synthetic Steroid Hormones at Beef Cattle and Dairy CAFOs
Edward Kolodziej, University of Nevada, Reno

This cooperative research project focused on the transport pathway through which steroid hormones move from the CAFO to receiving waters to evaluate risks to aquatic organisms in the receiving waters. The research method involved a complex filtration process that moved a water sample through several steps until a derivatized steroid compound could be isolated and analyzed via GC/MS/MS. EPA's National Health and Environmental Effects Laboratory (NHEERL) tested samples for *in vitro* estrogenic and androgenic activity. Estrone was found in 88 percent of the samples. However, little correlation was noted between the various sites, or between the amount of hormones present and the hormone activity found. Florisil cleanup has improved sample quality, and hypothesis driven sampling methods may be implemented in the future. Rainfall simulator test plots were studied to determine if occurrence and transport could be predicted at the field sites, and whether rangelands could be used to study CAFOs, since they have a direct link to surface waters, have little dilution, are flood irrigated, and are subject to variable manure management. A significant amount of steroid hormones was found to be transported off of these test plots when tested at 5, 20, 40, and 60 minutes. Future research will focus on groundwater testing and modeling. Tile drain and groundwater samples will be collected. Researchers will work to identify the most effective soil column/subsurface transport experiment and determine if surrogates can predict where hormones will be detected. The growth in the organic and natural beef markets has resulted in a significant decrease in the use of steroid implants in California. As a result, there is likely a much smaller amount of synthetic steroids in waters in California today compared to 10 years ago.

Discussion

A participant asked if Dr. Kolodziej knew of any studies on physiological effects in wildlife that measure hormone concentrations in nanograms per liter. Dr. Kolodziej responded that the number used for a predicted null effect of estrogen is approximately 1 nanogram/liter estradiol equivalent. The 1-10 nanogram/liter level is the break point where effects in aquatic organisms can be expected.

A participant noted that Dr. Kolodziej had stated that estrone was found in 88 percent of the field samples collected, but he did not say that they were typically above the predicted no effect concentration (PNEC); the PNEC for estrone and estradiol, however, would be different. What was the typical concentration he was referring to? Dr. Kolodziej responded that only 10 to 15 percent of the samples were above PNEC. There was a 5:1 difference between estradiol equivalency of estrone and estradiol, so estrone was prevalent, but often below PNEC. The participant was concerned that the PNEC has not yet been established for all hormones; she thought that the levels were probably a lot higher. She asked why EPA did not find a correlation with their bioassays if they were running them on unconcentrated samples. Dr. Wilson noted that the samples were eluted in 10 mL of solvent and then further concentrated. In the concentration process, during which the solvent was dried and resuspended, it is possible that less than

100 percent of the hormones present were redissolved. After the extraction, there still is potential for interference. It is possible that EPA lost some of the activity due to the process. Now that EPA is conducting research with estradiol equivalents, it would be useful to revisit the data using some of the new testing techniques to find more specific levels. The participant asked specifically about 17α-estradiol. Dr. Wilson responded that EPA usually ran tests on 17β-estradiol so she did not have that data, but she expected that 17α-estradiol would be slightly less potent.

Assessing Occurrence, Persistence, and Biological Effects of Hormones Released From Livestock Manure
Jocelyn Hemming, University of Wisconsin at Madison

The overall project goal is to determine the presence, persistence, and biological effects of natural and synthetic hormones that may be released into the environment from CAFOs, and evaluate the effects of different animal manure handling practices on the fate and activity of these compounds. The specific objectives include: identifying and quantifying the suite of estrogenic, androgenic, and progesteronic compounds associated with animal farming; characterizing the environmental transport and fate of natural and synthetic steroid hormones that accompany animal manure from farms in Wisconsin; evaluating how various animal manure handling/management strategies impact the transport, fate, potential exposure, and associated effects of steroid hormones discharged from farms; and investigating the ecological effects associated with steroid hormones in animal manure from farms using reproductive, developmental, and gene expression endpoints in fathead minnows (FMs). A variety of soil and water samples were taken from currently operating private farms. Hormones of interest included natural and synthetic estrogens, androgens, and progestins. Data collection is ongoing, but results to date suggest that rain events and snow melts are likely important in hormone mobilization. Compared to other samples, snow melt samples had the highest concentrations of progesterone. E-screen bioassays were performed to determine preliminary potency of the target compounds. Results to date show that increasing the concentration decreases proliferation; 17β-trenbolone was found to be the most potent compound in the E-screen assay. Results from transgenic yeast assays did not have adequate sensitivity. FM assays did not show a significant effect of diluted manure on reproduction, but high doses of trenbolone acetate increased mortality in FM embryos. Future work will include continued sampling; finalizing extraction and clean-up steps for solid samples; characterizing the target hormone suite with HPLC-MS/MS and hormonal activity with *in vitro* bioassays; and investigating the effects of other steroids associated with CAFO effluent, especially on FM oocyte maturation, embryonic development, and shorter exposures on gonad development and gene expression.

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

This project is testing the hypothesis that a mass balance for total estrogen equivalents from swine CAFOs can be predicted based on quantitative input and modeling of estrogen concentrations throughout a CAFO facility. Operational elements that may impact hormone concentration, fate, and transport also will be identified. Livestock farms are a putative source of natural steroid hormones in the environment. In North Carolina, swine farming generates about 19 million tons of waste per year and this amount is expected to grow. Estrogens are excreted in swine urine and feces. Most of the swine waste in North Carolina is held in lagoons and then applied to fields as fertilizer. This project aims to provide an estimate of total estrogen production and composition, identify the fate and stability of estrogens in lagoons, assess off-site transport of estrogens following spray field applications, and develop a Bayesian network model to characterize causal relationships for a total facility estrogen budget in a probabilistic manner. A kickoff meeting was held in April 2008. To date, researchers have identified a new field site and developed a sampling strategy for this site; developed analytic procedures; completed phase 1 of sampling; and started model development. Future work includes further refining the Bayesian model, beginning phase 2 of sampling in the lagoon, and beginning phase 3 sampling of the spray fields.

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

The State of Georgia produces 2 million tons of broiler litter each year. The first objective of this project is to determine the variability of hormone concentrations in broiler litter. Findings to date show hormone concentrations to be higher in caked litter (as compared to whole litter), and increasing the number of flocks from one to three appears to increase hormone concentrations in caked litter but not in whole litter. Broiler litter samples were taken from two different houses and were examined to determine if the age of the broiler had an effect on hormone levels. Samples from both houses showed little to no effect on hormones based upon the age of the broiler. The second project objective is to determine the effect of storing broiler litter in stack houses on hormone concentrations. Two of five houses have been sampled to date and preliminary results from one stack house show an approximate 50 percent reduction in all hormones after 4 weeks. The third project objective is to evaluate the effect of soil temperature and water content on the decomposition of estradiol and testosterone in soil when mixed with broiler litter. Preliminary results show that water content and temperature interact to affect the mineralization of estradiol. Ten percent of estradiol was decomposed in 100 days. Preliminary results for testosterone show that the mineralization in the Sedgefield soil (poorly drained) is high even under relatively dry conditions. Forty percent of testosterone was decomposed in 20 days. The fourth objective involves comparing estradiol and testosterone transport in intact and disturbed columns using stock solutions of hormones mixed with broiler litter. Preliminary work on this objective has recently begun. Researchers also are evaluating the effect of runoff occurring at different times after litter application on hormone concentrations in runoff. Analysis of the data has begun, but results are not yet available. The effect of grassland aeration on concentrations of hormones in runoff from large field plots also will be evaluated. Broiler litter has been applied twice to aerated soil, but there have not yet been rains to stimulate runoff.

Discussion

Dr. Kolodziej asked if the researchers had measured any androgens besides testosterone. Dr. Cabrera said that they had not yet tested other androgens; the researchers are currently working to determine if it is possible to clean the samples adequately to search for other androgens. Dr. Kolodziej noted that the literature shows androgen levels higher than those in the data presented and asked Dr. Cabrera to explain this discrepancy. Dr. Cabrera said that for one of the stack houses sampled, preliminary data show much higher levels of testosterone than the data presented today. The samples with the high levels of androgens are the ones that have not been cleaned out for years; this may be due to accumulation of androgens over the years.

Transport, Fate, and Ecological Effects of Poultry Manure-Associated Contaminants
Lance Yonkos, University of Maryland

The goals of this controlled field runoff study were to: compare contaminants in runoff under competing cropping strategies; investigate the persistence of contaminants in receiving waters; and investigate the contaminants' effects on fish. Two research fields treated with poultry litter were studied, one that was turbo tilled, and one that was not tilled. Because synthetic steroids are not used in poultry farming, the focus was on determining the presence of the endogenous hormones estradiol, estrone, estriol, and testosterone. Runoff was collected from both fields, and water in a retention pond was sampled for 3 weeks. After a rain event, fish in the retention pond died quickly, and the ammonia level was found to be more than 6 mg/L (taken directly from the no till runoff flume it was over 30 mg/L.) Fathead minnows (FMs) then were exposed to both fresh and preserved water from the flume and the receiving pond for 4 to 19 days. Plasma vitellogenin, estrone, and estradiol were measured. In an additional test, largemouth bass (LMB) were collected from six lakes in the Delmarva Peninsula and examined for testicular oocytes. Preliminary results indicate that: turbo till reduces lateral transport of nutrients (and probably other

contaminants) from fields; poultry litter-associated runoff is estrogenic; exposure to nearly lethal runoff and surface water treatments induced only moderate levels of vitellogenin; and incidence of testicular oocytes in field-collected LMB was 33 to 100 percent, but severity was very minor. More conclusions can be made after additional data are gathered and analyzed.

Discussion

A participant asked if pesticides, versus ammonia, were ruled out as a cause of the fish kill. Dr. Yonkos responded that he did not measure for pesticides. The only pesticide applied to the field is Roundup®, which persists for approximately 2 days and would have been applied 3-4 weeks prior to the runoff event.

A participant noted that corn is planted in the area, and atrazine is a pesticide commonly used on corn. Dr. Yonkos responded that atrazine is not used on these fields, but is in use in some places in the Delmarva Peninsula. Dr. Yonkos stated his belief that it is ammonia that killed the fish in less than 5 hours rather than pesticides, given that the ammonia concentration was extremely high.

A participant noted that there are similar fields in North Carolina, and there is a problem with historical applications of arsenic from 50 to 60 years ago. Dr. Yonkos responded that the concentration of arsenic in poultry litter is approximately 40 mg/kg, which is well below the acute toxicity level.

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Participants List

David Anick
North Carolina State University

Catherine Aubee
Murray State University

Barry Baldigo
U.S. Geological Survey

Dieldrich Bermudez
U.S. Environmental Protection Agency

Lindsey Blake
U.S. Environmental Protection Agency

Jennifer Brennan
University of California at Davis

Miguel Cabrera
University of Georgia

Jenna Cavallin
U.S. Environmental Protection Agency

Tom Chandler
University of South Carolina

Kathy Edwards
North Carolina State University

Charles Eirkson
U.S. Food and Drug Administration

Doug Ensley
Fort Dodge Animal Health

Lee Ferguson
University of South Carolina

Elaine Francis
U.S. Environmental Protection Agency

Piero Gardinali
Florida International University

Sarah Gerould
U.S. Geological Survey

Sonya Havens
University of Wisconsin at Madison

Jocelyn Hemming
Wisconsin State Laboratory of Hygiene

Somporn Kamolsiripichaiporn
Rutgers, The State University of New Jersey

Kristen Keteles
U.S. Environmental Protection Agency

Bushra Khan
Purdue University

Tohren Kibbey
University of Oklahoma

Won-Seok Kim
University of South Florida

Edward Kolodziej
University of Nevada, Reno

Alan Kolok
University of Nebraska at Omaha

Seth Kullman
North Carolina State University

Susan Laessig
U.S. Environmental Protection Agency

Ronald Landy
U.S. Environmental Protection Agency

Jim Lazorchak
U.S. Environmental Protection Agency

Gerald LeBlanc
North Carolina State University

Linda Lee
Purdue University

Tim Loes
St. Cloud State University

Dalma Martinović
U.S. Environmental Protection Agency

Greg Mayer
Texas Tech University

Meghan McGee
St. Cloud State University

Marc Mills
U.S. Environmental Protection Agency

Monica Mion
University of South Florida

Tom O'Farrell
U.S. Environmental Protection Agency

Michael Quinn
U.S. Army Center for Health Promotion

Mary Reiley
U.S. Environmental Protection Agency

Clifford Rice
U.S. Department of Agriculture

John Sanseverino
The University of Tennessee, Knoxville

Nora Savage
U.S. Environmental Protection Agency

Heiko Schoenfuss
St. Cloud State University

Michael Sepaniak
The University of Tennessee, Knoxville

Maria Sepulveda
Purdue University

Jan Sharp
McLaughlin Gormley King Company

Weilin Shelver
U.S. Department of Agriculture

Daniel Snow
University of Nebraska–Lincoln

Ted Van Vleet
University of South Florida

Steve Wharton
U.S. Environmental Protection Agency

Vickie Wilson
U.S. Environmental Protection Agency

Lance Yonkos
University of Maryland

Contractor Support:

Denise Hoffman, C.M.P.
The Scientific Consulting Group, Inc.

Jen Hurlburt
The Scientific Consulting Group, Inc.

.