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Detection, Occurrence and Fate of Emerging Contaminants in Agricultural Environments

Daniel D. Snow¹, Shannon L. Bartelt-Hunt², Samuel E. Saunders³, Shannon L. Devivo³, and David A. Cassada⁴

Agricultural settings are affected by a unique set of environmental contaminants typically associated with land use. Nutrients and sediments from run-off and erosion have historically been, and continue to be, studied and understood with respect to their impacts to aquatic environments. Studies involving newer classes of contaminants, such as pharmaceuticals and steroids, are becoming more prevalent as methods for measuring these compounds become available. These “emerging” contaminants clearly have potential to enter the environment and cause known or suspected adverse ecological or human health effects. Release of these contaminants to the environment has occurred for quite some time, but methods for their detection at environmentally-relevant concentrations have only recently become available.

Studies involving emerging contaminants typically focus on the environmental fate and effects of surfactants, antibiotics and other pharmaceuticals, steroid hormones and other endocrine-disrupting compounds (EDCs), fire retardants, sunscreens, disinfection byproducts, new pesticides and pesticide metabolites, and naturally-occurring algal toxins. Detection of these and wastewater-related contaminants in environmental matrices (water, wastewater, soils and sediments) is particularly challenging because of the low detection limits required, the complex nature of the samples, and difficulty in separating these compounds from interferences. New extraction and clean-up techniques, coupled with improvements in instrumental technologies provide the needed sensitivity and specificity for accurate measurement.

The objective of this paper is to review the literature published in 2007 evaluating the detection, fate, and occurrence of emerging contaminants, with a particular focus on emerging contaminants in agricultural systems.
Relevant contaminants are EDCs (particularly hormones and anabolic steroids), antibiotics and other pharmaceuticals associated with wastewater, antibiotic resistance genes in bacteria and prions. Studies on pesticides and flame retardants are not reviewed unless they were evaluated in the same study.

**Analytical Methods for Emerging Contaminants**

**Reviews.** Recent reviews have helped summarize developments and the wide variety of analytical methods available for emerging contaminants now available. Nikolaou et al. (2007) provide a general review of the environmental occurrence of pharmaceuticals, including antibiotics and steroid hormones, in water and wastewater. Methods used to detect these compounds are summarized as well as potential sources in the environment.

Automation of sample analysis is of particular interest in helping to speed sample analysis and improve reproducibility in the measurement. Rodriguez-Mozaz et al. (2007) provide a comprehensive review of on-line solid phase extraction (SPE) coupled to liquid chromatography-mass spectrometry listing over 200 references related to this field. The authors compare advantages and limitations of off-line versus automated pre-concentration techniques for a wide variety of sorbents and instrumentation. They also discuss the use of biosensor technologies for monitoring emerging contaminants such as steroids, pesticides and pharmaceutical compounds.

**Analysis of Hormones and Anabolic Steroids.** Studies are underway to understand the importance of potential inputs from agricultural sources natural and synthetic steroid hormones. A variety of methods were published in 2007 using gas chromatography-mass spectrometry as well as liquid chromatography-mass spectrometry methods. The US Environmental Protection Agency hosted a workshop in August 2007 entitled “Fate and Effects of Hormones from Concentrated Animal Feeding Operations” (CAFO) to promote research in this area. Among other presentations at the workshop, researchers presented plans for seven projects funded through the EPA Science to Achieve Results (STAR) program in 2007.

Hutchins et al. (2007) was a presenter at the EPA CAFO workshop and described analysis of free and conjugated estrogens in lagoon wastewater in a related paper. Concentrations of estradiol, estrone and estriol as well as the conjugated forms of these steroid hormones was measured in samples of wastewater lagoons from cattle, swine and poultry operations. Free hormones were concentrated by SPE followed by derivatization gas chromatography-tandem mass spectrometry (GC/MS/MS). Solids were extracted by ultrasonication with a mixture of acetone and methanol followed by the same analysis used for liquid extracts. Conjugated forms of the estrogens were concentrated by SPE using graphitized carbon cartridges. After several clean-up steps, extracts were analyzed on a triple quadrupole liquid chromatograph-tandem mass spectrometer (LC/MS/MS) using electrospray ionization. Free estrogens were generally higher in swine wastewater (1000-21000 ng/L), compared to poultry (1800-4000 ng/L), dairy (370-550 ng/L), and beef (22-24 ng/L) whole lagoon samples. Traces of estrogen sulfate conjugates at low ng/L concentrations were observed in some lagoons and were
thought to account for up to one-third of the total estrogens detected.

Kolodziej and Sedlak (2007) describe results for a method of analysis of steroids using GC/MS/MS in water samples from small creeks impacted by cattle grazing. Co-author D. Sedlak was also a presenter at the EPA CAFO workshop. Twelve-liter samples were pressure filtered and extracted using 90mm reverse phase C18 SPE disks. Recovery of natural estrogen, androgen and progesterone hormones averaged between 72-106% with method detection limits near 0.2 ng/L. Free steroid hormones were detected in 88% of the surface water samples in a small California agricultural watershed at concentrations up to 44 ng/L. Estrone and estradiol were the most frequently detected steroid hormones in the study and concentrations were comparable to those from municipal wastewater treatment plant effluent.

Hájková et al. (2007) compare several GC/MS approaches in the analysis of five un-derivatized natural and synthetic steroid hormones extracted from river sediments. Standard, single quadrupole GC/MS, low-pressure GC/MS, GC with a time-of-flight (TOF) analyzer, and orthogonal GC-TOF instrumental designs were compared. Steroid estrogens were extracted from samples of river sediments using several different mixtures of polar and non-polar solvents and ultrasonication, followed by a comparison of clean-up procedures using reverse-phase, polymeric and graphitized carbon SPE. Instrumental analysis of un-derivatized steroids in the extracts was compared under four different configurations. The polymeric SPE clean-up provided the best recoveries of fortified samples, and lower-pressure GC/MS provided reasonable sensitivity with detection limits from 1-5 ng/g. The use of a wide bore (0.53um) capillary column connected to an uncoated fused silica restriction column before the quadrupole permits injection of larger sample volumes in comparison to the standard capillary column, seems to provide better peak shapes and improved detection limits. Two-column separation with the TOF analyzer provided better sensitivity in full-scan mode than the quadrupole analyzer used allowing spectral confirmation of unknowns.

Isotope dilution GC/MS has been widely used in mass spectrometric methods to improve quantification of analytes. Stanford and Weinberg (2007) describe an in-line solid-phase extraction and clean-up of steroid estrogens and nonylphenols extracted from ground water, wastewater and soil by gas chromatography with tandem mass spectrometry. Filtered wastewater samples from septic tanks were spiked with deuterium labeled analogues of 17β-estradiol, 17α-ethylaryl estradiol and nonylphenol prior to extraction with a strong anion exchange (SAX) followed by a polymeric (Strata-X™) SPE cartridge. The use of two types of cartridges and additional wash steps provided the best recovery of all target compounds in wastewater, while extraction of ground water did not require the SAX cartridge. Soil samples were extracted with acetonitrile using ultrasonication, separated by centrifugation, and the supernate mixed with water prior to cleanup using the wastewater procedure.

Sediments can serve as both sources and sinks for steroid hormones in aquatic systems. Several methods for
extraction and analysis of these contaminants in sediments were published in 2007. Labadie and Hill (2007) utilized microwave assisted solvent extraction (MASE) for quantitative extraction of estrone, 17β-estradiol and 17α-ethynyl estradiol from river sediments impacted by wastewater effluent. Wet sediment was spiked with labeled internal standards, and butylated hydroxytoluene (BHT) added to prevent degradation of estrogens during extraction. After extraction with methanol, raw extracts were purified using polymeric (Strata-AX™) cartridges followed by a second cleanup step using silica gel. The addition of a 0.22 µm membrane filtration step with extracts in a mixture of water and acetonitrile greatly reduced ion suppression in sediment extracts. Extracts were analyzed by both LC/MS/MS and liquid chromatography time-of-flight mass spectrometry (LC/TOF-MS) with electrospray ionization and negative ion detection mode. Recovery of estrogens averaged between 92 and 107% from river sediment spiked at 10 ng/g with better precision at a lower extraction temperature. Method detection limits using the LC/TOF MS ranged between 0.2 and 0.5 ng/g while the quadrupole LC/MS/MS provided greater sensitivity and ranged between 0.015 and 0.040 ng/g for the estrogens in sediments.

A similar method for extraction and analysis of estrogens in river sediments was published by Matejicek et al. (2007). Five estrogens and some conjugated forms were extracted using MASE with aqueous methanol at 100°C. BHT was added as well as copper to remove sulfurous compounds. Several different extraction temperatures, and combinations of water:methanol were evaluated for the MASE, as well as approaches to extract clean-up using SPE. Extracts were analyzed on an ion trap LC/MS/MS using electrospray ionization, negative ion detection and multiple reaction monitoring (MRM). The best recoveries and sensitivity of the method was found with a 25:75 mixture of water and methanol, and cleanup with a polymeric weak anion exchange (WAX) cartridge. Recoveries of free and conjugated estrogens in spiked river sediment ranged between 83 and 107% with detection limits below 1.0 ng/g on a 1-gm dried sample. Trace concentrations of natural estrogens and conjugates were detected in sediments from two rivers in the Czech Republic at concentrations near 2.5 ng/g for estrone.

Kjaer et al. (2007) describe analysis and leaching of estrone and 17β-estradiol in soils fertilized with swine manure. Tile drainage water beneath fertilized test plots was sampled, extracted and analyzed using a previously described SPE and derivatization GC/MS/MS method. Manure samples were extracted using automated pressurized liquid extraction method with a both a reverse phase and silica gel clean-up. Several hundred ng/g of estrone and estradiol were measured in the swine manure and traces of both compounds (up to 68 ng/L for estrone) were detected in the tile drainage water.

Sources of natural and steroid hormones to aquatic systems includes both agricultural and municipal wastewater. Salvador et al. (2007) describe a method for analysis of natural and synthetic estrogens in wastewater using on-line SPE LC/MS/MS with improved specificity and sensitivity using chemical derivatization. A 1.0 milliliter sample was automatically loaded onto an on-line
polymeric (Oasis™ HLB) cartridge, followed by a wash and then a mixture of dansyl chloride in a carbonate buffer loaded onto the cartridge. After 4 minutes to allow on-column derivatization, switching a multi-port valve allowed removal of derivatizing reagent, followed by another wash. The derivatized analytes were automatically eluted from the SPE column onto a reverse phase analytical column for separation and analysis by electrospray ionization LC/MS/MS in positive ion mode. The effects of derivatization time, dansyl chloride concentration, and organic solvent in washes were evaluated. Detection limits were reported to be below 1.0 ng/L and recoveries of 2 natural and one synthetic estrogen range from 80 to 95%.

Zuo et al. (2007) report a method for analysis of five estrogens in river water with microwave assisted derivatization (trimethylsilyl-derivatives) followed by GC/MS. Microwave heating was found to be more efficient than conventional heating. Estrogens were extracted from spiked river water using a C18 bonded silica SPE cartridge. Recoveries ranged from 78 to 107% and detection limits were estimated to be below 1 ng/L.

Vulliet et al. (2007) describe a method for the determination of the 12 steroid sex hormones in water and apply these methods for the investigation of effluents from STPs in the Lyon area of France. Recoveries of natural and synthetic estrogens, androgens and progesterone was compared in sample extraction using two polymeric and a bonded silica SPE phases. Detection was on a single quadrupole LC/MS both with electrospray and APCI positive ion modes, and extract clean up using silica gel was required for wastewater extracts. Method detection limits were reported to be in the low ng/L range.

Derivatization has been used in many cases to convert steroid hormones to more volatile forms for GC/MS and analysis and to improve sensitivity by LC/MS. Lin et al. (2007) compared three derivatization regents for improving sensitivity for electrospray ionization and APCI LC/MS analysis of estrogens. Drinking, river water and wastewater were extracted using a reverse phase SPE and portions of each eluate was spiked with know amounts estrogens to test dansyl chloride, 2-fluoro-1-methylpyridinium, p-toluenesulfonate (FMPTS), and pentafluorobenzyl bromide (PFBBr) derivatizing reagents. Derivatives were separated on a reverse phase column and analyzed on a triple quadrupole LC/MS/MS system with electrospray and APCI modes. Both dansyl chloride and PFBBr derivatives improved signal considerably over underivatized forms of the steroids, though severe matrix suppression occurred in wastewater extracts. APCI negative ionization detection of PFBBr derivatives was less susceptible to ion suppression and more appropriate for analysis of complex matrices.

Immunoassay kits are commonly-used for screening samples and estimation of steroid concentrations in biological matrices. Farre et al. (2007) compare results for 17β-estradiol and 17α-ethynylestradiol concentrations measured by immunoassay to analysis by triple quadrupole LC/MS/MS and a newer ultra-pressure liquid chromatography (UPLC) interfaced with a quadrupole time of flight (Q-TOF) mass spectrometer. Twenty eight natural and spiked samples (ground water, river, wastewater
influent and effluent) were tested by the three methods. The immunoassay test was used on some samples directly, while reverse phase SPE followed by a aminopropyl SPE cleanup was used for the instrumental analysis. Electrospray ionization negative ion detection was used for both the triple quadrupole and high resolution Q-TOF mass spectrometers. Reasonably good agreement was found for estrogen levels between the immunoassay test and both instrumental methods, though the ELISA kit was found to overestimate concentrations in complex matrices. The method detection limits for the estrogens tested were in the range of 0.4 to 2 ng/L using the triple quadrupole system and around 5 ng/L using the UPLC Q-TOF approach. The high resolution Q-TOF proved useful for exact mass characterization of unknowns and identification of false positives in sample extracts.

A suite of 30 estrogenic contaminants were measured in municipal and pulp mill influent and effluent using a method described by Fernandez et al. (2007). Forty to 500 milliliter samples were spiked with labeled internal standards and extracted with dichloromethane. Extracts were evaporated, purified with deactivated florisil, and derivatized with bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 10% trimethylchlorosilane (TMCS). Derivatized compounds were analyzed using a GC with a high resolution magnetic sector mass spectrometer. Method detection limits were estimated at 1 ng/L. Synthetic estrogens, 19-Norethindrone and 17α-ethynylestradiol were frequently detected in raw and treated wastewater samples. A modified toxicity identification evaluation (TIE) on SPE extracts of wastewater samples was reported, using an yeast estrogenic assay and estrogenic fractions analyzed using a low resolution ion trap GC/MS. Estrogenic activity could not be correlated with the presence of natural estrogens in the fractions however, suggesting that other compounds may be the cause.

Salste et al. (2007) employ bioluminescent yeast assay and LC/MS/MS to help characterize estrogenic fractions of wastewater effluent discharge. Aqueous samples were extracted using polymeric SPE (Oasis HLB™), cartridges washed with mixtures of methanol, water, and ammonium hydroxide, and eluted with a mixture of methanol and MTBE. Semi-preparative HPLC was used to fractionate freeze-dried wastewater residues. LC/MS/MS analysis was conducted on a triple quadrupole instrument using negative ion detection and electrospray ionization. Detection limits were reported to be between 0.1 ng/L for estrone to 5 ng/L for 17α-ethynylestradiol. Estrone occurred at the highest concentration in the effluent and was the major contributor to the estrogenic activity, equivalent to 4–7 ng/L of 17β-estradiol based on the bioluminescent yeast assay.

Wang et al. (2007) describe a new type of SPE for estrone extraction using commercially-available multi-walled carbon nanotubes. SPE packing was prepared and aqueous samples of estrone-fortified water analyzed by enzyme linked immunooassay. Specific polyclonal antibody for estrone (A-E1) and a broad-spectrum antibody for estrone, estradiol and estriol (A-E2) were produced and used to analyze extracts with detection limits between 0.04 to 0.2 µg/L.
Steroid Hormones and Wastewater Treatment. Because municipal wastewater effluent is thought to be a major contributor of estrogenic substances to the environment, many studies are underway to examine the effect of wastewater treatment on steroid hormones. Esperanza et al. (2007) examined the fate of seven steroid hormones in a wastewater treatment system. Aqueous samples were extracted using reverse phase SPE flowed by elution and alumina cleanup. Steroid hormones extracted from solids and semisolids in 3 sequential steps using shaking with methanol and centrifuging over a 10-hour period. Extracts from the solids were purified using the reverse phase SPE and alumina cleanup, with a final purification step using a fraction collector and normal phase HPLC. A 2-step derivatization was used to convert steroid hormones into forms suitable for analysis by GC/MS. Method detection limits were estimated at 1-2 ng/L for aqueous samples and 5-10 ng/g for solid samples. Removal efficiencies averaged ~90% for natural estrogens but only 42% for ethynyl estradiol, while the removal efficiency for the other steroids measured appeared to be near 100%.

Cicek et al. (2007) measured concentrations of estrogens at different points in a wastewater treatment plant. Aqueous samples were filtered and extracted using a reverse phase silica SPE cartridge, prior to analysis by derivatization with N-Methyl-N-(trimethylsilyl)trifluoro-acetamide and pyridine and analyzed by GC/MS/MS. Filters and solids were extracted using accelerated solvent extraction (ASE). Degradation of estrone, estradiol and ethynyl estradiol was estimated by the change in concentrations over the course of the treatment plant. Estrone and estradiol removal efficiency appeared to be greater (75-90%) than that of the synthetic estrogen ethynylestradiol.

Bila et al. (2007) studied the effect of ozone treatment on 17β-estradiol degradation. Water samples were extracted using reverse phase SPE, eluted and derivatized with BSTFA, and analyzed by GC/MS. Byproducts of ozone treatment were characterized and tentatively identified by GC/MS. The yeast estrogenic assay was used to screen treated water containing estradiol byproducts for estrogenic activity. Results indicate that ozonation can be an effective treatment for removal of estrogens and estrogenic activity.

Estrogen and conjugated estrogen removal in membrane bioreactor wastewater treatment systems was investigated by Hu et al. (2007). Aqueous samples were extracted using polymeric SPE (Oasis HLB™) and solids freeze dried and Soxhlet extracted using methylene chloride. Two natural estrogens, 17α-ethynylestradiol, bisphenol-A, and 4-nonylphenol were analyzed by LC/MS/MS on a triple quadrupole mass spectrometer using negative ion detection and electrospray ionization. Detection limits were estimated at 1 for most compounds except for the estrone 3 glucoronide (5ng/L). The results of the LC/MS/MS analysis were compared to overall estrogenicity as measured using a YES assay.

Passive Samplers. Polar organic contaminant integrative samplers (POCIS) are a new method for monitoring pharmaceuticals and steroids in aquatic
environments. The devices permit in-situ continuous extraction of contaminants over an extended period and are designed to simulate exposure of aquatic organism. POCIS have some advantages over grab sampling, especially in systems where contaminant concentrations are low and fluctuate rapidly. One disadvantage is that it is difficult to obtain accurate concentration estimates using POCIS. Togola and Budzinski (2007) determined uptake rates for several pharmaceutical compounds, effectively calibrating these devices for several groups of primarily human pharmaceuticals. The POCIS were then exposed in a river receiving wastewater inputs and grab samples collected and analyzed for the same contaminants. SPE and a cation exchange cleanup was used with GC/MS to analyze grab samples for 14 different pharmaceuticals.

Kolok et al. (2007) used POCIS and caged fish to monitor the occurrence and effects of steroid hormones in agricultural watershed dominated by livestock feeding operations. Eleven natural and synthetic hormones were analyzed in POCIS extracts exposed to river water at four different locations. Three estrogens, two androgenic steroids and 2 progestagens were detected in POCIS using LC/MS with electrospray ionization. No association between estrogenic effects in caged fish was found with levels and types of steroid hormones recovered from the POCIS.

Analysis of Pharmaceuticals. Babia et al. (2007) describe a method using thin layer chromatography (TLC) and videodensitometry for the simultaneous determination of several pharmaceuticals. Samples were pre-concentrated using polymeric SPE (Oasis HLB™) cartridges before application to the TLC plate. TLC separation and development was followed by detection using videodensitometry. Method validation data using 100-ml wellspring and wastewater samples is presented with detection limits ranging from 1 to 200 g/l. Narrow linearity and relatively low sensitivity issues limit this method to highly contaminated wastewater.

Botitsi et al. (2007) present a SPE LC/MS/MS method for the determination of trimethoprim, diclofenac and several sulfonamides in effluent wastewaters. Pre-concentration of 50-ml wastewater samples utilizing polymeric (Oasis HLB™) SPE cartridges resulted in detection limits of < 10 ng/L and recoveries of > 70% using positive electrospray ionization. Application of the method to effluent samples from four wastewater treatment plants in Greece resulted in detection of trimethoprim, diclofenac, and sulfamethoxazole at concentrations up to 400 ng/l.

Automated sample preparation is increasingly employed to improve sensitivity, reproducibility, and processing time for emerging contaminants. An LC/MS/MS method using on-line SPE is described by Feitosa-Felizzola et al. (2007) for the analysis of several classes of antibiotics (macrolides, sulfonamides, fluoroquinolones and tetracyclines) in wastewater. Quantitative recoveries were obtained for fortified reagent water at sample volumes less than 80 mL using polymeric (Oasis HLB™) SPE cartridges. Recoveries for analytes in wastewater samples ranged from 40 to 120% with detection limits between 1 and 46 ng/L, which represented an improvement by a factor of 10 over off-line SPE methods.
Gibson et al. (2007) describe a GC/MS method for the analysis of acidic pharmaceuticals and endocrine disruptors. Acidic extraction using polymeric (Oasis HLB™) SPE cartridges was followed by separate elution and derivatization procedures for both classes of compounds. Recoveries in wastewater ranged from 68 to 114% at the lowest concentration studied (10 g/L). Detection limits were analyte class specific and ranged from 0.25 and 1.0 ng/L for the acidic pharmaceuticals to between 0.005 and 0.05 ng/L for the estrogens.

Kim and Carlson (2007a) describe an off line SPE method with LC/MS/MS for the analysis of antibiotics in water and sediment. Water samples were extracted using a polymeric (Oasis HLB™) SPE cartridge. Sediment samples were extracted with either McIlvaine or ammonium hydroxide buffer solution and EDTA followed by centrifugation, filtration, and subsequent cleanup using SPE. Recoveries from water samples were quantitative (70-124%) for all analytes except minocycline (< 30%) with relative standard deviations between 1 and 13%. In sediments, recoveries were more variable (relative standard deviations of 16-27%) and ranged from 32 to 128 % for all analytes except for minocycline (< 30%). Detection limits were 0.01-0.04 g/L in water for all analytes except for monensin, salinomycin, and narasin (0.001-0.003 g/L) while for sediments, detection limits were 0.3-3.6 g/kg.

Loos et al. (2007) utilized an LC/MS/MS method for a survey of pharmaceutical and polar herbicide contamination in Lake Maggiore in Northern Italy. SPE extraction with polymeric (Oasis HLB™) cartridges and subsequent concentration of the eluate was followed by electrospray ionization and multiple reaction monitoring (MRM) detection. Detection limits for a 100-ml sample were 0.05-0.10 ng/L with recoveries of >60% for most analytes. Contaminate concentration averages and variabilities from lake, tap, river, and rain water sampled from the survey area are presented.

Pharmaceutical contamination from pig, chicken, and turkey manure and manure fertilized soil in Austria was the focus of a risk assessment study by Martinez-Carballo et al. (2007) Samples were analyzed using LC/MS and LC/MS/MS. Selected tetracyclines, sulfonamides, trimethoprim, and fluoroquinolones were extracted from animal manure using ultrasonication in aqueous buffers followed by both SPE and liquid-liquid extraction cleanup. Severe ion suppression was observed and required a 1:10 dilution of most sample extracts. Because of extract dilution a conservative method quantification limit was set at 100 ng/g with matrix-dependent recoveries ranging from 61 to 105%.

Because municipal biosolids are often used as a soil conditioner on agricultural lands, the occurrence of emerging microcontaminants in the material is under close scrutiny. A pressurized liquid extraction (PLE) system was used by Nieto et al. (2007) to extract selected antibiotics, including several macrolides and sulfonamides, ranitidine, omeprazole and trimethoprim from sewage sludge. Sample extracts were filtered and then analyzed by LC/MS using positive ion electrospray ionization. Detection limits of 2-11 ng/g dry weight were obtained with recoveries >74% for all analytes except for ranitidine at 54%. Results
of application of the method to sewage sludge samples from two domestic treatment plants were presented.

Song et al. (2007) describe a method using LC/MS/MS for the determination of several veterinary antibiotics (amprolium, carbadox, monensin, and tylosin) in surface runoff water. Solid phase extraction using polymeric (Oasis HLB™) cartridges was followed by tandem mass spectrometric analysis in positive ion electrospray mode. Analyte specific detection limits of <35 ng/L were obtained for a 30-mL sample with recoveries of 89-113%. Amprolium and monensin were frequently detected in runoff samples from a livestock farm at concentrations up to 288 ng/L.

Stackelberg et al. (2007) analyzed water and sediment samples from drinking water treatment plants to determine removal efficiencies of 113 pharmaceutical and other organic contaminants at various stages during water purification. Compound dependent methods utilizing LC/MS/MS and LC/MS (both in positive ion electrospray mode) as well as GC/MS were used to estimate reduction in concentration of each contaminant. The study concluded that while substantial contaminant reduction occurred during treatment of the influent water, the purification process was incomplete. Detectable concentrations of 21 compounds were found in several treated wastewater samples with most individual samples containing more than 3 contaminants.

Stanford and Weinberg (2007) describe a method utilizing isotope dilution GC/MS/MS for the analysis of derivatized steroid estrogens and nonylphenols extracted from septic, soil and water samples. Matrix dependent extraction and SPE clean-up conditions were used to prepare the samples for derivatization with bis(trimethylsilyl)-trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS). Relative responses between the sample matrices for each analyte and its isotope labeled analog were between 11-30%. Detection limits of 2–4 ng/L for estrogens and 500 ng/L for nonylphenols were obtained under optimal conditions for a 500 mL water sample.

Watkinson et al. (2007) utilized LC/MS/MS to access the removal of antibiotic contaminants in wastewater treatment plants. Sample contaminants were extracted with polymeric (Oasis HLB™) SPE cartridges and positive ion detection with electrospray ionization. Conventional and advanced treatment methodologies were compared for removal efficiency. Nitrate concentration and conductivity were found to be potential indicators of antibiotic concentration in each treatment method, respectively. The study also found that while the removal of contaminants from wastewater treatment facilities is efficient, low g/L concentrations of antibiotics remain in the effluent after treatment.

A rapid method for the LC/MS/MS analysis of 14 sulfonamides in wastewater was described by Ye et al. (2007). Solid phase extraction with Oasis HLB™ cartridges was followed by positive ion detection and electrospray ionization MS analysis. Recoveries ranged between 22 and 87 % with detection limits estimated at 1 ng/L for all sulfonamides except sulfathiazole which had a detection limit of 3 ng/L. Sulfamethazine, sulfamethoxypyridazine, and sulfamethoxazole were found
most frequently in field studies of effluents from municipal and aquaculture wastewater outlets.

Zhang et al. (2007) describe a method for the determination of 16 polycyclic aromatic hydrocarbons (PAHs), 28 polychlorinated biphenyls (PCBs), and 12 pharmaceuticals and personal care products (PPCPs) extracted from river water. Contaminants were extracted using multi-phased extraction disks and eluted before analysis by GC/MS. PPCPs were analyzed after derivatization with BSTFA while the PAH and PCB analytes were analyzed separately without derivatization. Detection limits near 3 ng/L were obtained with recoveries ranging from 44 to 121% for all analytes. Low ng/L concentrations of contamination were found for various PPCPs, total PAHs, and total PCBs when the method was applied to Mississippi river water.

**Contaminant Fate and Occurrence**

Endocrine disrupting compounds (EDCs) enter the environment primarily through wastewater treatment plant effluent and agricultural practices. Direct excretion and land-application of livestock waste may contribute significantly to the load of EDCs present in the environment.

**Occurrence of EDCs in Agricultural Systems.** Hutchins et al. (2007) analyzed lagoon samples for estrogens and estrogen conjugates. Eight lagoons were sampled at selected commercial swine, poultry, and cattle CAFOs in the south central U.S. All lagoons are used directly for land application. Total free estrogen levels of estrone, 17α-estradiol, 17β-estradiol, and estriol were highest in swine primary lagoons at concentrations ranging from 1000 to 21000 ng/L. Poultry primary lagoons contained free estrogens in the range of 1800 to 4000 ng/L, while dairy secondary lagoon concentrations ranged from 370 to 550 ng/L. Beef secondary lagoon samples had total free estrogen levels ranging from 22 to 24 ng/L. Some lagoons also contained estrogen conjugates such as estrone-3-sulfate, 17β-estradiol-3-sulfate, 17α-estradiol-3-sulfate, and 17β-estradiol-17-sulfate. These conjugates can be readily deconjugated to produce the active free estrogens. Estrogen conjugates may significantly contribute to the overall estrogen load from agricultural sources. Overall, the results from this study indicate that animal wastes from CAFOs may have a significant impact on estrogen loads in the environment, regardless of the livestock present.

Livestock that have direct access to surface waters can also excrete steroids directly into the agricultural watershed. Kolodziej and Sedlak (2007) used gas chromatography-tandem mass spectrometry to measure steroid concentrations in small creeks impacted by rangeland grazing (2007). Samples were collected from sites representative of cattle-grazing rangelands in the western U.S. Eighty-eight samples were collected from sampling locations at frequencies that allowed the authors to evaluate the effects of precipitation, streamflow, animal density, and creek accessibility on steroid concentrations. Samples were analyzed for a suite of estrogens, androgens, and progestins. All steroid analytes were detected in at least one sample. Estrone was detected in 78% of the samples with a maximum concentration of 38 ng/L. The most potent estrogen, 17β-estradiol, was detected in 18% of
the samples with a high concentration of 1.7 ng/L. Estrogen concentrations were converted to 17β-estradiol equivalents using a potency factor of 0.2 for estrone and 0.05 for 17α-estradiol. Based on these equivalents, 9 out of 88 samples contained concentrations higher than the predicted no-effect concentration of 1 ng/L of 17β-estradiol. Testosterone was detected in 11% of the samples at concentrations less than 2.3 ng/L. Androstenedione was detected in 18% of the samples with a high concentration of 44 ng/L. Progesterone was detected in only 5% of the samples, but its concentration was generally higher than the concentrations of the other detected steroids for that sample. These hormones may be carried downstream to other organisms.

Fate of EDCs in the Environment. A possible route for hormones released into stream water is migration through river bed sediments into soil and possibly groundwater. Labadie et al. (2007) conducted a study to determine whether substantial concentrations of estrogens could migrate from surface sediments through river bed sediments. Sediment core samples were taken from two sites and dated using 210Pb dating. One site consisted of top layers of coarse, organic-rich to fine, organic-poor sediment with bottom layers of clay. The second site consisted of fine sediment throughout. Vertical profiles of estrogens and estrogenic activity were determined for each sample. At both sites, estrone was detected in sediment layers more than 120 years old, suggesting that estrone could migrate through sediment. At the first site, estrogenic activity was detected down to 15 cm with a large peak just above the clay layer. The authors conducted an experiment to determine if changes in sediment properties could be responsible for the spike in estrogenic activity just above the clay layer. They concluded that sorption properties and different vertical flow rates through the different layers were not responsible for the peak, but it is possible that differences in permeability and advective flow result in a higher flux of estrone through the more permeable layers. Estrogenic activity decreased with increasing sediment depth at the second site. This indicates that degradation and sorption rates were higher than transport rates through the pores. With deep groundwater tables, estrogens pose little threat since they will be degraded before reaching the water. However, shallow water tables may receive some contamination if flow rates are higher than degradation and sorption rates.

While hormones may be degraded before reaching the water table, they may also be transported through the soil via preferential flow pathways. In order to determine the leaching of estrogenic compounds through root zones, Kjaer et al. (2007) applied pig slurry to two tile-drained loamy field sites. Concentrations of estrone and 17β-estradiol were then determined following typical storm events over a one-year period. Estrogens were found to leach from the root zone into the tile drains at both sites. Concentrations of estrone exceeded the lowest observable effect level in 3 out of 27 samples across the two sites, and nearly half of all samples had a detectable concentration of estrone. The maximum detected level of estrone was 68.1 ng/L. Estrone was also detected up to 11 months after application of the slurry. At one site, 17β-estradiol leached on only a few occasions, and never exceeded the LOEL.
At the second site, it only leached on one occasion with a concentration of 1.8 ng/L. These results indicate that under field conditions, leaching may be influenced by preferential transport.

A laboratory study was conducted by Fan et al. (2007a) to determine the fate and transport of testosterone in undisturbed soil. Hamar soil samples were collected using beveled stainless steel cylinders and Teflon plates to ensure that the soil samples were identical to those under field conditions. Batch experiments and miscible displacement experiments were performed on the soil. During the batch experiments, \(^{14}\)C-activity initially decreased before steadily increasing for the duration of the experiment. Other studies reporting the same trend have attributed it to the presence of metabolites in the aqueous phase. However, due to a lack of radioactivity left in the aqueous phase, thin layer chromatography could not be used to determine the metabolites of testosterone. Other possible reasons for this trend include desorption, mineralization, biodegradation, and photodegradation.

Two parallel batch experiments conducted to determine the mechanism responsible for the trend indicate that mineralization and biodegradation are responsible for the increase in \(^{14}\)C activity. Nonequilibrium was used to model the results of the batch experiments, and the real and simulated results show that testosterone is quickly sorbed onto soil particles, testosterone remaining in the aqueous phase was quickly biodegraded or mineralized, and decrease of testosterone in the aqueous phased resulted in desorption of testosterone back into the aqueous phase. The miscible displacement experiments indicated that only small amounts of testosterone and its metabolite eluted from the soil columns. Most of the \(^{14}\)C label was sorbed to the top 1 to 5 cm and only 0.6 to 1.74% of the mass recovered inside the column was from the lower 5 cm. Testosterone also continued to degrade and mineralize even after the soil was extruded from the column.

Stumpe and Marschner (2007) conducted a study to determine the mineralization of 17\(\beta\)-estradiol and testosterone in soils with different properties and land uses. Four soils were used in the study: soil with and without sewage sludge application and soil from fields with either freshwater or wastewater irrigation. Soil samples were supplemented with 1 mg/kg and mineralization was determined over 23 days. A second experiment used soil from control sites pre-incubated with 0.1 mg/kg of unlabeled steroids for two weeks before application of the labeled hormones at 1 mg/kg. A third experiment used the same soils with the steroids applied in an aqueous solution or in wastewater and incubated for 21 days. Results showed that testosterone was mineralized much more than 17\(\beta\)-estradiol in all four soils, which is consistent with the results of previous studies. Similarly, 17\(\beta\)-estradiol is more strongly sorbed to soil. Long-term application of sewage sludge had no effect on hormone mineralization, while long-term irrigation with wastewater retarded the mineralization of testosterone for the first 7 days of incubation. Based on these results, it may be concluded that 17\(\beta\)-estradiol mineralization is low and that it or its metabolites may accumulate in soils. Testosterone may be less persistent, but may leach into groundwater due to lower sorption.
Kim et al. (2007) conducted a study to determine the sorption of testosterone and androstenedione. Four sorbents (two soils and two sediments) were used in the study with total organic carbon contents ranging from 2.10 to 45.7% weight. NaN$_3$ was used to inhibit biological activity throughout the experiment. Completely mixed batch reactors were used under both rate-limiting and equilibrium conditions. A reverse-phase HPLC was used to detect the hormones in the CMBRs. The sorption data was fit to the Freundlich sorption model. Measured aqueous solubility limits were outside the reported ranges, possibly due to variations in temperature and solution chemistry conditions. The data collected from the experiment was used for the sorption experiments since the data was collected under the same conditions as the sorption experiment. The two soils used as sorbents exhibited slightly greater sorption capacity for testosterone while the two sediments used had higher sorption capacities for androstenedione. Results indicate that androstenedione is slightly more soluble than testosterone. The researchers suggest that short-term experiments underestimate sorption capacities for steroids and that one to three weeks may be required for sorption to reach equilibrium. Results from this study indicate that low concentrations of androgens and estrogens in the environment may sorb more readily to soils and sediments than previously thought, retarding their movement through the ecosystem.

Incubation experiments were conducted by Fan et al. (2007b) to determine the fate and occurrence of 17β-estradiol and testosterone in agricultural soils under aerobic and anaerobic conditions. The authors evaluated four different soil microcosms: native soil under aerobic conditions, native soil under anaerobic conditions, autoclaved soil under aerobic conditions, and autoclaved soil under anaerobic conditions. Liquid scintillation counting was used to determine the concentrations of the hormones in the soil. Under aerobic and anaerobic conditions, testosterone was mineralized to a much greater extent than 17β-estradiol. Similarly, 2% of the testosterone was methanogenized to $^{14}$CH$_4$ in native soil under anaerobic conditions, but no $^{13}$CH$_4$ or $^{14}$C-labeled volatile organic compounds were detected for the 17β-estradiol experiments. Results using the autoclaved soil indicate that microbes play an important role in the transformation and degradation of both hormones. This experiment also shows that testosterone mineralization rates are much higher than 17β-estradiol rates under both aerobic and anaerobic conditions.

Stumpe and Marschner (2007) also found total mineralization for both hormones was higher under aerobic conditions than anaerobic conditions, possibly due to different degradation pathways, the number of available electron acceptors, and the difference in microorganism species involved in the mineralization under the two conditions. Results from this study indicate that 17β-estradiol can be transformed into a polar compound via processes that do not involve microbial activity, but can only be degraded to estrone by biological processes. Similarly, testosterone can only be degraded through microbial processes.

**Treatment of EDCs in Agricultural Systems**. With the possibility that steroids released into the...
environment may reach water supplies before being degraded, it is important to determine possible treatment mechanisms. Many CAFOs in the United States have treatment systems for livestock wastes that are designed for nutrient removal. Shappell et al. (2007) measured steroid concentrations in wastewater samples from a lagoon-constructed wetland system at a swine farrowing facility. Estrogenic activity was determined using an in vitro E-screen assay while testosterone concentrations were measured using liquid chromatography/tandem mass spectrometry. Nutrient removal was also measured to ensure that the wetlands were functioning in accordance with literature reports. Samples were also taken from a manure pit, two lagoons, and a storage pond to determine the effectiveness of the wetland system in removing steroids. Results showed that the estrogenic activity was decreased by 83-93%, and contained effluents with concentrations of estrogenic activity below the lowest equivalent 17β-estradiol concentration known to have an effect on fish. This indicates that constructed wetlands may be an effective treatment mechanism to reduce the estrogenic activity of applied swine wastewater.

**Occurrence of Veterinary Antibiotics.** There were new reports of antibiotic detection in 2007. Managaki et al. (2007) reported the first evidence of widespread veterinary antibiotics in Vietnam. Erythromycin, trimethoprim, and various sulfonamides, were detected in the Mekong Delta, which receives runoff from approximately 3.7 million pigs and 35 million poultry birds along with the raw sewage of 20 million people. The authors report the detection of a variety of antibiotics in the Tamagawa River in Japan, a mostly urban watershed, for comparison purposes. Sulfamethazine was detected in relatively high concentrations in the Mekong Delta, where it is heavily used exclusively in agriculture. Gulkowska et al. (2007) detected tetracycline, erythromycin, norfloxacin, and trimethoprim in Hong Kong coastal waters and Martinez-Carballo et al. (2007) detected chlorotetracycline, enrofloxacin, and ciprofloxacin in arable Austrian soils.

Kim and Carlson (2007a) followed-up previous studies of antibiotic incidence in Northern Colorado. Temporal analysis of a wide range of antibiotics tested showed that the highest concentrations in both water and sediment samples occurred in the winter, when flows and temperatures were low. Sediment samples had greater detection frequencies and higher concentrations than water samples of the same site. The highest concentrations of veterinary antibiotics were mainly found downstream of agricultural activity. In a modeling study, Schneider et al. (2007) used a geo-referenced European database to develop scenarios of veterinary antibiotic exposure. They found two new scenarios not currently included in the European Union’s veterinary medicinal product assessment models.

**Fate of Antibiotics in Soil, Manure, & Water Environments.** Antibiotic sorption and transport in soil and water environments were the focus of numerous reports in 2007- too many to review in detail in this paper. Sulfonamides alone were investigated in at least 10 reports. Kahle and Stumm (2007a) determined the sorption properties of sulfathiazole in two papers. Sorption to sterile manure, compost, and humic acid was determined to be strongly affected by contact time and pH, with sorption
continuing after an initial fast sorption period of 1 day. Sorption to inorganic sorbents (clay and ferrihydrite) was an order of a magnitude lower than the organic matter and still highly dependent on pH (Kahle and Stamm (2007b). Kurwadkar et al. (2007) also found a strong pH dependency for sorption of sulfathiazole and sulfamethazine to three sand and loam soils.

Burkhardt and Stamm (2007) reported the depth distribution of three sulfonamides applied to a loamy grassland soil. Antibiotic concentrations down to 30 to 50-cm depth were as high as the top 5 cm regardless of whether applied in manure or aqueous solution. Stoop et al. (2007) conducted a field study of sulfonamide runoff from cropland to surface water. Results suggest a worst-case scenario of 0.5% runoff loss for sulfonamides, lower than predicted by previous sorption results, but in agreement with previously observed in-stream concentrations. Wehrhan et al. (2007) reported extensive column studies of sulfadiazine. Breakthrough curves were fitted best with a three-site sorption model.

Accinelli et al. (2007) found half-lives of around 20 days for sulfamethazine and sulfachloropyridine in silt loam and sandy soils. Liquid swine slurry increased degradation, and the applied antibiotics did not appear to affect soil microorganism activity. In contrast, Blackwell et al. (2007) calculated a sulfachloropyridazine dissipation half-life of 4 days for a sandy loam. Sulfachlorophyridazine was detected in surface run-off and soil water at the field site. De Liguoro et al. (2007) conducted field-scale studies of sulfadimethoxine fate in a complete cycle from fresh feces and bedding to cropland and drainage water. The initial half-life in calf bedding was 24 hours, while the half-life in stable manure was 64 days. No sulfadimethoxine was found in soil amended with manure or water in drainage ditches. Holtge and Kreuzig (2007) traced the fate of sulfamethoxazole and a metabolite in soil (Holtge and Kreuzig 2007).

Blackwell et al. (2007) determined oxytetracycline fate in soil, with a dissipation half-life of 22 days and very low concentrations were detected in surface runoff but not in soil pore water. Pils and Laird (2007) evaluated sorption of tetracycline and chlorotetracycline to clays and humic substances. The antibiotics were found to strongly sorb to clays and to a lesser extent humic substances. Clay-humic complexes had the weakest sorption. Sassman and Lee (2007) studied sorption and degradation of monensin and lasalocid for eight soils. Half-lives were less than 4 days, and manure amendments did not significantly alter degradation, although microbial degradation was the major degradation pathway. Sassman et al. (2007) also studied sorption and degradation of tylosin and its degradation products. Pico and Andreu (2007) reviewed the literature on fluoroquinolones in soil, and Kreuzig et al. (2007) investigated the fate of benzimidazole antiparasitics in manure and soil.

**Degradation of Veterinary Antibiotics.** Degradation processes are important components of any antibiotic mass-balance or exposure model. Lamshoft et al. (2007) radioactively traced sulfadiazine and its metabolites and degradation products after administration to pigs. Four metabolites were determined, and only 4% remained in the pig after 10 days, with sulfadiazine accounting for 44% of
the excreted radioactivity. Storteboom et al. (2007) investigated antibiotic fate in high- and low-maintenance horse manure composting systems. Half-lives of chlortetracycline, tylosin, and monensin were 4 to 15 days for high-intensity and 8 to 31 days for low-intensity. Arikan et al. (2007) reported a half-life of 3 days for oxytetracycline in a composting study. The composting process was not affected by the presence of oxytetracycline, but removal mechanisms could not be determined. In another study, Kakimoto et al. (2007) found that amoxicillin did significantly decrease the composting of human feces even at only 10 µg/g dry weight.

The photodegradability of antibiotics in aqueous and soil environments can be an important factor in environmental fate. Thiele-Bruhn and Peters (2007) observed significant photodegradation of various tetracyclines and sulfonamides in sterile water and soil surfaces. Rate coefficients varied with antibiotic and soil sorptive properties. Pouliquen et al. (2007) found significant photolysis of oxytetracycline in freshwater and seawater. Verma et al. (2007) observed significant photodegradation of tetracycline in prairie waters exposed to light and UV radiation. Hu and Coats (2007) estimated a half-life of 200 d for tylosin A (the parent compound) in water exposed to light, and a half-life of 7 d in soil exposed to light, with sorption and photodegradation as the most important degradation factors evaluated at a limited the UV spectrum. Werner et al. (2007) conducted similar studies over the full UV spectrum and found much faster degradation kinetics for tylosin. A stable photoisomer was observed to be in near 1:1 equilibrium with tylosin within minutes. The photoisomer was less active than tylosin A in inhibiting E. coli growth.

Abellan et al. (2007) studied the photocatalytic degradation and mineralization of sulfamethoxazole with TiO2 and UV light. In the studied aqueous suspension, 82% degradation of sulfamethoxazole and 23% mineralization was observed. The partitioning and photodegradation of ciprofloxacin, primarily a human drug, in the presence of fine and coarse particulate organic matter were investigated by Belden et al. (2007).

**Environmental Toxicology and Plant Uptake.**

Two studies evaluated the toxicity of monensin on aquatic organisms. One found that monensin is not toxic to floating and submersed freshwater macrophytes at up to 100 µg/l exposure for 35 days McGregor et al. (2007). Another found that monensin does not pose a risk to zooplankton; effects were seen only at an exposure of 500 µg/l (Hillis et al. (2007). Kim and Carlson (2007b) evaluated the aquatic toxicity of six sulfonamides and trimethoprim among other drugs. Acute median lethal concentrations for a marine bacterium, freshwater invertebrate, and fish were in the mg/l range. Because of the dose required, these antibiotics likely do not pose an acute risk to these representative species. One study did find antibiotic toxicity at lower concentrations. Tetracycline was found to significantly inhibit protein production in river water bacteria at 10 µg/l and in wetland water at 1000 µg/l (Verma et al. (2007). It is unclear from this study why there was a large difference in toxicity between the two environments.

Plant uptake of antibiotics could reduce yield and expose consumers to low levels of veterinary antibiotics,
but might also be used in phytoremediation. Farkas et al. (2007) investigated the phytotoxicity of chlortetracycline (CTC) on pinto beans and maize. A significant increase in the plant stress proteins glutathione S-transferases (GST) and peroxidases occurred in maize but not in pinto beans, possibly explaining previous results of stunted growth of beans cultivated in antibiotic-contaminated soils. In vitro experiments found that GST induced glutathione to form stable conjugates with CTC, suggesting that maize might be able to detoxify CTC in contaminated soil. In an unrelated study, Park and Choung (2007) found that GST made tetracycline, sulfathiazole, and ampicillin readily biodegradable.

Grote et al. (2007) demonstrated the uptake of CTC and sulfadiazine in wheat from manure-fertilized soil. Root concentrations reached 1.1 mg/kg and 0.5 mg/kg for CTC and sulfadiazine, respectively, and an average of 0.043 mg/kg of CTC was detected in the wheat grain.

Dolliver et al. (2007) observed the uptake of sulfamethazine in corn, lettuce, and potato grown in manure-amended soil. Concentrations, as measured by an ELISA analysis, were between 0.1 and 1.2 mg/kg dry weight, and total plant uptake represented less than 0.1% of the total antibiotic applied.

Antibiotic Resistance Genes (ARG). In 2007, a large number of studies investigated ARG occurrence and fate in confined animal feeding operation (CAFO) waste slurry lagoons, manure composting, and manure-applied soil. Occurrence in aquaculture environments was also a focus. Gilchrist et al. (2007) reviewed and highlighted the role CAFOs might play in the spread of antibiotic resistance to humans and animals and argued for a ban on the use of antibiotics for growth-promotion, similar to the one recently enacted in Europe. Progress was made in 2007 to further define the relationship between agricultural antibiotic use and the spread of ARGs in the environment. Nevertheless, this effort has been hindered by heterogeneity of soil bacterial populations, the multitude of potential horizontal gene transfer mechanisms (plasmids, transposons, integron gene cassettes, phages, integrating conjugative elements), the poorly understood interrelationships between ARGs, the variety antibiotics in use, and the ever increasing number of known ARGs. It is important to note that current culture-based studies may be biased since only a small percentage of soil and fecal bacteria are cultivable. On the other hand, non-culture-based studies (e.g. real-time PCR) do not indicate what species and strain with which the ARGs are associated.

Perhaps the most commonly studied ARGs are the tetracycline ARGs (tet). Macauley et al. (2007) investigated tet resistance in a swine CAFO lagoon facility which had previously only used the antibiotic bacitracin. Even so, they were able to culture 85 species with tet resistance, including 17 new species. Stine et al. (2007) cultured 60 species with tet genes in a swine CAFO lagoon. Chlortetracycline had been used at the facility for over 20 years. This study also found tet genes in bacteria from feed, fresh feces, lagoon water-amended soil, and up- and downstream surface water. However, the authors did not find evidence of wide dissemination of the specific tetracycline-resistant strains cultured from the lagoon. Thus,
horizontal-gene transfer may be a more important process for ARG spread.

Two other studies found evidence of CAFO impacts on ARG incidence. Sapkota et al. (2007) found elevated resistance to erythromycin and tetracycline in culturable enterococci in downstream surface water and elevated clindamycin-resistance in downstream groundwater. Unfortunately, antibiotic use data at the facility was unavailable. Koike et al. (2007) found tet genes in two swine CAFO lagoons and downstream groundwater. No temporal patterns could be discerned from three-years of sampling. Although sequencing studies found agreement between E. coli isolates from the lagoons and the groundwater, the groundwater also appeared to have an indigenous tet gene pool.

Peak et al. (2007) further investigated the relationship between antibiotic use and ARG prevalence in lagoons by studying eight cattle CAFO lagoons in the U.S. using six tet genes are ARG indicators. Quantified by real-time PCR, the total tet genes were significantly higher in the ‘high-use’ lagoons, where tetracycline was heavily used, as compared with ‘mixed-use’ lagoons, where tetracycline was only used therapeutically, and ‘no-use’ lagoons. Tet gene levels were positively correlated to feedlot size and lagoon 16S-rRNA gene levels, but interestingly, only a weak correlation was found between lagoon tetracycline levels and tet gene levels. Summer tet levels were 10-100 times lower than autumn levels, when a prophylactic tetracycline dose is often given to newly weaned calves. Chen et al. (2007) found a positive correlation between tet genes and erythromycin genes (erm) in manure and lagoon samples. Using six newly-developed real-time PCR assays, the authors detected erm genes in swine manure at much higher levels than bovine manure. While lagoon- and biofilter-treated samples did not have significantly reduced erm gene abundance, composted samples did. These results agree with an earlier study of tet genes.

Pei et al. (2007) tested the effects of aerobic and anaerobic dairy lagoon water treatment on tet(W) and tet(O) as well as sulfonamide (sul) and macrolide (ere, msr) genes in lab studies. Responses varied for each gene, temperature, and aerobic condition. After the initial spike of antibiotics, tet and sul genes generally increased and then decreased to initial levels, while ere(A) and msr(A) remained constantly low throughout. In some reactors, tet genes increased even if biocide was added along with antibiotics.

Several other 2007 studies focused on ARGs in manure systems. Patterson et al. (2007) found significant differences in total of 23 tet genes in manure samples from across Europe. Swine manure from no antibiotic-use farms had significantly lower tet genes than manure from heavy-use farms, even if these farms did not use tetracycline. Kobashi et al. (2007) also found 15 different tet genes in bacteria isolated from fresh feces and manure in Japan. Duriez and Topp (2007) studied the antibiotic resistance of E. coli populations in fresh and stored manure over 6 months. A majority of the isolates were shared between the two environments, and antibiotic resistance genes, which varied monthly, were detected for many antibiotics not used at the facility.
Guan et al. (2007) studied the transfer of two different plasmids between E. coli strains in fresh and composting chicken manure. Transfer occurred in both environments except the 50°C compost pile, where the plasmids were likely destroyed. Storteboom et al. (2007) investigated ARGs in the composting environment. In a pilot study with horse manure, levels of the measured tet genes (W and O) exhibited varied responses to composting, with tet(W) increased and tet(O) decreased after 140 days. In a field study with cattle and dairy manure, both genes significantly decreased after 6 months. They also found significantly higher levels of the tet genes in cattle manure compared with dairy manure, which was higher than horse manure, mirroring the antibiotic use for those species.

The occurrence of antibiotic resistance in soils amended with manure was the topic of three studies in 2007. Ghosh and LaPara (2007) did not find significant differences in the chlortetracycline-resistance in bacteria isolated from three pristine soils, from soils at three therapeutic-use only farms, and from soils at three high-use farms. However, one other high-use farm which allowed manure to accumulate outside an animal pin did have significantly higher resistance. Tet genes were rarely detected in the bacteria isolated from the soils, except those from the one high-use farm. In two controlled studies, Heuer and Smalla (2007) and Binh et al. (2007) investigated sulfadiazine and amoxicillin effects on soil ARGs. Two soils spiked with swine manure and sulfadiazine showed significantly higher levels of sul genes after 2 months compared to control soils not spiked or spiked only with manure. Integrons introduced by manure established in both soils, but soil type and sulfadiazine affected the integron composition. Amoxicillin resistance was only significantly increased in one of two soils spiked with manure and amoxicillin. Nevertheless, an extremely diverse number of plasmids conferring resistance were exogenously isolated from soil samples.

Research continued in 2007 on antibiotic resistance in aquaculture environments. In two studies, Akinbowale et al. (2007a) surveyed resistance in Australian trout farms, where antibiotic use is nominally prohibited. Widespread resistance to numerous antibiotics was found in Pseudomonas and Aeromonas spp. isolated from water samples. Four tet genes were also found in bacteria isolates, and tet(A), tet(D), and tet(M) were found in transferable R-plasmids Akinbowale et al. (2007b).

Agerso et al. (2007) detected tet(E) resistance plasmids in Aeromonas spp. in aquaculture. Integrons and their associated gene cassettes were found in Aeromonas spp. isolated from South African fish farms Jacobs and Chenia (2007).

**Prions in the Environment.** Prion diseases, also called transmissible spongiform encephalopathies (TSEs), are a group of fatal neurodegenerative diseases and include bovine spongiform encephalopathy (BSE or ‘mad cow’ disease), scrapie of sheep and goats, chronic wasting disease (CWD) of deer, elk and moose, and Creutzfeldt-Jakob disease in humans. Strong evidence suggests that the sole infectious agent of prion diseases is comprised of PrPSc (i.e. the prion protein), an abnormally-folded isoform of a normal cellular protein (PrPc). The misfolded conformation of PrPSc conveys
distinct biological and physicochemical properties to PrPSc, including resistance to proteolysis, increased hydrophobicity, and a propensity for aggregation. It is known that prions are long-lived in the environment and unusually resistant to inactivation. Scrapie and CWD are of particular environmental concern as they are horizontally transmissible and can remain infectious after years in the environment.

**Environmental Transmission & Infectivity.** VerCautern and colleagues analyzed elk wallowing activity in Colorado for its potential for CWD transmission (VerCauteren et al. (2007)). Results indicate that wallows are used too infrequently to account for a significant amount of transmission. However, preliminary studies of mineral lick activity suggested they may be more important in CWD transmission. Another 2007 study documented an unusual long-distance movement of a white-tailed deer in a CWD area in Wisconsin (Oyer et al. (2007)). Such movements could play a role in CWD spread if the host is shedding prions. In addition, a potent reservoir of infectivity could be established if the host subsequently dies in an area previously without CWD.

Transmission of prion diseases through oral ingestion of a prion-soil mixture has long been speculated and was the subject of two studies in 2007. Using hamster bioassays, each study confirmed that such a prion-soil mixture was infectious via the oral route (Johnson et al. (2007); Seidel et al. (2007)). Seidel et al. (2007) found that PrPSc remained infectious after burial in biologically-active soil for 29 months. They also reported the first use of the protein misfolding cyclic amplification (PMCA) method to amplify prions in a soil mixture, a potential first step towards a detection method for prions in the environment. Johnson et al. (2007) attempted to isolate PrPSc bound to soil particles and determine its infectivity. Their results indicate that prions bound to soil are infectious and may in fact be more infectious than unbound PrPSc. However, further studies are needed confirm and explain this result.

Also in 2007, Kincaid and Bartz (2007) found that prions were 10-100 times more efficiently transmitted in hamsters via the nasal cavity than by oral inoculation, underscoring the potential for nasal inhalation as an environmental route of infection. Results from a study by Scherbel et al. (2007) indicate that PrPSc could be shed in the feces of animals that orally ingested prions. Bovine gastrointestinal microbiota were able to degrade PrPSc below Western Blot detection limits, but did not completely remove infectivity as measured by bioassay. If this result correlates to CWD, the ingestion and subsequent excretion of infected tissue by predators or scavengers could spread CWD to previously uninfected areas. In a similar study, Huang et al. (2007) found that PrPSc in scrapie-infected sheep tissue degraded below the limits of Western Blot detection in a compost pile after 108 days. Thus, composting might be an effective way to degrade prion-infected carcasses. However, the composted tissue could have remained infectious, as bioassays were not performed.

**Prion Sorption and Transport in Soil.** The relationship between the prion protein and soils and minerals was the subject of a number of 2007 studies.
Previous results have indicated a strong uptake and a subsequently difficult extraction of PrPSc by all soils, clay minerals, and sand. Cooke et al. (2007) developed and applied an alternative extraction method for murine PrPSc from soils using Sarkosyl detergent. However, the extraction efficiency of the method may be comparable to previously developed SDS methods. The authors found that elution of PrPSc from clay soil required loss of the N-terminal, which agrees with previous results that the N-terminal might play an important role in binding PrPSc to clays. Another study investigated the influence of pH and ionic strength on PrPSc sorption to quartz sand Ma et al. (2007). Using a purified source of hamster PrPSc, the authors found prion sorption was maximal at a pH corresponding to the estimated isoelectric point of PrPSc aggregates (pH ≈ 4). Increased prion sorption was observed with increasing ionic strength and plateaued at an ionic strength of 0.1 M. Specific mechanisms for prion association with quartz sand were not determined.

One study took a first look at prion transport in soil (Cooke and Shaw 2007). A layer of contaminated soil containing model (non-infectious) recombinant ovine prion protein (recPrP) was placed in the unsaturated portion of a soil column and unsaturated pore water was sampled as a function of time. RecPrP was detected in the original contamination layer throughout the 9-month experiment, even in columns with active soil microbial populations. The maximum distance of recPrP transport was 1 cm, indicating that the recPrP was strongly sorbed to soil and underwent limited transport in soil systems. In another study, Rao et al. (2007) used catechol as a model of soil humic mineral complexes and recPrP as a prion model to study the interaction of prion proteins with organo-mineral complexes. Using UV-Visible and FT-IR measurements, they found a strong attraction between recPrP and the solid aggregates that formed when the catechol polymerized. However, the results of these two studies using recPrP may not correlate well to actual the environmental behavior of the infectious prion protein, because recPrP is noninfectious, unglycosylated, and is not subject to the in vivo biochemical environment.

Taking a potentially more relevant approach, Genovesi et al. (2007) developed a method to detect directly any PrPSc that was bound to soil particles. The method involves washing soil particles after equilibration with infected brain homogenate, incubation with guanidinium and primary and secondary antibodies, and then loading the soil particles into a 96-well plate and detecting any bound PrP by chemiluminescence. This study also confirmed that the soil-bound PrPSc remained infectious using a prion-susceptible cell line. This method avoids the extraction/desorption steps of previous methods and may be more applicable to the study of prion sorption.

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