Detection, fate, and bioavailability of erythromycin in environmental matrices

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Detection, fate, and bioavailability of erythromycin in environmental matrices

by

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A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Toxicology

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THESIS ABSTRACT

This thesis focuses on the environmental effects of the macrolide antibiotic erythromycin from livestock production practices and its possible influence in aquatic ecosystems. The veterinary antibiotic, erythromycin, is used for disease prevention and growth promotion in livestock. Erythromycin has been frequently detected in streams and sediments in the U.S. The widespread use of veterinary antibiotics in agriculture has led to increased concern about their environmental fate. For this research, a microcosm system was utilized to monitor the fate (including movement, binding, and degradation) along with bioavailability of the compound to aquatic invertebrates. The bioavailability study included the development of a surrogate model using a passive sampling device to assess erythromycin in aquatic systems with and without sediment. Analytical procedures to detect erythromycin in water and sediment were improved to assist in quantification. Results from the environmental fate study indicate that erythromycin has an affinity to bind to particulate matter and sediment and is influenced by biotic processes especially mineralization. An aged sediment microcosm system was incubated with erythromycin for 0, 1, 3, and 8 weeks which demonstrated its ability to be sequestered, but addition of surface water columns to the aged residues influenced the mobility of erythromycin into water and from extractable sediment residues. Erythromycin has been shown to be less bioavailable to aquatic organisms, compared to sulfamethazine, but additional studies with sediment containing a manure amendment could possibly influence its ability to affect non-target organisms through exposure and uptake.
CHAPTER 1
THESIS ORGANIZATION AND LITERATURE REVIEW

Thesis Organization

The organization of this thesis is arranged into five chapters. Chapter 1 serves as a general introduction and review of the current literature on erythromycin in the environment. This chapter includes background information on environmental fate of erythromycin in aquatic and terrestrial systems. Chapter 2 discusses the current analytical methods for detecting erythromycin in aquatic and sediment systems and details of improvements to methodologies used in subsequent chapters. Next, Chapter 3 describes the results attained from an erythromycin surface water fate study, which examined the fate of erythromycin in a sediment and water–containing microcosm in the presence and absence of microorganisms, manure, and nutrients for 7 to 63 days. In addition, an aged sediment study was conducted with erythromycin followed by the addition and subsequent incubation of water to determine the movement and persistence of the compound in surface water systems. Chapter 4 presents the bioavailability of erythromycin in the environment utilizing an aquatic microcosm system approach. The study included the use of aquatic worms, *Lumbriculus variegatus* and the surrogate model C₈ Empore® disks to determine if erythromycin concentrates within the worms or the Empore® disks. Finally, Chapter 5 presents general conclusions from this research and acknowledgements.

Abstract

The focus of this thesis is to determine the potential impact of the veterinary antibiotic erythromycin from livestock production through assessment of its fate and bioavailability within the environment. Erythromycin is used extensively in poultry and livestock production to prevent disease and aid in growth promotion and feed efficiency. This compound has been detected in surface water systems and sediments within the U.S. The increased usage of antibiotics and their detection in the environment, both in soil and water systems, has led to an enhanced interest in their fate, effects, and bioavailability in
these environmental matrices. Continued influx of antibiotics into the environment has raised concerns regarding possible development of bacterial resistance and potential effects on aquatic and terrestrial organisms. The work described within this thesis employed laboratory studies to evaluate the fate (mobility, dissipation, binding, and degradation) of erythromycin in surface water systems with and without sediment and its bioavailability to aquatic oligocheates. Additionally, an alternative model using a passive sampling device was developed as a possible surrogate for organisms taking up the drug.

Background

Entry and detection of antibiotics in the environment

Agriculture is a vital constituent of the United States economy with livestock production of swine, cattle, and poultry constituting a large component of the U.S. agricultural industry. In 2007, there were 2.26 billion acres of total land area in the United States, with over 922 million acres of farmland in the U.S. which accounts for 40.8% of total land use. Of the 922 million farmland acres, 408 million acres were pastureland (44.3%), and 406 million acres were cropland (44.1%). The number of farms in 2008 was estimated to be 2.2 million, accounting for $182 million in crop sales and $139 million in livestock sales (USDA-ERS, 2009). The regular use of antibiotics has been implemented by livestock producers to maximize sales by increasing weight gain and for disease prevention (Bunyan et al., 1977; Whiteker et al., 1977; Feighner and Dashkevicz, 1987; McKean, 2004). An estimated 4.5 million pounds of antibiotics are prescribed annually for medical treatment, and 21.7 million pounds are sold for use in farm and companion animals (Phillips, 2004). According to Hayes et al. (1999), antibiotics are prescribed for disease prevention and growth promotion in swine with these compounds included in more than 90% of starter feeds, 75% of grower feeds, 50% of finishing feeds, and 20% of sow feeds. In addition, antibiotics are used in cattle and poultry production at similar rates. Mellon et al. (2001) estimated 24.6 million pounds of antimicrobials are used non-therapeutically in livestock each year, with roughly 10.3 million pounds in hogs, 10.5 million pounds in poultry, and 3.7 million pounds in cattle. Antibiotics have been extensively detected in measurable quantities in the environment,
and entry is often in their original form as they are excreted in urine and feces of livestock. The waste from livestock which includes antibiotics is utilized as fertilizer for farm fields and pastures causing non-point entry of the antibiotics into the environment (Kay et al., 2005; Davis et al., 2006). Manure is commonly applied by injection or waste incorporation into soil causing the potential for antibiotic residues and nutrients to move closer to tile drainage systems which often leads to agricultural water flow from land into nearby waterways. The tile drainage systems or runoff from agricultural fields may act as potential sources of antibiotic contamination in the water systems.

The widespread usage of antibiotics in humans and livestock and their detection in environmental matrices, such as water and soil, has led to an interest in the fate and effects of these compounds and their transformation products. Although antibiotic residues have been well studied in meat and excrement samples, little focus has concentrated on their environmental fate (Tolls, 2001). Antibiotics may be excreted in their original forms directly into the environment or as manure or stored sludge in field application (Davis et al., 2006). Research on antibiotics in the environment has yielded some information on sorption and mobility of a few classes of antibiotics, while mobility and degradation of antibiotics in the environment has been studied very little (Rabolle and Spliid, 2000).

Antibiotics have been found in water, sediment, and manure-containing environmental samples. A wide variety of antibiotic classes have been detected in the environment at a broad range of concentrations. In many of the studies tetracyclines, sulfonamides, and macrolides were the most frequently detected compounds. Previous studies have focused on tetracyclines and sulfonamides, while the most commonly examined macrolides have been tylosin and roxithromycin. In a study conducted by Hirsch et al., (1999) 18 antibiotic substances were found in water samples acquired from sewage treatment plant effluents and surface water with results indicating a erythromycin degradation product, roxithromycin, and sulfamethoxazole as the most commonly detected antibiotics at concentrations up to 6 µg L⁻¹. A survey study conducted by the United States Geological Survey in 2002 reported that antibiotics were found in 48% of 139 streams tested (Kolpin et al., 2002). In this study the highest concentration of
antibiotics detected were sulfamethoxazole at 1.9 µg L⁻¹, followed by erythromycin with 1.7 µg L⁻¹, and lincomycin at 0.73 µg L⁻¹. An additional study also conducted in 2002 found antibiotics in 31% of samples collected near swine farms and 67% of samples near poultry farms with tetracyclines and macrolides (e.g. erythromycin, tylosin) identified at the highest concentrations. Mixtures of antibiotics were detected in 13% of water samples examined (Campagnolo et al., 2002). A study conducted in 2003 detected sulfonamides and trimethoprim in the majority of water samples with 10 ng L⁻¹ average detection and a 71 ng L⁻¹ peak of sulfamethoxazole, while concentrations of trimethoprim ranged between 40 and 50 µg L⁻¹, peaking at 98 µg L⁻¹. The most prevalent macrolide antibiotic detected in water samples from this study was erythromycin, as dehydraterythromycin, at concentrations above 50 ng L⁻¹ with peak concentrations ranging between 130 ng L⁻¹ and 300 ng L⁻¹ (Christian et al., 2003). Kim and Carlson (2007) found six tetracycline-type compounds measured at concentrations between 0.02 – 0.18 µg L⁻¹, while the sulfonamide, sulfamethoxazole, was the most frequently detected of this class with 0.11 µg L⁻¹. In this study the dehydrated form of erythromycin was the most frequently detected macrolide with the highest concentration detected at 0.45 µg L⁻¹, with an average concentration of 0.12 µg L⁻¹ found in water samples. The macrolide erythromycin has been found in sediment samples at levels of 17 µg kg⁻¹ and in aquatic samples at concentrations between 6 and 20 µg L⁻¹ (Hirsch et al., 1999; Davis et al., 2006). Antibiotics have been detected in surface water samples and waste water effluents with tetracyclines, macrolides, and sulfonamides being the most frequently detected antibacterial compounds.

In addition to the detection of antibiotics in surface waters, these compounds have also been found in sediment systems and manure slurries. A study conducted by Christian et al. (2003), found sulfadimidine at 1 mg kg⁻¹ in manure and 15 µg kg⁻¹ in soil using a fast immunnoassay. Kim and Carlson (2007) reported tetracyclines and macrolides having the highest concentrations in sediment samples with reported concentrations ranging between 2.1 µg kg⁻¹ to 24.3 µg kg⁻¹. Erythromycin and tetracyclines were detected in the highest amounts from sediment samples ranging between 82 µg kg⁻¹ to 128 µg kg⁻¹ in an environmental monitoring study (Davis et al.,
2006). The highest concentrations of antibiotics in liquid manure samples has been measured at 20 mg kg\(^{-1}\) for sulfonamides, 11 mg kg\(^{-1}\) for salinomycin, and 43 mg kg\(^{-1}\) for tiamulin (Schlüsener et al., 2003). Another antibiotic found to have strong sorption in soils was tylosin, exhibiting sorption distribution coefficients between 42 to 65 ml g\(^{-1}\) in soils containing 0.5 to 4% organic matter (Hu and Coats, 2009). This study also demonstrated the affinity for tylosin to adsorb to manure resulting in a K\(_d\) value of 285 ml g\(^{-1}\) for a 5 mg L\(^{-1}\) tylosin solution and sediments examined containing manure amendments displayed recoveries of less than 2.5% from soil columns (Hu and Coats, 2009). These studies show the influence of soil properties and manure on recovery and detection of antibiotics in the environment. Although detection of erythromycin in the environment has been examined, little information regarding the amount actually applied to fields has been discussed and few studies have focused on its fate and effects in the aquatic ecosystem. The fate and effects of veterinary antibiotics in aquatic and terrestrial ecosystems are still not well understood and additional studies are needed to better understand their fate (mobility, dissipation, binding, and degradation) and bioavailability in the environment.

**Movement and transport of erythromycin in the environment**

Erythromycin (Figure 1) is commonly produced by *Streptomyces erythreus* and consists of a 14–member macrocyclic lactone ring containing two β-glycosidic linked sugars: D-desosamine and L-cladinose (Flynn et al., 1954; Morimoto et al., 1990; Kanfer et al., 1998). The molecular weight of erythromycin is 733.9 g, pK\(_a\) of 8.8, and a K\(_{ow}\) of 3.06 has been reported (Flynn et al., 1954; Jacobsen et al., 2004; Gros et al., 2006). This antibiotic is considered a wide-spectrum antibiotic and is effective against gram-positive and some gram-negative bacteria. Erythromycin is commonly prescribed for the treatment of Mycoplasmas, *Haemophilus influenzae*, Chlamydia species, and Rickettsia. The mode of action of erythromycin is through binding to the 23S rRNA molecule in the 50S subunit of the bacterial ribosome, which then blocks the elongation in growing peptide chains, thus inhibiting protein synthesis (Mazzei et al., 1993; Prescott et al., 2000). This antibiotic belongs to the macrolide antibiotic class, and its structure and
function are similar to the veterinary antibiotic tylosin (Figure 2) (Kolz et al., 2006). Elimination of erythromycin occurs through bile and feces at a rate of 50-67% and with urinary excretion at 5-10% (McArdell et al., 2003). Tylosin and erythromycin are widely used in global livestock production (Massé et al., 2000). These two macrolide antibiotics are frequently detected in surface waters in the U.S. (Kolpin et al., 2002).

Macrolides are excreted as the parent compound ordinarily, but many metabolites are common, including various group substitutions, as well as acidic and basic products (Kanfer et al., 1998). The parent compound, erythromycin A, is partially transformed to a dehydrated form, anhydroerythromycin, by acidic and basic reactions in aquatic environments (Kurath et al., 1971). This compound is readily adsorbed by soil particles, especially clay, allowing the rate of degradation to be enhanced (Kim et al., 2004). The half-life of erythromycin has been experimentally determined to be 11.5 days in sediment (McArdell et al., 2003). Erythromycin A is converted to anhydroerythromycin through a loss of a water molecule and to erythromycin A enol ether through acid-catalyzed reactions in aqueous solutions. The rate of conversion of erythromycin A into either of these forms is increased when pH is decreased (Atkins et al., 1986; Cachet et al., 1988). Kurath et al. (1971) demonstrated the degradation of erythromycin A into 8,9 anhydroerythromycin (also referred to in the literature as erythromycin-H2O and dehydrato-erythromycin) (Figure 3) with subsequent hydrolysis to form anhydroerythromycin A (Figure 4). This compound (anhydroerythromycin A) has been shown to metabolize into an inactive 6,9; 9,12-spiroketal metabolite (Morimoto et al., 1990).

Veterinary antibiotics are excreted as an active form in manure, which is applied to fields allowing the transport of antibiotics through many routes into the environment including drift, accidental direct input, possible leaching, surface run-off from manure fertilized fields, incorporation into sediment matrices, and uptake by terrestrial and aquatic organisms (Christian et al., 2003; Schlüsener and Bester, 2006). It has been suggested that these antibiotics may have an affinity for clay particles, organic matter, or manure in soil, which could affect their degradation and leaching (Rabølle and Spliid, 2000). In a 2004 study erythromycin’s affinity for adsorption and degradation was
assessed in clay soils and indicated erythromycin was rapidly adsorbed to clay particles and protonation of erythromycin occurred (Kim et al., 2004). Erythromycin was found to have the highest concentrations in sediment during a 10 or 20 minute interval in a simulated rainfall event compared to other antibiotics examined including tetracycline, chlortetracycline, sulfathiazole, sulfamethazine, tylosin, and monensin. Erythromycin was determined to have the highest relative loss associated with sediment, with greater than 50% loss due to runoff and erosion in the rainfall study compared to other antibiotics examined (Davis et al., 2006). Although few studies are available in regards to erythromycin’s environmental fate, other antibiotics have been examined including sulfonamides and the macrolide, tylosin. Tylosin was determined to strongly adsorb with no detection in any leechate from either a sandy loam soil, loamy sand soil, or a sand soil. Results indicated that tylosin leached to a depth of 5 cm in the sandy loam soil and 25 cm in sandy soil using a 30-cm column (Rabølle and Spliid, 2000). Additional studies examining tylosin’s adsorption in soils state its ability to strongly adhere to soil particles and its presence in topsoil (Hu and Coats, 2007; Sassman et al., 2007). Tylosin has also been detected in the environment as metabolites and photoreaction products (Isotylosin 1 and 2) in water samples with antibacterial activity ranging between 31% to 83% of tylosin A (Hu et al., 2008). The increase in detection of antibiotic degradation products in water and sediment has enhanced the need to understand the fate of antibiotics in the environment.

Although some environmental fate information has been published on sulfonamides and macrolides, few studies have examined erythromycin as it enters the environment through manure waste applied to agricultural fields. Additional information is needed on the fate and bioavailability of this compound in the environment due to its widespread use in animal production. Currently only rough extrapolations using data generated from tylosin could be applied to determine erythromycin’s environmental fate. Further studies using erythromycin are needed to assess its fate and bioavailability to facilitate a better understanding of the behavior of this compound in the environment and any potential risks.
Effects of antibiotics on organisms

Antibiotics have been detected in water and sediment systems in the environment, and the fate of some antibiotics have been examined. However, few studies have focused on the bioavailability of antibiotics to aquatic and sediment–dwelling organisms to determine their direct and indirect effects on organisms and the environment. Bioavailability is defined as the amount of a chemical within a system that is available to partition into the various biotic components (organisms) from the abiotic components, (water, sediment, soil, and air) in the system (Sijm et al., 2000). The study of bioavailability of compounds in the environment is important due to the potential toxicity of compounds to organisms within the environment and their availability for biodegradation.

Effects of antibiotics to organisms are primarily assessed through toxicity, growth, and reproduction studies. The effects of antibiotics on microbes has provided evidence of little change in population sizes, but erythromycin exposure may affect the composition in regards to the types of bacterial strains present within a system (Kim and Cerniglia, 2005). Clarithromycin effects in marine bacteria demonstrated little toxicity in growth and survival (Yamashita et al., 2006). However, in this same study effects on daphnid reproduction were seen at concentrations of 6.3 µg L⁻¹ and above, showing the potential for macrolides to affect invertebrates. Baguer et al., (2000) examined the effects of the antibiotic tylosin on springtails and earthworms and found sensitivity with reproduction and growth of the organisms, but overall toxicity was not observed. Presently, few studies have examined effects of antibiotics on non-target invertebrates (e.g. daphnids, springtails, aquatic worms, earthworms) and additional studies focusing on bioavailability and bioconcentration of antibiotics could aid in addressing exposure levels.

Assessment of bioavailability of compounds in water and sediment systems is through the use of the common benthic oligochaete worm Lumbriculus variegatus. This test organism is ideal for studies including both water and sediment as their primary routes of exposure are direct uptake from water and sediment and uptake from sediment through ingestion (U. S. Environmental Protection Agency, 2000). Lumbriculus
Lumbriculus variegatus are ideal due to their ease of culturing in the laboratory setting, widespread distribution throughout the world, and representation of a common invertebrate group. Procedures for using these organisms in bioavailability studies based on ingestion have been developed to standardize the comparison of chemicals in sediments using these organisms (Leppänen and Kukkonen, 1998; Weston et al., 2000). Numerous studies utilizing *Lumbriculus variegatus* have previously focused on the bioavailability of pesticides (e.g. permethrin, cyfluthrin), polycyclic aromatic hydrocarbons (PAHs) (e.g. pyrene), and polychlorinated biphenyls (PCBs) (Conrad et al., 2002; Yang et al., 2006; Yang et al., 2007; You et al., 2007). Due to the adsorption characteristics of antibiotics, it is postulated that these compounds could bioconcentrate and bioaccumulate in sediments and possibly in non-target sediment–dwelling organisms. Additional research is needed to determine the potential for bioavailability of antibiotics in the environment.

In recent years alternatives to traditional bioavailability studies using conventional test organisms have been explored to assess bioavailability of compounds found in the environment. Some of the alternatives include solid phase micro-extraction (SPME) fibers, octadecyl-modified silica disks, and Tenax-TA extraction beads, and the most common substitute, C8 Empore disks (Freidig et al., 1998; Tang et al., 1999; Sijm et al., 2000; Krauss and Wilcke, 2001; Leppänen and Kukkonen, 2006). These alternatives are aimed at combating the expensive cost and large amount of time needed when using live test organisms, and instead intend to provide a more cost-efficient and chemical-based analysis (Tang et al., 1999; Krauss and Wilcke, 2001). The biomimetic devices, which include solid phase micro-extraction (SPME), Tenax-TA beads, and C8 Empore disks, aim to simulate the uptake and bioavailability of a compound through absorbing or partitioning of a compound onto the sampler from the aqueous phase to measure the available compounds in the system (Sijm et al., 2000). The development of a surrogate model in place of or in addition to traditional test organisms to assess bioavailability of antibiotics in the environment may help to address where antibiotics, including erythromycin, bioconcentrate and bioaccumulate within the environment. Additional studies are needed to begin to develop a surrogate system to use as a comparison to traditional bioavailability tests in risk assessments.
Potential for the development of bacterial resistance in the environment

Recently, the detection of antibiotics have occurred in water and sediment systems in the environment and the entry of these compounds could affect the bacterial populations and alter their ecological functions in water, sediment and soil. The additional influx of antibiotics into the environment has raised concerns that they could cause antibiotic–resistant strains of bacterial populations to increase, leading to adverse effects in aquatic and terrestrial organisms, including humans. Resistance in bacterial populations in the environment is not new, but understanding the potential for the development of resistant bacteria from the use of antibiotics as growth promoters is beginning to be examined (Hirsch et al., 1999; Kümmerer, 2009). The development of resistance in bacteria occurs primarily through two mechanisms, “inherited” and secondary resistance. Inherited resistance occurs through bacterial cell division, while secondary resistance involves the transfer of plasmids between microorganisms. It has been demonstrated that low concentrations of antibiotics may influence cell function and genetic expression of antibiotic resistance (Ohlsen et al., 1998; Salyers, 2002). Various microorganisms inhabit the water, soil and sediment environments, but variability exists due to physiochemical properties, pH, moisture, nutrients, light, and temperature present in each niche (Kümmerer, 2009). Bacteria are important to invertebrates as nutrients and symbionts within their gut (Dubilier et al., 1995; Wetzel, 2001). Resistant bacteria have been found to naturally occur in the aquatic environment at low levels (Kümmerer, 2009). However, development of resistance in the environment is not well understood. Although, the studies presented in the subsequent chapters of this thesis do not investigate the issue of bacterial resistance development, it is of increasing concern and information was provided in this chapter to address resistance mechanisms in general.

Problem formulation and research objectives

A wide variety of classes of antibiotics have been detected in surface waters and sediments in the environment at low levels. One of the most common antibiotic classes consistently detected are the macrolides, including roxithromycin, tylosin, and erythromycin. Most studies examining these compounds have focused on their detection,
with few studies investigating their transport, persistence, and degradation in the environment. Although some data has been recently published detailing the fate (mobility, dissipation, binding, and degradation) of tylosin and the sulfonamide, sulfamethazine, little research has been conducted regarding the macrolide erythromycin. Antibiotics have been shown to enter the aquatic environment primarily through surface runoff and leaching. These compounds may have an affinity for clay particles, organic matter, or manure in soil, which could affect their environmental fate. Previous studies conducted with erythromycin on the fate of the antibiotic in the environment have primarily focused on its mobility in clay soils and transport involving soil loss and erosion. In addition to understanding the environmental fate of erythromycin, future studies should also focus on its bioavailability, including bioaccumulation and bioconcentration, within non-target organisms and surface water systems containing sediment. Surrogate systems for determining bioavailability of erythromycin should also be studied to assist in developing alternatives to employing studies using live organisms and as an improvement in comparing field data to lab studies. Further information pertaining to the environmental fate and bioavailability of erythromycin in the environment is needed to improve the understanding of this compound’s importance in the environment and to non-target organisms.

The specific objectives of this project are:

1. To improve the quantification of erythromycin from water and sediment samples through extraction methodology for subsequent studies with this compound.
2. To evaluate the transformation/dissipation and bioavailability of erythromycin in an aquatic microcosm in the presence and absence of microorganisms, organic matter (manure), and sediment utilizing fresh and aged residues.
3. To determine the bioavailability and movement of erythromycin in surface water systems using *Daphnia magna*, *Lumbriculus variegatus*, and the surrogate model C₈ Empore™ disks.
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Figure 1 – Chemical structure of erythromycin

Figure 2 – Chemical structure of tylosin
Figure 3 – Chemical structure of erythromycin-H₂O

Figure 4 – Chemical structure of anhydroerythromycin
CHAPTER 2

OPTIMIZATION OF ANALYTICAL METHODS TO IMPROVE DETECTION OF ERYTHROMYCIN FROM WATER AND SEDIMENT

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Abstract

Analytical methods to improve the detection of erythromycin in water and sediment were developed to optimize for erythromycin’s recovery of extractable and bound residues from the aquatic environment. Comparison of solid phase extraction (SPE) and sediment extraction methods were analyzed to determine optimal recovery of erythromycin from water and sediment. SPE methods examined included previously reported methods for macrolide and sulfonamide antibiotics with recoveries ranging from 75.5% to 94.7% for the various methods examined. Results indicated the best SPE method for the concentration of erythromycin from water was a method previously used to quantify tylosin published by Kolz et al. (2006) with 94.7% recovery. Extraction of erythromycin was performed from sand employing various solvents and buffers to determine the best methods for extraction of the compound from sediment. Two sandy loam pond sediments, one each from Iowa and Oklahoma, were examined in this study utilizing the best erythromycin extraction recoveries from sand. Various extraction times were also examined, and all extraction procedures were performed in duplicate. The greatest recovery of $^{14}$C-erythromycin in the Iowa sediment using 0.3 M ammonium acetate at pH 4.2: acetonitrile (15:85, v/v) solution, using a 20-minute shake or an 85-minute/ overnight settling followed by a 15-minute shake the following day, with recoveries of 84 ± 3.4%. While the Oklahoma sediment yielded the
greatest recovery (86.7 ± 3.5%) of $^{14}$C-erythromycin with 0.3 M ammonium acetate at pH 7: acetonitrile (30:70, v/v) with a 60-minute shake time.

**Introduction**

Non-therapeutic use of antibiotics in livestock accounts for 24.6 million pounds of antibiotics in feeds including 10.3 million pounds in hogs, 10.5 million pounds in poultry, and 3.7 million pounds in cattle (Mellon et al., 2001). In recent years, detection of various classes of antibiotics in several environmental matrices have been reported. In 1999, 18 antibiotics were detected in water samples from sewage treatment plant effluents and surface water (Hirsch et al., 1999). A survey study in 2002 reported the detection of antibiotics in 48% of 139 streams examined (Kolpin et al., 2002). One antibiotic commonly detected in water is erythromycin, which has been detected in water samples at concentrations between 50 ng L$^{-1}$ to 20 µg L$^{-1}$ (Hirsch et al., 1999; Kolpin et al., 2002; Christian et al., 2003; Davis et al., 2006). Additionally, antibiotics have been detected in sediment systems and manure slurries. In one study erythromycin had one of the highest detection levels for antibiotics, between 82 µg L$^{-1}$ and 128 µg L$^{-1}$ (Davis et al., 2006). Concentrations of a broad range of classes of antibiotics in manure slurries have been detected ranging between 11 mg kg$^{-1}$ and 43 mg kg$^{-1}$ (Schlüsener et al., 2003). The rise in the amount of papers addressing veterinary antibiotics detection and fate in the environment emphasizes the need for analytical methods to be optimized to enhance their detection in environmental matrices including water and sediment.

Erythromycin is a common macrolide antibiotic frequently detected in streams and sediment in the United States at a wide range of concentrations. Due to an increase in occurrence and detection of this antibiotic in surface water bodies, additional information regarding the occurrence and fate of erythromycin is needed to better understand erythromycin’s environmental fate. Erythromycin is an antibiotic used in livestock and poultry production to aid in growth promotion, feed efficiency, and disease prevention. This wide-spectrum antibiotic is produced by *Streptomyces erythroes* and consists of a macrocyclic lactone ring with two β-glycosidic-linked sugars (Figure 1) (Flynn et al., 1954; Morimoto et al., 1990; Kanfer et al., 1998). Excretion of erythromycin by animals occurs as parent compound or metabolites. Environmental entry of this compound occurs from
injection or waste incorporation of fertilizer in soil, leading to the potential for antibiotic residues and nutrients to enter water and sediment (Jacobsen et al., 2004). Recent studies have indicated antibiotics have the ability to leach into ground water, run off into surface water, and possibly enter drinking water (Tolls, 2001).

Improvement of analytical methods for the measurement of erythromycin in environmental matrices is needed to enhance the selectivity and sensitivity of detecting this compound at environmentally relevant levels. Prior to identification of parent compound and metabolites present in samples, clean up and concentration of erythromycin must occur due to the low levels found in most environmental samples. This step is primarily achieved through solid-phase extraction (SPE) procedures, which aims to concentrate the analyte in a water sample into a smaller volume before quantification and identification of sample components. Many of the SPE methods that have been previously utilized focus on incorporating multiple columns in tandem, various pH adjustments, and high flow rates yielding recoveries from water samples ranging between 45% and 100% for the various antibiotics examined (Shao, 2001; Kolpin et al., 2002; McArdell et al., 2003; Schlüsener et al., 2003; Blackwell et al., 2004; Kolz et al., 2006; Schlüsener and Bester, 2006; Blackwell et al., 2008; Velicu and Suri, 2008; Shao et al., 2009). However, these methods are rather expensive, time consuming, and recoveries vary for the various antibiotic classes examined in multiple class studies. Common SPE steps for macrolide antibiotics include pH adjustment prior to analysis and the use of buffers to assist in increasing recoveries (Abuin et al., 2006; Kolz et al., 2006; Blackwell et al., 2008). Recovery of erythromycin in experiments has been demonstrated to vary widely, from 40% - 95% (Billedeau et al., 2003; Heller and Nochetto, 2004; Yang and Carlson, 2004; Abuin et al., 2006). Due to recovery of erythromycin being highly variable from one published SPE method to another, optimization of the SPE step is needed to improve quantification to increase the reliability of analytical detection methods.

Another important aspect regarding detection of antibiotics in the environment is the ability to recover extractable residues from sediment samples. The extraction of antibiotics from soils and sediments is often more difficult due to the organic matter, moisture, and clay contents. Recoveries of macrolide antibiotics from soils with and without manure
amendments ranged between 43% and 86% (Rabølle and Spliid, 2000; Schlüsener and Bester, 2006; Blackwell et al., 2008). Improved extractability and reliable extraction procedures for quantifying erythromycin from sediment and soil systems will aim to improve recovery rates and potentially yield better initial extractability data for environmental fate studies.

Current methods for the detection of erythromycin focus on its detection in conjunction with other antibiotics primarily from water samples, with few studies examining sediment systems. The aim of this study was to optimize SPE clean-up and sediment extraction procedures of erythromycin from water and sediment. In this study, we examined three previously published solid-phase extraction methods, a sulfamethazine method and two tylosin methods, plus a variation on one of the tylosin methods. Extraction of erythromycin in sand and pond sediment from Iowa and Oklahoma was examined. Sand was used to determine the optimal pHs of the buffers utilized, and the best recovery from each buffer extraction solution was then tested on the two pond sediments. All analyses were performed employing radiotracer analysis with $^{14}$C-erythromycin. The overall goal was to improve recovery of erythromycin and enhance quantification of this compound from environmental matrices.

**Materials and Methods**

*Chemical, Reagents, and Standards*

Radiolabeled erythromycin was purchased from American Radiolabeled Chemicals (St. Louis, MO) with a specific activity of 55 mCi mmol$^{-1}$. Erythromycin was $^{14}$C-labeled on one of the methyl groups of the desosamine sugar. Acetonitrile, methanol, ethyl acetate, citric acid, ammonium acetate, potassium hydroxide, ammonium hydroxide, glacial acetic acid, hydrochloric acid, and Ultima Gold liquid scintillation cocktail were purchased from Fisher Scientific (Pittsburgh, PA). Solvents utilized were HPLC grade and included methanol and acetonitrile. The sediment extraction buffers and solutions were made fresh daily and included 0.2 M sodium phosphate:acetonitrile (15:85, v/v), 0.2 M citric acid:acetonitrile (50:50, v/v), and 0.3 M ammonium acetate:acetonitrile (15:85, v/v and 30:70 v/v).
Sand Preparation

Commercial sand (Lowe’s) was utilized in this study and was washed six times prior to use with nanopure water and sifted to remove fine particulate matter. Sand was completely dried prior to utilization in experiments.

Collection and Composition of Sediments

Sediments utilized in this study included pond sediment attained from Iowa (IA) and Oklahoma (OK). The IA sediment for experiments was collected from the Iowa State University Horticulture Research Station Pond (Gilbert, IA), while the OK sediment was from the Oklahoma State University Agronomy Experimental Pond Facility located at Lake Carl Blackwell in Stillwater, Oklahoma. Collection of sediment samples was conducted manually by inserting a soil auger 10 -15 cm (depth) into the pond sediment. Sediment was brought to the laboratory and stored at 4°C prior to use. Sediment characterizations for the two sediments were conducted, and properties are listed in Table 1.

Solid-Phase Extraction (SPE)

Samples contained 50 ml of distilled water in a 100-ml glass jar spiked with 50 µl of 0.94µCi ml⁻¹ of ¹⁴C-erythromycin. Total concentration per jar was 0.003 µCi of ¹⁴C and 0.04 ug of erythromycin. After water samples were spiked they were shaken on a rotary shaker at 300 rpm for 5 minutes. Erythromycin was extracted and concentrated from water using HLB solid-phase extraction cartridges (6 cc, Oasis HLB®, Waters Corporation, Milford, MA). Extraction methods examined for the quantification of erythromycin from water were compared using previously published methods for tylosin and sulfamethazine, with a modified tylosin SPE procedure examined based upon Hu and Coats (2007) and Hu et al., (Hu and Coats, 2009) tylosin SPE methods. All SPE methods, including conditioning and elution solutions and amounts, utilized in this study are listed in Table 2. Each extraction method was performed in triplicate (n=3). Samples were passed through the cartridges at a flow rate of 5 ml min⁻¹. Radioactivity was counted after SPE extraction in 1-ml aliquots of sample extract waste water and sample elution concentrate plus 15 ml of Ulitma Gold cocktail.
**Sand and Sediment Extraction**

Samples consisted of either 20 g dry weight sand or 36.75 g wet weight sediment (20 g dry weight) in a 250-ml French square bottle and were analyzed in duplicate replications (n=2). Each sample was spiked with a solution of $^{14}$C-erythromycin that contained 0.14 µCi of $^{14}$C and 2 µg of erythromycin, mixed, and allowed to incubate for 15 minutes. Total concentration of $^{14}$C-radiolabelled erythromycin per sample was 0.014 µCi. Extractable erythromycin was removed from samples by addition of solvent to each substrate sample, followed by shaking on an orbital shaker at 300 rpm, centrifugation for 12 minutes at 350 g (for sediment samples) and collection of supernatant. A second solvent extraction was performed with the substrate utilizing an additional 40 ml of extraction solution with repeated shaking, centrifuging, and collection as described previously. An array of extraction solutions were examined for their ability to recover erythromycin, as described in Table 3. A variety of extraction times were examined for incubation of solvent with the substrates including 20, 30, 60, and an 85-minute shake followed by an overnight incubation with an additional 15 minute shake the next day. The total volume of each extract sample after extraction was 80 ml. Extracts were concentrated to 1 ml under N$_2$ flow at 15 psi and 50°C. The 1-ml samples were reconstituted to a 10-ml final volume with the extraction solvent, using acetonitrile for extraction solutions containing a buffer solution. Radioactive counts were conducted on a Beckman 5000ce LSC using 3 ml of extract sample and 12 ml of Ultima Gold cocktail.

**Results**

*Comparison of SPE procedure methods*

The erythromycin recoveries from water for the SPE conditioning and elution methods examined are displayed in Table 4. The lowest recoveries of erythromycin occurred utilizing the modified tylosin method (75.5%) and the Henderson (2008) method displayed 78.8% recovery. While the best recoveries of erythromycin were shown using the Hu and Coats (2007) and Kolz et al. (2006) methods, with 88.7% and 94.7% respectively.
**Erythromycin extraction recoveries from sand**

Solvent extraction of $^{14}$C-erythromycin from sand yielded lower recovery amounts compared to those with the inclusion of buffers, including 10.6% with ethyl acetate, 19.1% for acetonitrile, and 48% with a 70% methanol solution. Recovery was 43.9% for the acidified solvent acetonitrile: acetic acid (96:4, v/v). Various buffered extraction solutions were mixed with acetonitrile to determine optimal pH for $^{14}$C-erythromycin extraction from sand. Results are displayed in Table 3 for all extraction solutions examined with sand. The greatest recovery of $^{14}$C-erythromycin from sand with 99.3% occurred with acetonitrile: 0.3 M ammonium acetate buffer (85:15, v/v). An extraction solution of acetonitrile: 0.2 M sodium phosphate buffer (85:15, v/v) demonstrated the best recovery with this buffer at a pH 3.2. For the 70:30 mixture ratio of acetonitrile: ammonium acetate the highest recovery of erythromycin was observed with 84.9% for pH 7.

**Erythromycin extraction recoveries from Iowa and Oklahoma sediments**

Four solutions were examined for their extraction of $^{14}$C-erythromycin residues from both Iowa and Oklahoma pond sediments, investigating various extraction shake times of 20, 30, and 60 minutes, plus an 85-minute shake time with the sample settling overnight, followed by a 15-minute shake; results are displayed in Table 5. Iowa sediment showed recoveries of 25.7% with a 70% methanol solution, 66.6% using ACN: 0.2 M sodium phosphate pH 3.2 (85:15, v/v), 84% with ACN: 0.3 M ammonium acetate pH 4.2 (85:15, v/v), and 80.5% for ACN: 0.3 M ammonium acetate pH 7 (70:30, v/v). The two lowest recoveries at 20 minutes were not further examined for either pond sediment using an increase in shake time, which occurred with the 70% methanol and ACN: 0.2 M sodium phosphate pH 3.2 (85:15, v/v). The ACN: 0.3 M ammonium acetate pH 4.2 (85:15, v/v) solution was examined utilizing additional extraction shake incubation times and yielded recoveries of 78.8% and 70.8% for 30 and 60 minutes, while the 85-minute shake followed by an overnight sample-settling and a 15-minute shake yielded 84.3% recovery of erythromycin. The ACN: 0.3 M ammonium acetate pH 7 (70:30, v/v) had recoveries of 47.9%, 73.3%, and 73% with the additional shake times examined (30, 60, and 85 minutes).
For the Oklahoma pond sediment the same extraction solutions and shake times were examined (Table 5). The recoveries at 20 minutes were 36.5%, 52.9%, 71.6%, and 64.7% for 70% methanol, ACN: 0.2 M sodium phosphate pH 3.2 (85:15, v/v), ACN: 0.3 M ammonium acetate pH 4.2 (85:15, v/v), and ACN: 0.3 M ammonium acetate pH 7 (70:30, v/v), respectively. An increase in recovery of 14C-erythromycin residues was demonstrated with the increase in extraction shake times for the ACN: 0.3 M ammonium acetate pH 4.2 (85:15, v/v) solution yielding 71.7%, 79.4%, and 81.1% at 30, 60, and 85-minute timepoints. Meanwhile, the ACN: 0.3 M ammonium acetate pH 7 (70:30, v/v) solution showed recoveries of 46.9%, 86.7%, and 78.2% for 30, 60, and 85-minute timepoints, respectively. Similar to the Iowa sediment, the Oklahoma sediment showed the lowest erythromycin recovery with the 30-minute incubation of ACN: 0.3 M ammonium acetate pH 7 (70:30, v/v) solution.

**Discussion**

SPE methods are commonly employed techniques for the extraction of antibiotics, especially macrolides, from water samples. Many of the methods utilize multiple cartridge types and pH adjustment for improved recoveries (Billedeau et al., 2003; Yang and Carlson, 2003; Shao et al., 2009). One SPE cartridge that has been commonly used with macrolide antibiotics is the Oasis HLB® type, which has been demonstrated to yield recoveries ranging between 64% - 94% for erythromycin (Billedeau et al., 2003; Yang and Carlson, 2003; Yang and Carlson, 2004; Abuin et al., 2006; Radjenovic et al., 2009; Shao et al., 2009). The compound erythromycin is a weak basic antibiotic (pKₐ of 8.8) which can be transformed into ionic and lipophilic forms influencing its ability to be retained on SPE cartridges. For all of the methods examined in this study the Oasis HLB® cartridge was utilized yielding recoveries of >75%. Oasis HLB® cartridges are designed to retain both non-polar and polar compounds to improve extraction of a wider array of compounds, compared to other SPE cartridges (Oasis, 2008). This ability to retain a wider array of compounds possibly aids in the retention of erythromycin and its metabolites, including ionized forms. The methods which had the lowest recoveries for erythromycin were the Henderson (2008) method and the modified tylosin method with recoveries of 78.8% and 75.5%. The Henderson (2008) method yielded >95% efficiency for sulfamethazine, but was not shown to have similar
results with erythromycin. This variation is likely due to the difference in antibiotic classes, chemical structures, and $pK_a$ of the compounds, which influence the behavior of the chemicals in the environment. The Hu and Coats, (2007) and Kolz et al. (2006) methods displayed improved recoveries compared to the two previous methods due to the method’s specificity for tylosin, another macrolide antibiotic. The Kolz et al. (2006) method demonstrated the greatest recovery of erythromycin due to the pH-adjustment of water samples to above 9.4 pH using 0.5 M potassium hydroxide, which influences the chemistry of the compound, especially the $pK_a$ in relation to binding of the compound to the cartridge packing and interactions with the elution-step solution. Adjustment of sample pH prior to SPE influences the retention of the erythromycin on the sorbent and may be due to increasing the pH above the $pK_a$ of the basic compound causing an increase in the amount of erythromycin retained on the Oasis HLB® cartridge, thus increasing the elution amount. The Kolz et al. (2006) method has been shown to enhance the extraction of erythromycin and may aid in improved detection of the compound from water.

A comparison of methods to enhance extraction of erythromycin from matrices revealed improved recovery through the use of lower-pH solutions tested with the two pond sediments. Both sediments examined were classified as sandy loam with the Iowa sediment having a higher pH and a greater percentage of organic matter compared to the Oklahoma pond sediment. Organic matter content in sediment influences many properties of that matrix, including pore-space size which increases with an increase in organic matter. This may influence the binding of erythromycin in sediment and account for the slightly lower recoveries in Iowa pond sediment compared to Oklahoma sediment. Another difference between the two pond sediments was pH, and it has been shown to influence charges of ions in sediments through increasing cation-anion interactions with the pH increase. Cation exchange capacity (CEC) may also influence erythromycin’s ability to bind in various matrices, as organic matter, silt content, and clay content additions increase the CEC value increases in soil, which influences the adsorption of chemicals. The pH may affect the binding of erythromycin in the sediment accounting for the use of different extraction solutions with the Iowa sediment compared to the Oklahoma sediment. The parameter of pH may affect the ability of ionic (hydrophilic) and non-ionic (lipophilic) compounds to bind in
sediment through influencing the ability for adsorption to occur. Examination of the soil pH’s in this study may effect the adsorption of erythromycin to sediment particulate matter, where lower pH’s would allow for decreased binding accounting for higher extraction of erythromycin from pond sediment. For the sandy loam sediments examined in this study, it can be concluded that the extraction solutions and times examined yielded optimal recoveries of 84% and 86% erythromycin from the Iowa and Oklahoma sediments, respectively.

**Conclusions**

In water samples pH adjustment is important in extraction and concentration methods of erythromycin. When pH was changed to above 9.4, which was above the pKₐ of erythromycin, an increase in extraction was observed. SPE methods that work well with other macrolide antibiotics, worked better than methods for non-macrolide antibiotics (e.g. sulfamethazine). Additionally, pH and organic matter content may influence erythromycin’s ability to sequester in the two sediments examined in this study. The pH and amount of organic matter was higher in the Iowa sediment which may explain why a lower recovery was observed compared to the Oklahoma sediment. Furthermore, the solution that worked the best to extract erythromycin from sediment was the same for both sediments examined, except in regards to pH and shake time. These slight variations could influence adsorption of the erythromycin to particulate matter pertaining to cation exchange capacity within the sediment. Additional studies are needed to understand the interactions between erythromycin and particulate matter including its fate and bioavailability within the sediment system.

**Acknowledgments**

The authors of this article would like to thank Dr. Keri Carstens and Beth Douglass for their technical assistance. Additional thanks to Naomi Cooper for providing the Oklahoma State University pond sediment. Funding for this study was provided by a USDA-CSREES-NRI grant #IOW05091.

**References**


Figure 1 – Chemical structure of erythromycin

The molecular weight of ERY is 738.1g, pKₐ of 8.8, and Kₐₑ₃ of 1.4 to 4 (Flynn et al., 1954; Morimoto et al., 1990; Kanfer et al., 1998)
Table 1 – Sediment properties for Iowa and Oklahoma pond sediments

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>Moisture Content (%)</th>
<th>Organic Matter (%)</th>
<th>Sand (2000 - 50 µm) (%)</th>
<th>Silt (&lt;50 - 2 µm) (%)</th>
<th>Clay (&lt;2 µm) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iowa</td>
<td>8.1</td>
<td>33.2</td>
<td>2</td>
<td>60</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>7.2</td>
<td>43</td>
<td>1.2</td>
<td>55</td>
<td>32.5</td>
<td>12.5</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 ml methanol</td>
<td>3 ml Acetonitrile:</td>
<td>5 ml Acetonitrile</td>
<td>5 ml Methanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 ml - 0.5M HCl</td>
<td>Glacial Acetic Acid</td>
<td>3 ml Acetonitrile</td>
<td>5 ml Ultra pure water</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 ml Ultra pure water</td>
<td>(96:4, v:v)</td>
<td>5 ml Ultra pure water</td>
<td>4 ml 0.5M KOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rinse</td>
<td>3 ml Ultra pure water</td>
<td>3 ml Ultra pure water</td>
<td>4 ml 2% Acetonitrile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 ml methanol-water-ammonium hydroxide (60:38:2, v:v:v)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elution</td>
<td>3 ml Methanol</td>
<td>3 ml Acetonitrile:</td>
<td></td>
<td>2 ml acetonitrile:</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glacial Acetic Acid</td>
<td></td>
<td>Glacial Acetic Acid (98:2, v:v)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(96:4, v:v)</td>
<td></td>
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</table>
Table 3 – Recovery of erythromycin from sand utilizing different extraction solutions at varying pHs

<table>
<thead>
<tr>
<th>Extraction Solution</th>
<th>pH</th>
<th>Recovery (%)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td></td>
<td>19.1 ± 3.4</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td></td>
<td>10.6 ± 0.7</td>
</tr>
<tr>
<td>Methanol (70%)</td>
<td></td>
<td>48 ± 1.4</td>
</tr>
<tr>
<td>Acetonitrile: Glacial Acetic Acid (96:4)</td>
<td></td>
<td>43.9 ± 3.7</td>
</tr>
<tr>
<td>Acetonitrile: 0.2M Potassium Phosphate dibasic (60:40)</td>
<td>8.86</td>
<td>74.9 ± 4.8</td>
</tr>
<tr>
<td>Acetonitrile: 0.2M Sodium Phosphate (85:15)</td>
<td>3.2</td>
<td>87.2 ± 7.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>85.6 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>86.5 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>84.5 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>87 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>67.1 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>74.1 ± 1.1</td>
</tr>
<tr>
<td>Acetonitrile: 0.3M Ammonium Acetate (85:15)</td>
<td>4.2</td>
<td>99.3 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>93.7 ± 0.4</td>
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<tr>
<td></td>
<td>6</td>
<td>93.4 ± 5.7</td>
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<tr>
<td></td>
<td>7</td>
<td>91.3 ± 5.1</td>
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<td></td>
<td>8</td>
<td>81.5 ± 2.3</td>
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<td></td>
<td>9</td>
<td>77.4 ± 4</td>
</tr>
<tr>
<td>Acetonitrile: 0.3M Ammonium Acetate (70:30)</td>
<td>4.2</td>
<td>74.9 ± 10.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>79.2 ± 8.3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>69.2 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>84.9 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>83.1 ± 8.8</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>77.5 ± 2.9</td>
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</table>

\(^a\) Standard error is listed as percentages with n=2
<table>
<thead>
<tr>
<th>Method</th>
<th>Recovery (%)</th>
<th>Standard Error</th>
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<tbody>
<tr>
<td>Henderson, 2008</td>
<td>78.8</td>
<td>± 4.4</td>
</tr>
<tr>
<td>Modified Tylosin Method</td>
<td>75.5</td>
<td>± 4.3</td>
</tr>
<tr>
<td>Hu and Coats, 2006</td>
<td>88.7</td>
<td>± 4.7</td>
</tr>
<tr>
<td>Kolz et al., 2006</td>
<td>94.7</td>
<td>± 4.9</td>
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Table 5 – Sediment recoveries from Iowa (IA) and Oklahoma (OK) pond sediment for various extraction incubation times

<table>
<thead>
<tr>
<th>Extraction Solution</th>
<th>Extraction Incubation Time (Minutes)</th>
<th>20</th>
<th>30</th>
<th>60</th>
<th>85/15</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA Sediment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol (70%)</td>
<td></td>
<td>25.7 ± 1.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Acetonitrile: 0.2M Sodium Phosphate pH 3.2 (85:15)</td>
<td></td>
<td>66.6 ± 0.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Acetonitrile: 0.3M Ammonium Acetate pH 4.2 (85:15)</td>
<td></td>
<td>84 ± 3.4</td>
<td>78.8 ± 3.1</td>
<td>70.8 ± 2.9</td>
<td>84.3 ± 3.6</td>
</tr>
<tr>
<td>Acetonitrile: 0.3M Ammonium Acetate pH 7 (85:15)</td>
<td></td>
<td>80.5 ±1.7</td>
<td>47.9 ± 0.2</td>
<td>73.3 ± 2.8</td>
<td>73 ± 0.8</td>
</tr>
<tr>
<td>OK Sediment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol (70%)</td>
<td></td>
<td>36.5 ± 1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Acetonitrile: 0.2M Sodium Phosphate pH 3.2 (85:15)</td>
<td></td>
<td>52.9 ± 3.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Acetonitrile: 0.3M Ammonium Acetate pH 4.2 (85:15)</td>
<td></td>
<td>71.6 ± 9.8</td>
<td>71.7 ± 8.9</td>
<td>79.4 ± 2.6</td>
<td>81.1 ± 3.7</td>
</tr>
<tr>
<td>Acetonitrile: 0.3M Ammonium Acetate pH 7 (85:15)</td>
<td></td>
<td>64.7 ± 2.7</td>
<td>46.9 ± 3.8</td>
<td>86.7 ± 3.5</td>
<td>78.2 ± 8.2</td>
</tr>
</tbody>
</table>
CHAPTER 3
FATE OF ERYTHROMYCIN IN SURFACE WATER MICRO COSMS, INCLUDING FRESH AND AGED SEDIMENT SYSTEMS

Ashley M. Jessick¹, Thomas B. Moorman², and Joel R. Coats¹

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A paper to be submitted to a peer-reviewed scientific journal for publication

Abstract

The detection of antibiotics in water and sediment systems is of concern due to the potential adverse effects which could be associated with their environmental fate. Currently, few studies have examined the fate of the macrolide antibiotic, erythromycin, within sediment-containing systems. The focus of this study was to evaluate the fate of erythromycin in the environment using ¹⁴C-erythromycin microcosms consisting of pond water and submerged pond sediment. Total recovery of ¹⁴C-erythromycin in microcosms ranged between 90.1% and 48% throughout the 63-day study. Sediment-containing systems displayed a reduction in the amount of erythromycin in surface water by day 7, which corresponded to an increased amount detected in sediment. The levels of erythromycin in sediment demonstrated a decrease in extractable residues throughout the study correlating with an increase in bound residues. Biodegradation, as demonstrated by ¹⁴CO₂ evolution, accounted for less than 7% of ¹⁴C-erythromycin within the microcosm.

The availability of aged erythromycin was evaluated by incubating sediment with and without a manure amendment with ¹⁴C-erythromycin for 0, 1, 3, or 8 weeks. After residues were aged in these matrices, water and sediment microcosms were assembled and movement and availability of erythromycin in sediment was evaluated after 1, 3, 7, and 14 days. Results indicated differences in residues from aged sediment, with and without manure additions, in extractable residues at day 7 and 14. The addition of water to sediment after aging indicated
the potential for manure to enhance the availability and movement of erythromycin residues from sediment. There was a greater release of erythromycin to the water overlying the manure-treated sediments at 0 and 1 week than the unamended sediment at 1 and 2 weeks.

**Introduction**

Antibiotics continue to be an emerging contaminant of concern due to their increase in usage and detection in the environment. Various classes of antibiotics have been found in environmental sampling studies, and concentrations have been measured in a broad range within various matrices including water, soil, sediment, and manure (Hirsch et al., 1999; Campagnolo et al., 2002; Kolpin et al., 2002; Christian et al., 2003; Davis et al., 2006; Schlüsener and Bester, 2006; Hu and Coats, 2007; Kim and Carlson, 2007; Hu and Coats, 2009). The presence of these compounds in the environment could potentially affect many aspects of ecosystem function including alteration of bacterial populations leading to nutrient cycle impacts, potential adverse effects to aquatic and non-target organisms, and possibly influence human health (Halling-Sørensen et al., 1998; Kümmerer, 2009). One environmental entry point of antibiotics that is of concern is through land application of manure (Rabølle and Spliid, 2000; Schlüsener et al., 2003; Schlüsener and Bester, 2006).

Antibiotics are administered to livestock and poultry for treatment of infections as well as for disease prevention, growth promotion, and feed efficiency (Hayes et al., 1999; Thiele-Bruhn, 2003). The majority of the antibiotics administered to agricultural animals are excreted as parent compound, due to low absorption rates (Addison, 1984; Boxall et al., 2003). Manure produced from these animals is ultimately applied to farmland by injection or waste incorporation as fertilizer to improve crop growth and development (Tolls, 2001; Boxall et al., 2003; Christian et al., 2003; Jacobsen et al., 2004). Detection of antibiotics in water, sediment, soil, and manure samples has been prevalent over the past few years, with tetracyclines, sulfonamides, and macrolides being the most frequently detected antimicrobial compounds.

One of the most frequently detected antibiotic classes in the environment are the macrolides, which includes tylosin and erythromycin. Erythromycin’s structure is comprised of a 14-member lactone ring with two sugar groups with a molecular weight of 733.9, a \( pK_a \) of 8.8 and a \( K_{ow} \) of 3.06 (Flynn et al., 1954; Morimoto et al., 1990; Bryskier et al., 1993;
Kanfer et al., 1998). This antibiotic is effective against most gram-positive and some gram-negative bacteria, and its mode of action is through blocking elongation of peptide chains, which inhibits protein synthesis (Kanfer et al., 1998). Elimination of erythromycin occurs through bile and feces at a rate of 50-67% and with urinary excretion at 5-10% (McArdell et al., 2003). The high excretion rates of erythromycin may allow for environmental entry of the compound through manure application to agricultural fields, which could enter water and sediment systems through runoff events.

The United States Geological Survey in a 2002 study found 48% of 139 streams tested contained antibiotics, and the second most frequently detected antibiotic from this study was erythromycin (Kolpin et al., 2002). Another study conducted in 2002 found antibiotics in 31% of samples collected near swine farms and 67% of samples near poultry farms with tetracyclines and macrolides (e.g. erythromycin, tylosin) identified at the highest concentrations (Campagnolo et al., 2002). One of the most prevalent macrolide antibiotics detected in water samples has been erythromycin, commonly as dehydrato-erythromycin, ranging in concentrations between 50 ng L$^{-1}$ and 300 ng L$^{-1}$ (Campagnolo et al., 2002; Kolpin et al., 2002).

In addition to the detection of antibiotics in surface waters, these compounds have also been found in sediment systems and manure slurries. Macrolides have been found in sediment samples with reported concentrations ranging between 2.1 µg kg$^{-1}$ to 24.3 µg kg$^{-1}$ (Kim and Carlson, 2007). Erythromycin was detected in sediment samples ranging between 82 µg kg$^{-1}$ to 128 µg kg$^{-1}$ in an environmental monitoring study and was determined to have the highest relative loss with greater than 50% loss due to runoff and erosion in a rainfall study compared to other antibiotics examined (Davis et al., 2006). Another macrolide antibiotic found to have strong sorption in soils was tylosin, which demonstrated affinity to adsorb to manure and sediments examined, as evidenced by recoveries of less than 2.5% from soil columns (Hu and Coats, 2009). These studies found macrolide antibiotics in multiple environmental matrices, with tylosin and erythromycin being widely detected. However, less information regarding erythromycin’s environmental fate is known compared to tylosin and further research is needed to understand its behavior within the environment.
Erythromycin has been detected in sediments (82 µg kg\(^{-1}\) to 128 µg kg\(^{-1}\)) at markedly higher concentrations compared to water systems (50 ng L\(^{-1}\) to 300 ng L\(^{-1}\)), which could be due to the amount of aged residues that are sequestered within the sediments (Christian et al., 2003). It has been suggested that macrolide antibiotics, including erythromycin and tylosin, may have an affinity for clay particles, organic matter, or manure in soil, which could affect their degradation and leaching (Rabølle and Spliid, 2000). This compound is readily adsorbed by soil particles, especially clay, allowing the rate of degradation to be reduced (Kim et al., 2004a). The half-life of erythromycin in soil has been experimentally determined to be between 11.5 and 20 days (Gavalchin and Katz, 1994; Schlüsener and Bester, 2006).

Although few studies have focused on aged antibiotic residues in sediments, many studies have examined other organic compounds including herbicides and insecticides (Johannesen and Aamand, 2003; Saghir et al., 2007; Raich-Montiu et al., 2010). Some of the herbicides and insecticides have been shown to bioaccumulate in organisms within sediment and affect non-target organisms, but the significance of bioavailability of these aged residues within the environment is influenced by sediment characteristics (particles size, pH, clay content, and organic matter content) which affect adsorption and desorption rates of those compounds (Coats et al., 1989; Saghir et al., 2007). Erythromycin has the potential for transport in the environment through runoff from manure-treated fields leading to erythromycin’s entry into water, which in turn leads to erythromycin in sediment where it may persist and age, and be bioavailable for uptake by terrestrial and aquatic organisms to some unknown extent (Schlusener and Bester, 2006).

The overall aim of this study was to investigate the fate of erythromycin in a pond water and pond sediment microcosm through examination of its ability to bind to organic particulate matter and sediment, to partition between water and sediment, and of its abiotic and biotic degradation within the environment. This paper examines erythromycin’s movement within water and sediment microcosms, specifically to improve the understanding of erythromycin’s environmental fate and to simulate the impact of erythromycin run-off, which commonly occurs with manure from agricultural field application. Aged erythromycin residues in sediment were examined by aging erythromycin in sediment treatments for 0, 1,
3, or 8 weeks. Next, water and sediment microcosms were assembled containing aged erythromycin residues. These studies focus on erythromycin and its movement within the environment using runoff and erosion experimental fate designs through employing microcosm systems to evaluate sorption, degradation, and persistence.

**Material and Methods**

**Chemicals**

Acetonitrile (HPLC grade), acetic acid, ammonium acetate, sodium hydroxide, erythromycin, ashless cellulose powder, and Ultima Gold scintillation cocktail were purchased from Fisher Scientific (Pittsburgh, PA). Carbosorb E and Permafluor E+ scintillation cocktails were purchased from Perkin and Elmer (Waltham, MA). $^{14}$C-radiolabeled erythromycin was purchased from American Radiolabeled Chemicals (St. Louis, MO); the $^{14}$C-label was present in one of the methyl groups bonded to the nitrogen of the desosamine sugar of the erythromycin molecule.

**Pond Water, Pond Sediment, and Manure Collection**

Pond water and sediment were collected from the Iowa State University Horticulture Research Station (Gilbert, Iowa). Sediment was manually collected by inserting a soil auger 10 – 15 cm (depth) into the pond sediment. Sediment composition was determined as 60% sand, 28% silt, 12% clay, 2% organic matter, and a pH of 8.1. Alkalinity was 103 mg ml$^{-1}$ and total hardness was 150 mg ml$^{-1}$. Sediment moisture was 47% prior to use. Water and sediment samples were transported to the lab and were stored at 4°C until use (< 7 days).

Fresh manure was obtained from the Iowa State University Swine Nutrition Farm (Iowa State University) from antibiotic-free pigs on a corn-soybean-based diet. Manure was kept at 4°C until use (< 7 days).

**Environmental Fate Experimental Design and Analysis**

This study consisted of four different microcosm treatments: pond water only (PW), pond water overlying pond sediment (PWS), autoclaved pond water overlying autoclaved pond sediment (APWS), and pond water with dilute swine manure overlying pond sediment (PWS+M). The APWS treatment aimed to measure sorption and non-biotic processes, while the PWS treatment focused on the impact of sediment and biodegradation, and the PWS+M
examined the impact of manure associated with runoff through simulating agricultural field application. All treatments utilized in this study were selected to investigate erythromycin’s potential environmental entry systems and their detection in matrices (water, sediment, and manure), with the APWS treatment serving as a control.

One week prior to set-up 4 L of pond water and 1200 g of pond sediment were autoclaved three times at 121°C in one-hour cycles for use in the APWS treatment at one-day intervals. The PW treatment consisted of 200 ml of pond water, while the APWS treatment had 64.8 g (50 g dry wt.) autoclaved pond sediment and 185.2 ml autoclaved pond water. For the PWS and PWS+M treatments the microcosm was comprised of 73.5 g (50 g dry wt.) pond sediment and 176.5 ml pond water. Microcosms were assembled in wide-mouth 470-ml jars (Ball Corp., Broomfield, CO), 50 g dry weight of sediment and 200 ml pond water per jar and were incubated for 7, 14, 28, or 63 days. Each jar served as a replicate with four replicates per treatment and timepoint.

Sediment was allowed to settle one hour prior to 14C-erythromycin addition. The treatment solution utilized in this study was comprised of labeled and non-labeled erythromycin which was added to each treatment replicate. Treatment spiking solution was prepared with 85 mg of non-labeled erythromycin to obtain a concentration of 0.425 mg ml⁻¹ in a 200 ml volumetric flask and 171 µl of 0.1 mCi 14C-radiolabeled erythromycin (specific activity of 55 µCi mmol⁻¹). Each treatment replicate received 2.35 ml of the treatment spiking solution yielding final concentrations of 5 mg L⁻¹ and 0.201 µCi per jar.

For the PWS+M treatment a manure slurry was prepared by adding 33 g of manure to 100 ml distilled water to get a 33% slurry solution. The slurry was stirred for 40 minutes to break up large chunks, and 0.6 ml of slurry was added to each replicate. This addition gave the treatments a murky appearance compared to the treatments without the manure amendment. The autoclaved treatment was assembled and analyzed in a laminar flow hood using sterile equipment. All treatments were maintained in a 24°C environmental chamber with a 12:12 photoperiod. The pH of water in all treatments was monitored weekly and did not vary significantly throughout the course of the study.

Mineralization of 14C-erythromycin was tracked throughout the study by using sodium hydroxide solution traps for CO₂ evolution. A 25-ml high-density polyethylene vial
was glued onto the inner surface of each jar and was filled with 10 ml of 0.5 M sodium hydroxide. Traps were changed on Day 3, 7, 14, 21, 28, 35, 42, 49, and 56 of the study. Three milliliters of each sodium hydroxide sample was mixed with 12 ml Ultima Gold cocktail, mixed, and was counted for radioactivity on a Beckman Coulter 6500 liquid scintillation counter (LSC, Fullerton, CA).

After 7, 14, 28, and 63 days of incubation the distribution of $^{14}$C in water and sediment was determined. Treatment water was removed from each replicate jar and $^{14}$C-erythromycin radioactivity was counted on the LSC using 1 ml of water with 15 ml Ultima Gold cocktail. Next, the water samples were filtered through 0.2-µm, 47-mm diameter nylon filters (Fisher Scientific). Following filtering, water samples were extracted using Oasis® HLB cartridges (6 cc, Waters Corp., Milford, MA). Cartridges were conditioned using the Kolz et al., (2006) solid phase extraction method. Recovery of $^{14}$C-residues of applied $^{14}$C-erythromycin was determined to be 94.7% ± 4.9 from pond water utilizing this method.

Sediment was extracted with 100 ml of acetonitrile: 0.3 M ammonium acetate at pH 4.2 (85:15, v/v), and each sample was shaken on an orbital shaker for 85 minutes at 300 rpm. Samples were allowed to settle overnight at room temperature followed by siphoning off the liquid extract. A second 100-ml aliquot of acetonitrile: 0.3 M ammonium acetate at pH 4.2 (85:15, v/v) was added to each sediment sample and shaken on an orbital shaker for 15 minutes at 300 rpm followed by centrifuging and decanting. Each sediment extract sample was concentrated to a volume of 1 ml under nitrogen flow at 15 psi, at 50°C and reconstituted to a final volume of 10-ml with acetonitrile. A 3-ml aliquot of sediment extract for each sample was mixed with 12 ml Ultima Gold cocktail and counted for radioactivity on the LSC. Extracted sediment samples were allowed to dry in a fume hood for 24 hours. Dried sediment was sieved through a 5-mm sieve, followed by a 2.5-mm sieve to remove any large non-combustible material. Sieved sediment samples were ground using a mortar and pestle. Next, sediment pellets were constructed with 0.5 g dried, ground sediment and 0.5 g ashless cellulose powder (1:1 ratio). Sediment pellets were oxidized using a Packard Model 307 oxidizer (Perkin Elmer, Waltham, MA) with a two-minute combustion time. Following oxidation, sediment sample vials containing reagents were counted for radioactivity on the LSC to determine bound $^{14}$C-erythromycin residues.
Aged Sediment Experimental Design

Two metal pans were filled with 2.87 kg of pond sediment, and to one of the pans a manure slurry was then added. The manure slurry contained 57.4 g of manure dissolved in 50 ml of distilled water and was stirred for 50 minutes until thoroughly mixed, followed by addition to one container of sediment. Each sediment pan was spiked with 97.5 ml of treatment solution which was made by dissolving 24.2 mg of non-labeled erythromycin and 303 µl of a 0.1 mCi \(^{14}\)C-erythromycin into 200 ml of treatment solution. Erythromycin residues in the sediment were aged for 0, 1, 3, or 8 weeks prior to microcosm assembly. Microcosms were assembled after the designated timepoints which included 36.75 g (25 g dry weight) of sediment (either with or without manure amendment), and they were topped with 88.25 ml of distilled water in a 250-ml French square bottle. Microcosms were incubated for 0, 1, 3, 7, or 14 days and were performed in replicates of four (n=4). All aged sediment and water columns were maintained in a 24° C environmental chamber with a 12:12 photoperiod.

Water column replicates were sacrificed at the specified timepoints, at which water was removed from treatment containers and SPE was performed as discussed in the environmental fate experimental design and analysis section. Next, sediment was extracted using 50 ml acetonitrile: 0.3 M ammonium acetate at pH 4.2 (85:15, v/v) followed by bound residue analysis with the protocols outlined in the previous experiment environmental fate experimental design and analysis section.

Statistical Analysis

Statistical analysis was performed using SigmaStat 3.0 (Chicago, IL) employing ANOVA analysis with Bonferroni or Dunn’s analysis to compare data treatments and time points. Significance level was determined as P ≤ 0.05 for all analyses. Linear regression and least squares analysis were conducted with SigmaPlot 10 (Chicago, IL) to determine dissipation kinetics in water from treatment samples.
RESULTS AND DISCUSSION

Freshwater Microcosm Study

Mass Balance

Mean balance of $^{14}$C-residues recovered from $^{14}$C-erythromycin applied for treatment microcosm components are listed in Table 1. The pond water (PW) treatment recovery ranged between 89.9% and 81% throughout the course of the study. The APWS treatment displayed a decrease in mean $^{14}$C total recovery throughout the course of the study except with a small increase from day 28 to 63. The various microcosm components for the APWS system showed a decrease in $^{14}$C-erythromycin recovery in treatment water and slight increases in extractable and bound sediment residues. For the PWS treatment, a decrease in recoverable mean $^{14}$C-erythromycin between day 7 and 63 occurred, 72.7% to 44.7%. Microcosm components for the PWS treatment displayed a decrease in radioactive residues for treatment water and extractable sediment residues, but an increase with sediment bound residues. The PWS+M treatment displayed similar $^{14}$C-erythromycin residue patterns in all microcosm components to the PWS treatment. ANOVA analysis indicated significant differences between the treatments examined ($p = 0.034$).

Dissipation Kinetics

$^{14}$C-erythromycin residues in surface water remained fairly constant in the PW treatment throughout the study (Figure 1). The PW treatment was significantly different in the quantity of $^{14}$C residue in surface water compared to the sediment-containing systems, with a greater amount present in the PW treatment compared to all other treatments examined. In treatments APWS, PWS, and PWS+M a sharp decrease in $^{14}$C-erythromycin was noted between day 0 and day 7, with 33% to 19% remaining in water by day 7 and continued to decrease by day 63 with <10% remaining in the water portion of the microcosm. The pH of the water throughout the course of the study did not vary greatly between day 0 and 63.

Dissipation kinetics in water was examined, and results indicated that erythromycin dissipates from water via a one-compartment model for all treatments (Equation 1). A $DT_{50}$
was also calculated for the dissipation of erythromycin for the treatments examined, using Equation 2 for all treatments.

\begin{align}
(1) \quad & C = C_0 e^{-kt} \\
(2) \quad & DT_{50} = 0.693/k
\end{align}

The variables used in the equations above represent the following:
- $C$ = erythromycin concentration at time $t$
- $C_0$ = initial erythromycin concentration in rapidly degrading portion
- $k$ = first-order rate constant for erythromycin
- $t$ = time (days)

Table 2 lists dissipation models and values for the calculated parameters listed above. Erythromycin dissipates from water to 50% of applied by 5.8 days for APWS and PWS treatments, while it takes 5 days for the PWS+M treatment. In the PW treatment erythromycin takes about 365 days to reach 50% of applied. The first-order, one-compartment model was used for the APWS, PWS, and PWS+M treatments because the $r^2$ value was greater than 0.7 with small differences in F and p values, and this model has been utilized in pesticide risk assessment (Beulke and Brown, 2001). However, with these treatments better $r^2$ values were obtained with a three-parameter modified single, exponential decay model for APWS, PWS, and PWS+M treatments and an exponential linear combination model with PW, but these models do not have a mechanistic interpretation as do first-order or two-compartment dissipation kinetic models.

The dissipation of erythromycin from water is mostly due to its partitioning into sediment in the APWS, PWS, and PWS+M treatments. The total recovery of $^{14}$C-residues was examined to determine when the microcosm treatments containing sediment reached 50% of applied by plotting the $\log_{10}$ of total percentage recovered in microcosms at 7, 14, 28, and 63 days versus time and fitting a linear trendline to obtain treatment specific equations listed in Table 3. Results indicate that the $DT_{50}$ for the PWS+M treatment is shorter than the PWS treatment, 38 and 45 days, respectively. In the APWS treatment it was calculated to
take 155 days to reach 50% of applied $^{14}$C-erythromycin residues remaining within the system, which demonstrates the important role microorganisms have in degrading and utilizing erythromycin in the environment.

**Mineralization of erythromycin**

The inclusion of sediment and manure increased $^{14}$CO$_2$ evolution from mineralization in pond water (Figure 2). The PWS and PWS+M treatments displayed similar trends in CO$_2$ evolution with a lag phase between days 0 and 7, followed by an exponential growth phase between days 7 and 35. After day 35, $^{14}$CO$_2$ in these treatments began to plateau through day 63. These treatments were similar in total amounts of $^{14}$CO$_2$ evolved throughout the study, with significant differences seen between the APWS compared to PWS and PWS+M ($P = 0.023$). The PW and APWS treatments were similar in the amount of $^{14}$CO$_2$ evolved, with <2% of applied $^{14}$C-radiolabel detected in these treatments.

Mineralization is a common microbial process and the amount of mineralization occurring in the PWS and PWS+M microcosms may indicate a wide distribution of erythromycin-degrading microorganisms within the pond ecosystem. This distribution may be due to an increase in the density of microbial populations degrading erythromycin including erythromycin’s ability to influence the development and reproduction of microorganisms, especially those capable of degrading erythromycin including some gram-negative microorganisms (Kim et al., 2004b; Kim and Cerniglia, 2005). Kim et al., (2004b) demonstrated that the $^{14}$C-radiolabeled methyl group is more readily hydrolyzed compared to $^{14}$C-radiolabeled groups of the macrocyclic lactone ring, which the higher cumulative mineralization rates in this study were 10% to 15% for PWS+M and PWS treatments, respectively.

$^{14}$C-erythromycin in sediment

Erythromycin was found to move from the water column to the sediment in the microcosms. Figure 3 displays the results for extractable and bound $^{14}$C-residues in pond sediment. The APWS treatment displayed a plateau of bound $^{14}$C-residues throughout the 63-day study, possibly due to the small amount of biotic processes occurring within the
system. A slight increase in extractable residues between day 7 and day 63 was seen in the PWS and PWS+M treatments, most likely due to erythromycin interacting with clay and organic matter causing binding to occur. In addition, this decrease in extractable residues may be attributed to microorganisms utilizing erythromycin and subsequently incorporating it as biomass. In the PWS and PWS+M treatments extractability of erythromycin decreased between day 7 and day 63. Comparison of bound ^14^C-residues within PWS demonstrated a linear increase from day 0 to day 63, and a decrease in extractable ^14^C-residues between day 0 and day 63. In contrast, the PWS+M treatment showed a slight decrease between day 7 and 14 in extractable erythromycin residues, followed by a sharp reduction between day 14 and 63. Bound residue in the PWS+M treatment showed an increase from day 0 to day 28.

Erythromycin accumulated in sediment based on the amounts found in sediment components (extractable and bound) with a decrease in extractable residues and bound residues increased throughout the course of the study in the non-autoclaved systems. We found 40% to 50% erythromycin in sediment components after 7 days, with slightly more erythromycin in the manure-containing treatment. These studies demonstrate that erythromycin accumulates in sediment with the potential for degradation to occur through biotic processes. However, additional experiments are needed to better understand the degradation pathway and the influence of sorption parameters.

**Aged Sediment Study**

**Aged Erythromycin Residues in Sediment**

The extractable residues in sediment prior to assembly of surface water columns are shown in Figure 4. Data for sediment extracted after water column assembly timepoints is displayed in Figure 5. Erythromycin aged in sediment and aged in sediment with manure were analyzed at the experimental timepoints indicating erythromycin has the potential to bind in sediment, with a good portion of the compound being extractable throughout the study with the addition of water. Extractability of ^14^C-erythromycin from aged sediments was assessed prior to the assembly of water columns, demonstrating a decrease between 1-week, 3 week, and 8 weeks aged with no difference seen between the two matrices examined.
Erythromycin’s ability to bind in sediment and the influence of manure was also assessed indicating a linear decrease as water incubation time increased with both matrices at the fresh time point, 1 week aged sediment with manure, and 3 week aged sediment. Sediment aged for 3 weeks displayed a linear decrease in extractable $^{14}$C-erythromycin from day 0 to day 7, followed by a sharp decrease between day 7 and 14. Eight-week aged matrices with surface water displayed an increase from day 0 to day 1, a slight decrease between day 1 and day 3, a slightly sharper decrease between day 3 and day 7, and between day 7 and day 14 the concentration of erythromycin began to equilibrate and level off.

Statistically significant differences were found in extractable residues including fresh sediment compared to 3 and 8-week aged sediment ($P < 0.001$ and $P = 0.008$). Week 1 aged sediment was different from week 8 aged sediment treatment ($P = 0.029$). No significant differences were seen in sediment with manure amendment treatments at the various time points examined (0, 1, 3, and 8 weeks). Comparison between sediment and sediment with manure amendment treatments aged 0, 1, 3, and 8 weeks showed a significant difference ($P=0.006$). The extractable $^{14}$C-residue results from the aged study are different compared to the fate study, which may be due to the incorporation route of the manure and erythromycin in each study. In the aged study the manure was mixed directly into sediment versus the fate study which utilized a manure slurry added to water representing manure runoff from rainfall. The manure incorporation route utilized in this experiment could also represent antibiotics which enter river sediment that is overlain with fresh stream water. This difference in manure and residue environmental entry routes could suggest erythromycin’s potential to be more bioavailable in sediment with direct incorporation through injection into sediment compared to spreading manure on the surface of agricultural fields.

_Erythromycin’s Bioavailability in Water_

The percentage of $^{14}$C-erythromycin moving into surface water from the aged erythromycin residues in sediment is shown in Figure 6. More $^{14}$C-erythromycin was released into surface water from the manure-containing sediment treatment compared to the sediment only matrix. In the sediment only system, fresh and 3-week-aged $^{14}$C-residues yeilded higher amounts of erythromycin in surface water compared to 1 and 8-week aged $^{14}$C-residues by day 14 (Figure 6a). The sediment with manure amendment matrix showed
more erythromycin in fresh and at 1 week for day 14 compared to 3 and 8-week aged samples. No significant differences were seen between aged $^{14}$C-residue treatments in water component of microcosms for 1, 3, 7, and 14 days.

Addition of manure to sediment influenced the availability of $^{14}$C-erythromycin residues in surface water with greater amounts seen in the water portion for fresh and 1-week aged treatments. Overall, aged residues in sediment with manure showed an increase in $^{14}$C-erythromycin into water between day 0 and 7 for all treatments (Figure 6b). A statistical difference was observed with extractable sediment aged residues at 7 and 14 day incubation times with surface water ($P = 0.021; P = < 0.001$). By day 14 a decrease in erythromycin from water was seen with 3 and 8-week aged sediment with manure amendment, compared to an increase in fresh and 1-week aged treatments. Examination of extractable $^{14}$C-residues from sediment with manure amendment incubated for 0, 1, 3, and 8 weeks indicated no significant difference between surface water incubation timepoints (1, 3, 7, and 14 days) with ANOVA analysis ($P = 0.08$). Statistical analysis indicated that there is a significant difference between aged erythromycin in sediment with manure after 7 and 14 days of water incubation in fresh and 1-week aged compared to 3 and 8-week aged treatments ($P = < 0.001; P = < 0.001$).

**CONCLUSIONS**

The fate study indicated that erythromycin dissipates in surface water systems with an increased half-life in the pond water system (365 days) compared to the sediment-containing systems (APWS, PWS, and PWS+M) with around 5 to 6 days for 50% dissipation in water to occur. The dissipation values calculated in water indicates that erythromycin is readily adsorbed in sediment. In comparison, in the total microcosm system study the time to reach 50% of applied $^{14}$C-residues was found to be 38 to 45 days in non-autoclaved treatments, demonstrating the tendency of sediment to sequester erythromycin, potentially due to sediment composition, pH, and biodegradation. Biotic processes influence the sorption and degradation of erythromycin in sediment systems as observed by the increased CO$_2$ evolution in non-autoclaved sediment-containing systems. The PWS and PWS+M treatments displayed higher mineralization rates compared to PW and APWS treatments, and the
increase is due to microorganisms present in the sediment and their efficiency to convert residues to biomass and CO₂ as they decompose erythromycin and it’s residues within the microcosms.

Aged sediment systems showed erythromycin has the ability to partition into water from aged sediment, with manure influencing the partitioning in fresh and 1-week aged sediment samples. Erythromycin was found to be extractable from aged sediment and manure-containing sediment samples with a slight decrease observed with an increase in water incubation time. The different manure applications (water compared to sediment) employed in the fate and aged residues studies may indicate the influence of manure on the bioavailability of erythromycin. Manure incorporated directly in sediment, similar to direct injection of manure in agricultural fields, could potentially allow erythromycin to be more bioavailable in the sediment and could eventually allow increased detection of erythromycin in water samples. When manure is directly added into water in an environmental microcosm, as with a manure slurry in the fate study, a decrease in the amount of ¹⁴C-residues in treatment water and sediment extracts is observed possibly due to sequestration of this compound in sediment pore water spaces or adsorption. Further studies are needed to understand the bioavailability of erythromycin in the environment to non-target organisms due to this compound adsorbing into sediment and also movement into water from aged residues in sediment. Additional studies to better recognize the potential for metabolites to form in environmental components are needed, which may aid in a better understanding of erythromycin’s fate in water and sediment.

**ACKNOWLEDGEMENTS**

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REFERENCES


Table 1 – Mass balance of $^{14}$C-erythromycin residues in treatment microcosm components

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<td>Total Recovery</td>
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<td>PWS+M$^d$</td>
<td>Treatment Water</td>
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<td>Sediment - Extractable</td>
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<td>Total Recovery</td>
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</table>

$^1$ Values shown are mean % of applied radioactivity ± standard error

$^2$ Day post addition of $^{14}$C-erythromycin added to water portion of microcosm

$^a$ Pond Water

$^b$ Autoclaved Pond Water and Autoclaved Pond Sediment

$^c$ Pond Water and Pond Sediment

$^d$ Pond Water with Manure Slurry and Pond Sediment
Table 2 – Dissipation kinetics for erythromycin in treatment water of surface water microcosm systems

<table>
<thead>
<tr>
<th>Treatment</th>
<th>F value</th>
<th>Dissipation Model</th>
<th>k</th>
<th>r²</th>
<th>p-value</th>
<th>Half-life (days)</th>
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<tbody>
<tr>
<td>PW</td>
<td>1.9088</td>
<td>C=C₀e^{-kt}</td>
<td>0.0019</td>
<td>0.32</td>
<td>0.32</td>
<td>365</td>
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<tr>
<td>APWS</td>
<td>44.73</td>
<td>C=C₀e^{-kt}</td>
<td>0.1187</td>
<td>0.9986</td>
<td>0.007</td>
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<tr>
<td>PWS</td>
<td>44.73</td>
<td>C=C₀e^{-kt}</td>
<td>0.1895</td>
<td>0.9891</td>
<td>0.007</td>
<td>5.8</td>
</tr>
<tr>
<td>PWS+M</td>
<td>61.54</td>
<td>C=C₀e^{-kt}</td>
<td>0.1386</td>
<td>0.9889</td>
<td>0.0043</td>
<td>5</td>
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Table 3 – Equations for calculating 50% remaining $^{14}$C applied within sediment containing microcosm treatments including days when 50% is reached.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Linear Trendline Equation</th>
<th>$r^2$</th>
<th>50% of $^{14}$C remaining in microcosm (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APWS</td>
<td>$y = -0.0009x + 1.8438$</td>
<td>0.3675</td>
<td>155</td>
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<tr>
<td>PWS</td>
<td>$y = -0.0037x + 1.8692$</td>
<td>0.91</td>
<td>45</td>
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<tr>
<td>PWS+M</td>
<td>$y = -0.0057x + 1.9189$</td>
<td>0.9288</td>
<td>38</td>
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</tbody>
</table>
Figure 1 – Percentage of $^{14}$C-ERY remaining in surface water
Figure 2 – Cumulative mineralization of $^{14}$C-erythromycin from microcosm treatments
Figure 3 – Percentage of extractable and bound $^{14}$C-residues derived from applied $^{14}$C-erythromycin (a) extractable $^{14}$C-residues (b) bound $^{14}$C-residues
Figure 4 – Extractable aged $^{14}$C-erythromycin residues (% of applied $^{14}$C) in sediment matrices with and without manure amendment prior to assembly of surface water columns.
Figure 5 – Extractable $^{14}$C-ERY (% of applied $^{14}$C) in sediment and sediment with manure amendment (50:1, v/v) followed by addition of distilled water and incubation for 1, 3, 7, or 14 days; (a) fresh (b) Aged 1 week (c) Aged 3 weeks (d) Aged 8 weeks
Figure 6 – Percentage of $^{14}$C-ERY in surface water released from sediments treated with $^{14}$C-erythromycin at zero (fresh), 1, 3, and 8 weeks previously (a) Pond sediment system (b) Pond sediment + manure (50:1, v/v by weight) system
CHAPTER 4

DETERMINATION OF ERYTHROMYCIN’S POTENTIAL TO BIOCONCENTRATE IN THE TRADITIONAL AQUATIC INVERTEBRATES, LUMBRICULUS VARIEGATUS AND DAPHNIA MAGNA, AND DEVELOPMENT OF A SURROGATE MODEL IN ENVIRONMENTAL MATRICES

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Abstract

The bioavailability of ^14^C-radiolabeled erythromycin was evaluated in aquatic microcosm systems with and without the addition of pond sediment using C₈-Empore™ extraction disks and the aquatic worm, Lumbriculus variegatus, for comparison. An aquatic microcosm was also examined using Daphnia magna to assess the potential of erythromycin to bioconcentrate in non-target aquatic organisms. After 14 days of exposure, disks, worms, and daphnids were extracted, and uptake of ^14^C-erythromycin residues in tissues was determined. Comparisons of relative uptake indicated a steady-state bioconcentration factor of 2.2 for L. variegatus compared to 18 for disks in aquatic systems, which was reached between day 3 and day 7. The disk bioconcentration factor (BCF) was 8.2 times the worm bioconcentration factor without normalization to lipid and carbon content. In the pond sediment-containing microcosms, worms exposed to 0.05 and 0.5 ppm demonstrated a mean BCF of 0.04, compared to 0.13 for the 5 ppm treatment. The disks showed a peak “BCF” at day 3 in sediment-containing microcosms with no significant differences seen between concentrations and timepoints examined. The daphnids showed the highest erythromycin BCF at day 3, followed by a decrease in tissue content of the compound through day 14.
These organisms had higher BCF values at 0.005 ppm with a maximal BCF of 8 for 0.005 ppm at day 3, compared to the higher concentrations examined (0.05, 0.5 and 5). The higher BCF in daphnids in aquatic microcosms compared to the worms is potentially due to the daphnids being fed throughout the study, system type (renewal vs. static), and/or tissue permeability. The comparative data presented could be used to evaluate antibiotic compounds in aquatic environments to better assess their bioavailability to non-target organisms within water and sediment.

**Introduction**

The widespread usage of antibiotics in humans and livestock and their detection in environmental matrices, such as water and soil, has led to an interest in the fate and effects of these compounds and their transformation products. Although antibiotic residues have been well studied in meat and excrement samples, few studies have focused on their effects on non-target organisms in the environment (Tolls, 2001). A wide variety of antibiotic classes have been detected in the environment (water, sediment, and manure-containing samples) at a broad range of concentrations (Kolpin et al., 2002). One antibiotic class of concern in environmental matrices are the macrolides (e.g. tylosin, erythromycin) due to their occurrence rates in water and sediment (Hirsch et al., 1999; McArdell et al., 2003; Davis et al., 2006; Kim and Carlson, 2007; Hu and Coats, 2009).

One widely used macrolide in global livestock production is erythromycin produced by *Streptomyces erythreus* and consisting of a 14–member macrocyclic lactone ring containing two β-glycosidic linked sugars: D-desosamine and L-cladinose (Flynn et al., 1954; Morimoto et al., 1990; Kanfer et al., 1998). Chemical properties of this compound are reported with a molecular weight of 733.9, pKₐ of 8.8, and a Kₐ of 3.06 (Bryskier et al., 1993; Jacobsen et al., 2004; Gros et al., 2006). Elimination of erythromycin occurs through bile and feces at rates of 50-67% and with urinary excretion at 5-10% (McArdell et al., 2003). The excreted waste containing antibiotics is utilized as manure to fertilize agricultural fields, whereby antibiotics could be released into the environment through run-off and sediment erosion processes eventually entering water and sediment systems (Tolls, 2001; Boxall et al., 2003). Erythromycin has been detected in the environment, but few studies
have focused on its effects to non-target organisms in the environment through bioconcentration and bioavailability.

To assess the potential risk of a chemical in the environment, bioavailability (used as a determining factor) is commonly estimated through calculating bioconcentration and bioaccumulation factors. Bioavailability is the portion of an applied chemical available for uptake within a system, while bioconcentration is used to determine the portion in an organism’s tissue and bioaccumulation is the amount of a chemical in a microcosm component (e.g. sediment) (Sijm et al., 2000). Many factors contribute to bioavailability of a compound including adsorption, uptake, elimination routes, and biotransformation (Leppänen and Kukkonen, 2006; Saghir et al., 2007). Bioavailability of compounds differ for a wide variety of organisms, and detailed comparisons of specific classes of compounds utilizing multiple model organisms are needed to better understand their effects (Kelsey et al., 1997).

One common organism used in toxicity and bioavailability studies is *Lumbriculus variegatus*, an aquatic oligocheate, due to its ease of culturing, global distribution, and representation of a common invertebrate group (USEPA, 2000). Due to *L. variegatus’* ability to burrow in and ingest sediment, it is an ideal organism in sediment bioassays with its primary routes of exposure through uptake from water and sediment components. Another ideal test organism to assess bioavailability in aquatic systems is *Daphnia magna*, due to their wide availability, inexpensive cost, sensitivity to a wide range of toxic substances, little genetic variability due to parthenogenetic reproduction, fast reproduction rates, and short life cycle to minimize the length of testing (Lewis and Horning, 1988). Standard toxicity test methods have been established with *D. magna* to aid in reproducibility and static-renewal aquatic toxicity tests are commonly utilized in order to reduce the possibility of loss of toxicants through volatilization and adsorption (USEPA, 2002). Although these organisms aid in determining the toxicity and effects of numerous compounds, these bioavailability assays are expensive and time-consuming, and many alternatives to traditional bioassays have been examined.

Alternatives to traditional test organisms have been developed in order to be more cost efficient and utilizes a chemical based approach, with such materials including solid-
phase extraction, \( C_{18}/C_8 \) disks, and Tenax extraction beads (Kelsey et al., 1997; Freidig et al., 1998; Tang et al., 1999; Krauss and Wilcke, 2001; Vinturella et al., 2004; Yang et al., 2006). These materials have been designed to simulate uptake and bioavailability in a passive diffusion manner through absorption of the compound onto the sampler (Sijm et al., 2000). These methods tend to overestimate bioavailability compared to traditional test organisms, but may be helpful in studying slower portioning compounds and aged residues (Ehlers and Loibner, 2006). One alternative to traditional bioavailability assays that works well in comparison to \( L. \) variegatus in estimating antibiotics in the environment are \( C_8 \) Empore™ disks, due to the similarity in lipophilicity and that the burrowing behavior can be simulated (Henderson, 2008). These disks may better measure compounds from environmental matrices, such as soil or sediment and be able to aid in an enhanced understanding of the fraction available to organisms in a soil or sediment environment.

Assessing the bioavailability of antibiotics may be difficult, providing precise bioavailability data may also be challenging due to their polar characteristics and variability in concentration and equilibrium within the environment. The goal of this study was to determine accumulation (uptake and bioconcentrate) of erythromycin in \( Daphnia magna \) and \( Lumbriculus \) variegatus tissues from environmental exposure routes (water and/or sediment). Comparison with the \( L. \) variegatus studies were conducted with \( C_8 \) Empore® disks to develop a possible surrogate model for assessment of bioconcentration.

**Materials and Methods**

*Chemicals and Standards*

Acetonitrile (ACN) and methanol (MeOH), both HPLC grade, were purchased from Fisher Scientific (Pittsburgh, PA). Additionally, ammonium acetate, acetic acid, ashless cellulose powder, ammonium hydroxide, sodium hydroxide, potassium hydroxide, and Ultima Gold liquid scintillation cocktail were bought from Fisher Scientific. Carbosorb E and Permafluor E+ cocktails were purchased from Perkin Elmer (Waltham, MA). Erythromycin standard was purchased from Sigma-Aldrich (St. Louis, MO). Radiolabeled erythromycin with specific activity of 55 mCi mmol\(^{-1}\) was purchased from American
Radiolabeled Chemicals (ARC) (St. Louis, Mo). Pure water was obtained from a Barnstead Nanopure filtration system.

**Pond Sediment Collection**

Pond sediment was collected from the Iowa State University Horticulture Research Station (Gilbert, IA). Sediment was obtained by inserting a manual auger 10 -15 cm (depth) into the sediment. The pond sediment was characterized as 60% sand, 28% silt, 12% clay, 2% organic matter, alkalinity of 103 mg ml$^{-1}$, and a pH of 8.1. Sediment was kept at 4°C in the lab until use (< 7 days).

**Test Organisms**

*Daphnia magna* utilized in bioavailability studies were cultured in the laboratory at 25°C in individual 25-ml cups using a 16:8 photoperiod cycle. Cultures were maintained in reconstituted moderately hard water (80-100 mg/L as CaCO$_3$) prepared according to USEPA standard methods (USEPA, 2002) and were fed a mixture of *Pseudokirchneriella subcapitata*, yeast, cereal leaves and trout chow (YCT) (USEPA, 2002). Culture water was renewed daily.

*Lumbriculus variegatus* were reared in the laboratory at 74°C ± 0.5°C in water containing aquaria utilizing an unbleached shredded paper substrate on a 16:8 photoperiod cycle. Tanks were aerated and *L. variegatus* were fed shrimp pellets 3 times weekly. Adult worms weighing 6 mg ± 2.4 mg were chosen for utilization in the study.

Empore™ disks (C$_8$) purchased from 3M Corporation (St. Paul, MN) were used in this study as a surrogate model for comparison to *L. variegatus*. Previous work in our lab indicated the C$_8$ Empore™ disks were the best for studying the antibiotics, sulfamethazine and erythromycin, due to adsorption, extraction, and quantification applications (Henderson, 2008).

**Daphnia magna Aquatic Exposure Experimental Design**

The *D. magna* aquatic exposure tests were conducted utilizing the same controlled environmental conditions as described above for culturing following USEPA standard methods (USEPA, 2002). For this study, 10 *D. magna* were placed into 150 ml beakers containing 100 ml moderately hard water and were incubated 0, 3, 7, and 14 days with four
replicates per treatment. Treatment solutions were prepared daily by dissolving erythromycin (Sigma-Aldrich, St. Louis, MO) in moderately hard water and test treatments included four exposure concentrations (0.005 ppm, 0.05 ppm, 0.5 ppm, 5 ppm), a solvent control, and a moderately hard (MH) control. Each replicate was spiked with 0.1506 ml of a 4.98 μCi/ml stock with a final activity of 0.15 μCi per replicate. Test solutions were renewed every 24 hours, and all water quality parameters (pH, temperature, dissolved oxygen, conductivity, and water hardness (as total CaCO₃)) were measured before and after renewal of test solutions. Dissolved oxygen and temperature was measured using a Model 550A Dissolved Oxygen Meter (YSI Incorporated Yellow Springs, OH, USA). pH and conductivity were measured with Accumet® portable AP62 pH/mV and AP65 Conductivity meters respectively (Fisher Scientific Inc, Pittsburg, PA).

At the conclusion of each timepoint examined, *D. magna* (10 organisms per treatment replicate) were removed from MH water and placed into a 2-ml centrifuge tube, to which 0.5 ml of acetonitrile: glacial acetic acid (96:4, v/v) was added. After tissue extraction, 0.05 ml of tissue extract was mixed with 15 ml of Ultima Gold liquid scintillation cocktail and counted on a Beckman 6500e liquid scintillation counter (LSC; Fullerton, CA). Water components from experiments were also counted for radioactivity on the LSC using 1 ml water and 15 ml Ultima Gold LSC cocktail. Concentrations utilized in the treatments were corrected for specific activity prior to calculating bioconcentration factors.

**Lumbriculus variegatus Aquatic Exposure Bioassay Experimental Design**

Sample set-up was comprised of 100 ml glass jars containing 50 ml ultrapure water with 5 adult *L. variegatus* (approximately 6 mg each) added on Day 0. Each sample was dosed with a mixture of non-labeled and ¹⁴C-radiolabeled erythromycin to achieve concentrations of 0.05, 0.5, and 5 mg L⁻¹ in treatment water. The amount of erythromycin per jar was calculated to be 0.72, 0.52, and 5 μg L⁻¹. All samples were incubated in the dark at 24°C and 4 replications per treatment (0.05, 0.5, and 5 mg L⁻¹) and timepoint (1, 3, 7, and 14 days) were performed. Worms were not fed during the course of the study.

On day 1, 3, 7, and 14 of the study worms were removed from treatment water and placed into 5 ml of ultrapure water for 6 hours to allow depuration of their gut contents to occur. After 6 hours 5 ml of clearance water was removed and mixed with Ultima Gold...
cocktail. Clearance water samples were counted for $^{14}$C-erythromycin residues using the liquid scintillation counter. Next, *L. variegatus* tissue extraction was performed on worms. Treatment water was extracted utilizing the solid-phase extraction procedure discussed below.

**Surrogate Aquatic Exposure Bioassay Experimental Design**

$C_8$ Empore™ disks were rinsed with 10 ml of ACN: acetic acid (96:4, v/v) and allowed to dry. Next 10-ml additions each of ACN: acetic acid (96:4, v/v) and ultrapure water were passed through the disks followed by the addition of one moist disk to each test system containing 50 ml ultrapure water. One disk was used per replicate with four replicates per treatment and timepoint. Test systems were treated with 0.05, 0.5, or 5 mg l$^{-1}$ 14C-erythromycin as described for the *Lumbriculus variegatus* aquatic exposure bioassay. Test systems were incubated in the dark at 24 °C ± 1 °C for 1, 3, 7, or 14 days. Disks were removed from treatment systems at the specified timepoints and were eluted twice with 10 ml ACN: acetic acid (96:4, v/v) using a Kontes® disk solid-phase extraction manifold. Next, disks were transferred to a 300-ml wide-mouth jar containing 10 ml ACN: acetic acid (96:4, v/v) and were allowed to soak for 24 hours to remove any remaining 14C-erythromycin residues. Disk eluate and soak samples were combined for radioactivity analysis. Once the $C_8$ Empore™ disks completed the elution and soak steps, they were allowed to dry and were stored at 4 °C until bound residue analysis could be performed as described in the $C_8$ Empore™ disk bound-residue analysis section. Treatment water was extracted utilizing the solid-phase extraction procedure described below. Disks were analyzed for bound 14C-erythromycin residues with the $C_8$ Empore™ disk bound-residue protocol listed below.

**Lumbriculus variegatus Sediment Exposure Bioassay Experimental Design**

Test systems were comprised of 66.5 g (50 g dry weight) pond sediment weighed into wide-mouth pint jars with 133.5 ml ultrapure water to equal 167.5 ml water per jar. There were four replications per treatment and timepoint. Pond sediment was allowed to settle one hour prior to addition of 10 *L. variegatus* (approximately 6 mg each) to each test system replicate jar. Once worms were added each jar was incubated one hour before dosing with 14C-erythromycin solution. To each replicate jar, 50 µl of 2.16 µCi ml$^{-1}$ 14C-erythromycin
was added, with total radioactivity of 0.14 µCi per jar. Amounts of erythromycin in sediment-containing microcosms were determined to be 0.06 µg, 0.51 µg, and 5.01 µg for the 0.05, 0.5, and 5 ppm concentrations with each replicate receiving 500 µl of the specified concentrations. Aquatic microcosms were incubated in the dark at 24 °C ± 1 °C for 1, 3, or 7 days.

At the 1, 3, and 7 day timepoints, microcosms were sacrificed. Water was removed from the top of the sediment, and SPE was performed as outlined in the solid phase extraction (SPE) procedures below. The remaining water and sediment was transferred to a glass Petri dish to sift for the L. variegatus. Recovered worms were rinsed with ultrapure water, followed by a 6-hour gut clearance period described for the L. variegatus aquatic exposure bioassay and tissue extraction utilizing the L. variegatus tissue extraction procedures. After the removal of worms, sediment was dried and sediment bound-residue analysis was performed.

**Surrogate Sediment Exposure Bioassay Experimental Design**

C8 Empore™ disks were conditioned as previously described. Test systems were comprised of 66.5 g (50 g dry weight) pond sediment and 133.5 ml ultrapure water to equal 183.5 ml water per wide-mouth pint jar. One C8 Empore™ disk was buried 3 mm into the pond sediment allowing two-thirds of the disk to float in the water to simulate L. variegatus behavior. Dosing concentrations, incubation timepoints, and replicates were the same as discussed in L. variegates sediment exposure bioassay section.

Surrogate-containing sediment microcosms were sacrificed on day 1, 3, and 7 timepoints. Water was siphoned from the sediment and SPE was performed. The C8 Empore™ disk was removed from each treatment and placed into 30 ml ultrapure water for 30 minutes to remove excess sediment. After the rinse step disks were eluted and soaked as discussed above. Next, disks were oxidized to determine 14C-erythromycin bound residues remaining as described in the C8 Empore™ disk bound residue analysis section.

Once water and disks were removed from the pond sediment, extraction of the sediment was performed to determine extractable 14C-erythromycin residues as described above. After sediment extraction was performed, sediment was dried and 14C-erythromycin bound residue analysis was conducted.
**Lumbriculus variegatus Tissue Extraction Procedures**

Following gut clearance, worms were ground in 5 ml MeOH for 10 minutes followed by a 15 minute shake on an orbital shaker at 300 rpm. Next ground *L. variegatus* tissue samples were centrifuged at 350 g for 5 minutes and tissue sample aliquots were counted for radioactivity on the LSC.

**Solid-Phase Extraction (SPE) Procedures**

Extraction of treatment water from *L. variegatus* and surrogate aquatic bioassays was performed utilizing solid phase extraction (SPE) cartridges (6 cc, Oasis HLB®, Waters Corporation, Milford, MA). Cartridges were conditioned with 3 ml of ACN: acetic acid (96:4, v/v), 3 ml ACN, and 3 ml ultrapure water (Barnstead Nanopure Ultrapure Water System, Thermo Fisher Scientific, Waltham, MA). Water samples were loaded onto SPE cartridges at a flow rate of 3.8 ml min⁻¹. Cartridges were allowed to air dry for 5 minutes and were eluted using 3 ml of acetonitrile: acetic acid (96:4, v/v). Final volume of extracts was 5 ml using ultrapure water. Recovery for SPE was determined to be 75.5% ± 4.3 using this method.

Treatment water from sediment exposure bioassays were extracted with Oasis HLB® SPE cartridges (6 cc) using the method from Kolz et al., (2006), a brief description of the method is discussed. The SPE method utilized 5 ml MeOH followed by 4 ml 0.5 M potassium hydroxide for the conditioning step. After water sample application, cartridges were rinsed with 3 – 1 ml aliquots of MeOH: ultrapure water: ammonium hydroxide (60:38:2, v/v) and cartridges were allowed to air dry for 5 minutes. Cartridges were eluted with 4 – 0.5 ml aliquots of ACN: acetic acid (98:2, v/v). Recovery of erythromycin utilizing this method was determined to be 94.7% ± 4.9. Water extracts were counted for ¹⁴C-erythromycin residues on the LSC.

**Sediment Extraction Procedures**

After water was siphoned off sediment containing samples and *L. variegatus* or disks were removed from sediment 100 ml of ACN: 0.3 M ammonium acetate pH 4.2 (85:15, v/v) was added to each sample and shook on an orbital shaker for 85 minutes at 300 rpm.
Samples were allowed to settle overnight at room temperature followed by siphoning of liquid extract. A second 100-ml aliquot of ACN: 0.3 M ammonium acetate pH 4.2 (85:15, v/v) was added to each sediment sample and shook on an orbital shaker for 15 minutes at 300 rpm followed by centrifuging and decanting. Each sediment extract sample was concentrated to a volume of 1 ml under nitrogen flow at 15 psi, 50°C and reconstituted to a final volume of 10 ml with acetonitrile. A 3-ml aliquot of sediment extract for each sample was mixed with 12 ml Ultima Gold cocktail and counted for radioactivity on the LSC.

**C₈ Empore™ disk Bound Residue Analysis**

Dried C₈ Empore™ disks were folded in one-half-inch squares and were oxidized using a Packard Model 307 oxidizer (Perkin Elmer, Waltham, MA) with a two-minute combustion time. After oxidation, sample vials containing Carbosorb E and Permafluor E+ reagents were counted for radioactivity on the LSC.

**Sediment Bound Residue Analysis**

Dried sediment samples were sieved through a 5-mm sieve to remove any large non-combustible material, followed by processing through a 2.5-mm sieve. Next, sediment samples were ground into a fine powder by mortar and pestle. Sediment pellets were assembled using a 1:1 ratio of sediment and cellulose powder to produce a 1-g sediment pellet using a Parr pellet press. Triplicate replications (n=3) of sediment samples were oxidized using the sample oxidizer with a two-minute combustion time. Following oxidation, sediment sample vials containing reagents were counted for radioactivity on the LSC.

**Calculations and Statistical Analysis**

Bioconcentration factors were calculated by quantifying the total radioactivity (as erythromycin equivalents) in worm, disk, or daphnid extracts and dividing this by the initial concentration in each microcosm (aquatic or sediment containing). Concentrations in the organisms’ tissues were calculated as ppm. Equations detailing the BCF calculations are listed below:

\[
(1) \quad \text{BCF or BAF} = \frac{C_{\text{organism}}}{C_{\text{microcosm}}}
\]
(2) Daphnids = \frac{C_{\text{daphnids}(n=10)}}{C_{\text{water}}}

(3) Worms = \frac{C_{\text{worms}(n=5 \text{ or } 10)}}{C_{\text{microcosm}}}

(4) Disks = \frac{C_{\text{disks}(n=1)}}{C_{\text{microcosm}}}

Statistical analysis was conducted between concentrations and timepoints examined in all studies using ANOVA analysis, with significance level determined as \( P \leq 0.05 \) for all analyses.

**Results and Discussion**

*Bioavailability of erythromycin to Daphnia magna*

Concentration of \(^{14}\text{C}\)-erythromycin residues in tissue samples were calculated as 0.027 \( \mu \text{g} \), 0.072 \( \mu \text{g} \), 0.522 \( \mu \text{g} \), and 5.02 \( \mu \text{g} \) for the 0.005 ppm, 0.05 ppm, 0.5 ppm, 5 ppm microcosms. The amount of \(^{14}\text{C}\)-erythromycin residues in *D. magna* tissue accounted for 1% or less of the total applied amount. Significant differences in the BCF (Figure 1) were seen between the initial treatment concentrations at the three timepoints examined using ANOVA analysis (day 3, \( P = 0.007 \); day 7, \( P = 0.004 \); day 14, \( P = 0.007 \)). Tissue concentration of erythromycin in *D. magna* peaked at day 3, followed by a subsequent decrease throughout the remainder of the study (Figure 1). This decrease is most likely due to the excretion mechanisms and rate at which the organism can clear erythromycin. Although the *D. magna* accumulate erythromycin in their tissue, they most likely possess mechanisms to aid in its elimination. The initial 0.005 ppm concentration led to the highest BCF at day 3 with a factor 6.9, while the 0.005 ppm and 0.5 ppm solutions resulted in BCFs near 3.8. The lowest BCF was observed with 5 ppm with a maximal BCF of 0.1 achieved at day 3.

*Bioavailability of erythromycin in L. variegatus and C\(_8\) Empore™ disks in aquatic microcosms*

In Figure 2, BCF values are presented for each concentration examined with *L. variegatus* and C\(_8\) Empore™ disks. The total amount of erythromycin was calculated as 0.074 \( \mu \text{g} \), 0.52 \( \mu \text{g} \), and 5.04 \( \mu \text{g} \) for the 0.05 ppm, 0.5 ppm, and 5 ppm treatments, respectively. *L. variegatus* took up less than 3\% of total applied \(^{14}\text{C}\)-residues, compared to between 68\% and 95\% in C\(_8\) Empore™ disks throughout the study. No toxicity was observed
in microcosms containing worms throughout the course of the experiment. Erythromycin in *L. variegatus* in aquatic systems (Figure 2a) demonstrated an increase between day 0 and 7, followed by a decrease throughout the remainder of the study. Maximum (BCF = 2.2) was reached near day 7 in worm tissue. Higher BCF values were determined (without normalization) with steady state reached around day 7 (BCF = 18) in C$_8$ Empore™ disks (Figure 2b). The disks concentrated 8.2 times the amount of erythromycin compared to the worms. The *L. variegatus* were most likely able to degrade or eliminate the small amount of erythromycin through biological processes, however aquatic exposure yielded higher mean BCF factors compared to sediment exposure routes (Table 1). The majority of $^{14}$C-residues were measured in the water for the worm samples and in the disks for the treatments containing disks. This difference may be due to the larger surface area in the disks compared to the worms or the capability of the worms to eliminate erythromycin.

**Bioavailability of erythromycin in *L. variegatus* and C$_8$ Empore™ disks in sediment microcosms**

Figure 3 displays bioconcentration factors for C$_8$ Empore™ disks and *L. variegatus* in sediment-containing microcosms for all concentrations. Total percentage uptake of applied $^{14}$C-erythromycin residues was less than 0.5% in *L. variegatus* and ranged between 1.5% and 25% in C$_8$ Empore™ disks throughout the study. The mean BCF factors of $^{14}$C-erythromycin residues in sediment containing microcosms are listed in Table 1, demonstrating less than 0.1 in *L. variegatus* and ranged between 1.6 and 2.6 in C$_8$ Empore™ disks. The amount of worms recovered from the sediment-containing microcosms is displayed in Figure 4. Day 7 worm recovery was statistically different compared to day 0 and day 3 ($P = 0.012$), however no difference was observed with concentrations. In the sediment treatments containing worms, the majority of the $^{14}$C-residue is found in the surface water with little accumulation in sediment observed. Disk-containing sediment treatments displayed a decrease in the amount measured in the water, and an increase in the amount found in the sediment and sampling devices.
Comparison of erythromycin’s ability to bioconcentrate in various organisms and matrix influences

Erythromycin was shown to bioconcentrate more in *D. magna* compared to *L. variegatus* in aquatic systems, and peak BCF values were observed in *D. magna* at day 3 compared to day 7 in *L. variegatus*. The differences observed between these two organisms may be due to the test system used and the permeability and composition of each organism’s tissue. For the *D. magna* study organisms were fed, which could have influenced the exposure route by additionally allowing ingestion of food which could contain erythromycin from the water.

Comparison between the aquatic bioassay and sediment bioassay utilizing *L. variegatus* and C₈ Empore™ disks demonstrates the influence of sediment and differences between live organisms and surrogate models. In both the aquatic bioassay and sediment bioassay, the C₈ Empore™ disks had higher uptake and BCF compared to *L. variegatus*. This could be due to *L. variegatus*’ ability to eliminate harmful or toxic substances through excretion and the difference in surface area of the worms and disks utilized in the experiments. Differences in surface area of C₈ Empore™ disks to *L. variegatus* yielded a ratio of 8069.5:1 comparing one disk to one worm. The large difference in surface area needs additional consideration in the development and interpretation of surrogate models, as they would tend to overestimate concentrations of erythromycin.

Erythromycin had less bioconcentration and decreased uptake in *L. variegatus* compared to sulfamethazine. The mean uptake of erythromycin in aquatic worm bioassays was 0.005 µg, 0.02 µg, and 0.3 µg at the 0.05 ppm, 0.5ppm, and 5 ppm concentrations, while for the same concentrations the mean uptake by worms of sulfamethazine was found to be 0.052 µg, 0.016 µg, and 0.101 µg (Henderson, 2008; Henderson et al., 2009). Greater uptake was also seen with sulfamethazine in worms exposed to sediment with 1.614 at the for 3.03 ppm concentration compared to 0.02 µg of erythromycin in worms at 5 ppm. Comparison of the surrogate C₈ Empore™ disks between uptake and bioconcentration aquatic and sediment systems with erythromycin and sulfamethazine additionally reflected sulfamethazine’s higher rates compared to erythromycin. In the C₈ Empore™ disks with sulfamethazine in aquatic systems uptake was 141.4 µg at 5 ppm compared to 28.5 µg with sulfamethazine.
Differences between the bioconcentration of erythromycin and sulfamethazine could be due to the differences in chemical structure, degradation ability of microorganisms to degrade the antibiotic, and the differences in $K_{ow}$ values. The $K_{ow}$ of erythromycin is 3.06 compared to 0.25 for sulfamethazine, which would predict erythromycin would bioconcentrate more in biota and be more hydrophobic. This information is important for understanding erythromycin’s ability to bioconcentrate within various aquatic organisms to aid in ecological risk assessment of antibiotics.

**Conclusions**

Results from this study indicate erythromycin has a low potential to bioconcentrate in *D. magna* and *L. variegatus*, while $C_8$ Empore™ disks concentrate it 8.2 times more than worms in aquatic systems. However, the $C_8$ Empore™ disks adsorbed less erythromycin in sediment-containing systems compared to disks in aquatic systems and worms in sediment-containing systems. The differences between disks and worms in sediment-containing systems were more similar compared to the aquatic system, but additional development of a surrogate to estimate BCF of antibiotics in sediment is needed to yield improved data regarding interactions of sediment-dwelling organism with sediment components. The difference between the *L. variegatus* and $C_8$ Empore™ disks in the microcosms examined could be due to the rate of elimination of *L. variegatus*, and the pH of the water and sediment could influence chemical interactions and degradation of erythromycin. The mean uptake and BCF values determined for erythromycin in this study are lower than those determined for sulfamethazine, indicating erythromycin is bioconcentrated less by aquatic organisms (Henderson, 2008; Henderson et al., 2009). However, due to erythromycin’s environmental movement primarily through soil/sediment erosion after manure application, additional studies examining the influence of erythromycin to sediment-dwelling organisms in sediment-containing manure amendments is needed to better understand bioconcentration and persistence of erythromycin in the environment.

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References


Table 1 – Mean uptake and mean bioconcentration factors (BCF) of erythromycin in *L. variegatus* and C$_8$ Empore™ disks in aquatic and sediment microcosm bioassays

| Aquatic *L. variegatus* |  |  |  |
|------------------------|------------------|------------------|
| Treatment exposure concentration (mg/L) | Mean uptake (µg) | Mean BCF |
| 0.05                   | 0.005            | 1.38            |
| 0.5                    | 0.02             | 1.68            |
| 5                      | 0.3              | 2.5             |

| Aquatic C$_8$ Empore™ Disks |  |  |  |
|-----------------------------|------------------|------------------|
| Treatment exposure concentration (mg/L) | Mean uptake (µg) | Mean BCF |
| 0.05                         | 0.24             | 18.2            |
| 0.5                          | 2.82             | 21.3            |
| 5                            | 28.5             | 22              |

| Sediment *L. variegatus* |  |  |  |
|--------------------------|------------------|------------------|
| Treatment exposure concentration (mg/L) | Mean uptake (µg) | Mean BCF |
| 0.05                      | 0.0002           | 0.043           |
| 0.5                       | 0.001            | 0.043           |
| 5                         | 0.02             | 0.13            |

| Sediment C$_8$ Empore™ Disks |  |  |  |
|-----------------------------|------------------|------------------|
| Treatment exposure concentration (mg/L) | Mean uptake (µg) | Mean BCF |
| 0.05                         | 0.02             | 1.64            |
| 0.5                          | 0.24             | 2.61            |
| 5                            | 1.62             | 2.47            |
Figure 1 – Bioconcentration factors post addition of $^{14}$C-residues in *D. magna* in water-only microcosm system
Figure 2 – Bioconcentration factors post addition of $^{14}$C-residues in aquatic microcosms (a) *L. variegatus* (b) C$_8$ Empore™ disks
Figure 3 – Bioconcentration factors post addition of $^{14}$C-residues in water overlying sediment microcosms (a) L. variegatus (b) C$8$ Empore™ disks
Figure 4 – Number of *L. variegatus* recovered from sediment-containing microcosms
CHAPTER 5
GENERAL CONCLUSIONS

Each chapter of this thesis contains an individual conclusion summarizing the findings from that chapter. The goal of this chapter is to provide a summary of general conclusions regarding erythromycin’s analytical quantification methods, fate and bioavailability in environmental matrices. The specific objectives of this thesis were:

1. To improve the quantification methods of erythromycin from water and sediment samples through extraction methodology for subsequent studies with this compound.
2. To evaluate the transformation/dissipation and bioavailability of erythromycin in an aquatic microcosm in the presence and absence of microorganisms, organic matter (manure), and sediment utilizing fresh and aged residues.
3. To determine the bioavailability and movement of erythromycin in surface water systems using *Daphnia magna*, *Lumbriculus variegatus*, and the surrogate model C₈ Empore™ disks.

Results from the analytical improvement of erythromycin from water and sediment indicate that pH adjustment is an important factor in assisting in binding erythromycin to the sorbent of the Oasis HLB® cartridges. Modification of the pH of the water sample to 9.4 which was above the pKₐ allowed additional binding of erythromycin to the sorbent which yielded the highest recovery of 94.7%. Extraction of erythromycin from sediment was demonstrated to also be influenced by the pH of the extraction buffer examined. Various buffers were examined at a variety of pH’s with Iowa and Oklahoma sediments and could influence the cation exchange capacity. The major differences between the two sediments examined were pH and organic matter content. Soil pH may influence the extent of adsorption of erythromycin in the two sediments as lower pH sediments may bind less of the compound compared to higher pH sediments. Organic matter content may also influence interactions of compounds within sediment as pore-space size increases with an increase in organic matter content. The extraction solution which worked the best for quantifying
erythromycin from the Iowa sediment was acetonitrile: 0.3M ammonium acetate pH 4.2 (85:15, v/v) using an 85-minute shake, allowing it to settle overnight prior to siphoning the extract, followed by a second extraction with a 15-minute shake time. In contrast, the Oklahoma sediment had the highest recovery using an acetonitrile: 0.3M ammonium acetate pH 7 (85:15, v/v) with 60-minute extraction shake time.

A study investigating the fate of erythromycin in surface water microcosms revealed the effect sediment has in environmental matrices. The fate of this antibiotic was examined in pond water (PW); autoclaved pond water and autoclaved sediment (APWS); pond water and pond sediment (PWS); and pond water, pond sediment, and manure amendment (PWS+M). In the sediment-containing treatments the amount of 14C-residue decreased throughout the course of the study in treatment water. The extractable 14C-residues displayed a slight increase in the APWS treatment, but decreased throughout the study in PWS and PWS+M treatments. Bound 14C-residues in sediment-containing treatments were found to increase during the study with larger amounts seen in PWS and PWS+M microcosms. Mineralization of erythromycin was determined for all microcosm treatments as 14CO2, with the PWS and PWS+M treatments displaying higher cumulative amounts compared to the PW and APWS treatments. This increase in 14CO2 indicates that biotic processes influence the degradation and sorption of erythromycin in sediment. Dissipation kinetics were also examined with the various treatments, and a one-compartment model worked best for the treatments demonstrating DT_{50}’s of 5 days in PWS+M, 5.8 days in APWS and PWS treatments, and 365 days in PWS+M. The shorter half-lives seen with the sediment-containing treatments show the influence sediment has on erythromycin in the environment and the subsequent sequestration that occurs. The autoclaved system showed little difference from the PWS treatment, most likely indicating that sediment sequestration influences erythromycin’s fate compared to biotic degradation in regards to dissipation kinetics.

In addition to the fate study, an aged erythromycin study was conducted to assess the impact of aged antibiotic residues in sediment followed by incorporation into surface water systems. This study showed greater amounts of 14C-residues in surface water containing sediment with manure compared to sediment without manure. Extractable 14C-residues in sediment prior to assembly of surface water columns indicated a decrease in residues
between fresh and 8-week aged systems in sediment with and without manure amendment. There was no significant difference observed between the two matrix types examined. After assembly of surface water columns occurred, extractability of sediment matrices was examined to understand the movement of erythromycin from sediment into the water column. Results indicated differences in fresh sediment compared to 3 and 8-week sediment only treatments, but no differences were observed in treatments containing manure at the various aging timepoints (0, 1, 3, and 8 weeks). Comparison between sediment and sediment with manure amendment treatments aged 0, 1, 3, and 8 weeks showed a significant difference (p=0.006). The extractable $^{14}$C-residue results from the aged study are different from the fate study, which may be due to the incorporation route (waste incorporation versus surface runoff) of the manure and erythromycin in each study. This difference in manure and residue environmental entry routes could suggest erythromycin’s potential to be more bioavailable in sediment with direct incorporation into sediment compared to spreading manure on the surface of agricultural fields.

Bioconcentration of erythromycin was examined in D. magna, L. variegatus, and C. C-residue results from the aged study are different from the fate study, which may be due to the incorporation route (waste incorporation versus surface runoff) of the manure and erythromycin in each study. This difference in manure and residue environmental entry routes could suggest erythromycin’s potential to be more bioavailable in sediment with direct incorporation into sediment compared to spreading manure on the surface of agricultural fields.

In addition to examining two toxicity test model organisms in aquatic systems, L. variegatus were exposed to erythromycin in water containing pond sediment. When BCF values were compared for L. variegatus between the two microcosm systems, lower uptake and BCF values were observed in sediment-containing systems compared to water-only systems. The differences are most likely due to erythromycin dissipation from water and disks. Results indicate that this antibiotic accumulates more in D. magna compared to L. variegatus in aquatic (water-only) microcosms, but the accumulation is short lived in both organisms as lower BCF values were observed at the end of the studies. The differences in accumulation of erythromycin in D. magna and L. variegatus tissues is possibly due to the composition of the external cuticles, lipid and protein in daphnids, compared to higher lipophilicity in worms. Another factor which could account for the differences observed between the two test organisms within aquatic bioassays is due to the daphnids being fed throughout the course of the study allowing for exposure of erythromycin through ingestion. Differences in elimination rates between the two organisms could also contribute to the differences in BCF values calculated.
moving into the sediment portion of the system, where a smaller amount is bioavailable to sediment-dwelling organisms as observed in the increase in bound erythromycin residues within the sediment systems (data not shown). Differences between the aquatic and sediment systems showed the same results of higher amounts in disks in aquatic systems compared to sediment systems. Recovery of *L. variegatus* from sediment decreased by day 7, possibly due to toxicity, worm age, or difficulty in locating them within sediment. Sediment was demonstrated to have an impact on erythromycin’s fate and bioavailability to sediment-dwelling organisms, and the C₈ Empore™ disks did not concentrate much of erythromycin. Additional studies are needed to better understand erythromycin’s bioavailability and effects to non-target sediment organisms, and a better surrogate system should be developed, possibly using Tenax® extraction beads.

The development of a surrogate model to compare BCF values was attempted using C₈ Empore™ disks and comparing their “BCF” to *L. variegatus* BCF values in aquatic and sediment-containing microcosms at three different concentrations of erythromycin. The disks displayed higher “BCF” values compared to the worms, and could be used to help estimate exposure levels. However, *D. magna* and *L. variegatus* had very small amounts of erythromycin uptake and low bioconcentration, indicating little accumulation of this compound in the tissues of these organisms. Erythromycin was also found to bioconcentrate less than sulfamethazine, which could be due to erythromycin’s higher $K_{ow}$ and differences in structures. The low impact on these organisms may be due to their metabolism, elimination, and excretion methods. Erythromycin has a $K_{ow}$ of 3.06 which would indicate that this compound is hydrophilic and should have small bioconcentration in aquatic life, which was shown in this study.

The results obtained from these studies will be useful in improving the detection of erythromycin from sediment and assisting in the development of ecological risk parameters for antibiotics in the environment. Methods developed and utilized throughout this thesis will aid in future studies by assisting in improved quantification methods for erythromycin from many matrices. Erythromycin was found to partition from water into sediment where the incorporation of manure could influence its fate and bioavailability. Future studies are needed to better understand manure’s influence upon sediment in environmental systems.
through exposure to non-target organisms, which could be examined through aging erythromycin in sediment and exposing sediment-dwelling organisms, with and without incorporating a water component. An improved surrogate model is also needed for sediment systems to assist in better quantifying amounts and exposure levels to sediment-dwelling organisms.
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