1 **Effects of solid-liquid separation and storage on monensin attenuation in dairy waste**
2 **management systems**

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10
11 **Abstract**

12 Environmental release of veterinary pharmaceuticals has been of regulatory concern for
13 more than a decade. Monensin is a feed additive antibiotic that is prevalent throughout the dairy
14 industry and is excreted in dairy waste. This study investigates the potential of dairy waste
15 management practices to alter the amount of monensin available for release into the
16 environment. Analysis of wastewater and groundwater from two dairy farms in California
17 consistently concluded that monensin is most present in lagoon water and groundwater
18 downgradient of lagoons. Since the lagoons represent a direct source of monensin to
19 groundwater, the effect of waste management, by mechanical screen separation and lagoon
20 aeration, on aqueous monensin concentration was investigated through construction of lagoon
21 microcosms. The results indicate that monensin attenuation is not improved by increased solid-
22 liquid separation prior to storage in lagoons, as monensin is rapidly desorbed after dilution with
23 water. Monensin is also shown to be easily degraded in lagoon microcosms receiving aeration,
24 but is relatively stable and available for leaching under typical anaerobic lagoon conditions.

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Introduction

Monensin is a polyether ionophore antibiotic used exclusively for veterinary applications, specifically in poultry and cattle to treat coccidiosis and enhance growth (Fig. 1) (Russell and Houlihan, 2003). Monensin is also used in lactating dairy cattle to increase milk productivity, making monensin one of the few feed additives approved for lactating cattle in the United States (US FDA, 2004; NMFP, 2015). While standards on antimicrobial use have typically been more stringent in other countries than the US, monensin continues to be used as a growth promoting feed additive worldwide (Maron et al., 2013). Due to its high toxicity profile and the largely unknown fate in the environment relative to other antimicrobials, monensin is 1 of 13 veterinary active ingredients listed as high priority for detailed risk assessment out of 83 investigated (Capleton et al., 2006).

The potential for high monensin use in dairies raises concerns of significant monensin loading into the environment through manure management. Dairy manure, which contains excreted antibiotics and metabolites, is often collected by flushing freestalls. Solids are then separated from diluted manure through a variety of techniques. Solids are commonly separated by settling basin sedimentation, which uses gravity to remove the solids. Mechanical screening of manure is another common technique, often followed by further separation by settling. After solid-liquid separation, the liquid fraction is stored in a lagoon and often applied to forage crop fields as a soil amendment. This practice is concerning in California, where dairies are one of the state's most prevalent concentrated animal feeding operation (CAFO) industries (NASS, 2014).

Further, most of California's dairies are located in the San Joaquin Valley (CDFA, 2009), a topographically flat region of predominantly alluvial and fluvial unconsolidated aquifers with some areas of shallow water tables, making them particularly vulnerable to contamination.

There are two potential pathways for monensin to reach groundwater: by direct leaching from the production area and through the waste management system, such as lagoon storage and manure application to fields. Previous research in a California dairy system has shown that contamination of groundwater via waste storage is considerable, while liquid manure application to irrigated crops is not a significant source of monensin to groundwater (Watanabe et al., 2008; Hafner et al., 2016). Once released into the soil, processes for reduction of available monensin appear to be rapid and include degradation and sorption to soil (Carlson and Mabury, 2006; Davis et al., 2006; Sassman and Lee, 2007; Song et al., 2010).

Reducing monensin concentrations in the lagoon will reduce a major groundwater contamination pathway. The primary goal of this study is to investigate the effects that waste management practices have on final aqueous monensin concentrations in manure slurries. In order to fully assess monensin distribution in groundwater beneath dairies, additional samples were collected from the two dairy systems monitored in Watanabe et al. (2008). Groundwater, flush lane, and lagoon water samples were collected during four sampling events over two years. Laboratory manure microcosms were then constructed to determine the potential effect of waste management practices on monensin attenuation. Given that monensin is nearly exclusively excreted in the feces (Donoho et al., 1978), the effect of solid-liquid separation by screening (2 mm) on final lagoon monensin concentrations is investigated. Short-term microcosms (<48 h) were constructed to evaluate the effect that settling basin detention time has on final monensin concentration. Long-term microcosms were used to determine these same effects on a time scale

more relevant to lagoon storage (weeks), and included a treatment for the effects of aeration on monensin transformation.

Materials and Methods

2.1 Dairy Study Sites

Two dairy farms in the San Joaquin Valley of California were studied. Detailed descriptions of the site hydrogeology are given in Harter et al. (2002). Groundwater levels in the study area range from 2 to 5 m, with a well-drained sandy loam as the dominant surface soil texture. Monitoring wells (MWs) are 5 cm in diameter and 7 to 10 m in depth, with screening below 2 or 3 m. Wells capture recent recharge from irrigated fields, lagoon leachate, or corral recharge. Dairy maps and monitoring well locations are provided in Supporting Figure S1.

On both dairies, concrete-lined freestalls are flushed with lagoon water three to four times per day to collect waste. Flushed manure then undergoes solid-liquid separation before collection of the liquid fraction into a lagoon. Dairy I separates solids by mechanical screen and settling basins, while Dairy II utilizes only settling basins.

Groundwater, flush lane, and lagoon water samples were collected during four sampling campaigns in the fall (Oct. 17–18, 2006), spring (April 26 and May 22, 2007), summer (Sept. 4–5, 2007) and winter (Jan. 2–3, 2008) from Dairy I and Dairy II. Due to restricted accessibility, not all wells were sampled at each sampling date. Methods of groundwater, flush lane, and lagoon water sampling are detailed in Watanabe et al. (2008).

2.2 Manure Collection

Manure for laboratory experiments was collected from the University of California Dairy Teaching and Research Facility in Davis, CA. Treatment samples were obtained from milking cows consuming monensin at a dose of 6 mg lb⁻¹ dry matter. Manure without monensin was also collected from an organic dairy farm in Orland, CA, for use as a negative control. The feed rations for both dairies are described in the Supporting Material. Fresh manure was shoveled into buckets lined with plastic bags and stored at 4 °C. Treatment dairy manure was collected directly from the bedding while the organic control manure was collected at the end of the flush lane. Total solids (TS) was determined by taking three representative samples (~25 g) from both the treatment and control manure and oven drying at 105 °C, cooling in a desiccator, and weighing remaining solids. The process was repeated until samples differed by <50 mg over one hour of drying (Eaton et al., 1995). The bedding material in the treatment manure (sand) was removed from final samples prior to determining TS. Fecal samples were then diluted with 18.2 MΩ·cm water to reach an initial TS of 3% for use in all experiments. Diluted manure for screened treatments was passed through a 2 mm sieve with gentle stirring and light pressing of solids, and TS was determined for the final screened suspension. Due to the volume of diluted manure required for long-term lagoon microcosms with and without aeration, two separate dilutions were made and distributed throughout the time points.

2.3 Chemical Analysis

Chemical preparation for dairy site samples is described in Watanabe et al. (2008). The analysis of monensin and nigericin by liquid-chromatography mass-spectrometry for the microcosm study is detailed in the Supplemental Material.

Quality control for dairy site sample analysis is described in Watanabe et al. (2008). Quality control for microcosm experiments was evaluated by performing spike-recovery experiments on manure extracts. Diluted manure was screened to <2 mm (2.3% TS organic control, 1.2% TS treatment), centrifuged in 40 mL fluorinated ethylene propylene (FEP) Nalgene Oak Ridge tubes (20 min at $20,500 \times g$), and filtered to 0.3 μm using stacked glass fiber filters (1.3 μm , 0.7 μm , 0.3 μm) on glass frit filtration assemblies. Extracts (25 mL) were fortified to $6.6 \mu\text{g L}^{-1}$ with monensin and $6.4 \mu\text{g L}^{-1}$ with nigericin. Nigericin is an ionophore not used by the dairy industry, and was therefore appropriate to use as a surrogate to evaluate analyte loss. Samples were mixed for 15 min at 130 rpm on a platform shaker (New Brunswick Scientific, Edison, NJ) before solid-phase extraction (SPE) with Waters Oasis hydrophilic-lipophilic balance (HLB) cartridges (3 mL, 60 mg). Cartridges were conditioned with 3 mL methanol and equilibrated with 6 mL water before sample addition. Cartridges were then washed with 6 mL water before drying and eluting with 2 mL methanol. Recovery samples for each treatment were prepared in triplicate, with triplicate unfortified samples. Monensin recovery from filtered organic manure was 87.6% (4.3% CV), and recovery from filtered treatment manure was 87.1% (9.3% CV). Nigericin recovery from filtered treatment manure averaged 82.2% (4.5% CV).

2.4 Extractable Monensin from Manure

The effect of the manure quantity on monensin concentration for both screened and unscreened manure was evaluated by three manure dilutions. Wet manure (90, 110, or 130 g) was weighed into a beaker and diluted to 500 mL with water, for final amounts of 18, 22, and 26% manure (wet weight). Each manure dilution was performed three times. As each solution was stirred, aliquots were removed for measuring TS (triplicate) and monensin. The remaining

manure slurry was then screened with a 2 mm sieve, and the filtrate analyzed as before. Monensin samples were centrifuged, filtered, and extracted by SPE as previously described, without the addition of nigericin. Total solids were determined as previously described, but on <10 g of wet sample.

Extractable monensin from wet manure was evaluated by multiple extractions of manure with either water or methanol. Water-extractable monensin was determined by combining about 10 g of wet manure with 30 mL water, briefly vortexing, and rotating end-over-end for 1.5 h. Samples were centrifuged for 20 min at $20,500 \times g$, and the supernatant removed and extracted by SPE as previous water samples. Water was added to tubes and the process repeated for a total of three water extractions. Similar protocol was followed for three methanol extractions, with the exception that the second and third extractions were centrifuged for 10 min at $14,200 \times g$ and $9,100 \times g$, respectively. Samples (10 g) were extracted with methanol (30 mL) in a sonicating bath for 10 min per extraction. After centrifugation, supernatant was decanted and 30 mL fresh methanol added to tubes. Samples were vortexed (30 s) and shaken to resuspend the solids, in addition to 5 min of sonication since the organic manure (negative control) was difficult to resuspend. This process was repeated for a total of three extractions.

2.5 Microcosms

Amber bottles (1-L) were filled with diluted manure for each treatment (unscreened, screened, organic screened control) for the short-term settling basin representation. Samples for each time point and treatment were prepared in triplicate for destructive sampling. At the stated time points, the sample bottles were shaken and aliquots were removed for centrifugation for 20 min at $20,500 \times g$ and filtered to 0.3 μm . Electrical conductivity (EC) and pH were measured on

the remaining manure in bottles on a stir plate. Filtered samples were then subsampled for SPE analysis (25 mL) with nigericin (1 µg) added to samples just before extraction, and the remainder of filtrate was stored at -20 °C until analysis for dissolved organic carbon (DOC), nitrate, and ammonium. Dissolved organic carbon and N analyses are detailed in the Supporting Materials.

Long-term microcosms were prepared similarly to short-term microcosms. Samples not receiving aeration were capped and stored at ambient temperature next to aerated samples. Aeration was achieved by bubbling compressed ambient air through 6 mm diameter glass pipettes at an approximate rate of $5 \pm 1 \text{ cm}^3 \text{ s}^{-1}$ into uncapped samples. Samples were checked periodically to adjust air input to each sample for consistency. Samples were analyzed similar to settling basin samples.

2.6 Statistics: Lagoon Microcosms

R software was used to determine statistical significance of values within treatments. The data was first evaluated with the Shapiro-Wilk test, Levene's test, then significance determined by Tukey's honest significant difference test ($\alpha = 0.05$).

Results and Discussion

3.1 Monensin on Dairies

Monensin was consistently detected in all of the lagoon water samples at Dairy I and Dairy II (Table 1). The average concentrations in the lagoons are $5.6 \pm 9.2 \text{ µg L}^{-1}$ at Dairy I and $4.1 \pm 2.3 \text{ µg L}^{-1}$ at Dairy II over all four sampling campaigns. The discrepancy in monensin measurements between sampling dates can be explained by both spatial variability within the lagoon and temporal variability. Lagoon water was collected from 12 different locations around

the lagoon and composited to reduce the spatial variability of the surface water, but water was not able to be collected from either the central or deeper parts of the lagoon. Temporal variability is less likely compared to antibiotics that are administered on sick animals, since monensin is administered daily as a feed additive. However, the observed seasonal variation of monensin in the lagoon can be attributed to rainfall dilution (October to April), summer evaporation, and irrigation needs (primarily summer). Though there is variability, monensin is consistently detected in lagoons on all sampling dates, representing a constant source of monensin available for leaching to groundwater.

Monensin was detected once in MW 7 near the lagoon and irrigated forage field at Dairy I, and sporadically in MW 1, 2, and 10 at Dairy II. At Dairy II, MW 1 is near freestalls, the feed storage area, and the lagoon, while MW 2 is close to freestalls and dry cow housing area. The range of concentrations of monensin in these wells was often lower than the lagoon wells on each dairy (MW 3), where monensin was most frequently detected. These findings emphasize that monensin contamination of groundwater on a dairy is most likely to result from waste management practices, particularly the lagoons. The lagoons on these dairies were constructed several decades ago, and the clay liners are known to be leaking. These lagoons continuously supply monensin-containing water to groundwater, while the production area receives only intermittent and spatially heterogeneous loading. Additionally, the production area and corrals have very low infiltration rates relative to lagoon, since the lagoon supplies a much larger pressure head to soil.

3.3 Extractable Monensin from Manure

Monensin extraction of manure for lagoon microcosms is examined in the context of both methanol-extractable monensin, more representative of total monensin, and water-extractable monensin, relevant to the dairy system. The total monensin recovered by three subsequent methanol extractions is 1351 $\mu\text{g kg}^{-1}$ (dry weight), while water extractions recovered only 429 $\mu\text{g kg}^{-1}$ (Fig. 2). The third extract of the methanol extraction contained only 3.6% of the total monensin removed from that method, suggesting that three extractions are nearly exhaustive for removal of methanol-extractable monensin by sonication. Water extraction only recovered 31.8% of the total methanol-extractable monensin after three washes. There was also a linear decrease in the amount of monensin removed with each methanol extraction (61.7%, 34.7%, 3.6%), while the first water extraction removed the most water-extractable monensin (70.2%) followed by two extractions of similar concentrations (17.1%, 12.7%). This suggests that most water-extractable monensin is removed into the liquid phase during the initial manure dilution on a dairy, while the methanol extraction demonstrates that there is much more monensin available for desorption during manure mineralization in a lagoon or possible transport on colloids.

3.4 Solid-Liquid Separation

Short-term (<36 h) microcosms were constructed to be representative of the chemical changes that happen in diluted manure immediately after flushing and in settling basins. Results indicate that the initial and final monensin concentration does not change significantly over a 36 h period, though concentrations increase slightly from 0 to 20 h (unscreened manure) and 0 to 25.6 h (screened manure) (Fig. 3). While statistically significant, this difference in concentration of $\sim 2 \mu\text{g L}^{-1}$ is not realistically important in a dairy setting. Most desorption of monensin from

manure occurs rapidly after dilution (Fig. 3), and settling basin detention time does not substantially affect the concentration of monensin entering the lagoons.

Long-term (0 to 36 d) microcosms were constructed in order to monitor changes in aqueous monensin concentration during lagoon storage. Samples without aeration demonstrate the stability of monensin in these anaerobic lagoons, particularly when viewed in the context of samples which received aeration (Fig. 4). However, the effect of manure screening on aqueous monensin concentrations is not consistent between short-term and long-term microcosm experiments. This inconsistency may be attributable to differences in the TS measured between the screened manure in each experiment. The average TS remained similar for unscreened samples between experiments (3.1–3.2%), and materials contributing to TS variability are less likely to affect monensin concentrations in these samples (e.g., undigested hay). The TS of screened samples instead consists of fine manure particles which may sorb more monensin, and increases up to 29.5% from the short-term to long-term experiments (from 1.2% to 1.3–1.5% TS) (Fig. 5). This increase in TS between the experiments could account for the reversal in effect of screening on aqueous monensin concentrations.

The role of TS percentage and screening was explored through comparing aqueous monensin concentrations from different manure dilutions (18, 22, and 26% manure, wet weight). The results clearly demonstrate that aqueous monensin is increasing as the initial manure (or final TS) increases, and that the concentration of monensin is not affected by screening of manure (Fig. 6). This is consistent with findings from the short-term microcosms, which establish that monensin is desorbed from manure rapidly following dilution. Therefore, the practice of screening diluted manure does not result in a decrease in the final aqueous monensin concentration in the liquid fraction. Similar results have been demonstrated for two classes of

steroid hormones (estrogens, androgens) in mechanically separated swine waste, which show no significant difference ($p>0.01$) in steroid concentration relative to total solids between raw and liquid manure after various separation methods (Hansen et al., 2015). Collectively, these results suggest that solids separation may not be sufficient for reducing environmental loading with agrochemicals, even those which would be expected to primarily associate with the solid fraction.

Methanol extractions of the dairy manure also reveal that a considerable amount of monensin remains in the solid portion relative to the amount desorbed. However, solid manure products are often composted before reuse as bedding material on dairies, a practice which has been demonstrated to reduce monensin concentrations in animal manure (Dolliver et al., 2008; Kang et al., 2013). This reinforces the postulation that management of liquid waste is most critical when assessing monensin fate on dairies.

3.5 Aeration on Monensin Dissipation

Monensin concentrations in aerated samples are corrected for evaporation of water over the time period, though final monensin concentration was greatly reduced in all aerated samples regardless of correction. The reduction of monensin in aerated samples is likely due to transformation, by either indirect oxidation or microbial transformations, as monensin has a very low Henry's constant (low volatility) and is known to undergo biotransformation (Thiele-Bruhn, 2003; Sun et al., 2014). Monensin is also known to undergo hydrolysis in acidic conditions; however, the pH in aerated samples increased to 7.5 and 8.3 (unscreened, screened) while non-aerated samples decreased to 6.4 and 6.5 after 5 wk (Fig. S2). The high pH of the aerated samples indicates that hydrolysis is not a transformation pathway (Sun et al., 2014). The

decrease in DOC (Fig. S3) and ammonium (Fig. S4) in aerated samples relative to capped samples also supports transformation by microbial activity. Dissolved organic carbon represents a readily available C source for microbes, released as CO₂ after respiration. Ammonium loss is presumed to be through rapid denitrification and gas loss, as nitrate detection was low when present at all.

The rate of average monensin transformation was determined for both aerated and non-aerated long-term microcosms. Monensin reduction in aerated samples was determined to be first-order, and the resultant reaction rate coefficients (k) for monensin in screened and unscreened samples are nearly identical (0.051 and 0.049 d⁻¹), leading to similar half-lives of 13.7 and 14.1 d, respectively. These half-lives can be verified in the data through two half-lives by calculating the amount of monensin expected at 14 and 28 days (Fig. 3). Samples without aeration do not exhibit the same reduction in monensin. However, for a crude comparison, analysis of screened and unscreened samples following first-order dissipation (screened $r^2=0.79$, unscreened $r^2=0.85$) again have similar rate coefficients (k = 0.004 and 0.005 d⁻¹), resulting in half-lives of 161 and 133 d, respectively. If analyzed by zero-order kinetics (screened $r^2=0.78$, unscreened $r^2=0.85$), half-lives are 122 and 103 d, respectively. While these results cannot be verified from the data, they again demonstrate that screened and unscreened samples behave similarly, and clearly show that aerated samples benefit from much faster monensin degradation. This is similar to observations of monensin biotransformation in mixed cultures derived from poultry litter and litter-amended soil, which demonstrated much faster transformation under aerobic versus anoxic conditions (Sun et al., 2014). In addition to aeration resulting in shorter half-lives, the potential for difference in reaction order (zero- versus first-order) will affect how monensin degrades in each system. The half-lives of first order reactions (i.e., aerated samples)

are independent of concentration, while zero-order half-lives increase as the initial concentration increases (Whitten et al., 2004). This means that degradation would slow with increased monensin inputs, though the reaction order of non-aerated samples cannot be ascertained from the existing data.

While aeration in the microcosms greatly reduces monensin, there are important considerations when applying microcosm data to the farm-scale. The first is aeration efficiency, as the ratio of bubble volume to slurry is much higher in microcosms than would be expected on a farm scale, accelerating aeration and the degradation of monensin in microcosms. Another consideration is water evaporation, which results in a more concentrated manure slurry with respect to leachable ions. However, the benefits of aeration for managing odors, nitrogen, and pathogens in the final manure slurry may outweigh the cost of a more concentrated slurry (Westerman and Zhang, 1997).

There are multiple methods of aerating manure slurries, including compressed air (as replicated here), mechanical mixing (surface and subsurface), pumped liquid, or a combination of compressed air and mechanical mixing (Cumby, 1987). The use of compressed air benefits from having a simple, low cost design and the ability to add to existing lagoons. However, aeration efficiency is hindered in large, shallow ponds due to low contact time of bubbles with the manure slurry as they rise. While smaller bubbles (ideally <5 mm) will rise more slowly with a higher gas-transfer surface area help overcome this issue, manure slurries also tend to clog small bubble diffusers, limiting their effectiveness (Cumby, 1987; Singleton and Little, 2006). Operating costs are also a limiting factor when considering the use of aeration on a farm scale (Westerman and Zhang, 1997). These limitations are significant and often preclude the widespread use of lagoon aeration. Similar aeration systems are commonly employed in lakes,

reservoirs, and ponds to manage anoxic conditions, often facing comparable issues of cost and fouling. Additional research into practical designs for aeration of dairy lagoons would be valuable.

Environmental Impact

Groundwater analyses from monitoring wells distributed around two California dairies identify lagoon leakage as the most likely source of groundwater contamination with monensin. However, investigation into manure solid-liquid separation techniques commonly used on dairies, such as manure screening and settling basins, reveals that monensin is not attenuating during waste separation processes. In fact, monensin is desorbed from manure almost immediately after dilution. The implications of this research on groundwater contamination extend to other abundant veterinary pharmaceuticals found in lagoon-stored manure on CAFOs (e.g., dairy and swine). Monensin is primarily excreted in feces and is relatively hydrophobic ($\log K_{ow}=2.75$) compared to many other agrochemicals (Thiele-Bruhn, 2003; Elanco Products Company, 1989) suggesting that the current waste management system is also likely inadequate for processing chemicals which are more water-soluble and/or excreted in urine. While there are drawbacks to all waste management practices, the elimination of waste storage lagoons or further treatment of lagoon water could remove a primary and direct source of many contaminants to groundwater. Composting of the whole manure or aeration of lagoons would increase biological activity and degradation of agrochemicals, such as monensin. Since common dairy waste management techniques are not effective for removing agrochemicals from manure, further research into lagoon treatments is needed for reducing contaminant concentrations in dairy lagoons and the underlying groundwater.

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References

- California Department of Food and Agriculture (CDFA). *California Agricultural Directory 2008-2009*. **2009** Available at https://www.cdfa.ca.gov/statistics/PDFs/ResourceDirectory_2008-2009.pdf (verified 6 May 2016).
- Capleton, A.C.; Courage, C.; Rumsby, P.; Holmes, P.; Stutt, E.; Boxall, A.B.; Levy, L.S. Prioritising veterinary medicines according to their potential indirect human exposure and toxicity profile. *Toxicol. Lett.* **2006** 163:213-223. DOI:10.1016/j.toxlet.2005.10.023
- Carlson, J. C.; Mabury, S.A. Dissipation kinetics and mobility of chlortetracycline, tylosin, and monensin in an agricultural soil in Northumberland County, Ontario, Canada. *Environmental Toxicology and Chemistry* **2006** 25 (1): 1-10. DOI: 10.1897/04-657R.1
- Cumby, T.R. A review of slurry aeration: Performance of Aerators. *J. Agric. Eng. Res.* **1987** 36: 175-206. DOI:10.1016/0021-8634(87)90073-4
- Davis, J. G.; Truman, C.C.; Kim, S.C.; Ascough, J.C.; Carlson K. Antibiotic Transport via Runoff and Soil Loss. *Journal of Environmental Quality* **2006** 35: 2250-2260. DOI:10.2134/jeq2005.0348
- Dolliver, H.; Gupta, S.; Noll, S. Antibiotic degradation during manure composting. *Journal of Environmental Quality* **2008** 37: 1245-1253. DOI:10.2134/jeq2007.0399
- Donoho, A.; Manthey, J.; Occolowitz, J.; Zornes, L. Metabolism of monensin in the steer and rat. *Journal of Agricultural and Food Chemistry* **1978** 26: 1090-1095. DOI: 10.1021/jf60219a005
- Eaton, A.D.; Clesceri, L.S.; Greenberg, A.E.; Franson, M.A.H. *Standard methods for the examination of water and wastewater*. Washington, DC **1995**: American Public Health Association.
- Elanco Products Company. Environmental assessment for the use of Rumensin premixes in the feed of beef cattle for the prevention and control of coccidiosis. **1989** Elanco Products Company, Indianapolis, IN.
- Hafner, S.C.; Harter, T.; Parikh, S.J. Evaluation of monensin transport to shallow groundwater after irrigation with dairy lagoon water. *Journal of Environmental Quality* **2016** 45 (2): 480-487. DOI:10.2134/jeq2015.05.0251
- Hansen, M.; Björklund, E.; Popovic, O.; Jensen, L.S.; Jacobsen, C.S.; Sedlak, D.L.; Halling-Sørensen, B. Animal manure separation technologies diminish the environmental burden of steroid hormones. *Environmental Science & Technology Letters* **2015** 2:133-137. DOI: 10.1021/acs.estlett.5b00059
- Harter, T.; Davis, H.; Mathews, M.C.; Meyer, R.D. Shallow groundwater quality on dairy farms with irrigated forage crops. *Journal of Contaminant Hydrology* **2002** 55: 287-315. DOI:10.1016/S0169-7722(01)00189-9
- Kang, D.H.; Gupta, S.; Rosen, C.; Fritz, V.; Singh, A.; Chander, Y.; Murray, H.; Rohwer, C. Antibiotic uptake by vegetable crops from manure-applied soils. *Journal of Agricultural and Food Chemistry* **2013** 61: 9992-10001. DOI: 10.1021/jf404045m
- Maron, D.F.; Smith, T.J.S.; and Nachman, K.E. Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey. *Globalization and Health*; **2013** 9:48. DOI: 10.1186/1744-8603-9-48

- McNab, W.W.; Singleton, M.J.; Moran, J.E.; Esser, B.K. Assessing the Impact of Animal Waste Lagoon Seepage on the Geochemistry of an Underlying Shallow Aquifer. *Environmental Science & Technology* **2007** 41: 753-758. DOI: 10.1021/es061490j
- National Agricultural Statistics Service (NASS). 2012 Census of Agriculture, United States Summary and State Data, *Volume 1, Geographic Area Series, Part 51. (AC-12-A-51)*. Washington, DC, **2014**: U.S. Department of Agriculture.
- National Milk Producers Federation (NMFP). National Dairy FARM Program: Farmers Assuring Responsible Management™ Milk and Dairy Beef Drug Residue Prevention Manual. **2015** Available at <http://www.nationaldairyfarm.com/sites/default/files/2016-Residue-Manual.pdf> (verified 6 May 2016).
- Russell, J. B.; Houlihan, A.J. Ionophore resistance of ruminal bacteria and its potential impact on human health. *FEMS Microbiology Reviews*; **2003** 27: 65-74. DOI: 10.1016/S0168-6445(03)00019-6
- Sassman, S. A.; Lee, L.S. Sorption and degradation in soils of veterinary ionophore antibiotics: Monensin and lasalocid. *Environmental Toxicology and Chemistry* **2007** 26: 1614-1621. DOI: 10.1897/07-073R.1
- Singleton, V.L; Little, J.C. Designing Hypolimnetic Aeration and Oxygenation Systems – A Review. *Environ. Sci. Technol.* **2006** 40 (24), 7512-7520; DOI: 10.1021/es060069s.
- Song, W.; Ding, Y.; Chiou, C.T.; Li, H. Selected Veterinary Pharmaceuticals in Agricultural Water and Soil from Land Application of Animal Manure. *Journal of Environmental Quality* **2010** 39: 1211-1217. DOI:10.2134/jeq2009.0090
- Sun, P.; Huang, C.; Pavlostathis, S.G. Inhibition and biotransformation potential of veterinary ionophore antibiotics under different redox conditions. *Environmental Science and Technology* **2014** 48: 13146-13154. DOI: 10.1021/es503005m
- Thiele-Bruhn, S. Pharmaceutical antibiotic compounds in soils – a review. *J. of Plant Nutr. Soil Sci.* **2003** 166 (2): 145-167. DOI: 10.1002/jpln.200390023
- US Food and Drug Administration. New Animal Drugs; Monensin. *Federal Register* 69 **2004** (227): 68783-68784. <http://www.fda.gov/ohrms/dockets/98fr/04-26091.pdf> (accessed 6 May 2016).
- Watanabe, N.; Harter, T. H.; Bergamaschi, B.A. Environmental occurrence and shallow ground water detection of the antibiotic monensin from dairy farms. *Journal of Environmental Quality* **2008** 37: S78-S85 DOI:10.2134/jeq2007.0371
- Westerman, P.W.; Zhang, R.H. Aeration of livestock manure slurry and lagoon liquid for odor control: A review. *Applied Engineering in Agriculture* **1997** 13 (2): 245-249. DOI: 10.13031/2013.21596
- Whitten, K.W.; Davis, R.E.; Peck, M.L.; Stanley, G.G. *General Chemistry*, Seventh ed.; Belmont, CA: Brooks/Cole – Thomson Learning, **2004**.

Table 1. Monensin concentrations found in dairy lagoon, flush lane, and groundwater samples. Monitoring wells not shown were either not measured or had below detection levels of monensin for all sampling dates. All samples are analyzed in triplicate. Numbers in parentheses represent standard deviations. BD = below detection, NC = not collected.

Location	Well ID	Oct 2006 [†]	Apr/May 2007 [†]	Aug/Sept 2007	Jan 2008
<i>Dairy I</i>					
Lagoon		NC	16.24 (1.26)	0.41 (0.03)	0.14 (0.001)
Flush lane		1.89 (0.66)	3.58 (0.16)	0.54 (0.04)	0.17 (0.003)
Calf hutch flush		0.64 (0.17)	0.42 (0.04)	0.20 (0.02)	0.44 (0.05)
Lagoon well	MW 3	NC	0.39 (0.02)	0.45 (0.01)	0.32 (0.02)
Field well	MW 7	BD	BD	BD	0.02 (0.004)
<i>Dairy II</i>					
Lagoon		3.91 (0.05)	6.34 (0.17)	1.08 (0.04)	5.13 (0.55)
Flush lane		7.14 (0.51)	3.37 (0.19)	1.17 (0.08)	NC
Freestall well 1	MW 1	BD	BD	BD	0.08 (0.01)
Freestall well 2	MW 2	BD	0.04 (0.001)	0.25 (0.01)	NC
Lagoon well 1	MW 3	0.36 (0.06)	0.30 (0.02)	0.07 (0.006)	NC
Lagoon well 2	MW 10	NC	0.07 (0.002)	0.13 (0.01)	NC

[†]Watanabe et al., 2008

Figure 1. Structure of monensin, an ionophore antibiotic administered to dairy cattle as a feed additive. Structure created with MarvinSketch, Marvin 15.4.20.0, 2015, ChemAxon (<http://www.chemaxon.com>)

Figure 2. Methanol- and water-extractable monensin from wet manure following three extractions. Monensin concentration from each manure extract is presented on a dry weight basis. Error bars represent the standard error of quadruplicate samples.

Figure 3. Monensin concentration in screened and unscreened manure in short-term microcosms. Error bars represent the standard error of triplicate samples. Time points that share letters are not significantly different ($\alpha = 0.05$). Statistical comparisons are made within treatment, such that uppercase and lowercase letters cannot be compared.

Figure 4. Monensin concentration in screened and unscreened manure in long-term microcosms. Aerated samples are corrected for evaporative loss. Error bars represent the standard error of triplicate samples. Time points that share letters are not significantly different ($\alpha = 0.05$). Statistical comparisons are made within treatment, such that uppercase and lowercase letters cannot be compared.

Figure 5. Total solids in organic control, screened, and unscreened manure in short-term and long-term microcosms. Long-term microcosms were constructed from two separate dilutions of manure. Error bars represent the standard error of triplicate measurements.

Figure 6. Monensin concentration and total solids in suspensions with 18, 22, and 26% initial manure (wet weight), with and without screening (2 mm). Error bars represent the standard error of triplicate samples.