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Environmental Occurrence and Shallow Ground Water Detection of the Antibiotic Monensin from Dairy Farms

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Brian A. Bergamaschi USGS

Pharmaceuticals used in animal feeding operations have been detected in various environmental settings. There is a growing concern about the impact on terrestrial and aquatic organisms and the development of antibiotic-resistant strains of microorganisms. Pharmaceutical use in milking cows is relatively limited compared with other livestock operations, except for the ionophore monensin, which is given to lactating cows as a feed. By weight, monensin can be the most significant antibiotic used in a dairy farm. This study investigates the potential of monensin to move from dairy operations into the surrounding ground water. Using two dairy farms in California as study sites, we twice collected samples along the environmental pathway—from flush lanes, lagoon waters, and shallow ground water beneath the dairies and beneath its associated manured fields. Monensin concentrations were determined using solid-phase extraction and liquid chromatography–tandem mass spectrometry with positive electrospray ionization. Monensin was detected in all of the flush lane and lagoon water samples. Theoretical maximum concentration estimated from the actual dosing rate and the theoretical excretion rate assuming no attenuation was one order of magnitude greater than observed concentrations, suggesting significant attenuation in the manure collection and storage system. Monensin was also detected, at levels ranging from 0.04 to 0.39 μg L^{-1}, in some of the ground water samples underneath the production area of the dairy but not from the adjacent manured fields. Concentrations in ground water immediately downgradient of the lagoons were one to two orders of magnitude lower than the concentrations detected in lagoons, suggesting attenuation in the subsurface. The data suggest the possibility of monensin transport into shallow (2–5 m) alluvial ground water from dairy management units, including manure storage lagoons and freestalls occupied by heifers, lactating cows, and dry cows.

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Pharmaceuticals of human and veterinary origin have been detected in various environmental matrices, such as surface water, ground water, soils, and sediments (Hamscher et al., 2002; Kolpin et al., 2002; Schlüsener et al., 2003), and the use of veterinary antibiotics in concentrated animal feeding operations (CAFO) is a growing concern. Antibiotics are used in livestock production to prevent and treat diseases and to promote growth and improve productivity. The Animal Health Institute reports that in the USA, 12.0 thousand tons of antibiotics were sold for animal use in 2006, 4.6% of which was for growth promotion (Animal Health Institute, 2007). The Union of the Concerned Scientists estimates that 11.2 thousand tons, or 70% of total annual antibiotics use in the USA, is for nontherapeutic purposes for cattle and swine (Mellon et al., 2001).

Most CAFOs collect wastewater in lagoons. Lagoon manure water and solid manure are typically applied to fields as fertilizer. Antibiotics and their metabolites excreted in feces and urine can enter the environment through lagoon water and manure application, overflow, and surface runoff (Boxall et al., 2003). Antibiotics released in the environment may affect terrestrial and aquatic organisms (Fernandez et al., 2004; Schmitt et al., 2004; Wollenberger et al., 2000) and may lead to the development of antibiotic-resistant strains of microorganisms (Chee-Sanford et al., 2001; Sengelov et al., 2003). Dairies are the dominant CAFO industry in California, where 1.78 million dairy cows produced 17.6 million tons of milk in 2006, generating 21% of the supply in the USA (California Department of Food and Agriculture, 2007).

Ionophores comprise a class of antibiotics exclusively used for veterinary purposes. They are used as anticoccidial feed additives for poultry and livestock, as growth promoters, and for improved feed efficiency in ruminants. Ionophores have antibiotic activities against Gram-positive microorganisms. They contain a number of cyclic ether and ketal units, have a carboxylic acid group, and form complexes with mono- and divalent cations. They interact with bacterial cell membranes and allow cations to pass through the membranes, causing cell death. According to a 2006 survey by the Animal Health Institute, the greatest quantity (5.1 thousand tons) of antibiotics sales in the USA were of those from a

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Abbreviations: CAFO, concentrated animal feeding operations; K_d, sorption coefficient.

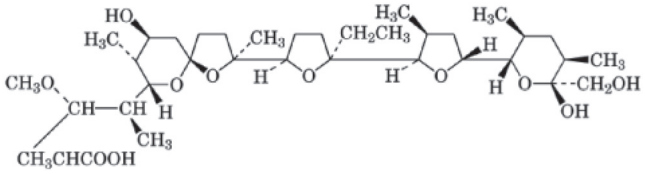


Fig. 1. Chemical structure of monensin A.

class of "ionophores, arsenicals, bambermycin, carbadox, and tiamulin" (Animal Health Institute, 2007).

Monensin is one of these ionophores. In the USA, monensin is the only feed additive permitted for use in lactating cows. The use of monensin for increased milk production was approved by the US Food and Drug Administration in November 2004 (Federal Register, 2004). Monensin has a high affinity for sodium ions. By active transport of cations across the cell membrane, monensin disrupts the ion gradient created by cells to control osmotic pressure and generate energy. The structure of monensin is shown in Fig. 1.

Where monensin is used as a feed additive for lactating cows, it is typically the most significant, by weight, of all the antibiotics used in dairy farms. It is unknown how universally the practice is adopted. Veterinarians estimate that approximately half of all dairy farms in California use monensin (Karle, personal communication, 2006). In the USA, milk from cows that are on antibiotics other than monensin can be sold only after a withdrawal periods specific to each antibiotic. In dairy operations, antibiotics are used for the treatment of sick cows, for dry cow therapy (e.g., use of intramammary antibiotics for the treatment and prevention of mastitis during the dry period right before calving when cows are not milked), and for disease prevention and/or growth promotion in heifers (adolescent cows before calving and milk production) and in calves, whose numbers and body weight are far less than that of lactating cows.

When monensin is released into the environment, it has the potential to persist and reach aquifers because hydrolysis is not typically observed and photolysis is slow (Elanco Products Company, 1989). However, the reported biodegradation rates indicate that rapid biological attenuation is possible. Reported half-lives range from less than 2 d (Sassman and Lee, 2007) to 13.5 d (Carlson and Mabury, 2006). The reported sorption coefficient (K_d) ranges from 0.915 to 78.6 (L kg^{-1}) for various soils at an aqueous phase concentration of 0.05 μmol L^{-1} (Sassman and Lee, 2007). Based on the K_d values, monensin is expected to be more mobile than tetracyclines and similar or less mobile than sulfamethazine in soil/water systems (Tolls, 2001). Monensin has been detected in river water and sediments that are affected by agricultural activities in Colorado (Cha et al., 2005; Kim and Carlson, 2006) and in surface water near agricultural sites in Ontario, Canada (Hao et al., 2006; Lissemore et al., 2006).

The lethal dose to 50% of the study population of monensin is highly species dependent, ranging from 2 mg kg^{-1} (oral, horse/donkey) to 100 (mg kg^{-1}, oral rat). These are generally much lower than the lethal dose to 50% of other antibiotics commonly used in dairy operations (e.g., oxytetracycline: 4800 mg kg^{-1}, oral, rat; penicillin G: 8000 mg kg^{-1}, oral, rat; sulfamethazine: 50 g kg^{-1}, oral, mouse) (U.S. National Library of Medicine, 2008). Hillis et al. (2007) observed changes in zooplankton populations as indirect result of the effects of monensin on the algal community with the no-observable effect concentration of 50 μg L^{-1}. Capleton et al. (2006) classified monensin as a high priority for detailed risk assessment based on high usage and high toxicity profile and with an unassessed potential to reach the environment.

The objective of this study was to assess the occurrence of monensin in the waste stream and its potential to enter the shallow ground water environment during normal dairy operation and in the absence of rainfall-driven transport. The study was conducted at two dairy farms in California. Both farms have approximately 1400 lactating cows, which are housed in freestalls. The quantities of monensin used in these farms were estimated through interviews with the farm owners. Potential monensin loading to dairy facilities and the associated forage fields were estimated for comparison to observed concentrations. We measured monensin concentrations in flush lane water, lagoon water, and in ground water beneath dairy production facilities and beneath the surrounding manured fields. To our knowledge, this is the first comprehensive assessment of monensin occurrence in wastewater and of monensin migration into shallow ground water.

Materials and Methods

Study Sites

The research dairies are located in the Central Valley of California. A detailed description of the site hydrology, hydrogeology, and dairy operations is given in Harter et al. (2002). Briefly, the dairies are located on the distal alluvial fans of the Stanislaus River and the Tuolomne River east of the northern San Joaquin Valley trough. Forage crops and almond orchards are the major commodities grown at and near the study sites. These commodities are irrigated with abundant surface water from storage reservoirs of the Sierra Nevada watersheds. Ground water levels in the study area tend to be shallow, ranging from 2 to 5 m at the study sites. This shallow portion of the alluvial aquifer consists of quaternary alluvial and sub-eolian deposits (Page and Balding, 1973). The sediments consist of alternating layers of sands, silty sands, and sandy silts and clays. At the study sites, the dominant surface soil texture is sandy loam. Soils are well drained. The climate is Mediterranean, with annual precipitation of 290 mm, practically all of which occurs between late October and early April. The area is characterized by featureless topography with slopes of less than 0.2%. Harter et al. (2002) showed that the average regional ground water flow rate is on the order of 5×10^{-7} m s^{-1}. Monitoring wells capture recent (weeks old to <2 yr old) recharge from irrigated fields, from lagoon leachate, or from corral recharge, within a source area that is typically from 150 m to several hundred meters long and a few meters to tens of meters wide (Harter et al., 2002).

The dairies house their animals in covered free-stalls equipped with flush-lanes for manure collection. There are 1450 lactating cows, 1400 heifers, and 250 dry cows at Dairy I. Dairy II has 1340 lactating cows, 1240 heifers, and 470 dry cows. These are larger

than the average California dairy herd, which was 908 milking cows in 2006 (California Department of Food and Agriculture, 2007), and much larger than the average dairy in the USA (93.4 milking cows in 2001) (USDA, 2002). The layout of the dairies and the sampling well locations are shown in Fig. 2 and 3. Cows are milked at milking barns three times per day at Dairy I and two times per day at Dairy II. Cows are primarily kept in freestalls but have access to adjacent corrals or exercise yards at certain times of the day. Freestall flush lanes are lined with concrete and flushed with lagoon water three to four times per day.

Before weaning, calves are kept in individual calf hutches. Calf hutches are on a raised structure at Dairy I, and the floor of the structure is flushed three times per day. Calf hutches at Dairy II are directly on the ground. Heifers are kept together in heifer freestalls.

All of the water used at the dairies is collected into lagoons. Solid waste is separated from the wastewater/manure before entering the lagoon, and it is used as bedding material after drying. The lagoon water is recycled and used to flush freestalls. Corral runoff during the rainy season (October–April) is collected and routed to the manure water lagoon. Off-side runoff is not permitted. Ultimately, manure solids and liquid manure water are land-applied as soil amendment and for fertilization of surrounding forage fields.

Quantifying Monensin Use

Monensin is typically purchased in the form of feed additive pellets amended with vitamins and minerals. Monensin is administered to heifers and cows by mixing the pellets in feed on a mass per animal basis, with the dose being specific to the age and milking status of the animal. Monensin use for each animal herd was estimated based on interviews with the dairy owners, and total usage was calculated by multiplying the dose by the number of heifers and cows treated. The calculation was verified by examining the dairy's purchase receipts over the preceding 6- to 9-mo period.

Ground Water, Flush Lane, and Lagoon Water Sampling

Ground water, flush lane, and lagoon water samples were collected during two sampling events in fall (17 and 18 Oct. 2006) and spring (26 Apr. 2007 and 22 May 2007). Not all wells were sampled at both dates due to restricted accessibility. Before sampling ground water, well water was purged with a stainless steel submersible pump and continuously monitored for field water quality parameters, including temperature, electrical conductivity, and dissolved oxygen. Ground water samples were collected after field water quality stabilized or after a minimum of 5 well volumes of water were removed. Duplicate samples were collected at approximately every 10th ground water sample for quality control.

Flush lane water samples were collected at the end of the lactating cow freestalls. Depending on the dairy, 8 to 12 individual samples were collected and composited in a large vessel, mixed, and subsampled for a final volume of 5 L. At Dairy I, flush water from calf hutches was also collected. Lagoon water (1 L) was collected with a pole sampler from the surface at 8 to 12 points

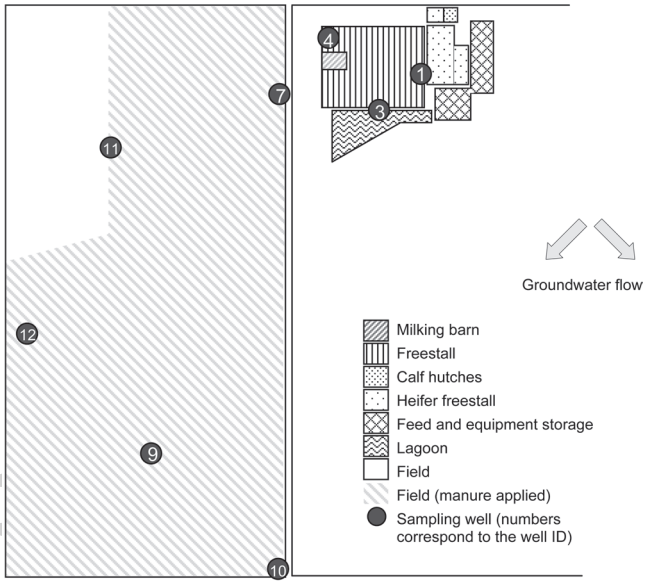


Fig. 2. Facility layout and sampling well locations at Dairy I.

around the lagoon to average any spatial variability. The samples were composited, and the final sample volume was decanted.

All ground water, flush lane, and lagoon water samples were collected in amber glass bottles with Teflon-lined caps and stored on ice for transport to the laboratory. Ground water samples were filtered through 0.3-μm glass fiber filters (Advantec, Dublin, CA) on site. Flush lane and lagoon water samples were transported to the laboratory on ice and centrifuged before filtration through a 0.3-μm glass fiber filter (Advantec, Dublin, CA). Quality control blanks were obtained using nanopure water (Barnstead International, Dubuque, IA) brought to the field and transferred into sampling bottles in the field. All samples were stored at 4°C until they were extracted. Samples were extracted as soon as practical and stored at −20°C until analysis. Extracts were analyzed within 3 mo of sample collection.

Chemical Preparation

Monensin concentrations in ground water, flush lane, and lagoon water samples were analyzed with a method adapted from Cha et al. (2005). Briefly, Oasis HLB cartridges (60 mg per 3 mL; Waters, Millford, MA) were used for solid phase extraction. Cartridges were preconditioned with 3 mL of methanol, 3 mL of 0.5 mol L^{-1} HCl, and 3 mL of water. Samples

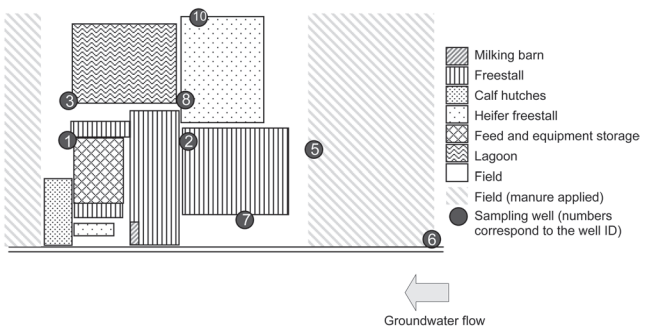


Fig. 3. Facility layout and sampling well locations at Dairy II.

Table 1. Properties of monensin A sodium.

Properties	Monensin A sodium
CAS† number	22373-78-0
Formula	$C_{36}H_{61}NaO_{11}$
Molecular weight	692.8601
Melting point	267–260‡
Water solubility	63 mg L^{-1} (pH 7)§, 0.85 mg L^{-1} (pH 9)§
Log K_{OW}¶	4.24 (pH 5)§, 2.75 (pH 7)§, 3.79 (pH 9)§
Vapor pressure	NA#
Henry's law constant	NA
pK_a	6.65 (66% dimethylformamide)§
Hydrolysis	no observed hydrolysis§
Photolysis	half-life 43.9 d§
Degradation in soil as microbial assay	half-life 5.8 d (with feces), 7.3 d (without feces)§
As radioactivity	7.4 wk††
As monensin A concentration	half-life 13.5 d‡‡ (laboratory conditions) half-life 3.3 d‡‡ (manure amended field) half-life 3.8 d‡‡ (manure free field) half-life 2.0, 1.3 d§§ (without manure) half-life 1.6 d§§ (with manure)

† CAS, Chemical Abstracts Service.

‡ Brimble (2004).

§ Elanco Products Company (1989).

¶ K_{OW}, octanol water partitioning coefficient.

NA, not available.

†† Fitted to the data set in Elanco Products Company (1989).

‡‡ Carlson and Mabury (2006).

§§ Sassman and Lee (2007).

were passed through cartridges at a flow rate of approximately 5 mL min^{-1}. The sample volume was 125 mL. After extraction, the cartridges were rinsed with 3 mL of water. The analyte was eluted with 5 mL methanol, and 12 μL of 1.0 mg L^{-1} simeton solution in methanol was added. The extract was evaporated to dryness at ambient temperature using nitrogen and reconstituted in 50 μL of methanol and 70 μL of water with 0.1% formic acid. All samples were analyzed in triplicate.

Monensin sodium salt (purity, 97%) was purchased from Acros Organics (Fair Lawn, NJ). The properties of monensin are shown in Table 1. The internal standard simeton was obtained from Absolute Standards Inc. (Hamden, CT). Optima-grade methanol and water with 0.1% formic acid were purchased from Fisher Chemical (Pittsburgh, PA). Water was treated with the Nanopure system (Barnstead, Dubuque, IA) or the milli-Q system (Millipore, Billerica, MA).

The liquid chromatography system was an Agilent 1100 LC (Palo Alto, CA) with a Gemini C18 column (50 × 2.0 mm) with 5 μm pore size (Phenomenex, Torrance, CA). The injection volume was 40 μL, and the flow rate was 0.30 mL min^{-1}. The liquid chromatography column temperature was maintained at 25°C. The mobile phase was methanol and water with 0.1% formic acid with a linear isocratic ratio of 80:20.

Mass spectra were acquired in positive ion electrospray on an 1100 Series LC/MSD Trap (Agilent, Palo Alto, CA). The drying gas was operated at a flow rate of 10 L min^{-1} at 350°C. The nebulizer pressure was 1.38 bar, and the capillary voltage was set at –2500 V. The ion trap induced fragmentation of the precursor compound at the amplitude of the excitation of 1 V. Mass spectra of the precursor and the product were collected. For monensin A, the precursor compound is the protonated sodium salt ion, $[M + Na]^+$ (mass over charge [m/z] 693.5), and the product compound is a sodiated sodium salt $[M + Na - H_2O]^+$ (m/z 675.5). Mass spectra were collected using an electron multiplier setting of 1550 V, an abundance target of 30,000, and a maximum accumulation time of 300 ms. Isolation width was 2.0 m/z, and fragmentation amplitude was 1.0 V. The m/z scan range examined was 100 to 1000 Da. The product ion $[M + Na - H_2O]^+$ (m/z 675.5) was used for selected reaction monitoring and quantitation in liquid chromatography–tandem mass spectrometry. Quantitation was based on the ratio of the base peak ion of the analyte to the base peak ion of the internal standard. A calibration curve was constructed for monensin spiked in water before extraction at a concentration range of 0.005 to 5.0 μg L^{-1}. For quantitation of October 2006 samples from Dairy II, a standard curve using responses of monensin only without the internal standard was used because there was an overlapping peak on the internal standard probably from dissolved organic matter.

The method detection limit was determined using the method recommended by USEPA (USEPA, 1999). Seven samples of 0.02 μg L^{-1} monensin in Nanopure water were analyzed by the method described previously. The sample SD was multiplied by Student's *t* variate for a one-sided *t* test at the 99% confidence level. The method detection limit was determined to be 0.009 μg L^{-1}.

To assess matrix effects, recoveries were determined in triplicate using (filtered) ground water samples from well 6 at Dairy II (Fig. 3) and Dairy II centrifuged lagoon water spiked with monensin at the additional concentration of 0.1 μg L^{-1}. Well 6 at Dairy II is located upstream of the dairy farm, and this ground water is considered representative of background conditions. The recoveries were 104.8 ± 6.6% CV for the ground water spike and 99.7 ± 6.5% CV for the lagoon spike, indicating quantitative recovery of the method. The matrix effects were considered minimal.

Results and Discussion

Our first task was to estimate the amount of monensin that theoretically would be found in the dairy wastewater streams. Before and during the study, Dairy I administered monensin to calves, heifers, and lactating cows, whereas Dairy II administered it only to dry cows. The total amount of monensin used was therefore significantly different between the dairies: Dairy I used a total of 389 g d^{-1}, and Dairy II used a total of 31 g d^{-1} (Table 2). To estimate the fraction of the administered amount that would have likely been excreted, we used the results of Donoho et al. (1978), which showed that after dosing ^{14}C-labeled monensin to two steers, 40 and 50% of the radioactivity in feces was counted from unmetabolized monensin. According to another study using ^{14}C-labeled monensin in three steers, 94, 88, and 102% of the radioactivity were excreted in feces and none in urine within 12 d after dosing (Herberg et al., 1978). Thus, if we assume that 50% of monensin is excreted unmetabolized, 195 and 15.5 g d^{-1} of monensin would have been discharged in Dairy

Table 2. Documented monensin and water use and estimated wastewater monensin concentrations (lagoon water concentrations).

Dairy	Monensin use	Water use estimate†	Lagoon volume	Retention time	Theoretical monensin concentration in lagoon‡
	g d^{-1}	m^3 d^{-1}	m^3	d	μg L^{-1}
Dairy I	389	792	6.66×10^4	84.1	246
Dairy II	31	373	8.98×10^4	241	42

† Estimated based on Meyer et al. (2006).

‡ Theoretical monensin concentration in lagoon = monensin use × excretion rate (50%) × retention time/lagoon volume. Assumed no attenuation.

I and Dairy II, respectively, almost entirely in the feces. This corresponds to total monensin concentrations in wastewater (solids and liquid combined) of 246 and 42 μg L^{-1} for Dairy I and Dairy II, respectively (Table 2), when considering the amount of water used in those dairies (Meyer et al., 2006). Although water use estimates are not precise, these concentrations offer a good order-of-magnitude estimate of maximum monensin concentrations to be expected in the wastewater stream.

Monensin in the Manure Management System

Monensin was detected in flush lane and lagoon water samples at both dairies. Concentrations in lagoon water ranged from 3.91 to 16.24 μg L^{-1}, in flush lane samples from 1.89 to 7.14 μg L^{-1}, and in calf hutches flush water from 0.42 to 0.64 μg L^{-1} (Table 3). Monensin concentrations in lagoon water samples in this study are slightly lower than the concentration that Sassman and Lee (2007) detected in beef lagoon water at 40 μg L^{-1}. The concentrations of monensin in lagoon water samples were similar between the two dairies despite the fact that Dairy I used 10 times more monensin than Dairy II. This may be due to the differences in solid separation efficiencies between the two dairies. Solid waste in the flush water system is separated before entering lagoons. Because monensin is excreted in feces, less monensin enters the lagoons if solid separation efficiency is high. At Dairy I, solid separation is achieved by mechanical separation, followed by a settling basin. On Dairy II, only a settling basin is used. Distribution of monensin between fecal solid and flush lane water needs further study.

Because this work is focused on the transport of monensin into the ground water environment, we focus here exclusively on the amount dissolved in water. Colloidal particles are thought not to be significantly transported into the subsurface due to the absence of macropores in the soil and subsurface (very low clay content). Detected concentrations of monensin in lagoon water are one order of magnitude smaller than the loading computed from monensin use assuming no attenuation in the waste transport and storage system (Table 2). The mechanisms that most likely attenuate the dissolved concentration of monensin are biodegradation and sorption to solids.

A lower limit of the approximate half-life of monensin in the waste storage system can be estimated by assuming a completely mixed and steady-state system, first-order biodegradation, and complete dissolution of monensin in the aqueous phase, but, for the moment, neglecting sorption and using the

Table 3. Monensin concentrations and properties of the water samples.

Sample	Monensin	pH	EC†	DO
	μg L^{-1}		dS m^{-1}	mg L^{-1}
Fall sampling, Oct. 2007				
Dairy I				
Flush lane	1.89 (0.66)‡	7.3	5.78	NA§
Calf hutches flush	0.64 (0.17)	7.4	1.22	NA
Ground water 1	BD¶	7.3	1.67	NA
Ground water 4	BD	7.2	1.44	NA
Ground water 7	BD	7.0	1.40	NA
Ground water 9	BD	7.2	0.99	NA
Ground water 10	BD	7.7	1.76	NA
Ground water 11	BD	7.1	1.80	NA
Ground water 12	BD	7.0	1.21	NA
Dairy II				
Flush lane	7.14# (0.51)	7.6	6.64	NA
Lagoon	3.91# (0.05)	7.8	6.13	NA
Ground water 1	BD	7.0	2.99	NA
Ground water 2	BD	7.3	2.23	NA
Ground water 3	0.36# (0.06)	7.1	5.24	NA
Ground water 5	BD	7.1	1.83	NA
Ground water 6	BD	7.3	1.24	NA
Ground water 7	BD	7.1	1.48	NA
Ground water 8	BD	7.3	1.78	NA
Spring sampling, Apr./May 2007				
Dairy I				
Flush lane	3.58 (0.16)	7.7	6.47	NA
Calf hutches flush	0.42 (0.04)	7.9	1.03	NA
Lagoon	16.24 (1.26)	7.6	7.50	NA
Ground water 1	BD	7.0	1.87	0.7
Ground water 3	0.39 (0.02)	7.0	3.72	0.4
Ground water 4	BD	7.2	1.58	1.7
Ground water 7	BD	7.0	1.51	0.7
Ground water 9	BD	7.0	1.26	0.5
Ground water 10	BD	7.4	1.92	5.4
Ground water 11	BD	7.0	1.83	2.0
Ground water 12	BD	7.1	1.20	0.7
Dairy II				
Flush lane	3.37 (0.19)	7.5	5.86	NA
Lagoon	6.34 (0.17)	7.5	4.91	NA
Ground water 1	BD	7.8	2.71	NA
Ground water 2	0.04 (0.001)	7.2	2.17	1.3
Ground water 3	0.30 (0.02)	7.4	5.95	0.7
Ground water 5	BD	7.0	2.09	1.1
Ground water 6	BD	7.1	1.21	1.1
Ground water 7	BD	7.1	1.33	0.9
Ground water 8	BD	7.2	1.95	1.0
Ground water 10	0.07 (0.002)	7.4	2.25	1.0

† EC, electrical conductivity; DO, dissolved oxygen.

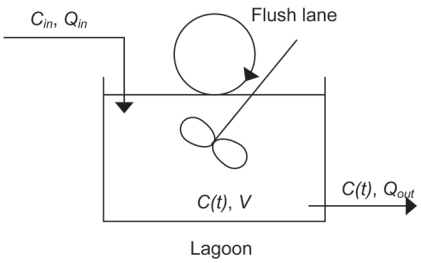
‡ All the samples are analyzed in triplicate. Numbers in parentheses represent SDs among the results from the three analyses.

§ NA, not analyzed.

¶ BD, below method detection limit.

Quantified using the standard curve of monensin only without the internal standard because of overlapping dissolved organic matter peaks on the internal standard.

estimated lagoon inflow and outflow rates (Fig. 4). At Dairy I and II, the values are 4.1 and 23 d, respectively. These results are consistent with other studies on monensin disappearance in soil: Carlson and Mabury (2006) observed monensin half-lives in soil to be 13.5 d in controlled laboratory study, 3.8 d in the field study without manure, and 3.3 d in the field study with manure addition. Sassman and Lee (2007) reported



C_{in} (μg/L): concentration of monensin in the inflow

$$= \frac{\text{monensin discharge (mg/d)}}{\text{water use(L/d)}}$$

Q (L/d): daily water use

Q_{in} (L/d): inflow

Q_{out} (L/d): outflow

$C(t)$ (μg/L): concentration of monensin in the lagoon at time t

V (L) : lagoon volume

<Asumptions>

- $C(t)$ is uniform throughout the lagoon.

- $Q = Q_{in} = Q_{out}$

- Biodegradation in the lagoon is first order with a rate constant k(d^{-1})

- The system is at steady-state

$$\lim_{\Delta t \to 0} \frac{\Delta C}{\Delta t} = \frac{C(t+\Delta t) - C(t)}{\Delta t}$$

Fig. 4. Estimation of biodegradation half-lives and assumptions.

half-lives in soil to be less than 2 d with and without manure addition. Sassman and Lee (2007) reported the disappearance of monensin A under abiotic conditions. Our results suggest that the attenuation rates may be similar in soils and in wastewater.

Another likely component of the observed loss is partitioning to particles. Possible sorption mechanisms to particles include hydrophobic partitioning, cation exchange, cation bridging at clay surfaces, surface complexation, and hydrogen bonding (Tolls, 2001); however, it is not clear which mechanism, if any, dominates monensin partitioning to solids. The octanol water partitioning coefficient of the monensin sodium complex at pH 7 is 2.75 (Table 2). This is not particularly high, suggesting that hydrophobic partitioning is unlikely to dominate sorption. Monensin forms a strong pseudo-macrocyclic complex with sodium (Huczynski et al., 2007; Martinek et al., 2000). When the complex is formed, charges of the functional groups of the monensin molecule are shielded, making the molecule more hydrophobic. In this state, there are no obvious ionized functional groups available to interact with charged mineral surfaces. Sassman and Lee (2007) observed pH-dependent sorption of monensin; however, speciation-dependent partitioning did not fully predict monensin sorption. They listed the coordinated complexation of monensin with inorganic cations of different sorption properties as a possible cause. Sassman and Lee (2007) reported K_d values of monensin ranging from 0.915 to 78.6 L kg^{-1} for soils and 50 L kg^{-1} for suspended solids in a beef lagoon. Davis et al. (2006) reported the pseudo-partitioning coefficient in a runoff system to be 6 L kg^{-1}. Assuming that the K_d value is 50 L kg^{-1} and based on the observed dissolved-phase concentrations, the solid phase concentrations in the study site lagoons would be 812 μg kg^{-1} (Dairy I, spring sampling), 197 μg kg^{-1} (Dairy II, fall sampling), and 317 μg kg^{-1} (Dairy II, spring sampling). Approximately 13% of the total monensin would be partitioned to the solid phase, and 87% would be in the aqueous phase at equilibrium. Hence, even after accounting for sorption, degradation rates must be close to the above estimates to explain the observed order-of-magnitude difference between total excreted and total recovered monensin on the dairy. However, we did not independently confirm these solid phase concentrations. The extent and mechanisms of monensin sorption to solids remain to be studied in more detail.

Monensin in Ground Water

In ground water, monensin was detected in one of eight shallow monitoring wells at Dairy I and in three of eight shallow monitoring wells at Dairy II at concentrations ranging from 0.04 to 0.39 μg L^{-1} (Table 3). The detection of monensin in some of the ground water samples demonstrates that monensin has the potential to reach shallow alluvial ground water. Of the four wells with monensin above the detection limit, one well showed monensin at both sampling dates, one well showed monensin at one of the two sampling dates, and two wells were sampled only at the second sampling date.

Higher concentrations of monensin, ranging from 0.30 to 0.39 μg L^{-1}, were detected in ground water from well 3 at Dairy I and Dairy II. Well 3 at Dairy I was sampled only at the second sampling date, and well 3 at Dairy II was sampled at both sampling dates. The sources of these ground waters are the lagoons located immediately upgradient of these two wells (Fig. 2 and 3). Anaerobic lagoon water contains high levels of organic nitrogen and ammonium (NH_4–N) but almost no measurable nitrate (NO_3–N). In the ground water downgradient of the lagoons, high concentrations of dissolved nitrogen (>>10 mg N L^{-1}) occur in the reduced (NH_4–N) form (Table 3), indicating the presence of an anaerobic zone extending from the bottom of the lagoon to these wells and possibly beyond (Harter et al., 2002; Singleton et al., 2007). The lagoons are both over 30 yr old; they are lined with soil containing at least 10% clay but have been identified as leaking.

Ground water 2 at Dairy II is from a well close to lactating and dry cow freestalls, and ground water 10 is close to the lagoon and heifer freestalls. Monensin concentrations in these ground water samples were 0.04 and 0.07 μg L^{-1}, respectively, and were one order of magnitude lower than in ground water from well 3 and two orders of magnitude lower than the lagoon samples (Table 3). Unlike other wells, wells 2 and 10 were significantly turbid during the initial stages of well purging. Al-

though the detection of monensin suggests that corrals can be a source of monensin to shallow aquifer, the presence of turbidity suggest that direct leakage of surface runoff may have occurred, preventing a firm conclusion from these specific wells.

No monensin was detected in the monitoring wells affected by application of lagoon water on irrigated forage crops. This is consistent with the findings of Carlson and Mabury (2006), who did not detect monensin in soil samples from the 25- to 35-cm-depth zone after application in sandy loam soil at concentrations over 1 mg kg^{-1} with and without manure. Based on the absence of monensin in their samples, Carlson and Mabury concluded that biodegradation of monensin is likely to prevent it from reaching ground water.

There are several possible reasons monensin was detected in lagoon-effected wells but not in wells under fields where manure was applied. First, ground water from the lagoon wells at both of the dairies is subject to continuous leakage of lagoon water, whereas the fields were intermittently irrigated with lagoon water. Second, the monensin loading per recharge area was less in the field well source area than in the lagoon well source area. Finally, biodegradation rates may be different due to different soil bacterial consortia and redox conditions. Irrigation water is sufficiently high in dissolved oxygen to prevent pervasive anaerobic conditions in the subsurface of the field well source area, whereas an anaerobic zone exists at the bottom of the lagoon and extends for at least a few tens of meters into the shallow ground water (Harter et al., 2002; Singleton et al., 2007). Carlson and Mabury (2006) list possible differences in microbial community as a reason for differences in biodegradation half-lives in the laboratory (13.5 d) and in the field (3.3 and 3.8 d). Differences may exist between the predominantly anaerobic microbial community around the lagoon wells and the predominantly aerobic microbial community in ground water underneath fields with manure water applications. Effects of oxygen concentrations on biodegradation rates of monensin should be further studied.

Future research is necessary to confirm the detection and attenuation of monensin in waste streams and ground water in dairy farms and associated fields, especially with additional sampling events to investigate possible temporal/seasonal dynamics over a longer period of observation. In addition, monensin in the soil profile needs to be analyzed to determine whether attenuation is limited to upper soil layers or is likely to occur along the entire vadose zone–ground water path. To assess the effects and mechanisms of attenuation, biodegradation and sorption of monensin require further research. Determining the distribution of monensin between fecal solid and flush lane water is necessary to evaluate the effects of solid separation in controlling the amount of monensin entering the wastewater streams.

Conclusions

Major findings of this study are as follows: (i) Monensin persists at relatively high concentrations in the manure transport and storage system. Monensin was detected in flush lane water samples and in lagoon water samples at levels of 10^0 to 10^1 µg L^{-1}. (ii) Monensin levels measured in the manure transport and storage system are one order of magnitude lower than concentrations computed based on actual monensin usage and reported excretion-to-intake ratios. This suggests that there is significant attenuation in the waste handling and storage system. (iii) Monensin was detected in ground water samples within the production facility of dairy farms. It was detected in wells located within a shallow anoxic ground water zone associated with lagoon water recharge; there, detected concentrations were one order of magnitude lower than the source lagoon water, indicating some, but not complete, attenuation in the anoxic ground water environment. (iv) Monensin was not detected in ground water underneath fields that receive lagoon manure water. Monensin attenuation may be higher under predominantly aerobic subsurface conditions than under the anoxic conditions associated with the lagoon monitoring wells.

Table 4. Conditions used for biodegradation half-lives estimation.

	Dairy I	Dairy II
C_{in}, µg L^{-1}	0.246	0.042
Q, L	7.92×10^5	3.73×10^5
Retention time, d	84.1	240.6
V, L	6.66×10^7	8.98×10^7
$C_{steady\text{-}state}$, µg L^{-1}†	16	5
k, d^{-1}	0.17	0.03
Half-life, d	4.1	23

† $C_{steady\text{-}state}$ values are taken from detected concentrations in Table 3.

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References

Animal Health Institute. 2007. Trends in sales of lifesaving animal medicines continue. Available at http://ahi.timberlakepublishing.com/files/Media%20Center/Antibiotic%20use%202006.pdf (verified 2 July 2008). Animal Health Inst., Washington, DC.

Boxall, A.B.A., D.W. Kolpin, B.H. Surensen, and J. Tolls. 2003. Are veterinary medicines causing environmental risks? Environ. Sci. Technol. 37:265A–304A.

Brimble, M.A. 2004. Polyether antibiotics. *In* Kirk-Othmer encyclopedia of chemical technology. 5th ed. Vol. 20. John Wiley & Sons, New York.

California Department of Food and Agriculture. 2007. California Agricultural Resource Directory 2007. CDFA, Sacramento, CA.

Capleton, A.C., C. Courage, P. Rumsby, P. Holmes, E. Stutt, A.B.A. Boxall, and L.S. Levy. 2006. Prioritising veterinary medicines according to their potential indirect human exposure and toxicity profile. Toxicol. Lett. 163:213–223.

Carlson, J.C., and S.A. Mabury. 2006. Dissipation kinetics and mobility

of chlortetracycline, tylosin, and monensin in an agricultural soil in Northumberland County, Ontario, Canada. Environ. Toxicol. Chem. 25:1–10.

Cha, J.M., S. Yang, and K.H. Carlson. 2005. Rapid analysis of trace levels of antibiotic polyether ionophores in surface water by solid-phase extraction and liquid chromatography with ion trap tandem mass spectrometric detection. J. Chromatogr. A. 1065:187–198.

Chee-Sanford, J.C., R.I. Aminov, I.J. Krapac, N. Garrigues-Jeanjean, and R.I. Mackie. 2001. Occurrence and diversity of tetracycline resistance genes in lagoons and ground water underlying two swine production facilities. Appl. Environ. Microbiol. 67:1494–1502.

Davis, J.G., C.C. Truman, S.C. Kim, J.C. Ascough, and K. Carlson. 2006. Antibiotic transport via runoff and soil loss. J. Environ. Qual. 35:2250–2260.

Donoho, A., J. Manthey, J. Occolowitz, and L. Zornes. 1978. Metabolism of monensin in steer and rat. J. Agric. Food Chem. 26:1090–1095.

Elanco Products Company. 1989. Environmental assessment for the use of Rumensin premixes in the feed of beef cattle for the prevention and control of coccidiosis. Elanco Products Company, Indianapolis, IN.

Federal Register. 2004. New Animal Drugs; Monensin. 69 Federal Register (26 Nov. 2004). Available at http://www.epa.gov/fedrgstr/EPA-IMPACT/2004/November/Day-26/i26091.htm (verified 23 June 2008).

Fernandez, C., C. Alonso, M.M. Babin, J. Pro, G. Carbonell, and J.V. Tarazona. 2004. Ecotoxicological assessment of doxycycline in aged pig manure using multispecies soil systems. Sci. Total Environ. 323:63–69.

Hamscher, G., S. Sczesny, H. Hoper, and H. Nau. 2002. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal. Chem. 74:1509–1518.

Hao, C., L. Lissemore, B. Nguyen, S. Kleywegt, P. Yang, and K. Solomon. 2006. Determination of pharmaceuticals in environmental waters by liquid chromatography/electrospray ionization/tandem mass spectrometry. Anal. Bioanal. Chem. 384:505–513.

Harter, T., H. Davis, M.C. Mathews, and R.D. Meyer. 2002. Shallow ground water quality on dairy farms with irrigated forage crops. J. Contam. Hydrol. 55:287–315.

Herberg, R., J. Manthey, L. Richardson, C. Cooley, and A. Donoho. 1978. Excretion and tissue distribution of monensin-C-14 in cattle. J. Agric. Food Chem. 26:1087–1090.

Hillis, D.G., L. Lissemore, P.K. Sibley, and K.R. Solomon. 2007. Effecs of monensin on zooplankton communities in aquatic microcosms. Environ. Sci. Technol. 41:6620–6626.

Huczynski, A., M. Ratajczak-Sitarz, A. Katrusiak, and B. Brzezinski. 2007. Molecular structure of the 1:1 inclusion complex of monensin A sodium salt with acetonitrile. J. Mol. Struct. 832:84–89.

Kim, S.C., and K. Carlson. 2006. Occurrence of ionophore antibiotics in water and sediments of a mixed-landscape watershed. Water Res. 40:2549–2560.

Kolpin, D.W., E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, and H.T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. Environ. Sci. Technol. 36:1202–1211.

Lissemore, L., C. Hao, P. Yang, P.K. Sibley, S. Mabury, and K.R. Solomon. 2006. An exposure assessment for selected pharmaceuticals within a watershed in Southern Ontario. Chemosphere 64:717–729.

Martinek, T., F.G. Riddell, C. Wilson, and C.T. Weller. 2000. The conformations of monensin-A metal complexes in solution determined by NMR spectroscopy. J. Chem. Soc. Perk. T. 22:35–41.

Mellon, M., C. Benbrook, and K.L. Benbrook. 2001. Hogging it: Estimates of antimicrobial abuse in livestock. Union of Concerned Scientists, Washington, DC.

Meyer, D., B. Reed, C. Batchelder, I. Zallo, P.L. Ristow, G. Higginbotham, M. Arana, T. Shultz, D.D. Mullinax, and J. Merriam. 2006. Water use and winter liquid storage needs at central valley dairy farms in California. Appl. Eng. Agric. 22:121–126.

Page, R.W., and G.O. Balding. 1973. Geology and quality of water in the Modesto-Merced area, San Joaquin Valley, California. Water-Resour. Invest. 6:85. USGS, Washington, DC.

Sassman, S.A., and L.S. Lee. 2007. Sorption and degradation in soils of veterinary ionophore antibiotics: Monensin and lasalocid. Environ. Toxicol. Chem. 26:1614–1621.

Schlüsener, M.P., M. Spiteller, and K. Bester. 2003. Determination of antibiotics from soil by pressurized liquid extraction and liquid chromatography-tandem mass spectrometry. J. Chromatogr. A. 1003:21–28.

Schmitt, H., P. Van Beelen, J. Tolls, and C.L. Van Leeuwen. 2004. Pollution-induced community tolerance of soil microbial communities caused by the antibiotic sulfachloropyridazine. Environ. Sci. Technol. 38:1148–1153.

Sengelov, G., Y. Agerso, B. Halling-Sorensen, S.B. Baloda, J.S. Andersen, and L.B. Jensen. 2003. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. Environ. Int. 28:587–595.

Singleton, M.J., B.K. Esser, J.E. Moran, G.B. Hudson, W.W. McNab, and T. Harter. 2007. Saturated zone denitrification: Potential for natural attenuation of nitrate contamination in shallow ground water under dairy operations. Environ. Sci. Technol. 41:759–765.

Tolls, J. 2001. Sorption of veterinary pharmaceuticals in soils: A review. Environ. Sci. Technol. 35:3397–3406.

U.S. National Library of Medicine. 2008. ChemIDPlus Advanced. Available at http://chem.sis.nlm.nih.gov/chemidplus/ (verified 23 June 2008). U.S. National Library of Medicine, Bethesda, MD.

USDA. 2002. Part II: Changes in the United States Dairy Industry, 1991–2002. USDA:APHIS:VS,CEAH, National Animal Health Monitoring System, Fort Collins, CO.

USEPA. 1999. Title 40: Protection of Environment, Part 136 Guidelines Establishing Test Procedures for the analysis of pollutants. USEPA, Washington, DC.

Wollenberger, L., B. Halling-Sorensen, and K.O. Kusk. 2000. Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna*. Chemosphere 40:723–730.