EVALUATION OF VETERINARY PHARMACEUTICAL AND IODINE FOR USE AS A GROUNDWATER TRACER IN HYDROLOGIC INVESTIGATION OF CONTAMINATION RELATED TO DAIRY CATTLE OPERATIONS

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Abstract

Standard groundwater tracers such as Rhodamine WT, Fluorescein, Eosin and Tinopal CBX effectively provide a snapshot of hydrological conditions over a brief period of time and in a tightly controlled setting. However, in complex environmental situations with multiple potential sources, groundwater hydrologists are often seeking groundwater tracers that have extended longevity in the natural environment and the ability to directly pinpoint source locations. After reviewing operations of the nearby dairy it was determined that emerging contaminants, specifically two bovine veterinary pharmaceuticals (antibiotics), cephalixin sodium (CEPNa) and cephalixin benzathine (CEPB), and a sanitation agent, elemental Iodine (I) may have potential as extended longevity groundwater tracers if analytical methodology could be established. Initially, sample analysis indicated that cephalixin is undetectable in unconcentrated samples of lagoon wastewater at parts per billion (ppb) concentration; pre-concentrated samples which utilized solid phase extraction allowed for better detection at part per trillion level. Concentrated samples from one of the two lagoon cells sampled (cell #3), detected cephalixin at 13.14 ppt level, while cell #1 failed to detect any cephalixin present. Controlled laboratory testing later indicated that in a wastewater environment cephalixin degrades to approximately 20% of initial concentrations within 4 days, with complete degradation within 6 days. Degradation patterns in surface water and groundwater samples were less dramatic and at slower rates. Degradation curves of the surface and groundwater samples indicate that concentrations of cephalixin are still detectable for approximately 25 days. Unconcentrated Iodine samples collected in lagoon cells ranged from 50.896 ppb and 1,704.55 ppb with variations determined to be a result of the primary inflow of the lagoon. Cephalixin's use as

a long term groundwater tracer does not seem to be an immediate option. Further research may reveal that its degradation products are potentially useful as a tracer. In some instances, such as catastrophic discharges of large volumes of milk when samples can be collected and analyzed quickly, the use of cephalixin as an environmental tracer may prove possible. The validity of pharmaceutical iodine as a groundwater tracer appears to be much greater than that of cephalixin. Iodine was detected in all of the environmental samples including the highly organic and anaerobic environment of the dairy wastewater lagoon. This study concludes that iodine is capable of surviving the hostile wastewater environment. If sufficient data is collected to determine natural background levels, iodine may prove useful in determining hydrological connections between iodine laden dairy effluent and the underlying groundwater.

Introduction

Investigation of the hydrologic environment in the vicinity of a fitness center in Southeast Missouri began in the summer of 2010. This investigation included a series of water traces utilizing the standard fluorescent dyes such as fluorescein and Rhodamine WT to evaluate potential sources of bacteria entering the center's well. The sources included surface water infiltration surrounding the wellhead, onsite septic system failures and a nearby dairy operation (Pierce, 2012).

After reviewing operations of the nearby dairy it was determined that additional groundwater tracers may be present in the form of veterinary pharmaceuticals and sanitizing agents. A two-fold investigation was then initiated. The first phase of the investigation was to determine if these pharmaceuticals/sanitizing agents were detectable in a laboratory environment. Once the analysis methodology was proven to be successful,

the potential for their survival in natural environments or highly organic and anaerobic conditions of dairy wastewater lagoons needed to be determined. The second phase was to then test the applicability of the pharmaceuticals and sanitizing agents as long term groundwater tracers in the natural environment.

Background

Literature reviews and research revealed that the potential for water tracing using emerging contaminants such as bovine pharmaceutical compounds, trace elements and other chemicals used in sterilization and milking process may be possible (Boxall et al., 2003; Drewes et al., 2003; Jones, 2009).

Emerging contaminants, specifically two bovine veterinary pharmaceuticals (antibiotics), cephalixin sodium (CEPNa) and cephalixin benzathine (CEPB), and a sanitation agent, elemental Iodine (I) are detectable in groundwater samples (Santschi, et al; 1999) and have potential for use as long-term groundwater tracers for hydrogeologic investigations where dairy operations are a potential source of contaminant (Boxall et al., 2003; Drewes et al. 2003; Jones, 2009).

Through the course of several conversations the State of Missouri's dairy inspector indicated that the pharmaceutical products cephalixin sodium (CEPNa) and cephalixin benzathine (CEPB) were routinely used for treatment of dairy cows at the nearby dairy operation. According to literature the vast majority of cephalixin is excreted unchanged in the urine. Most excretion occurs within 8 hours of application and the process of excretion completed within 72 hours (Jones, 2009). Additionally, the inspector indicated that iodine is used as a sterilization agent for the cleaning of teats both before and after milking at the dairy operation. It too is washed to the lagoon and thus has the potential to reach the natural environment in a similar manner to the cephalixin.

Further literature research indicated that some pharmaceuticals and iodine are detectable in groundwater samples. (Santschi et al., 1999) Therefore, if analyzable and detectable in environmental samples, CEPNa, CEPB, and iodine may potentially be used as tracers to investigate the groundwater hydrology of the CAFFC area to determine whether the contamination in the affected well is related to the dairy operations.

If a hydrologic connection between the CAFFC well and the nearby dairy operation exist then testing should reveal cephalixin, used in the dairy operation, to be present in CAFFC water samples. Additionally, iodine levels should be elevated in water samples when compared to natural background iodine concentrations.

To determine if Cephalixin was readily detectable Dr. Honglan Shi at Missouri University of Science and Technology was contracted to develop liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods to analyze water samples for the bovine antibiotic. In addition Dr. Shi developed protocol for inductively coupled plasma mass spectrometry (ICP-MS) to analyze for total iodine concentrations. Upon completion, the methodological process was put into service to analyze for these constituents in the field samples associated with the CAFFC well investigation.

Cephalixin and Iodine Analysis Development

The results of the cephalixin analysis methodology development were mixed. Dr. Shi was able to successfully develop a reliable, fast and simple method for detecting the base Cephalixin (CEP) molecule in water samples to the part per trillion (ppt) level. However difficulties were also observed in the analysis of field samples, stability studies showed that the Cephalixin degrades to form a degradant (DACEP) in all sample matrices (water and lagoon effluent). In a series of further test conducted on spiked samples the degradation was shown to be highest in the lagoon samples with complete degradation of CEP to DACEP within six days when left at room temperature. Figure 1 highlights these degradation rates. Analysis of samples in both groundwater and surface water matrices also show similar degradation patterns, but at a slower rate. Testing revealed that the currently analyzable degradants was also unstable and quickly degraded to undetectable levels.

The ICP-MS analysis methodology for total iodine in water samples was previously developed by Dr. Shi and the MS&T laboratory in 2009 and would be used for the investigation (Shi and Adams, 2009).

Field Sampling and Investigation

As part of the initial dye tracing, a well survey was conducted by MGS to locate and inventory any groundwater wells servicing residence, farms, or

Figure 1. Cephapirin degradation patterns by matrix.

businesses in the vicinity of the CAFFC. This survey was expanded to additional locations to help establish a more representative natural Iodine background for the area.

Following the survey, wells in the vicinity of the CAFFC site were sampled and analyzed for the presence of cephapirin and iodine. Wastewater samples were

collected from each cell of dairy's two-cell wastewater lagoons. A representative population of inventoried wells was selected for sampling and analysis to establish natural background levels of iodine. As well as a small nearby spring and surface water samples from Williams Creek and the small tributary below the dairy operation. A total of nineteen water samples were initially collected for analysis. Two additional samples were collected at a later date to allow for the SPE and pre-concentration analysis. Figure 2 is a map showing all sample locations.

Samples were collected using procedures to meet MDNR standards and the methodology needs for Dr. Shi's analysis. Water samples were collected in pre-cleaned, 4-ounce, Teflon- capped, wide-mouth amber bottles. For well water collection at homes, the faucet aerator was first removed (if present) and the water allowed to flow for approximately 5 minutes. Sample bottles were then filled. Surface water sample bottles were submersed and filled directly in the sampling bottle. For wastewater

Figure 2. Sample location map.

samples a reusable sample dipper was utilized and subsequently cleaned and decontaminated between uses to avoid cross-contamination. Field blanks, trip blanks, and duplicate samples were collected throughout the sampling event as a quality assurance measure. The collected samples were sealed and placed in an iced cooler then transferred to the University of Missouri Science and Technology laboratory using standard MDNR chain of custody protocol.

Results

Analysis of collected samples initially indicated no detection of cephalixin in the wastewater lagoons at part per billion (ppb) levels. A second round of sampling was conducted on the wastewater lagoons. At this time larger volumes of sample were collected so that a solid phase extraction (SPE) could be used to pre-concentrate the cephalixin and allow for better detection sensitivity to the PPT level. In this analysis large volumes of lagoon waste were reduced to approximately 10% of its original amount while still retaining the concentrations of cephalixin. In this second analysis a two liter sample taken from the taken from cell #3 of the dairy lagoon treatment system, detected 13.14 ppt of Cephalixin. Analysis of a sample from cell #1 failed to detect any cephalixin.

Iodine levels collected from the study area ranged from 4.939 ppb to 1704.55 ppb and are shown in Table 1. Iodine was detected in all eight drinking water wells with concentrations ranging from 4.939 to 24.712 ppb with a mean concentration of 12.532 ppb and a standard deviation of 6.672 ppb. Six surface water samples were collected and analyzed with concentrations ranging from 6.671 to 21.247 ppb. The mean concentration of iodine in surface water samples was 14.732 ppb with a standard deviation of 5.489 ppb. A single sample from a shallow spring was collected and determined to have 5.855 ppb iodine present.

Samples collected and analyzed from lagoon cell #1 and lagoon cell #3 contained 50.896 ppb and 1,704.55 ppb iodine, respectively. A considerable difference in iodine concentrations is obvious in the lagoon samples. Further investigation revealed that the lagoons are not successive and that the source area and influents of waste were different for each. The primary waste source for lagoon cell #1 is runoff from the animal feed lot and loafing areas. Lagoon cell #3 received

some barnyard runoff similar to cell #1. However, the influent of wash water from the milking parlor was also observed discharging into the cell. Since iodine is used to sanitize teats prior to milking its presence in the daily cleaning effluent is not uncommon and likely explains the large difference in iodine concentration between cell #1 and cell #3.

Conclusion

Due to the instability of cephalixin its use as a long term groundwater tracer does not seem to be an immediate option. Future research may at some point identify a specific cephalixin degradant product that has the stability and environmental longevity required for groundwater tracing, but at this time those degradants have yet to be identified. In some instances such as direct runoff, catastrophic lagoon failures or discharges of large volumes of milk, where samples can be collected and analyzed in a rather quick time frame, the use of cephalixin or its degradants as an environmental tracer may prove possible.

Table 1. Sample locations and iodine concentrations.

Sample ID	Sample Name	Concentration PPB
FB001	Field Blank	<0.2
TB001	Trip Blank	<0.2
WW001	Class Act Family Fitness Center	16.178
WW002	Koehler Engineering Well	11.472
WW003	University Farm Well	11.93
WW004	Volkerding Well	4.939
WW005	New Midway Dairy Well	24.712
WW006	Old Midway Dairy Well	6.255
WW007	Dave Brown Well	19.31
WW008	Wayne Eakins Well	5.457
SS001	Upper Williams Creek	6.671
SS002	Lower Williams Creek	9.216
SS003	Tributary at Shale Lane	21.247
SS004	Tributary just above confluence with Williams Creek	20.23
SS005	Tributary just below outfall of dairy lagoon #3	13.117
SS006	Tributary above dairy lagoon outfall (Ponco Lane)	17.913
SP001	Spring on University Farm	5.855
LG001	Lagoon Cell #1	50.896
LG002	Lagoon Cell #3	1704.55

The validity of iodine as an extended-longevity groundwater tracer appears to be much higher than the cephalixin. Iodine was detected in all of the environmental samples including the highly organic and anaerobic environment of the dairy wastewater lagoon. Iodine levels within dairy lagoons #1 and #3 were found to be 206% to 6898% higher than background samples. This study concludes that iodine is capable of surviving a hostile wastewater environment. If sufficient data is collected to determine natural background levels, the use of iodine may prove useful in determining hydrological connections between iodine laden dairy effluent and the underlying groundwater.

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