Title: Selection and Evaluation of Chemical Indicators for Waste Stream Identification

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Background/Need: Human and animal waste poses a threat to the quality of groundwater, surface water and sources of drinking water. This is especially of concern for private and public water supplies in agricultural areas of Wisconsin where land spreading of livestock waste occurs on thin soils overlaying fractured bedrock. Current microbial source tracking methods for reliable source identification requires the use of expensive and time consuming testing using polymerase chain reaction (PCR) techniques. Due to cost, these tests are often not an option for homeowners, municipalities or state agencies with limited resources. The Water and Environmental Analysis Laboratory (WEAL) sought to develop a method to provide a lower cost analytical technique to determine sources of fecal waste using fecal sterols/stanols, pharmaceuticals (human and veterinary) and human care/use products in ground and surface waters using solid phase extraction techniques combined with liquid chromatography and triple quadrupole mass spectrometry (LC/MS/MS).

Objectives: The objectives of this study were to identify a series of chemicals that can distinguish a source of fecal waste. The chemicals include fecal sterols and stanols, pharmaceuticals and personal care products (PPCPs), and artificial sweeteners. Once identified, analytical methods were assessed to provide necessary sensitivity (low part per trillion) in aqueous samples.

Methods: Groundwater samples from private wells that were suspected to be contaminated with fecal waste were acquired through WDNR field agents and the Wisconsin State Laboratory of Hygiene (WSLH). Samples were also obtained from known waste sources including livestock slurry tanks, municipal wastewater treatment facilities, and private septic systems. Groundwater samples were analyzed for biological contamination using polymerase chain reaction techniques at the WSLH. All samples were analyzed for fecal sterols/stanols, PPCPs and artificial

sweeteners were analyzed at the WEAL using solid phase extraction and LC/MS/MS. Analytical methods for chemicals are detailed in the report and biological methods are referenced.

Results and discussion: Limiting factors to this study were the small number of samples received and the apparent mix of human and bovine waste in 9 of 11 samples as reported by WSLH. Mixtures of waste obstructed the determination of source identification though fecal sterol ratio analysis. Six ratios of fecal sterols from five independent researchers were used in an attempt to identify sources as bovine, human or mixed waste. Of the 11 samples with MST data, 9 contained both human and bovine *Bacteroides*. If the assumption is made that the MST process is the most reliable method, the ratios R1 (Gourmelon) and R3 (Evershed) accurately predicted the waste as being from a mixed source in 78% of the samples. The PCR method referenced by WSLH (Layton et al., 2006) asserts a 100% positive identification (0% false positive) for the bovine-associated *Bacteroides* 16S-rRNA gene sequence by real time PCR. However, the human-associated *Bacteroides* analysis for the same gene sequence is apparently similar to that of swine and the authors state a 32% false positive detection to this process. The artificial sweeteners acesulfame and sucralose were found in 4 of 11 samples that were determined positive for human-associated *Bacteroides.* One sample determined as negative for human-associated *Bacteroides* contained saccharin. Saccharin has been used as an additive to swine feed and has been noted in literature references and feed labels. Other pharmaceuticals detected in groundwater samples are: caffeine, acetaminophen, sulfamethoxazole, and the bovine antibiotic sulfamethazine. Analysis of known sewage/septic waste samples confirm the presence of most target analytes, the stability of acesulfame and sucralose, and the degradation of caffeine and saccharin in the waste treatment process.

Conclusions/Recommendations: When compared to MST using PCR techniques, the scope and sensitivity of chemical analytes selected for waste stream identification were alone inadequate to determine a waste source. With the advancement of lower cost, increasingly sensitive LC/MS/MS technology, and the continued introduction of synthetic organic chemicals into human and livestock diets, chemical indicators that are specific to a waste stream can be continually inserted into these methodologies and evaluated for their effectiveness in identifying a waste source. Because of some uncertainty with determination of human-associated *Bacteroides*, chemical techniques should be used in combination with MST technologies as confirmatory methods until technologies advance to ensure reliable identification of waste sources.

Key words: groundwater, animal waste, human waste, septic systems, pharmaceuticals, fecal sterols, artificial sweeteners.

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