EXHIBIT H

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August 11, 2008

Professor L. Nicholas Ornston
Editor-in-Chief, *Applied & Environmental Microbiology*
American Society for Microbiology
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RE: Manuscript submission by Harwood *et. al.* (J.L. Weidhass, T.W. Macbeth, R.L Olsen, V.J. Harwood) entitled *Identification and Validation of a Poultry Litter-Specific Biomarker and 1 Development of a 16S rRNA Based Quantitative PCR Assay*

Dear Professor Ornston:

I write regarding the above referenced manuscript, attached as Exhibit 1, currently under submission to *Applied & Environmental Microbiology*. As the authors acknowledge, the work discussed in that article was undertaken on behalf of one side of an ongoing court case. The authors of the article are paid experts retained by the plaintiffs in that litigation. I represent the Tyson Foods companies, who are some of the poultry-producer defendants in that case. As the defendants in the case have worked with various experts, including Drs. Mansour Samadpour and Samuel Myoda, those experts have pointed out a number of considerations that cause them to question the validity of plaintiffs' work and their proposed article. In particular, there are a number of inaccuracies, material omissions, and flawed conclusions that should be of substantial concern to an objective observer. Because plaintiffs have involved you in their work, we thought you would appreciate us submitting a summary of some of these problems to you.

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Background

The litigation in question, *Oklahoma v. Tyson Foods, et al.*, is presently pending in federal district court in Tulsa, Oklahoma. The plaintiffs allege that the use of poultry litter as a fertilizer causes natural resource damages throughout the Illinois River Watershed (IRW). While the named plaintiffs are Oklahoma officials, the State is not itself funding the litigation. Rather, the Oklahoma Attorney General has retained private plaintiffs' lawyers who are funding and conducting the litigation in the hope of themselves recovering a substantial portion of any verdict as a contingency fee. Accordingly, the case is not like the usual government litigation where the state's interest is solely in reaching a just result. Rather, the attorneys advancing theories on behalf of the state have a substantial personal interest in obtaining a large verdict.

The authors of the article have been selected, retained and compensated by these private contingency fee lawyers. Dr. Olsen and his firm have already been paid more than $6 million in the case, and have been tasked with identifying potential bases for linking poultry production to alleged environmental injuries. Professor Harwood likewise has been tasked to develop a new method of microbial source tracking to link bacteria to poultry production. The remaining authors of the proposed article, Jennifer Weidhass and Tamzen Macbeth, are employees of North Wind, Inc., a laboratory working as part of the plaintiffs' litigation expert team.

The authors purport to have identified a previously unknown species of *brevibacterium*, closely related to *b. avium*, which they associate with poultry feces. This bacterium, they assert, contains a unique genetic sequence, which, when found in the environment, can serve as a "biomarker" for poultry-derived fecal contamination. The manuscript presents the authors' conclusions in relatively couched terms, observing that while their "results indicate that the watershed is indeed being impacted by the application of poultry litter . . . the magnitude of the impact . . . cannot be quantified." However, the authors have previously given sworn testimony about their work. In that testimony, the authors told a different story to the court, asserting that this work proves that poultry are the "dominant contributors to the fecal indicator bacteria loads" in the IRW and constitute a "substantial, serious and immediate threat to human health." The authors' work, however, supports neither conclusion.

As noted above, the defendants have retained Dr. Mansour Samadpour and Dr. Samuel Myoda to advise us in connection with microbial source tracking questions and to respond to the testimony advanced by Drs. Harwood and Olson. Drs. Samadpour and Myoda previously submitted a declaration to the Court addressing this research. For your convenience, I am

attaching this report as Exhibit 2. Drs. Samadpour and Myoda are currently preparing an additional report for the court.[1]

Discussion

With that background, let me turn to a few of the substantive points that have been made in court and in related sworn testimony. First, we believe that a necessary first step in a study such as this is to ensure the integrity of the sampling regime from which data will be drawn. I am attaching as Exhibit 3 a report prepared by Jay Churchill of Conestoga-Rovers, Inc., previously submitted to the court, which documents a portion of the sampling program undertaken by the plaintiffs. As Mr. Churchill relates, his staff documented numerous instances of deficient sampling, including drawing water and soil samples in close proximity to cattle contamination, failing to discharge water sources to clear potential surface contamination, failing to segregate soil samples, and failing to clean instruments properly.

Please note that, while the manuscript that plaintiffs submitted to you asserts that litter samples were drawn "through the entire depth of the litter," Mr. Churchill's team observed litter being collected with a triangular-shaped spade, resulting in samples biased towards the top, where fresher layers of litter reside. Also, please note the manuscript asserts that indicator bacteria were enumerated "according to standard methods." In fact, the plaintiffs' sampling regime violated both hold time and geomean standards. Indeed, samples collected in Oklahoma and Arkansas were shipped to a laboratory in California under unknown conditions where they were enumerated days, and in some instances, weeks, later. Moreover, plaintiffs' samples disproportionately reflect high-flow and other non-representative conditions.

Moving to the data itself, we believe you should be aware that the manuscript bases its conclusions on a limited and biased dataset. In Table 4, the authors present the results of the application of their qPCR assay to 46 environmental samples – 10 litter samples; 10 soil samples; and 26 water samples – to detect the presence or absence of their purported "biomarker." While the manuscript discusses only these 46 samples, as part of the litigation the authors tested more than 200 environmental samples, the results of which are far more ambiguous than the manuscript leads the reader to believe. Table 4 suggests that nearly all samples were positive, and that a majority were quantifiable. But in fact, a plurality of the

[1] The declaration attached as Exhibit 2 was prepared in response to expert materials submitted to the Court in support of a motion by the plaintiffs' for a preliminary injunction. Our opposition to that motion was prepared on an extremely short timeframe, and with limited discovery into the plaintiffs' case. I have therefore attached a brief errata sheet to the declaration noting a few errors.

authors' tests resulted in non-detection, and fewer than a quarter were quantifiable. The full range of test results is set out below.

	Total Samples	Quantifiable	Present	Non-Detect
Litter	10	10	0	0
Soil	40	6	29	5
Water	187	35	53	99
Totals	**237**	**51**	**82**	**104**

When deposed on July 18, 2008, as part of this litigation, Professor Harwood excused the omission of this substantial body of contrary data by stating that the manuscript had been drafted a year ago and therefore not all of the relevant data could be included. But the authors' correspondence demonstrates that the qPCR assay described by the manuscript had not yet been fully developed as of July 2007, that the majority of the plaintiffs' testing was undertaken in November 2007 through April 2008, and that the article in fact was being edited until shortly before its submission in June 2008. A copy of Dr. Harwood's recent deposition, which discusses the manuscript and her work in this litigation more generally, is attached for your review as Exhibit 4.

Based upon this dataset, the authors purport to have identified a new species of *brevibacterium* that allegedly carries a unique "biomarker" that might be used to identify poultry-derived fecal contamination. This "biomarker" was developed based on the 16s rRNA sequence. The DNA used, however, was not recovered from poultry feces directly, but rather from "used" poultry litter. Poultry litter is made of bedding material and bird excrement. The plaintiffs never tested poultry feces to confirm that the *brevibacterium* is actually present. This is significant given the fact that, as you are doubtless aware, many *brevibacterium* species have been associated with rice or decaying wood. It is therefore an open question whether this unknown bacterium is even a component of poultry feces. Accordingly, the authors cannot determine whether the previously-unknown bacteria is associated with poultry feces or some other environmental condition.

To be of any practical use as a marker for poultry-associated fecal contamination, the PCR assay must be specific to poultry, and must not reproduce DNA from other sources. In order to confirm the specificity of their assay, the authors tested it against fecal samples from cattle, geese, ducks, swine, and humans. But these tests are insufficient to assure the assay's specificity.

The authors provide no explanation as to why they limited their tests to these five alternate sources when the IRW contains hundreds of species of wildlife, all of which shed fecal indicator bacteria. Professor Harwood has testified that the plaintiffs focused on the dominant sources of indicator bacteria, yet admitted that they never actually calculated fecal loading from most animals in the watershed. Nor are the levels of *brevibacteria* identified in environmental samples high enough to warrant discounting other sources. Indeed, despite the fact that almost all of the authors' water samples were taken close to poultry operations, a plurality of samples resulted in non-detects, and those few that resulted in a quantifiable reading identified only a few hundred to a few thousand *brevibacteria*.[2] These figures are too low to discount any potential source.

Even with regard to those species examined, the authors' testing does not effectively rule them out as possible sources. As Professor Harwood candidly admitted at her deposition, the authors made no attempt to ensure that their samples were sufficiently large as to characterize accurately the populations of those various animals in the IRW. Lacking statistical significance, it cannot be said that the assay is specific to poultry feces. Indeed, we know this not to be the case as the assay identified the same DNA sequence in duck and goose feces.

Lack of statistical significance is characteristic of plaintiffs' work more generally. As Professor Harwood admitted at her deposition, the plaintiffs' lawyers made no effort to ensure that their experts' samples were gathered so as to be representative of the watershed they are now used to characterize. Rather, sampling locations were selected based on their proximity to poultry operations and on the perceived likelihood that they would they would yield high bacterial counts. Plaintiffs' qPCR testing appears to have been similarly selective. The plaintiffs tested four sets of samples, beginning with poultry litter and soil where poultry litter had been applied, and then moving out towards surface waters. While they appear to have originally intended to test all 550 samples, they suspended testing after results turned increasingly negative for their theory that the newly discovered bacterium is associated with poultry. The only further testing was a narrow and highly selective set of bacteria-laden "dirty" samples, selected to maximize the chances of finding the "biomarker." The chart below sets out the results of plaintiffs' testing by set.

[2] The authors present their findings in terms of numbers of gene copies of the 16S rRNA sequence identified through their process. *Brevibacteira sp.* have been found to carry 4-5 copies of the 16S gene. Because the authors have never cultured their newly-identified bacteria, it cannot be said for certain how many copies it carries. For simplicity's sake, we assume 5 gene copies.

Set	Date Reported	Total	Quantifiable	Present	Non-Detect
1	11/3/2007	67	26	28	13
2	12/4/2007	60	15	11	34
3	1/21/2008	77	8	19	50
4	4/24/2008	29	2	23	4

Finally, in order to serve as an effective indicator of poultry fecal contamination and of any associated health risk, a "biomarker" must share fate and transport characteristics with fecal indicator bacteria and/or pathogens derived from poultry feces. Yet, the authors made no effort to document any such relationship. The authors have also never cultured this newly discovered bacterium to discover its characteristics. *Brevibacterium* is a relatively easy organism to culture, and culturing allows the researcher to confirm the nature and properties of the organism. Failure to do so here is significant.

Having not actually cultured the *brevibacterium*, the authors have not studied its fate and transport characteristics, nor, as Professor Harwood admits, have they studied these characteristics of any other organism in the IRW. Moreover, no epidemiological work has been undertaken to demonstrate any health impact, and indeed the plaintiffs' efforts to identify pathogens such as *campylobacter* and *salmonella* in the IRW have largely come up empty.

Nevertheless, the authors present correlations between their *brevibacterium* and indicator organisms *enterococci* and *e. coli* in poultry litter and in water, and assert that these relationships demonstrate that the one indicates the presence of the other. This methodology suffers several substantial flaws.

First, the correlations themselves are based on far too few data points to be considered serious statistical work. The litter correlation, presented in Figure 3, is drawn from ten litter samples which, as discussed above, hardly characterize poultry litter across the million-acre watershed, and which were sampled in a biased manner. The water correlations, presented in Figure 4, appear to rely on only five of the 187 water samples actually taken.

Second, even if these correlations were individually valid, in order for the "biomarker" to be an effective indicator of poultry fecal contamination, a correlation must be demonstrated not only at individual locations, but rather consistently along the asserted "pathway". The fact that these organisms exhibit a correlation in poultry litter says nothing about their relationship in surface water miles away. The authors have not demonstrated any such correlation. Quite the contrary, the correlations they present are quite disparate. In the litter, the biomarker is said to

correlate to *enterococci* with an R^2 value of .75, and to *e. coli* with an R^2 of only .28. Yet in surface water, the correlations presented are .89 and .85 respectively. This substantial change in relationship from litter to water suggests that the respective bacteria have substantially different fate and transport characteristics, undermining the utility of the *brevibacteria* as an indicator organism. Moreover, as Professor Harwood admits, the authors took no steps to account for alternate sources of *enterococci* and *e. coli* in surface waters, rather using total counts in their correlation.

Third, as noted, the authors never tested poultry feces to confirm the presence of the *brevibacteria*. Table 4 reports remarkably high levels of *brevibacteria* in poultry litter, with readings ranging from 0.5 to 2.5 billion bacteria per gram of litter, eclipsing by several orders of magnitude the 1,200 *e. coli*/g and 52,000 *enterococci*/g measured in those same litter samples. It would be remarkable if a bacteria so ubiquitous in poultry feces has in fact gone unnoticed for so long by the many researchers who have studied poultry. Yet, if poultry feces do not contain this *brevibacteria*, and at levels correlating to the authors' sampling results, then the authors' thesis that the "biomarker" is an effective marker for poultry fecal contamination cannot hold.

Conclusion

The use of scientific evidence in litigation has not been without its critics. Indeed, federal courts are increasingly skeptical of science prepared at the request and under the direction of attorneys. This case, unfortunately, gives life to many of these concerns. Documents uncovered during the course of the litigation indicate that the plaintiffs' attorneys determined and outlined what their experts' scientific conclusions would be before the relevant experts were even hired or lab work begun, and decided that work which generated answers that did not support the attorneys' theories would be discontinued.

As noted, this article was created to be used as evidence in litigation. Accordingly, my client has a substantial interest in how the article is presented to others and reviewed. We would therefore be interested in providing you with more detailed information about the case and the expert work undertaken by the plaintiffs. Also, we respectfully request that you preserve any materials relating to the article, including any correspondence, records, data, or documents, relating to the same, as those may become evidence in the litigation.

I look forward to talking with you about this matter.

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Best regards,

Jay T. Jorgensen

encls.
cc w/ encls.: *Applied & Environmental Microbiology* Editors
Plaintiffs' Counsel

Identification and Validation of a Poultry Litter-Specific Biomarker and Development of a 16S rRNA Based Quantitative PCR Assay

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Running title: Brevibacterium marker for fecal source tracking of poulty

ABSTRACT

A poultry litter-specific biomarker was developed for microbial source tracking (MST) in environmental waters. 16S rRNA sequences that were present in fecal-contaminated turkey and chicken litter were identified by terminal restriction fragment length polymorphism (T-RFLP). Cloning and sequencing of potential targets from pools of *E. coli, Bacteroides* or total bacterial DNA yielded four sequences that were ubiquitous in poultry litter and also contained unique sequences for development of target-specific PCR primers. Primer sensitivity and specificity were tested by nested PCR against ten composite poultry litter samples and fecal samples from beef and dairy cattle, swine, ducks, geese, and human sewage. The sequence with greatest sensitivity (100%) and specificity (93.5%) has 98% identity to *Brevibacterium avium,* and was detected in all litter samples. It was detected at low level in only one goose and one duck sample. A quantitative PCR assay was developed and tested on litter, soil and water samples. Litter concentrations were $2.2*10^7$ - $2.5*10^9$ gene copies/g. The biomarker was present in a majority of soil and water samples collected in and near areas where litter was spread, reaching concentrations of 2.9×10^5 gene copies$\cdot g^{-1}$ in soil samples and 5.5×10^7 gene copies$\cdot L^{-1}$ in runoff from the edges of fields. The biomarker will contribute to quantifying the impact of fecal contamination by land-applied poultry litter in this watershed. Furthermore, it has potential for determining fecal source allocations for total maximum daily load (TMDL) programs and ambient water quality assessment, and may be useful in other geographic regions.

INTRODUCTION

Excessive land application of poultry litter as a waste disposal mechanism has been linked to eutrophication of water bodies (28, 35, 39), the spread of pathogens (15, 19, 21), air and soil pollution with metals (11, 33) and groundwater contamination with nitrate (5). Despite these known effects, land application is still the typically practiced disposal method for poultry litter even though viable and economically favorable alternative disposal practices are available (7, 20).

Identification of the source of fecal pollution contaminating a watershed is of particular interest for protection of water resources and the safety of recreational waters. For example, TMDL assessments require identification of the source of contamination, which is also necessary for remediation of impaired waters(44). Current methods for detecting the presence of fecal pollution, which carries an increased risk of the presence of pathogenic microorganisms, involve the cultivation of fecal indicator organisms such as fecal coliforms in the family *Enterobacteriaceae* (Oklahoma Administrative Code, Title 785, Chapter 46). The U.S. EPA and many states recognize *Escherichia coli* and enterococci as indicators of freshwater recreational water quality (42).

Drawbacks to the use of indicator organisms which limit the ability of researchers to pinpoint sources of fecal contamination include the non-specificity of the fecal coliforms to one source (25, 43), variable survival rates of various indicator organisms (1) and the growth or extended persistence of these indicator organisms after release to the environment (12, 45). These drawbacks have lead to research into alternative methods for the assessment of human health risk

55 from microbial pathogens in recreational waters that do not include the culturing of fecal
56 indicator organisms for identification and quantification of the source of fecal pollution (46).

57 A variety of microbial source tracking (MST) methods (for recent reviews see (17, 40, 47)) have
58 been proposed as an alternative to cultivation of fecal coliforms. Some of these genotypic
59 molecular based techniques have included library dependent methods (i.e., culture and isolate-
60 based) such as ribotyping (10, 31) and repetitive element polymerase chain reaction (REP-PCR)
61 (14). Library independent methods (i.e., detection of a genetic biomarker in extracted DNA)
62 have also been developed using discovery techniques such as suspension arrays (8), subtractive
63 hybridization (13, 26), and terminal restriction fragment length polymorphism (T-RFLP) (3),
64 among others. Host marker specific targets have included *Enterococcus faecium* (37),
65 *Bifidobacterium* and members of the *Bacteroidales* (3, 22, 38), among others. Relatively few
66 microbial targets specific to poultry fecal material have been identified. To date *Enterococcus*
67 *faecalis* (23), *E. coli* (10) and *Bacteriodes* (26) have been associated with poultry fecal material,
68 but only the *Bacteroides* biomarker (26) was specifically associated with poultry and not other
69 fecal sources The objective of this research was to identify a poultry litter-specific biomarker,
70 validate its specificity against other sources of fecal material from within and outside the
71 watershed and develop a 16S rRNA based real-time PCR assay for quantifying the biomarker in
72 environmental samples. This work was carried out as part of ongoing litigation in which the
73 plaintiff is the Oklahoma Attorney General.

74 **METHODS**

75 **Sample collection.** Litter samples were collected from ten separate facilities (poultry houses),
76 nine chicken and one turkey facility. Litter samples were collected from 18 locations within each

poultry house through the entire depth of the litter. The subsamples (total volume of 4 to 5 gallons) from each house were composited, homogenized and split (riffle splitter) before placement into a sterile whirl pack (approximately 500 mL) and shipped on ice to the laboratory for analysis. Litter application areas in fields (soils) were sampled by collecting 20 subsamples on a predetermined grid pattern across a uniform subarea of one to ten acres in size. The zero to two inch sample from six inch soil cores were composited, disaggregated, sieved to 2 mm, ground, homogenized and split. Vegetation, feathers, and rocks were removed. The split soil samples (500 ml) were transported on ice to the laboratory. Nontarget fecal samples for specificity testing were collected as composites from groups of individuals (Table 3). Samples from beef cattle were collected from ten grazing fields, of which five were within the watershed and five were outside the watershed. Two independent duplicate samples were collected for each field, and each duplicate consisted of feces from ten scats. A total of 200 beef cattle scats were collected and composited into 20 samples. Duck and goose samples were collected in the same fashion, consisting of composites from ten individual scats, and independent duplicates were collected for each area. For ducks, three landing areas inside the watershed and two outside the watershed were sampled, while for geese, two landing areas inside and three landing areas outside the watershed were sampled. A total of 100 scats for duck and geese were collected and composited into 10 samples for duck and 10 samples for geese. Composite samples of fecal slurries were collected from swine facilities, one inside the watershed and one outside (2 duplicate samples) and dairy cattle facilities (one inside the watershed and two outside (2 duplicate samples each) human residential septic cleanout trucks (3 samples) and influent of three separate municipal wastewater treatment plants (3 samples). A total of 20 g of each fecal sample other than litter from each site was collected and was placed in a 20 ml, sterile,

polystyrene tube containing 10 ml of 20% glycerol and shipped on dry ice to the laboratory. All fecal samples were homogenized in the glycerol before DNA extraction. Discrete water samples from larger rivers and lakes were collected using a Van Dorn water sampler or with a churn splitter for discrete or composite samples. Samples from larger rivers were typically composites of 3 samples collected on a transect across the width of the river channel. Samples from smaller rivers were collected using automated samplers. Samples collected during high flow events were composited based on flow volume. Base flow samples were collected as grab samples. River samples were placed into sterile 1-L polystyrene bottles in duplicate and shipped on ice to the laboratory where they were filtered. Runoff samples from the litter application areas (e.g. edge of field runoff samples) were collected during or as soon as possible after rainfall events. Samples were collected either with a passive runoff collector for composite samples or with a dip sampler for discrete samples. Runoff samples were placed into sterile 1-L polystyrene bottles in duplicate and shipped on ice to the laboratory where they were filtered. Groundwater samples were collected directly from existing homeowner's wells or from hydraulically driven shallow probes. Spring samples were collected as grab samples or by using a peristaltic pump. All samples were placed into sterile 1-L polystyrene bottles and shipped onice to the laboratory where they were filtered.

Enumeration of Indicator Bacteria. Indicator bacteria (fecal coliforms, *E. coli* and enterococci) were enumerated according to standard methods using multiple tube fermentation (MTF) and calculation of the most probable number according to according to SM-9221F or SM-9230 (APHA, 2005). MTF tubes containing *E. coli* were identified using broth cultures supplemented with (MUG) (SM-9221F) (2).

122 **Soil, Litter and Fecal Sample DNA Extraction.** Genomic DNA was extracted from soil, liter
123 and fecal samples with Bio101 Fast®Spin® DNA extraction kits (QBiogene, Inc.) following the
124 manufacturer's instructions. Typically 0.25 g of soil or litter was used in each extraction. DNA
125 was purified by size-exclusion chromatography. Sepharose CL-4B (Sigma-Aldrich) was
126 resuspended in Tris-HCL and sterilized by autoclave at 121 °C for at least 20 minutes. Micro-bio
127 spin columns (Bio-Rad Laboratories) were packed with 1 mL of Sepharose CL-4B through
128 centrifugation. Sepharose columns were then washed twice with Tris-HCl buffer (pH 8) and 50
129 to 150 µl of sample was added. Purified DNA was concentrated with ethanol precipitation and
130 re-eluted in 100 µL sterile water.

131 **Water Sample DNA Extraction.** Within 12 hours of receipt at the laboratory all water samples
132 were filtered through a sterile Supor-200, 0.2 µM filter and frozen at -80°C. Filters were then
133 shattered with sterile glass beads and vortexed vigorously for 15 minutes with sterile, DNase,
134 and RNase free water to remove solids and cells from the filters. The cell suspension was
135 removed from the centrifuge tubes by pipette and placed in a 2 mL bead beating tube from the
136 Bio101 Fast®Spin® DNA extraction kits. The cells were centrifuged at 20,000 x g for 10
137 minutes, and the supernatant was decanted. Genomic DNA was then extracted using the Bio101
138 Fast®Spin® DNA extraction kits (QBiogene, Inc). The extracted DNA was quantified using a
139 Nanodrop® UV-Vis Spectrophotometer.

140 **T-RFLP Analysis.** Extracted genomic DNA and/or cloned DNA was amplified with
141 phosphoramidite fluorochrome 5-carboxyfluorescein (FAM) labeled universal bacterial primers
142 8F-907R (16, 24), with *E.coli* genus specific primers (Tsen, et al. 1998), and *Bacteroidales*
143 specific primers (Bernhard and Field, 2000). All PCR primers targeted the 16S rRNA gene.
144 Triplicate PCR reactions were generated from each DNA extraction, combined and purified

145 using QIAquick PCR purification Kits (Qiagen). Approximately 200 ng each of PCR product
146 was digested at 37°C for 6 hours with the *Msp*I restriction enzyme (20μ/μL) (New England
147 BioLabs). Samples were denatured by heating to 95° C for 3 minutes followed by cooling to
148 4°C. The digested fragments were purified by ethanol precipitation.

149 **Primer Design.** Primers were designed using the ABI Primer Express v.2 program (Applied
150 Biosystems, Foster City, CA) and were targeted to variable regions between the potential
151 biomarker sequences and sequences of the top 20 closest related organisms in the GenBank
152 database. The BLAST search (Basic Alignment Search Tool,
153 http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) was used to check the specificity of each primer.

154 **PCR Assay Conditions.** PCR was used to amplify approximately 900 bp of the 16S rRNA genes
155 from *Bacteria* for clone library construction. Each 25 μL PCR reaction included 0.4 mg mL^{-1}
156 molecular-grade bovine serum albumin (BSA) (Sigma Chemicals), 1X PCR Buffer (Promega),
157 1.5 mM $MgCl_2$, 0.5 μM of both the forward (8F) (16) and reverse (907R) (24) primer
158 (Invitrogen), 1U Taq DNA polymerase (Promega), 0.2 mM dNTP (Invitrogen), 1 μL DNA
159 template, and molecular-grade water (Promega). Amplification was performed on a PerkinElmer
160 Model 9600 thermocycler using the following conditions: 94 °C for 5 minutes, 30 cycles of 94
161 °C (1 minute), 55 °C (45 seconds), and 72 °C (2 minute). A final extension at 72 °C for 7
162 minutes was performed and the PCR products were held at 4°C. Specificity of the PCR primers
163 to the poultry litter biomarker was evaluated with nested PCR by first amplifying non-target
164 fecal samples by universal bacterial primers 8F, 907R and then amplifying by the potential
165 poultry litter biomarker PCR primers. The nested PCR master mix and thermocycler conditions
166 were similar to the universal PCR with the following exceptions: 1) forward and reverse PCR

167 primers were specific to the potential poultry biomarker as shown in Table 2, 2) the annealing
168 temperature was 60 °C. Amplification by nested PCR was evaluated by gel electrophoresis.

169 **Clone Libraries.** Clone libraries were constructed from the original genomic DNA extracted
170 from the soil and litter samples and amplified with either universal bacterial primers 8F-907R
171 (16, 24), targeting the 16S rRNA genes of *Bacteria* or the *E. coli* genus specific primers V1SF-
172 V3AR (41). The TOPO ® Cloning Reaction methods from Invitrogen ™ were followed for
173 clone library construction. Two clone libraries were constructed (targeting *Bacteria* and *E. coli*)
174 from pooled DNA samples (i.e., 1 µl of genomic DNA extract from each sample was added to
175 the PCR reaction for inclusion into the clones) based on the abundance of the various potential
176 biomarkers as evidenced by the T-RFLP profiles.

177 **qPCR Assay Conditions.** Quantitative PCR (qPCR) was used to amplify 530 bp of the 16S
178 rRNA gene from *Brevibacterium spp.* DNA samples were diluted to final concentrations of 3
179 ng/µL DNA. Each 25µL qPCR reaction included: 1X SYBR Green Master Mix (Roche), 0.5 µM
180 of both the forward (LA35F) and reverse primer (LA35R) (Invitrogen), 5 % DMSO, 5 µL of
181 diluted sample DNA, and molecular-grade water (Promega). Amplification was performed in
182 triplicate on a Biorad Chromo4 thermocylcer using the following conditions: 50 °C for 2
183 minutes, 95 °C for 15 minutes, 45 cycles of 95 °C (30 seconds), 60 °C (30 seconds), and 72 °C
184 (30 seconds) with a plate read. The 45 cycles was followed by a final extension at 50 °C for 5
185 minutes. Immediately following the final extension was a melting curve from 70 °C to 90 °C, by
186 0.1 degree increments, holding for 5 seconds with a plate read. DNA standards ranging from
187 $6*10^{-15}$ to 10^{-21} ng/ul were prepared from serial dilutions of clone plasmid DNA containing the
188 sequence of interest and used to develop the standard curve and method detection limit. Gene
189 copy numbers were calculated from concentrations of positive control standards assuming 9.124

190 * 10^{14} bp/ul of DNA and one gene copy per genome. Detection limits for the qPCR assay were
191 approximately 2000 plasmid copies in *E. coli*/L water and 7.3 *10^{4} plasmid copies in *E.*
192 *coli*/gram of soil. Nested qPCR was performed by first amplifying DNA with the universal
193 bacterial 16S rRNA 8F (16) and 907R (24) primers. The production of PCR products was
194 confirmed on a 1.5% agarose gel. The 16S rRNA PCR products were purified with the QIAquick
195 PCR purification kit (QIAGEN) were subjected to qPCR as previously described using the
196 LA35F and LA35R primers for the poultry litter biomarker.

197 **Phylogeny.** The phylogeny of the LA35 clone was investigated using the following methods.
198 The clone sequences were assembled and aligned with BioEdit v. 7.0.5.3 and sequences were
199 checked for chimeras with the Ribosomal Database Project II Chimera Check program and
200 Bellerophon. The 16S rRNA sequences of the closest neighbors to the clone sequences were
201 downloaded for inclusion in the phylogenic analysis. Multiple sequence alignments were
202 constructed with Clustal W alignment tool and manually aligned in BioEdit. The bootstraps
203 (1000 resamplings), maximum likelihood and distance matrix analysis (Kimura), and the
204 reconstruction of the phylogenetic trees (FITCH) were performed with the Phylip 3.65 package
205 and in particular the programs SEQBOOT, DNAML, DNADIST, FITCH, CONSENSE, and
206 RETREE. The reconstructed phylogenetic tree was visualized with PhyloDraw V. 0.8 (Graphics
207 Application Lab, Pusan National University).

208 **RESULTS**

209 ***Identification of potential biomarkers by T-RFLP.*** A total of 20 T-RFLP profiles were
210 generated from the 5 subsamples of each of the two litter and two soil samples. The T-RFs
211 common among the subsamples and representing more than 1% of the community were selected

212 for cloning and sequencing (Table 1). A total of 3 *E. coli* T-RFs (i.e., T-RF 496.0, 498.9 and
213 500.8) and 3 *Bacteria* T-RFs (i.e., T-RF142.9, 147.3 and 158.9) were selected for cloning and
214 sequencing. Clone libraries were constructed from PCR products amplified with *E. coli* specific
215 primers (V1SF-V3AR) (41) or universal bacterial primers (8F-907R) (16, 24). A total of 300
216 plasmids from the clone libraries were randomly picked. T-RFLP analysis was carried out on
217 each plasmid insert to identify which plasmids contained the T-RFs of potential biomarkers.
218 Inserts containing the T-RFs of interest were sequenced and PCR primers were developed for
219 those sequences containing mismatches as compared to BLAST database results of the top 20
220 closely related organisms. In all 4 PCR primers for members of 4 genera were developed; a
221 *Brevibacterium spp.*, a *Rhodoplanes spp.*, a *Kineococcus spp.* and a *Pantoea ananatis* strain
222 (Table 2). Two *E. coli* T-RFs were from plasmids that did not contain mismatches between the
223 sequence of interest and the sequences of closely related organisms identified in a BLAST search
224 and therefore were not appropriate biomarkers.

225 **Evaluation of biomarkers against fecal samples.** The PCR assays developed for the 4 potential
226 biomarkers of poultry litter were tested for amplification against a variety of nontarget fecal
227 samples from within and outside the watershed (Table 3). Only the *Brevibacterium* clone LA35
228 appeared to be a potential candidate biomarker for poultry litter in that did not amplify in any
229 fecal samples with the exception of weak amplification in one duck and one goose sample from
230 outside the watershed when analyzed with a nested PCR approach (i.e. PCR with universal
231 bacterial primers and then with the *Brevibacterium* clone LA35 primers). The reconstructed
232 phylogenetic tree of the *Brevibacterium* clone LA35 in relationship to other *Brevibacterium* spp.
233 is presented in Figure 1.

Quantification of the poultry litter biomarker in environmental samples. A SYBR green qPCR protocol was developed and optimized using the LA35F and LA35R primers (Table 2) specific to the *Brevibacterium* clone LA35 poultry litter biomarker. The standard curve of the qPCR assay for the biomarker is presented in Figure 2. The detection limit of the qPCR assay was 6 gene copies/ul of extracted DNA.

Environmental samples from the potential poultry litter impacted watershed were tested for the presence of the biomarker with the qPCR assay (Table 4). A variety of samples from within the watershed were tested, some of which were expected to contain the biomarker (e.g., litter, contaminated soil, runoff samples), some of which had variable potential for higher biomarker levels (e.g., surface water), and some of which had lower potential for biomarker presence (i.e., groundwater samples).

The correlation between the poultry litter biomarker concentration (i.e., as quantified by qPCR) in water and litter samples and *E. coli* and *Enterococcus* as measured by most probable number is presented in Figures 3 and 4. In general the *Enterococcus* MPN counts were well correlated with the concentration of the biomarker in litter ($R^2 = 0.75$) and with the biomarker concentration in water samples ($R^2 = 0.89$). The correlation between *E. coli* concentrations and the biomarker in water samples was also strong ($R^2 = 0.85$) while *E. coli* was less tightly (but significantly) correlated with the biomarker in litter samples ($R^2 = 0.28$). Correlation of the biomarker with *E. coli* and *Enterococcous* spp. provides a line of evidence of the human health risk associated with the runoff from poultry litter application to fields although there is evidence that regrowth of these organisms is possible once they are introduced into the environment (36).

DISCUSSION

The *Brevibacterium* sp. poultry litter biomarker developed in this study was validated in terms of sensitivity (100%) against numerous positive (poultry litter) samples from different locations with the watershed and for specificity (93.5%) against composite non-target fecal samples. These practices are in accordance with recent critical reviews (34, 40) that strongly recommend MST method validation. Future efforts will attempt to extend the method validation outside the watershed and possible outside the region as this biomarker could be useful for identifying fecal pollution sources in other river systems and coastal waters.

The *Brevibacterium* clone LA35 poultry litter biomarker was most closely related to *Brevibacterium avium*, which is associated with bumble-foot lesions in poultry (32). *Brevibacterium* spp. were recently identified in spent mushroom compost that was originally derived from chicken litter and cereal straw (29). Additionally *Brevibacterium avium, Brevibacterium iodinum,* and *Brevibacterium epidermidis* were found to represent more than 7% of a 16S rRNA clone library originating from broiler chicken litter (27). Certain *Brevibacterium* spp. are associated with milk and cheese curds(6), human skin(9), and soils (30). *Brevibacterium* spp. have been associated with disease in humans although to date these opportunistic pathogens have only been isolated from immunocompromised patients (4, 9, 18).

As poultry litter is land-applied as a disposal practice (19, 33, 35), it was important to identify a marker that could survive the process of deposition on bedding and spreading on fields. Therefore, the T-RFLP screening process included both litter and contaminated soil samples. This strategy allowed for the rapid elimination of numerous targets that could be abundant in the poultry fecal material, but not as abundant in the litter and not present in the environment after

277 litter application. This strategy for marker identification is in contrast with the work by Lu and
278 colleagues (2007) where a genome fragment enrichment method was used to identify microbial
279 sequences specific to chicken feces. Based on the PCR assays developed from clone libraries of
280 the genome fragments, 6 to 40% of the chicken fecal samples collected from a wide geographic
281 region contained DNA that could be amplified by the various assays (26). In comparison the
282 LA35 biomarker was found in all the poultry litter samples tested, although it should be noted
283 that all of the samples were collected in the Oklahoma/Arkansas region.

284 The examination of environmental samples from within the poultry litter impacted watershed
285 suggest a correlation between the application of poultry litter to a field and concentration of the
286 biomarker in the receiving waters, as evidenced by the generally decreasing trend in biomarker
287 concentration with decreasing concentration of fecal indicator organisms. These results indicate
288 that the watershed is in fact being impacted by the application of poultry litter to fields within the
289 watershed. However, the magnitude of the impact as measured by the distribution of the
290 biomarker within the watershed cannot be quantified with the limited number of environmental
291 samples processed to date. Future work will include the testing of environmental samples from
292 within the watershed by the qPCR assay to evaluate the distribution of the poultry litter-specific
293 biomarker as compared to indicator bacteria, antibiotics and heavy metals. Additionally, testing
294 of the poultry litter-specific biomarker against more fecal samples from other watersheds and
295 additional avian fecal material will be conducted as the LA35 poultry litter biomarker was found
296 in low abundance (i.e., a nested PCR approach was required for detection) in two non-target
297 composite avian fecal samples (i.e., a duck and a goose sample) from outside the watershed.

298 **Conclusions**

In summary a novel biomarker of poultry litter was identified and a 16S rRNA based real-time PCR assay was developed for this biomarker. The specificity of the assay (93.5%) was tested against 31 separate non-target fecal samples and sensitivity was tested against10 target litter samples (100%). The field applicability of the assay was evaluated by testing for the biomarker in environmental samples expected to have variable concentrations of the biomarker, which we hypothesized would be correlated with the concentration of fecal indicator bacteria. A generally positive correlation was found between biomarker concentration and fecal indicator bacteria concentration which was particularly strong for enterococci. The research presented herein is the first identification of a *Brevibacterium* spp. for microbial source tracking studies and is among the first quantifiable method for tracking of poultry fecal sources in environmental waters.

ACKNOWLEDGMENTS

This research was conducted in connection with work performed as retained experts in a pending legal case brought by the State of Oklahoma against several poultry integrators. Drs. Harwood & Olsen have been retained to serve as expert witnesses by the State of Oklahoma and have provided testimony regarding this research.

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454 Table 1. Common T-RFs among replicates from two fecal-contaminated poulty litter samples

455 and two soils to which the litter had been applied.

456

	Number of subsamples tested (number containing T-RF of interest)			
T-RF	Litter A	Litter B	Soil A	Soil B
E.coli PCR products, digested with *MspI*				
496.0	4 (4)	5 (4)	5 (3)	5 (5)
498.9	4 (4)	5 (5)	5 (4)	5 (5)
500.8	4 (4)	5 (5)	5 (5)	5 (5)
Universal bacteria PCR products, digested with *MspI*				
80.1	4 (4)	5 (5)	5 (0)	3 (3)
130.9	4 (3)	5 (5)	5 (1)	3 (0)
142.9	4 (4)	5 (4)	5 (2)	3 (2)
147.3	4 (4)	5 (5)	5 (5)	3 (2)
158.9	4 (3)	5 (5)	5 (4)	3 (2)
165.0	4 (3)	5 (5)	5 (4)	3 (2)

*Underlined T-RFs correlate to those organisms for which PCR primers were developed

457

458

459 Table 2. Nucleotide sequences and targets of primers used in this study.

460

Primer	Target	Sequence (5'-3')	Position	Tm (°C)	T-RF
LA35F	*Brevibacterium*	ACCGGATACGACCATCTGC	166-184	57	147.3
LA35R	clone LA35	TCCCCAGTGTCAGTCACAGC	717-736	58	
SA19F	*Kineococcus*	TACGACTCACCTCGGCATC	163-181	56	158.9
SA19R	*spp.*	ACTCTAGTGTGCCCGTACCC	602-621	55	
SB37F	*Rhodoplanes*	AACGTGCCTTTTGGTTCG	143-160	56	142.9
SB37R	*spp.*	GCTCCTCAGTATCAAAGGCAG	616-626	55	
SA15F	*Pantoea*	CGATGTGGTTAATAACCGCAT	490-510	56	500.8
SA15R	*ananatis*	AAGCCTGCCAGTTTCAAATAC	668-688	55	

461

462 Table 3. Specificity of the poultry litter biomarker assay tested against fecal samples from within and outside the watershed.

463

Fecal sample (inside or outside watershed)	Number of samples tested (Number of samples containing potential biomarker)			
	Brevibacterium clone LA35	*Rhodoplanes* clone SB37	*Kineococcus* clone SA19	*Pantoea ananatis* clone SA15
Beef cattle (outside)	5 (0)	5 (2)	5 (1)	5 (0)
Beef cattle (inside)	5 (0)	5 (3)	5 (5)	5 (1)
Dairy cattle (outside)	2 (0)	2 (1)	2 (1)	2 (1)
Dairy cattle (inside)	1 (0)	1 (1)	1 (0)	1 (0)
Swine (outside)	1 (0)	1 (1)	1 (1)	1 (0)
Swine (inside)	1 (0)	1 (0)	1 (0)	1 (0)
Duck (outside)	2 (1)*	2 (2)	2 (2)	2 (2)
Duck (inside)	3 (0)	3 (1)	3 (1)	3 (2)
Goose (outside)	3 (1)*	3 (3)	3 (2)	3 (2)
Goose (inside)	2 (0)	2 (2)	2 (1)	2 (1)
Human sewage (outside)	2 (0)	2 (2)	2 (2)	2 (1)
Human sewage (inside)	4 (0)	4 (3)	4 (1)	4 (1)

* One duplicate amplified when analyzed with a nested PCR assay.

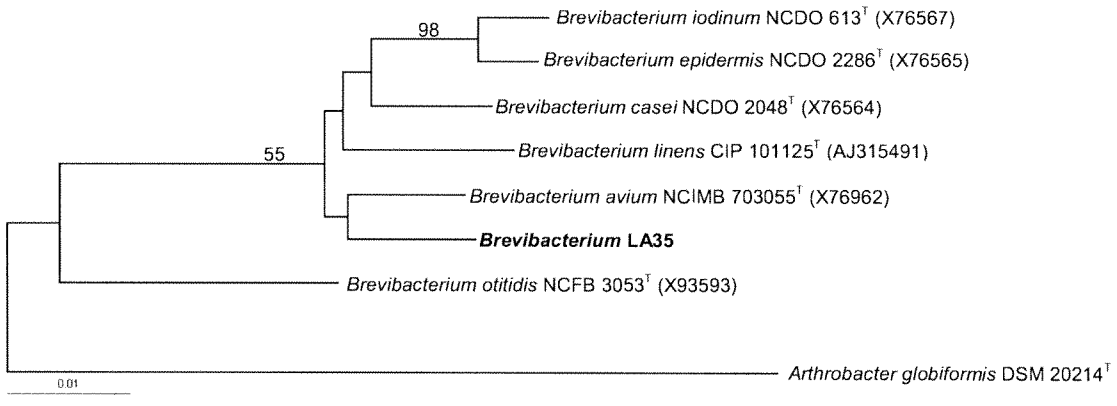


Figure 1. Reconstructed phylogentic tree of the *Brevibacterium* spp. based on 16S rRNA. Numbers at the nodes represent bootstrap values (i.e. the number of times this organism was found in this position relative to other organisms in 1000 resamplings of the data). Bootstraps less than 50% are not shown. The closest cultured organisms as reported in an NCBI BLAST search are reported. The distance bar represents a 1% estimated sequence divergence.

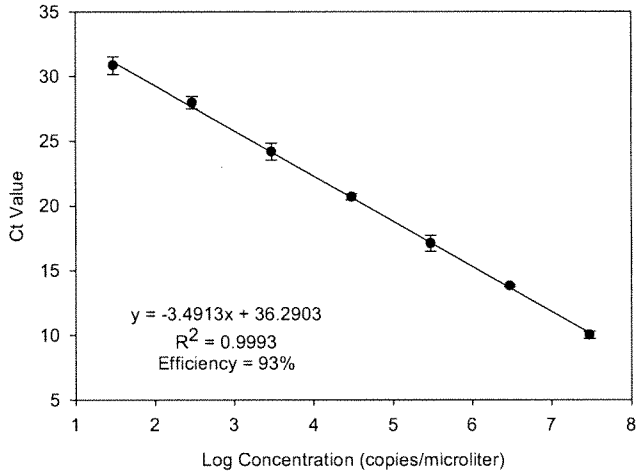


Figure 2. Standard curve of measured Ct values and standard deviations versus log plasmid biomarker concentration.

479 Table 4. Environmental samples tested for *Brevibacterium* clone LA35 poultry litter biomarker

480

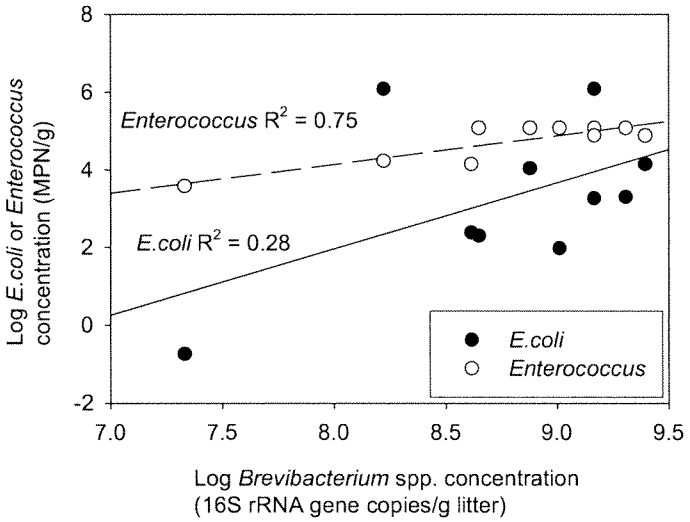
Sample type	Number samples tested	% of samples containing biomarker [a]	% of samples quantifiable [b]	Range of biomarker present (16S rRNA copies/L water or g soil or g litter)
Litter	10	100	100	$2.2*10^7 \pm 7.1*10^6 – 2.5*10^9 \pm 9.5*10^7$
Soil	10	100	50	$7.0*10^3 \pm 4.4*10^2 – 2.9*10^5 \pm 2.0*10^4$
Edge of field runoff	10	100	100	$2.6*10^3 \pm 1.2*10^2 – 5.5*10^7 \pm 5.3*10^6$
River	10	50	20	$2.9*10^3 \pm 8.6*10^2 – 3.2*10^4 \pm 6.8*10^3$
Groundwater	6	0	0	Not applicable

[a] indicates the percent of samples in which the biomarker was identified by qPCR or nested qPCR methods

[b] indicates the percent of samples for which a quantifiable number of biomarker genes were measured by qPCR

481

482



483

484 Figure 3. Correlation between the concentrations of poultry litter biomarker, *E. coli* and

485 *Enterococcus spp.* in poulty litter samples.

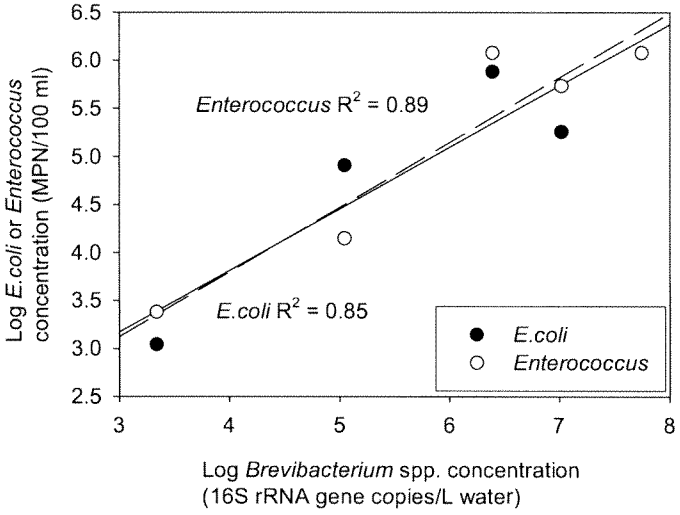


Figure 4. Correlation between the concentrations of poultry litter biomarker, *E. coli* and *Enterococcus* spp. in water samples.

IN THE UNITED STATES DISTRICT COURT
FOR THE NORTHERN DISTRICT OF OKLAHOMA

STATE OF OKLAHOMA, ex rel. W.A. DREW EDMONDSON, in his capacity as ATTORNEY GENERAL OF THE STATE OF OKLAHOMA AND OKLAHOMA SECRETARY OF THE ENVIRONMENT C. MILES TOLBERT, in his capacity as the TRUSTEE FOR NATURAL RESOURCES FOR THE STATE OF OKLAHOMA **PLAINTIFFS**

v. **CASE NO.: 05-CV-00329 GKF –SAJ**

TYSON FOODS, INC., TYSON POULTRY, INC., TYSON CHICKEN, INC., COBB-VANTRESS, INC., CAL-MAINE FOODS, INC., CAL-MAINE FARMS, INC. CARGILL, INC., CARGILL TURKEY PRODUCTION, LLC, GEORGE'S, INC., GEORGE'S FARMS, INC., PETERSON FARMS, INC., SIMMONS FOODS, INC. and WILLOW BROOK FOODS, INC. **DEFENDANTS**

Declaration of Dr. Mansour Samadpour and Dr. Samuel P. Myoda

1. Dr. Mansour Samadpour is President of IEH Laboratories and Consulting Group and Molecular Epidemiology, Inc. (IEH). Dr. Samuel Myoda is Vice President of IEH. Our CVs are attached hereto as Exhibit 1 and 2. In the last four years, Dr. Samadpour has testified in the following cases:

   Novak v. Kroger Co. of Michigan, 02-038264-NP (Mich. Cir. Ct.)

   Bar-S Foods v. Tiromat, et al., CJ-02-111 (Okl. Dist. Ct.)

   Metz v. Dalesio's of Lilttle Italy, 24-C-06-001426OT (Baltimore Cir. Ct.)

2. IEH was retained in November of 2007 by Defendants at an hourly rate of $400/hr for Dr. Samadpour and $350 for Dr. Myoda to serve as expert consultants regarding, but not limited to, microbial source tracking, microbiology, water quality, public health and related regulatory affairs. We have been asked to review the Plaintiff's testimony, the water quality data and other relevant parameters that influence water quality in the Illinois River Watershed (IRW) and to offer our scientific opinions on the water quality and factors that affect the water quality in the IRW.

3. Bacteria are microscopic, unicellular organisms that are prokaryotes, meaning that unlike our cells they do not contain a nucleus. There are many different physical and biological properties of these organisms, they have preferred habitats, and interact with their surroundings in many different ways. There are literally trillions upon trillions of bacteria in the environment and the overwhelming majority of them are not pathogenic. Bacteria are an integral part of our lives; they are used in food production, e.g. yogurt and cheese, in our digestive system, e.g. *E. coli* and enterococcus, in the soil, e.g. nitrogen fixing bacteria that are essential to the nutrient cycle and are also in the air and water. Bacteria are used to treat wastewater, to break down pollutants, e.g. bioremediation, and are used in drug development and production. There are a plethora of beneficial uses for bacteria.

4. Despite the wealth of knowledge regarding bacteria, in fact relatively little is known about the total universe of bacteria, and it is estimated that scientists have been able to culture (grow in the lab) < 2% of the bacteria that exist. There are a host of factors that affect the "fate and transport" of specific types of bacteria in the environment: how do different bacteria handle different environmental factors; what is the

relationship between bacterial transport and precipitation; what factors govern soil infiltration and filtration/sorption; what characteristics govern relative survival rates, predation rates, and growth rates; and the impact of factors such sunlight (UV radiation), temperature, sedimentation, humidity, pH, moisture content, etc.? These factors affect the fate and transport of each bacterium.

5. Developing strategies to protect public health has driven the study of microbiology. In the late 1800s/early 1900s, illness due to poor sanitation and water quality was commonplace, largely due to inadequate sewage treatment, hence the need to develop and install sewage treatment facilities and limit, if not prevent, fecal matter from entering the water. In order to evaluate the effectiveness of the treatment interventions and resulting water quality, a test was needed. The ideal approach is to test for the pathogens in the water directly; however, many of the pathogens were unknown, in relatively small quantities and hard if not impossible to test for at the time. Therefore, the indicator organism approach was embraced and because certain coliforms are virtually always found in feces, the presence of total coliforms was used as an indication that fecal contamination was probable. The total coliform group contains bacteria such as Citrobacter, Enterobacter, Escherichia (*E. coli*), Hafnia, Klebsiella, Serratia and Yersinia.

6. The problem with using total coliforms as an indicator of fecal contamination is that not all the bacteria in this group are found in feces. In an effort to increase the accuracy of the indicator organism approach, a subset of total coliforms - the faecal coliform group (aka fecal coliform) replaced total coliforms as a measure of water quality. The faecal coliform group contains bacteria such as *E. coli*, Citrobacter,

Enterobacter and Klebsiella. Although this was a better approach, it still did not eliminate the possibility that indicators would be found when in fact there was no fecal contamination (or pathogens) present. This is because in the faecal coliform group, *E. coli* is associated with feces however; organisms such as Citrobacter, Enterobacter and Klebsiella do not originate in feces and because the faecal coliform count does not differentiate which bacteria(s) are being quantified, it is not known which organisms are present/absent or where they came from.

7. In the early 1980s, realizing the shortcomings of the use of faecal coliforms as an indicator, the EPA set out to develop a better methodology to measure water quality. The technology was still not in place to directly detect all the potential pathogens so alternative indicator bacteria were evaluated. By definition, indicator(s) must be easy to detect, non pathogenic, exist in greater quantities than the pathogen and must live longer than the pathogens. An indicator would be useless if it did not persist in the environment at least as long as the pathogen. In addition, there should be a correlation of the indicator concentration with the pathogen concentration and the fate and transport properties of the indicator and pathogen should be identical/similar. Unfortunately, the EPA did not evaluate the correlation of indicator with pathogen or the fate and transport characteristics of each. The EPA did however; evaluate the indicator concentration versus number of reported gastrointestinal illnesses reported by swimmers using the water bodies for primary contact recreation although they did not attempt to identify which pathogens were causing illnesses. The general consensus in the scientific community is that the majority of the illnesses were caused by enteric viruses; the anecdotal evidence supports this conclusion as the studies were done at beaches that were impacted by wastewater discharges that

would typically carry human enteric viruses. The conclusions of the epidemiological studies resulted in issuance of the *Ambient Water Quality Criteria for Bacteria -1986* (EPA440/5-84-002) that recommended a water quality standard of a geometric mean of 126CFU *E. coli*/100ml (235CFU/100ml to 576CFU/100ml single sample maximum)or 33 CFU enterococcus/100mL (61CFU/100ml to 151CFU/100ml single sample maximum) for fresh water and 35CFU enterococcus/100ml (104CFU/100ml to 500CFU/100ml single sample maximum) for marine water (based on the EPA determination that the acceptable illness rate is 8 to 19 illnesses per 1,000 swimmers). After its issuance, the EPA recommended that all States use either *E. coli* or enterococcus instead of faecal coliforms as indicators of water quality and in 2000, the Beaches Environmental Assessment and Coastal Health (BEACH) Act required States adjacent to the Great Lakes and coastal states to adopt the 1986 Standards.

8. The recommendation to change indicators was met with resistance due to the reluctance to change for a variety of reasons, including but not limited to the fact that the correlation of illnesses to indicator concentrations was not as strong as some deemed appropriate and that the epidemiological studies were carried out in waters that were impacted by wastewater treatment plant discharges. One of the major objections was that in areas that have wastewater treatment plant effluent the illness rate versus indicator concentration would be higher due to the presence of human enteric viruses in the effluent. At the time of the studies, the EPAs intention was to do additional studies to determine if indicators that were derived from various sources did in fact hold a different correlation with illness rates. However, the studies were never carried out due to funding constraints. The indicators, both *E. coli* and enterococcus are shed from virtually all warm blooded animals, e.g. cattle, pigs, deer,

birds, wildlife, waterfowl, humans, pets, etc. In fact, wildlife, waterfowl and birds are major contributors of the *E. coli* and enterococcus that are found in surface waters. The issue of different sources was addressed with an EPA policy that stated a State could discount all indicator bacteria derived from nonhuman sources when making regulatory decisions. This policy was extremely important because States were in the process of developing total maximum daily load regulations (TMDLs) to address high levels of bacteria in surface waters throughout the country and finding that *E. coli* and enterococcus were ubiquitous in the environment. Relative to the EPAs recommended standards, surface waters throughout the country are out of compliance. For example, Delaware a state with three counties, one primarily urban, one primarily agriculture and one mixed lists approximately 97% of the State's waters on the 303(d) list as impaired due to high indicator bacteria levels. Coincidently, Delaware monitors approximately 97% of its surface waters. In Oklahoma, 5,847 miles of stream segments are listed as impaired due to high enterococcus levels, 3,118 miles due to high *E. coli* levels and 2,921 miles due to high faecal coliform levels. Of the lakes assessed, 34% did not meet the primary contact recreation standards (Tenkiller Ferry Lake is not impaired by bacteria). In Oklahoma, more stream segments are listed as impaired for enterococcus than any other water quality parameter. In the IRW there are no stream segments listed as impaired by faecal coliforms, 8.6 miles listed as impaired by *E. coli* and 97.2 miles listed as impaired by enterococcus. This represents 0% of the States impairments for faecal coliforms, 0.28% of the States impairments for *E. coli* and only 1.78% of the States impairments for enterococcus (Oklahoma 2006 303(d) list). The issue of high bacteria levels is prevalent throughout Oklahoma and is in no way confined to the IRW or areas that are used for poultry production or the application of poultry litter. The bacteria levels that are seen in the

IRW and throughout Oklahoma are typical of the levels seen throughout the country as evident by 303(d) listings and TMDLs that require reductions of up to and in some times greater than 90% of the indicator bacteria.

9. A TMDL is the appropriate regulatory mechanism that is in place to address impaired waters. They require monitoring, identification of the sources of a pollutant, and load allocations and pollution control strategies to remediate the problem.

10. In order to discount the nonhuman sources and determine what the sources were so that appropriate pollution control strategies (PCS) and best management practices (BMPs) could be developed, the science of microbial source tracking (MST) was developed (early/mid 1990s). MST was an extension of the principles used in track down studies such as those done by the CDC during an illness outbreak. Antibiotic resistance analysis (ARA, aka ARP – antibiotic resistance pattern) was one of the more widely used MST techniques. Initial studies reported high average rate of correct classifications (ARCC) as a measure of accuracy (using various calculation techniques including the holdout method of cross validation) and suggested that an ARCC of 60% to 70% was enough for water quality managers to base decisions on (Harwood et. al, 2000, *Classification of Antibiotic Resistance Patterns of Indicator Bacteria by Discriminant Analysis: Use in Predicting the Source of Fecal Contamination in Subtropical Waters*). Encouraged by the results of initial studies and due to the time constraints relating to the development of TMDLs and other regulatory pressures, ARA was quickly embraced by many as a mainstream technology and was widely used for MST. But as more studies were undertaken and ARA as well as other MST methods were challenged by the scientific community in method comparison studies in which

known samples were given blindly to participating labs, the majority of the methods performed poorly. In both the Southern Coastal California Water Research Project (SCCWRP)/EPA study and the USGS method comparison, ARA performed very poorly and had extremely high false positive rates (39% to 100%). Dr. Harwood participated in the SCCWRP study and utilized this methodology; her lab's performance reflected this deficiency. In addition, it was determined that the use of ARCC using techniques such as the holdout method of cross validation was not an adequate measure of the accuracy of the method (Harwood et. al., 2004, *Phenotypic library-based microbial source tracking methods: Efficacy in the California collaborative study* and Stoeckel and Harwood, 2007, *Performance, Design, and Analysis in Microbial Source Tracking Studies*). In general, library-based methods performed poorly, especially those that employed the population ecology approach to fingerprint analysis. The population ecology approach matches genetic patterns, e.g. ribotyping "fingerprints" by using mathematical algorithms to estimate the similarity between two fingerprints. In reality, two fingerprints can be very similar, often differing by only one band and be from bacteria originating from different sources. One method that is much more accurate is the molecular epidemiological approach in which only identical matches are considered to originate from the same source. This approach is used by the CDC and other regulatory agencies in track down investigations such as the determination of the source of disease outbreaks. IEH uses this analytical approach and performed the best in these method comparison studies.

11. In 2004, the EPA promulgated a rule that required the states included in the 2000 BEACH Act to adopt the 1986 bacteria standards and reversed the policy of discounting nonhuman derived bacteria indicators. However, many states that were not included

in the Act were still reluctant to switch from the faecal coliform standard. There was/is still a great deal of apprehension regarding the usage of E. coli and/or enterococcus as indicators, so much so that the 2000 BEACH Act mandated the EPA to reevaluate the standards. Dr. Myoda was invited to serve in spring of 2007 as an expert in the Experts Scientific Workshop discussing the critical research and scientific needs relating to the development of a new recreational water quality standard. Issues addressed in the workshop included but were not limited to the problem that bacteria data was not available until 24 hours after a sample was taken, there was no/weak correlations between the indicator(s) and many of the pathogens and that the correlation is variable based on the source of the indicators and the fate and transport characteristics of the indicators versus the pathogens is unclear.

12. When developing the appropriate indicator(s) to use, knowledge of the fate and transport characteristics of the indicator(s) and pathogens, both individually and as they relate to each other is critical. Individually, fate and transport is significant because only those pathogens that are present and viable in the water pose a potential public health risk to those recreating in the water. As the microbiological characteristics of each pathogen are significantly different, it is highly likely that their fate and transport characteristics will vary as well.

13. The most simplistic route of transport is direct deposition, e.g. cattle defecating in streams. Once the pathogen(s) (assumed to be carried in the feces of warm blooded mammals) is excreted over or in the water, the relevant questions are how long will the pathogen be viable and available. Indirect deposition of feces introduces many more variables affecting the fate and transport of the bacteria and or pathogen. First,

the fecal properties from different mammals vary substantially. One of the primary differences (aside from pathogen and indicator density) is moisture content. Very "wet" feces is more likely than "dry" feces to introduce pathogens into the environment. After defecation, the distance from the water plays an important role as well. Driven by heavy precipitation and transported primarily via surface runoff, the organisms may be washed into the surface water by sheet flow. During this transport, they are subjected to a variety of environmental factors including but not limited to UV disinfection, predation, temperature etc. that affect the proportion that will ultimately end up in surface water in which people are recreating. In the application of poultry litter, any indicator bacteria (as well as any other bacteria contained in the feces) are subjected to the conditions in the litter for great lengths of time prior to the application of the litter as a fertilizer/soil amendment. During this time, the composting processes along with natural die off kill a substantial portion of the bacteria. What bacteria may survive until application is then subjected to the aforementioned environmental factors and only a small portion (if any) will remain viable.

14. Resuspension from sand or sediment could also play an important role. There may be a reservoir of indicator(s) that could be reintroduced into the water column. Additional, regrowth of the indicator(s) could represent a source and confound the risk assessment/prediction.

15. Ideally, the indicator(s) chosen as the surrogate for the pathogens will have the same fate and transport characteristics of the pathogens themselves. However, since this is unlikely, it is important to know and relate the characteristics that are indicator(s)

specific to the pathogens so that the measurement of the indicator can be correlated to the concentration of the viable pathogens in the water and ultimately to public health risk.

16. Lastly, when studying microbiology, it is imperative that standard methods that have been accepted by the scientific community are followed. These methods should be approved by the appropriate authority such as the EPA, Standard Methods for the Examination of Water and Wastewater, and/or AOAC, etc. This ensures that, if the tests are carried out correctly, the results are reliable and reproducible. In addition to utilizing the proper testing method, sample collection must be carried out with equal rigor and quality controls. Issues such as hold time (normally included in the standard method) must be strictly adhered to or the results are invalid, e.g. exceeding the hold time on water samples that are being analyzed for bacteria concentrations could lead to higher counts due to regrowth. Statistically valid sampling plans must be followed; sample locations and the time the samples are taken must be randomly selected. A minimum number of samples must be taken to ensure that the testing reflects an accurate picture of the whole. Positive and negative controls must be used. Unless all these elements are included in a scientific study, the results are questionable if not invalid.

17. In reviewing the data it is apparent that standard methods were not followed in the Plaintiff's testing. In approximately 60% of the water samples, the 6 hour hold time mandated by the EPA for recreational water being tested for indicator bacteria (*E. coli*, enterococcus) was violated, in many cases by one to two days; therefore, this data is unreliable. In addition, the recreation water quality standard is based on a

geometric mean of no less than 5 samples taken within 30 days, a frequency that was not maintained during the Plaintiff's study. Sampling locations were not chosen randomly. It appears that the locations that the Plaintiff's thought it most likely to find what they wanted to find were chosen. Furthermore, it appears that the timing of sample collection was not randomized either, both with respect to the time of day samples were collected and the timing relative to flow conditions. Many of the samples were taken during high flow conditions during which bacteria counts will generally be higher than average due to resuspension and runoff. Based on all of these violations of standard methodologies, we believe that the data is unreliable and is biased and skewed in favor of the Plaintiff's position.

18. The CRA report revealed egregious violations in proper sampling protocols. These violations included but were not limited to samplers walking through feces into water that they then sampled, soil borers driven through feces and into the dirt when soil samples were taken and sampling tools not being disinfected between use. A review of the edge of field sample data reflects that the mean bacteria concentration for E. coli is 4,174 CFU/100ml, for enterococcus is 14,664 CFU/100ml, and for faecal coliforms is 6,371 CFU/100ml (see attachment A). Although there were a few samples reported to have concentrations of 1,600,000 CFU/100ml, those are atypical and represent outliers in the data set. However, even those outlying values are an order of magnitude below that of sewage influent (58,000,000 CFU *E. coli*/100ml, Miyanaga et. al, 2006, *Detection of Escherichia coli in the sewage influent by fluorescent labeled T4 phage*). In our view, the atypically high values are more consistent with samples taken in close proximity to a concentrated source of indicator bacteria, e.g.

cattle feces, than with runoff samples taken from areas affected by uniformly distributed indicator bacteria such as the application of poultry litter.

19. The indicator bacteria in the waters of the IRW originate from many sources. The loading from cattle is extremely significant. Typically, cattle will excrete 15 to 35 kg of feces per day. In the summer when the majority of primary contact recreation is occurring, the initial *E. coli* concentration in the feces will be approximately 3,000,000 *CFU E. coli*/gram, however after deposition the bacteria multiply and reach levels of approximately 48,000,000 CFU *E. coli*/gram (Sinton et. al, 2007, *Survival of Indicator and Pathogenic Bacteria in Bovine Feces on Pasture)*. Also in the summer months the cattle tend to congregate near and in the streams in order to cool off, increasing the possibility of direct deposition into and in close proximately of the streams. This means that each day one cow will contribute roughly 960,000,000,000 *E. coli* into the environment and with approximately 200,000 head of cattle in the IRW over 192,000,000,000,000,000 CFU *E. coli* will be introduced into the environment each day.

20. In addition to cattle, there are approximately 150,000 swine and wildlife (geese, ducks, deer, turkeys, etc) and birds that live throughout the watershed as well as the wastewater treatment plant effluent and septic system loads that are sources of indicator bacteria. Wildlife sources are a significant source of fecal material and indicator bacteria. The USGS reported that in Delaware County, Oklahoma, 45% of the *E. coli* sampled came from birds and 22% came from cattle (*Reconnaissance of the Hydrology, Water Quality, and Sources of Bacterial and Nutrient Contamination in the*

Ozark Plateaus Aquifer System and Cave Springs Branch of Honey Creek, Delaware County, Oklahoma, March 1999-March 2000).

21. The presence of indicator bacteria does not mean that pathogens are present. Those that could be present include bacteria such as salmonella, campylobacter and *E. coli* O157:H7. These pathogens are carried by a variety of hosts, e.g., *E. coli* O157:H7 are primarily found in cattle, salmonella in reptiles and poultry and campylobacter in cattle, swine and poultry. Other hosts could carry these pathogens as well. The Plaintiff contends that *E. coli* O157:H7 is shed from poultry. However, there is virtually no evidence that poultry carries *E. coli* O157:H7 and the Plaintiff never tested for or found it in the litter or in the environment. Campylobacter is an organism that grows well in the conditions typically found in the digestive system. However, it does not survive well in the environment. It will die when exposed to oxygen and will also readily dehydrate and die. The Plaintiff's edge of field (EOF) sampling revealed that there was no campylobacter running off of the fields where litter had been applied[1]. Salmonella was only reported in 3 EOF samples[1], the concentrations were very low, and the source of the salmonella was not determined.

[1] No campylobacter was found in EOF water samples. Using BioSep beads, campylobacter and salmonella was reported however, using that technology the bacteria sorb onto the beads and no concentration can be determined. In addition, the reported values were all 50/bead (with the exception of one 5/bead) which raises suspicions regarding the quantification accuracy)

22. The "biomarker" that the Plaintiff claims to be poultry specific is not specific to poultry. In the extremely limited sampling of known sources used to validate this claim, the biomarker was found to be carried by ducks, geese and cattle. When retested the cattle sample came back negative and the explanation given by Dr. Harwood was that it was most likely due to contamination in the laboratory. If contamination was occurring in the laboratory the reliability of all the test results are suspect except the duck and goose positive samples which were verified to be correct.

23. Absence of proof is not proof of absence. Only 24 cattle manure composites, 2 swine manure composites, 10 duck and 10 goose manure composites, 3 septic and 3 wwtp samples were tested to validate the specificity of the biomarker. Virtually all of the sources of the indicator bacteria in the IRW were not tested, e.g. birds, deer, wild turkeys, pets, wildlife, etc. Additionally, the manure composites were taken from 10 "patties" so it is impossible to know if one in ten or all ten in ten carried the biomarker. If only one in ten carried the biomarker, diluting it with 9 other patties may have reduced the concentration below the method detection limit. When the samples were taken, locations were not randomly selected and the geographic variability was not captured. It is very probable that the bacterial communities of animals living in close proximity to one another will be similar therefore, in order to capture a more representative sample of the watershed, 10 patties should be taken from 10 different farms, not 10 from the same farm (Hartel et. al, 2007, *Geographic sharing of ribotype patterns in enterococcus faecalis for bacteria source tracking*).

24. The "poultry biomarker" MST method is not a standard method nor has it been peer reviewed or third party tested, making it at best a research method and not one that

can or should be used for any regulatory action(s) or used to draw conclusions about the sources of indicator bacteria in the IRW (or anywhere else).

25. The method is based on the amplification of a particular genetic sequence that may or may not be from a live organism. The polymerase chain reaction (PCR) cannot differentiate between live or dead organisms. DNA can persist for long periods of time in the environment, creating positive PCR results long after the indicator or pathogen has been introduced into the environment and has died. Pathogens, in this case salmonella and campylobacter need to be alive to be infectious.

26. The genetic sequence used as the biomarker is a portion of the 16S rRNA gene. The known bacteria that has the closest sequence to this fragment is *Brevibacterium avium*. *Brevibacterium avium* was first identified and isolated from bumble-foot lesions in domestic fowl in 1999. *B. avium* can be cultured and is differentiated from other Brevibaterium species by both its genetic sequence and phenotypical traits e.g., temperature that it grows, utilization of arabinose, etc. It is perplexing that no attempt to culture this "new" bacteria species that the Plaintiff claims to have discovered was carried out. There is no evidence that this "new" organism is viable or pathogenic. Dr. Harwood stated that it is close to the pathogenic, *B. casei*, although genetic analysis indicates that it is much closer to *B. avium*. Being in the same genus as a pathogen does not mean that it will be a pathogen, e.g. the majority of E. coli is nonpathogenic although the nearly identical *E. coli* O157:H7 is highly pathogenic.

27. The Plaintiff claims that the levels of biomarker can be used to quantify the indicator bacteria originating from poultry. However, when developing the quantitative aspect of this assay, negative values were reported for the amount of DNA present. It is impossible to have negative amounts of DNA. Therefore the standard curves used in quantification cannot be correct and the quantitative values reported by the Plaintiff are also incorrect.

28. There is no correlation between the amount of the biomarker and the indicator organism concentration in the litter (see attachment 3). Assuming that all the other technical issues surrounding this new test could be overcome, without a correlation between indicator concentration and biomarker concentration in the litter it is impossible to extrapolate the amount of indicator bacteria originating from poultry in the waters of the IRW.

29. There is no public health linkage between the biomarker and illness rates. No epidemiological studies have been conducted to determine if there is a correlation between these two parameters.

30. Indicator bacteria are ubiquitous and originate from multiple sources in the IRW. Major contributors include cattle, swine and wildlife. The new "biomarker" that the Plaintiffs claim proves that poultry is the primary source of indicator bacteria is not specific therefore, it does not support that conclusion. In addition, there is no correlation between it and the indicator bacteria in the litter so it cannot be used as a quantitative tool. Based on the data that we have reviewed, there is no evidence that

poultry is the major contributor of indicator bacteria or that there is an imminent public health threat in the IRW.

I declare under penalty of perjury that the foregoing is true and correct.

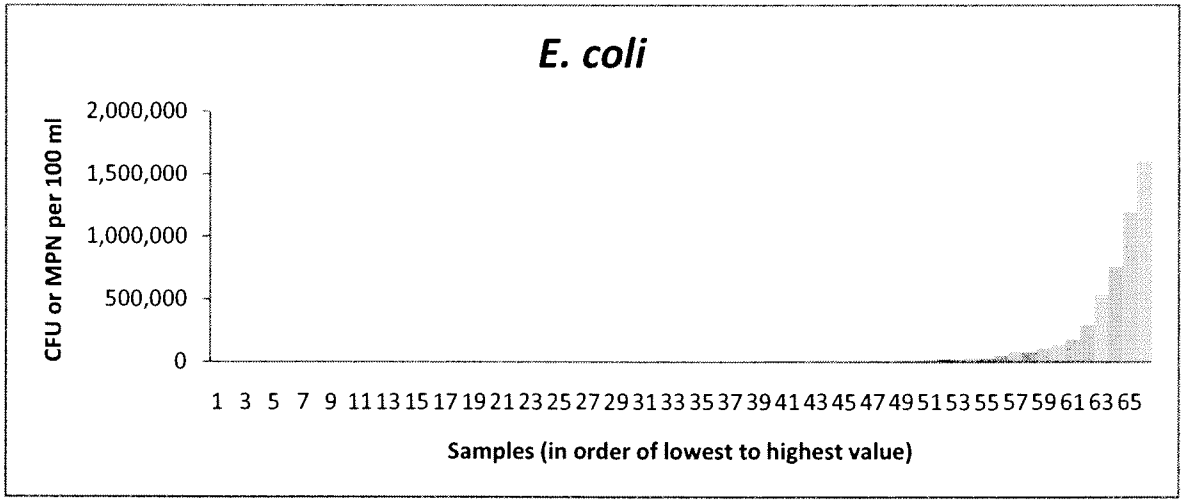
Executed on February 8, 2008

Sam Myoda

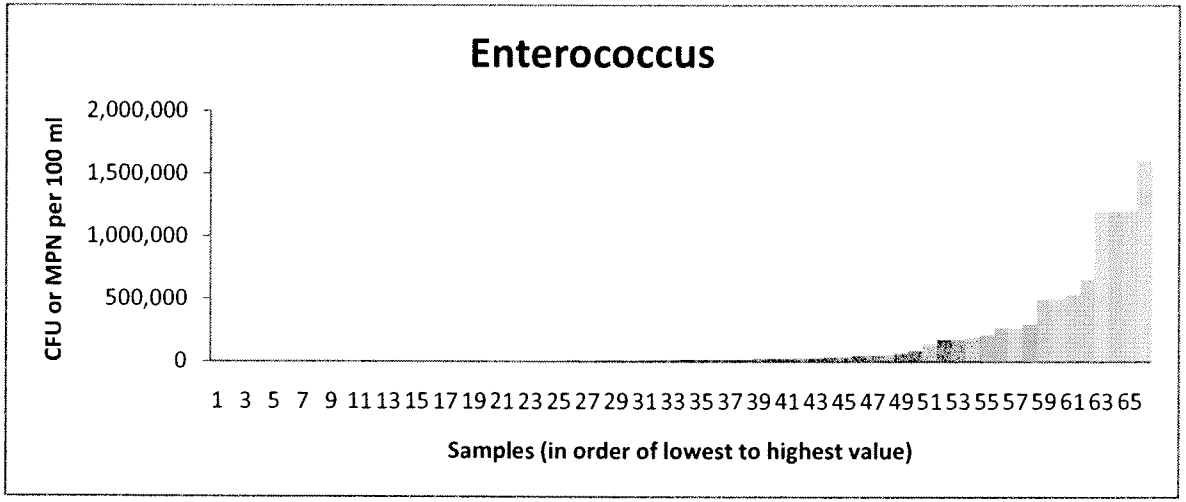
Dr. Samuel P. Myoda

Mansour Samadpour

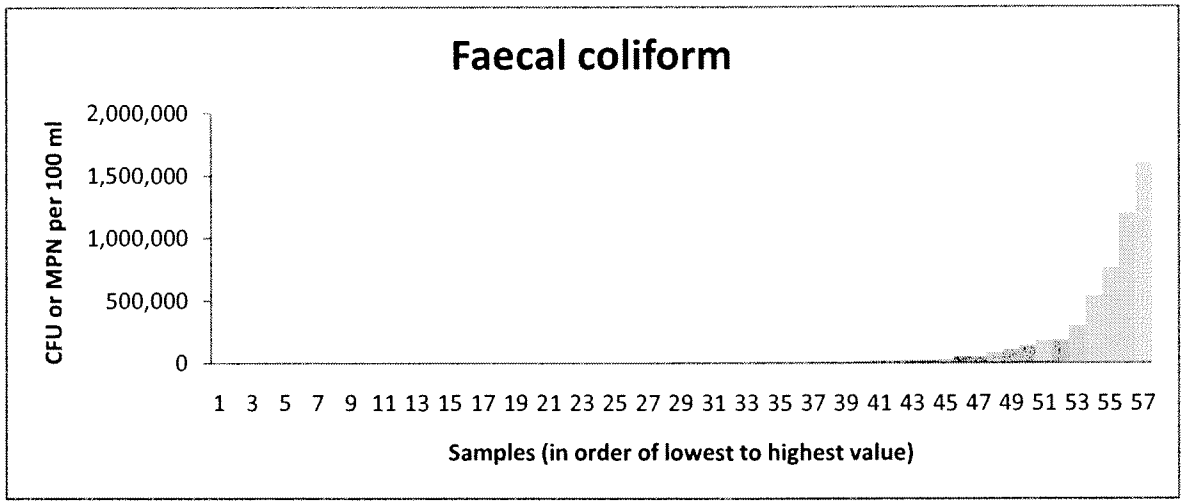
Dr. Mansour Samadpour



Edge of Field	
# of samples	66
	CFU or MPN/100 mL
Geometric mean	4,174
Minimum value	17
Maximum value	1,600,000
80% of all samples below	<24,000
90% of all samples below	<125,000
***Raw sewage ~ 58,000,000 CFU/100mL**	



Edge of Field	
# of samples	66
	CFU or MPN/100 mL
Geometric mean	14,664
Minimum value	110
Maximum value	1,600,000
80% of all samples below	<180,000
90% of all samples below	<503,000

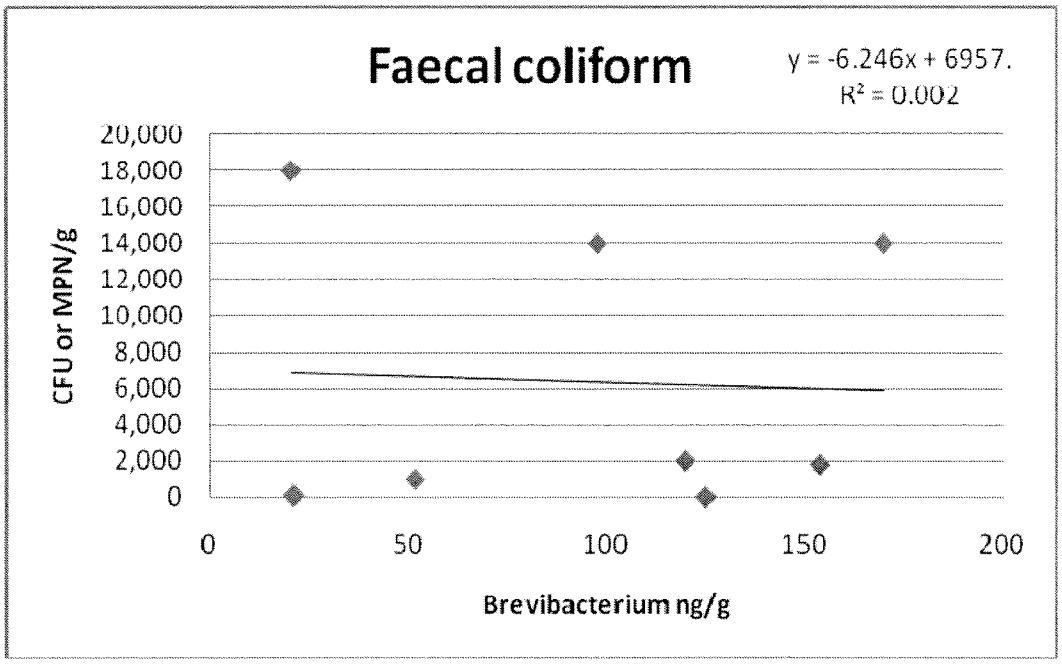


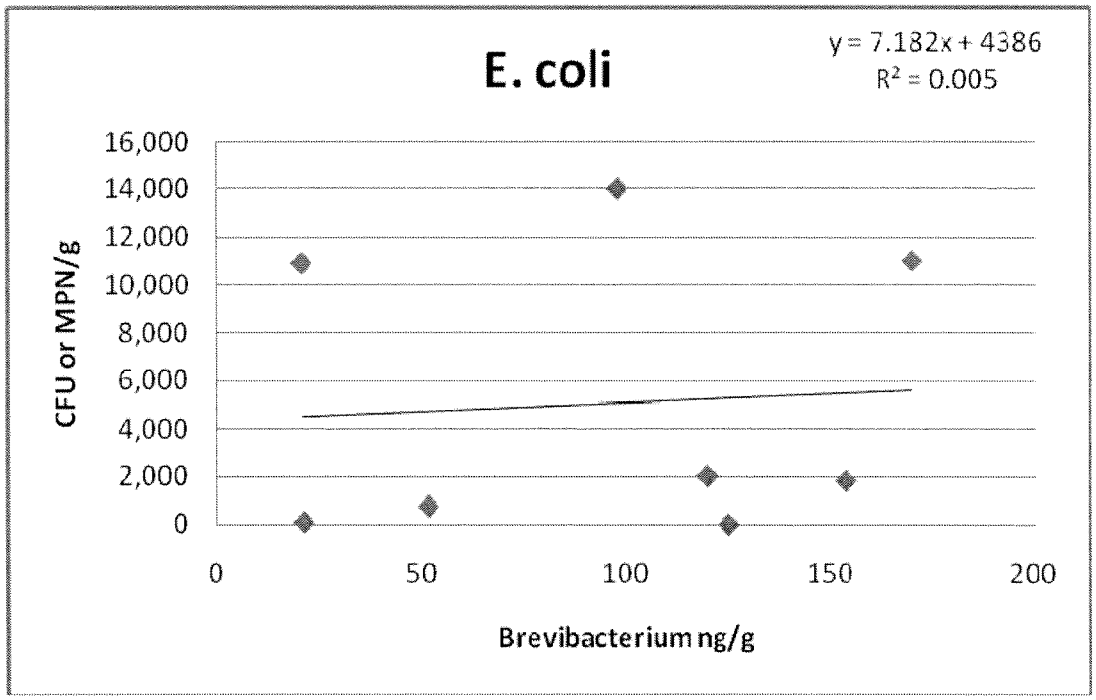
Edge of Field	
# of samples	57
	CFU or MPN/100 mL
Geometric mean	6,371
Minimum value	30
Maximum value	1,600,000
80% of all samples below	<46,000
90% of all samples below	<180,000

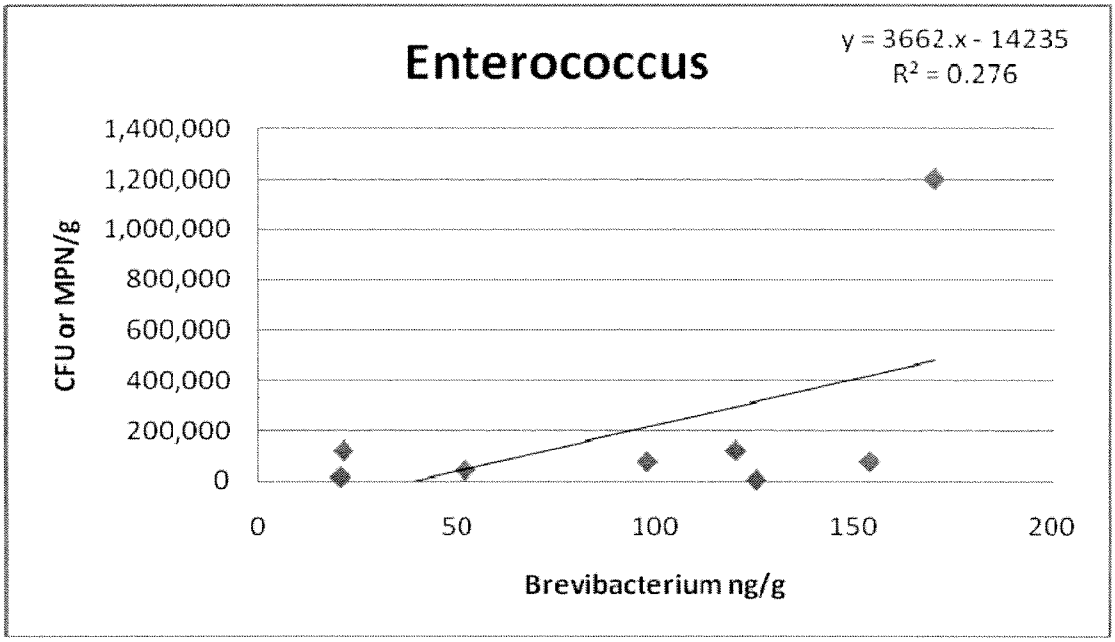
Litter Samples

No correlation between Brevibacterium and indicator organism concentrations

	Faecal coliform MPN or CFU/g	E. coli MPN or CFU/g	Enterococcus MPN or CFU/g	Brevibacterium ng/g
2/2/06	18,029	10,904	13,776	21
7/6/06	94	94	120,000	21.3
6/21/06	980	759	40,988	51.9
8/3/06	14,000	14,000	76,000	98.1
9/22/06	2,000	2,000	120,000	120.1
6/20/06	0.2	0.2	3,800	125.3
8/15/06	1,800	1,800	76,000	154
8/31/06	14,000	11,000	1,200,000	170.1




E. coli
y = 7.182x + 4386
R² = 0.005
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
CFU or MPN/g
0
50
100
150
200
Brevibacterium ng/g


Enterococcus
y = 3662.x - 14235
R² = 0.276
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
CFU or MPN/g
0
50
100
150
200
Brevibacterium ng/g

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Profile

Environmental engineer with extensive regulatory, business and laboratory experience combined with excellent analytical, computer, organizational, public relations, and sales skills. Wide-ranging knowledge of federal and state laws, rules, regulations, policies and procedures including but not limited to monitoring, permitting, compliance and settlements, e.g. Clean Water Act, Clean Air Act, CERCLA, SWDA, RCRA, SARA. Highly motivated and innovational with exceptional project management, training, multitasking and budgeting skills.

Education

University of Delaware, Newark, DE
B.S. Environmental Science (1995), *magna cum laude*
(ranked first in class within major)
Minors in Biology and Economics

University of Delaware, Newark, DE
Ph.D. Civil & Environmental Engineering (2001)

Professional Experience

Institute of Environmental Health/Molecular Epidemiology
Seattle, WA (6/2007 to present)
Vice President

- Expert witness for attorneys on cases such as the September 2006, spinach - *E. coli* O157:H7 outbreak
- Genetic track downs and pathogen detection and quantification
- Laboratory Director and water quality and water systems expert

Delaware Department of Natural Resources and Environmental Control,
Dover, DE (6/2001 to 6/2007, current DWR "Employee of the Year")
Environmental Engineer VI

- Responsible for all aspects of State environmental regulations and standards including authoring, adoption, implementation, compliance and permitting, e.g. TMDL, CAFO, NPDES, MS4, Title V, etc.
- Computer modeling of the fate of air and water pollutants and the effectiveness of reduction strategies, e.g. Watershed Nutrient, DO and pathogen modeling
- Contribute new designs, processes, and techniques, which are regarded as major advances in the field, e.g. established a genetics lab for bacteria source tracking (BST) and served as Director performing BST on all waters in the State of Delaware
- Program management, employee supervision and budgeting (operating and capital)
- Provide technical expertise, direction, guidance and assistance to department personnel, federal/state agencies, industry and private sector organizations
- Formulate long-range objectives, broad policies and strategies and develop and analyze performance measures

- Negotiate and manage consultant contracts, monitor progress and participate in soliciting, reviewing and screening consultants
- Represent the state and department on national and regional committees, e.g. EPA Experts Scientific Workshop on Critical Research Needs for the Development of new or Revised Recreational Water Quality Criteria (3/25-30/2007)
- Plan and implement special studies designed to analyze programs, projects, and services and make appropriate recommendations
- Negotiate settlements, e.g. >$200 million, Title V, air pollution reduction settlement
- Present, prepare and publish reports and documents, e.g. impact statements, technical reports, budget reports, monitoring results, training and safety guidance, QA and QC guidance and peer-reviewed journal articles

University of Delaware, Newark, DE (1997-2001)
Laboratory Manager, Research and Teaching Assistant

- Oversaw all operations within 4 laboratories, duties included technical advisement, scheduling, safety, equipment purchasing and maintenance, ordering and budgeting
- Safety officer
- Assistant to the Department Chairperson
- Taught and trained visiting professors, scholars and graduate and undergraduate students

Lawn Doctor of Newark, Newark, DE (1986-1993, 1995-1997)
Manager

Responsible for the majority of sales and production aspects of operations for three lawn care franchises serving thirty five hundred customers within a 400 sq. mile area. Supervised a staff of fifteen personnel and developed/implemented training programs to maintain multi-state pesticide certification. Interacted with the public on a daily basis.

Selected Accomplishments:

- Set individual and franchise sales records in five of seven years
- Developed IPM (integrated pest management) programs to reduce pesticide usage by 60%
- Spearheaded business expansion into new areas, resulting in the acquisition of a fourth franchise
- Designed employee safety procedures, reducing accidents and chemical exposure
- Expanded sales by initiating a tree and shrub maintenance program and an all-natural lawn maintenance program
- Won "Best Franchise in the Nation" award (1989, 1990, 1992)

Certifications OSHA 40-hour training
Ornamental Horticulture, Longwood Gardens
Delaware Department of Agriculture: categories 03, 5B, 06, 07

Selected Publications and Presentations

Hartel, P.G., **Myoda, S.P.,** Kuntz, R.L., Rodgers, K., Entry, J.A., VerWey, S.A., Schroder, E.C., Calle, J., Lacourt, M., Theis, J.E., Reilly, J.P., Fuhrmann, J.J. (2007), Geographic and Temporal Changes of Enterococcus Faecalis Ribotypes for Bacteria Source Tracking. *Journal of Water and Health (*accepted on 12 Dec 2006)

Samuel P. Myoda, C. Andrew Carson, Jeffry J. Fuhrmann, Byoung-Kwon Hahm, Peter G. Hartel, Helen Yampara-Iquise, LeeAnn Johnson, Robin L. Kuntz, Cindy H. Nakatsu, Michael J. Sadowsky and Mansour Samadpour (2003), Comparison of genotypic-based microbial source tracking methods requiring a host origin database *J Water Health*, 01, pp. 167-180

Jill R. Stewart, R. D. Ellender, Janet A. Gooch, Sunny Jiang, **Samuel P. Myoda** and Stephen B. Weisberg (2003), Recommendations for microbial source tracking: Lessons from a methods comparison study, *J Water Health*, 01, pp. 225-231

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Comparative Diversity of Fecal Bacteria in Agriculturally Significant Animals to Identify Alternative Targets for Microbial Source Tracking (ASM – 2003) J. M. Simpson, **S. Myoda**, D. J. Reasoner, J. W. Santo Domingo

Myoda, S.P., Huang, C.P. (2001) The Ultrasonic Disinfection and Organic Constituent Mineralization of Wastewater. Presenting at the Seventh International Conference on Advanced Oxidation Technologies for Water and Air Remediation, Niagara Falls, Canada (6/28/01)

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ABBREVIATED CURRICULUM VITAE

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B.S. Microbiology, University of Washington, Seattle, WA, 1981
M.S. Microbiology, University of Washington, Seattle, WA, 1987
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4. Professional Experience:
President, IEH Laboratories & Consulting Group, 2001-Present
President, Molecular Epidemiology, Inc., 2001-Present
Assistant Professor, Dept. of Environmental Health, University of Washington, 1993-2003
Research Associate, Dept. of Environmental Health, University of Washington, 1991-1993
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5. Summary of Publications:

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Research Reports:	>200
Invited Presentations:	65
Abstracts:	52

6. Membership in Professional Organizations:
American Society for Microbiology
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International Association for Food Protection
American Society of Animal Science
American Water Works Association
American Public Health Association
Water Environment Federation

7. Selected Activities include:
University of Washington Service: Admissions Committee
Nutritional Sciences Executive Committee
Nutritional Sciences Chair Search Committee
PTAP Committee

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Committee on Faculty Responsibilities
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Invited Presentations:

USDA, Manure Management Conference, Des Moines, IA
AMI, Molecular Epidemiology Workshop, Arlington, VA
Tyson Foods, Food Safety Workshop
AMI, Annual Conference
NMI, Annual Convention (Future of Food Microbiology)
AMI, Special Workshop on Epidemiological Approaches in Detection of Foodborne Outbreaks
Annual Food Safety Research Symposium, Recall of Fresh Meat Products

8. Selected Publications in Refereed Journals (1983-2007):

1. Field KG, **Samadpour M.** "Fecal source tracking, the indicator paradigm, and managing water quality."Water Res. 2007 Aug;41(16):3517-38. Epub 2007 Jun 27.

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Errata for Samadpour/Myoda Declaration

p.6	Observation that waterways in the IRW represent 1.78% of Oklahoma waterways impaired for enterococci should be approximately 1.25%.
p.12	Reference to 58 million *E. coli* in raw sewage should refer to 58 million total coliform, and the correct count for *E. coli* in raw sewage is 5.4 million cfu/100 ml.
p.17	The negative numbers referenced in paragraph 27 refer to total starting DNA, not total starting biomarker.
p.17	The correlations referred to in paragraph 28 and in attachment 3 were calculated using the wrong dataset. It remains the case that without demonstrated correlations in the litter, and indeed in poultry feces and in surface water, the "biomarker" is not a useful indicator of poultry fecal contamination.

REPORT OF SAMPLING OVERSIGHT OBSERVATIONS

ILLINOIS RIVER WATERSHED OKLAHOMA AND ARKANSAS

Prepared For:
Tyson Foods et al.

Prepared By:
Jay Churchill, P. Eng.
Conestoga-Rovers & Associates

DISCLAIMER:
SOME FORMATTING CHANGES MAY HAVE OCCURRED WHEN THE ORIGINAL DOCUMENT WAS PRINTED TO PDF; HOWEVER, THE ORIGINAL CONTENT REMAINS UNCHANGED.

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TABLE 1 LIST OF FIELD ISSUES OBSERVED 2006/2007, ILLINOIS RIVER WATERSHED - SOIL, WATER, AND LITTER SAMPLING

LIST OF APPENDICES

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1.0 INTRODUCTION

Conestoga-Rovers & Associates (CRA) was retained in June 2006 by Tyson Foods et al. to review documents associated with the Illinois River Watershed (IRW) Sampling Activities, including:

- Camp Dresser and McKee (CDM) Work Plan entitled "Soil and Litter/Manure Sampling Protocol" (CDM Work Plan);
- OSU Cooperative Extension Fact Sheets (Factsheets);
- CDM Standard Operating Procedure entitled "Residential Well Sampling" dated January 9, 2006, revised January 2007 and February 6, 2007;
- CDM Standard Operating Procedure entitled "IRW Groundwater Sampling" dated January 9, 2006, revised January 3, 2007;
- CDM Standard Operating Procedure entitled "Spring Water Sampling" dated June 20, 2005 revised February 5, 2007;
- USEPA Standard Operating Procedures (EPA SOP); and
- Oklahoma Water Resources Board (OWRB) Water Quality Monitoring Program – Field Sampling Protocol for Water Quality Assessments of Streams and Rivers. Draft revised April 27, 2004.

In addition, CRA personnel provided oversight of the field sampling activities conducted by CDM personnel. CDM was assisted by sampling personnel from a firm named Lithochimeia. The business relationship between CDM and Lithochimeia is not known to CRA. Additionally, the employment of the various sampling personnel is not known to CRA. Accordingly, hereafter, in this Report on Sampling Oversight Observations, general reference is made collectively to "CDM" personnel collecting samples.

CRA field observations were compiled in field books, video recordings, photographs, and located using Global Positioning System (GPS) coordinates. A summary of the sampling issues observed by CRA during oversight of CDM's collection of soil, groundwater, surface/spring water and litter samples pursuant to subpoenas and notice in 2006 and 2007 is listed in Table 1. The issues identified in Table 1 occurred on a routine basis and are the most significant and material violations of published standard operating procedures and protocols observed. In addition to the issues listed in Table 1, CRA personnel noted deviations from the above mentioned documents, which are detailed in this report.

Jay Churchill, P. Eng. of CRA has a degree in engineering, and over 20 years of professional experience in engineering, project management, design, and construction oversight of environmental projects throughout North America and in Puerto Rico. Mr. Churchill has collected numerous soil, sediment, surface water, groundwater, concrete core, wipe, sludge, and air samples in accordance with regulatory agency-approved work plans at numerous sites. Mr. Churchill additionally has technical expertise in the agricultural field related to conservation planning, agricultural waste management systems, land treatment practices, nutrient management, and soil and water quality. In recent years, Mr. Churchill has provided project management and technical expertise to CRA's Agricultural Services Group and has been instrumental in the preparation of detailed reports, Comprehensive Nutrient Management Plans, work plans for agri-environmental projects, completion of environmental assessments for agricultural operations, and design review. Mr. Churchill's curriculum vitae is presented in Appendix A.

2.0 ACTIVITIES

2.1 BACKGROUND

The principals of environmental sampling are based on published Standard Operating Procedures and Protocols. The reason the EPA has promulgated procedures and protocols is to provide consistent methods for sample collection, thereby ensuring sample integrity and reliable analytical results. For the purposes of the 2006/2007 IRW sampling activities OSU factsheets, the CDM Work Plan and CDM Standard Operating Procedures (CDM SOPs) provided procedures and protocols for CDM field personnel to follow during sampling activities, including:

- Scope, Overview, and Application of SOPs for Soil, Water, and Litter Sampling;
- Sampling Methods Summary;
- Sampling Procedures for Soil, Water and Litter;
- Sample Containers, Preservation Techniques, Quality Assurance/Quality Control (QA/QC);
- Documentation of Sample Collection and Handling; and
- Reporting of Analytical Data, QA/QC, and Corresponding Field Details.

CRA field personnel observed repeated and material violations of the aforementioned protocols during oversight of the CDM sampling activities. In many instances CRA field personnel observed activities that resulted in direct cross-contamination of samples, presented the potential for unrepresentative analytical results, and showed disregard for established protocols. The manner in which samples were collected would indicate that CDM field personnel lacked the necessary training and experience to conduct the IRW sampling activities. This was evident in the underlying actions and poor adherence to the CDM SOPs.

2.2 OBSERVATIONS OF SOIL SAMPLING ACTIVITIES

During the 2006/2007 IRW sampling activities CRA field personnel observed CDM soil sampling activities on 19 Litter Application Locations (LALs). These LALs are pastures and fields that CDM believe received poultry litter application in the past. The purpose of the sampling is to characterize environmental conditions occurring as a result poultry litter application. The unrepresentativeness of the soil samples with respect to poultry constituents is a concern with many of the LALs selected for soil sampling, due to the presence of cow manure in the fields.

In addition to the repeated and material issues identified in Table 1, CRA field personnel routinely observed cases where CDM actions were either inconsistent with written protocols or otherwise compromised the integrity of the samples. Observations during soil sampling activities included repeated and material actions which violated SOPs and would have resulted in cross-contamination between discrete sample depths, sample grids, and LALs. The following expand on material issues identified in Table 1:

- Sampling in fields where cow manure was present and sampling in close proximity to cow manure. This is a violation of the OSU Factsheet F-2207 which indicates "do not sample immediately after lime, fertilizer or manure applications because those samples do not reflect true soil fertility";
- Advancing the sample probe directly through cow manure during soil sampling, which would have resulted in cow manure and associated nutrients being introduced directly into the soil samples. This is supported by the fact that significant amounts of organic matter were observed in a number of the soil samples. This is a violation of Section III. A. 6. of the CDM Work Plan. This would result in cross-contamination of each subsequent sample until the probe was properly decontaminated;
- Dropping sample equipment in cow manure, resulting in contamination of gloves and sampling equipment prior to collecting soil samples. This is a violation of Section III. A. 6. of the CDM Work Plan. This would result in cow manure and associated nutrients being introduced into the soil samples;
- Cow manure visible on sample probe prior to sample collection. This is a violation of Section III. A. 6. of the CDM Work Plan. This would result in cow manure and associated nutrients being introduced into the soil samples;
- Failure to consistently and sufficiently decontaminate field equipment at the start of daily sampling, between grids, or after visible contamination from soil and cow manure. This is a violation of Section III. A. 6. of the CDM Work Plan. This would result in cross-contamination of contaminants from residual soils and cow manure between sample locations, sample grids, and composite sample depths;
- The sampling knife was not cleaned between sample locations and depths, and residual soil was routinely visible on the knife prior to sample collection. This is a violation of Section III. A. 6. of the CDM Work Plan. This would result in residual amounts of soil material from one sample interval being introduced to soil sample material for another depth interval;
- Stepping in fresh cow manure then on the corner of the sample triangle prior to advancing soil sampling probe. This is a violation of Section III. A. 6. of the CDM

Work Plan. This would result in cow manure and associated nutrients being introduced to the soil sample location and subsequent soil samples;

- Clearing vegetation and organic matter from sample location with nitrile gloved hands introducing surficial soil, vegetation, and organic matter to the gloves and to subsequent soil samples handled with nitrile gloves. This is a violation of Section III. A. 6 and Section III. A. 4. d. of the CDM Work Plan.
- Not changing soiled nitrile gloves between sub-samples after coming into contact with cow manure and shallower soil intervals. This is a violation of Section III. A. 6. of the CDM Work Plan. This would result in cross-contamination of the deeper soil samples with nutrients and bacteria from the shallower soil samples;
- Soiled nitrile gloves were not changed between individual sample grids or fields. This is a violation of Section III. A. 6. of the CDM Work Plan. This would result in cross-contamination between soil samples and sample grids;
- Touching soil samples directly with soiled nitrile and cotton gloves and non-gloved fingers. This is a violation of Section III. A. 6. of the CDM Work Plan. This would result in cross-contamination of the soil sample with material that is present on the gloves;
- CDM personnel placing bare fingers and soiled nitrile gloves inside sample bag. This includes placing sample bag labels on the inside of the sample bags prior to arriving at the site to commence sampling activities. This is a violation of Section III. A. 6. of the CDM Work Plan. This would result in the cross-contamination of the soil sample with material that is present on the gloves or bare fingers;
- Discarding residuals of soil samples directly on top of the location of the subsequent samples which is a violation of Sections III. A. 4. b. of the CDM Work Plan. This would result in mixing of soil from different sample depths;
- Tipping the sample probe to empty all remaining soil into sample bag. This is a violation of Sections III. A. 4. b. of the CDM Work Plan. This would result in mixing of soil from different sample depths;
- The soil probe tip was used to clear vegetation and organic matter the from the sample location prior to driving the sample probe but did not appear to be effective. In other instances vegetation and organic matter were not cleared from the sample location prior to driving the sample probe. At no time was a shovel used to clear vegetation and organic matter which is a violation of Section III. A. 4. d. and Section 3 of Exhibit E of the CDM Work Plan. This would result in the inclusion of vegetation and organic matter in the sample;

- Consistently, not all soil material in the sample probe was removed from the probe when collecting the sample from a 2-inch depth interval using the knife. As sample material from shallower depth intervals was dragged into plastic sample bags from the tip of the sample probe, soil material remaining in the sample probe from the deeper depth intervals also was dragged into the sample bags for the shallower samples, which is a violation of Section III. A. 4. b. of the CDM Work Plan. This would result in mixing of soil from different sample depths;
- Advancing the sample probe deeper than 6 inches and using the same sample knife to remove soils deeper than 6 inches in depth, which is a violation of Section III. A. 4. b. of the CDM Work Plan. This would result in mixing of soil from different sample depths;
- Recovering less than 6 inches of soil in the sample probe but still dividing the sample into three "2-inch" sample depth intervals, which is a violation of Section III. A. 4. b. of the CDM Work Plan. This would result in mixing of soil from different sample depths; and
- Insertion of sample probe tip into sample bag and holding sample bag against sample probe tip during filling of sample bag. Touching of sampling equipment to sample containers would result in sample contamination from nutrients and/or bacteria from different soil depths than the soil depth being collected.

When comparing the above noted actions of CDM field personnel with the aforementioned procedures and protocols, significant concerns arise. By violating generally accepted standards, CDMs actions significantly compromised sample integrity which undermines the representativeness of the analytical results obtained from the soil samples.

2.3 OBSERVATIONS OF GROUNDWATER AND SURFACE/SPRING WATER SAMPLING ACTIVITIES

During the 2006 IRW sampling activities conducted by CDM, CRA field personnel observed the collection of six groundwater samples and four surface/spring water samples pursuant to subpoena and notice. There were no groundwater or surface/spring water sampling activities observed by CRA during 2007. During 2006 sampling activities, CRA field personnel observed cases where CDM actions were either inconsistent with written protocols or were otherwise technically unsound, and which may have resulted in the collection of compromised or unrepresentative samples. The following expand on material issues identified in Table 1:

- Failure to adequately purge groundwater wells, which is a violation of Section 2.0 of the Residential Well Sampling and IRW Groundwater Sampling SOPs which states "Wells should be allowed to fun for fifteen minutes before parameters are recorded and samples collected." This would result in the collection of unrepresentative groundwater samples;
- Collection of groundwater samples directly from a garden hose, a potential source of bacteria and other contaminants, which is a violation of Section 4.1 of the Residential Well Sampling and IRW Groundwater Sampling SOPs. This would result in the collection of unrepresentative groundwater samples;
- Collection of groundwater directly from an un-sterilized spigot, a potential source of bacteria and other contaminants, which is a violation of Section 4.1 of the Residential Well Sampling and IRW Groundwater Sampling SOPs. This would result in the collection of unrepresentative groundwater samples;
- Collection of groundwater and surface/spring water samples in un-sterilized sample containers, a potential source of bacteria and other contaminants, which is a violation of Section 4.1 of the Residential Well Sampling, IRW Groundwater Sampling and Spring Sampling SOPs. This would result in the collection of unrepresentative groundwater samples;
- Collection of geochemical indicator measurements (e.g., temperature, pH, and conductivity) after sample collection, which is a violation of Section 3.0 of the Residential Well Sampling and Spring Sampling SOPs. This would result in fresh groundwater not being sampled;
- Collection of groundwater and spring/surface water samples in unpreserved sample containers and/or without field filtering, which is a violation of Section 4.0 of the Spring Sampling SOP. This would result in the collection of unrepresentative groundwater samples;
- Stirring up sediment by stepping in spring/pond prior to water sample collection and after walking down cow path with visible cow manure on it. This action resulted in suspended sediments, which may have additionally been contaminated with cow manure, being included in the surface/spring water samples, thereby contaminating the samples;
- Stirring up sediment prior to sample collection by allowing peristaltic pump to discharge directly upstream of surface/spring water sample location and in the vicinity of cow manure. This action resulted in the suspended sediments, which may have additionally been contaminated with cow manure, being included in the surface/spring water sample, thereby contaminating the sample;

- Sampling surface/spring water where cattle had been observed standing, and with visual evidence of cow manure prior, to the spring sampling. This would have resulted in collection of unrepresentative and possibly contaminated samples;
- Sampling of spring/pond with significant algae growth and visible cow manure in and around water source, would result in the collection of unrepresentative and possibly contaminated samples; and
- Not decontaminating Yellow Springs Incorporated (YSI) meter (used for collection of geochemical indicator measurements) before placing it in the spring/pond prior to obtain a spring/surface water sample collection. This could result in cross-contamination of water samples.

These technically unsound sampling procedures conducted by CDM undermine the representativeness of the analytical results obtained from both the groundwater and surface/spring water samples.

2.4 OBSERVATIONS OF LITTER SAMPLING ACTIVITIES

During the 2006/2007 IRW sampling activities conducted by CDM, CRA field personnel observed the collection of 17 litter samples pursuant to subpoena and notice. During sampling activities, CRA observed cases where CDM's actions were inconsistent with written protocols. The following expand on material issues identified in Table 1:

- Composite sample not mixed thoroughly, which is a violation of Section 8 of Exhibit E from the CDM Work Plan and OSU Factcheet F-2248 which reads, "Place subsamples in a plastic bucket, and mix thoroughly". This would result in unrepresentative and non-homogenous CDM or split samples;
- CDM personnel placing sampling equipment and fingers inside sample containers. Touching of sampling equipment inside sample containers would result in cross-contamination of samples from nutrients and/or bacteria present on the sampling equipment;
- Sub-samples collected in tracks of the catchers' cage handling machines, resulting in cross-contamination of samples from nutrients and/or bacteria outside the poultry house;
- Inconsistent number of sub-samples collected from each poultry house;
- Inclusion of litter that was dropped on the litter bed, and then picked back up in the composite sample could result in compromised samples;

- Use of a pointed spade for sub-sample collection resulted in a proportionately higher amount of litter material from the upper portion of the litter pack being included in the composite sample, which is a violation of OSU Factsheet F-2248, which states, "Collect the entire depth of the litter.." and Section IV. C. 3. of the CDM Work Plan which states, "All samples from litter areas shall be collected through the full thickness (surface to base) of the litter/manure";
- Inconsistent trimming of litter material on shovel, using a trowel, resulted in inconsistent litter volumes being collected from each sample aliquot in the poultry house. In addition, the trimming also resulted in a proportionately higher amount of litter material from the upper portion of the litter pack being included in the composite sample, as varying amounts of loose material from the lower portion of litter pack would fall from the shovel during the act of trimming;
- Soil (i.e., not litter) from beneath the poultry house included in composite sample violates OSU Factsheet F-2248, which states "....but be careful not to remove soil beneath the litter"; and
- Mixing of litter sample and filling sample jar with hands, which violates Section 1.1 of Exhibit D that states, "Mixing will be accomplished using a disposable, plastic sampling scoop or a decontaminated stainless steel spoon".

During the collection of a number of litter samples, CDM field personnel violated the,, aforementioned procedures and protocols, resulting in unrepresentative or compromised sample results.

2.5 ADDITIONAL OBSERVATIONS

Additional observations made by CRA field personnel during the 2006/2007 IRW sampling activities included repeated and material actions of CDM field personnel which were inconsistent with written protocols, including but not limited to the following:

- Use of multiple soil sample probes with different diameters during sampling activities, which is a violation of Section III. A. 4. c. of the CDM Work Plan. This would result in biased analytical results due to unbalanced portions of soil material being included in the composite samples;
- Decontamination blanks on every LAL were not collected, which is a violation of Section III. A. 5. c. iii of the CDM Work Plan. Accordingly, there is no evidence that CDM's equipment decontamination procedures were sufficient;

- Visible rust present on soil sample probe sampler was not removed prior to the first sampling grid each day. This would result in the introduction of metals into the soil samples and unrepresentative sample results;
- Soil sample collection without use of the sample triangle, which is a violation of Section III. A. 4. of the CDM Work Plan. This would result in biased soil sample locations;
- Soil sample locations in visible ground depressions, under tree canopy, and along heavy use areas, which would result in unrepresentative sample results;
- Dragging of sample probe along ground surface between sub-sample locations and grids, which would result in cross-contamination of the soil samples; and
- Failure to remove manufacturer sticker from shovel used to collect litter samples.

The above actions occurred on a routine basis over the course of CDM's field activities, thereby adding to the significance of concerns with the deviations from the CDM Work Plan.

3.0 CONCLUSIONS

The following conclusions are made based on CRA's observations of CDM's field activities during the 2006/2007 IRW sampling activities and a review of protocols and procedures outlined in the CDM Work Plan, Factsheets, and SOPs:

1. During the collection of samples of soil, groundwater, surface/spring water, and poultry litter by CDM, CRA routinely observed CDM field personnel deviating from CDM's written sampling protocols and procedures, or otherwise collecting samples using technically unsound procedures, which resulted in unrepresentative sample analytical results.
2. CDM field personnel actions during soil sampling allowed for cross-contamination between soil sample locations, sample grids, and composite sample depths.
3. CDM field personnel actions during groundwater and surface/spring water sampling allowed for collection of unrepresentative and contaminated samples.
4. CDM field personnel actions during litter sampling allowed for collection of unrepresentative samples.

All of Which is Respectively Submitted,

CONESTOGA-ROVERS & ASSOCIATES

Jay A. Churchill, P. Eng.

Date: February 6, 2008

TABLE 1

LIST OF FIELD ISSUES OBSERVED 2006 - 2007
ILLINOIS RIVER WATERSHED
SOIL SAMPLING

Farm Name	*Soil Sample*	*Cow Manure Observed in Field*	*Sample Probe in Bag*	*Improper Decontamination Soil On Sample Probe*	*Soil Visible on Gloves*	*Knife Dirty Prior to Sample Collection*	*Cow Manure in Proximity of Sample Location*	*Sampler Driven Through Cow Manure*	*CDM Staff Stepped on Triangle Corner*	*Sampling Knife Used to Scrape Soil Collected From Deeper Than 6 Inches*	*Nitrile/Non-Nitrile Gloved Fingers in Sample Bag*	*Vegetation in 0-2 Inch Sample*	*Vegetation Scraped From Ground Before Sampling Using Sampler Tip*	*Vegetation Not Scraped Prior to Sampling*	*Vegetation Pulled From 0-2 Inch Sample*	*Vegetation Included in 2-4 Inch Sample*	*Vegetation Included in Sample (Depth Not Noted)*
Bill Anderson Section 30	LAL 5-A	x	20	0	0	0	5	0	0	8	12	0	0	20	1	1	13
Julie Anderson-Chancellor Farm	LAL 5-B	x	20	0	3	1	12	3	0	8	3	2	20	0	8	0	3
Julie Anderson-Chancellor Farm	LAL 5-C	x	18	3	2	0	3	0	1	5	10	0	16	0	1	0	4
Julie Anderson-Chancellor Farm	LAL 5-D	x	19	1	1	3	10	0	0	0	2	1	18	1	11	0	2
Anderson Hen Farm # 41	LAL 6-A	x	20	0	1	2	1	0	0	14	2	2	20	0	4	0	0
Anderson Hen Farm # 41	LAL 6-B	x	18	0	1	5	4	0	0	14	0	0	6	0	3	0	0
Anderson Hen Farm # 41	LAL 6-C	x	19	1	2	1	1	0	0	11	3	0	14	10	6	0	1
Anderson Hen Farm # 41	LAL 6-D	x	20	0	4	10	1	0	0	19	4	6	2	10	7	1	5
Pigeon Family Farm	LAL 7-A	x	16	2	11	1	8	0	14	7	4	0	3	4	20	0	0
Pigeon Family Farm	LAL 7-B	x	16	2	8	1	13	0	11	9	1	1	0	6	20	0	2
Pigeon Family Farm	LAL 7-C	x	18	3	3	1	4	0	0	14	13	0	0	2	20	3	0
Pigeon Family Farm	LAL 7-D	x	16	0	6	2	2	0	0	11	10	0	0	0	20	0	0
Ren Butler Farm	LAL 8-A	x	20	0	0	0	6	0	4	20	10	0	16	20	0	1	5
Ren Butler Farm	LAL 8-B	x	20	1	0	0	4	0	4	17	6	0	20	0	0	0	0
Ren Butler Farm	LAL 8-C	0	20	0	7	0	0	0	13	19	0	0	20	1	2	0	0
Ren Butler Farm	LAL 8-D	x	20	1	11	0	5	0	7	19	0	3	20	0	9	0	2
Reed Farm	LAL 9-A	x	20	2	4	2	15	0	2	19	3	1	4	13	12	0	1
Reed Farm	LAL 9-B	x	19	3	4	1	19	0	2	17	1	2	5	12	8	0	1
Reed Farm	LAL 9-C	x	19	0	3	0	0	0	7	19	5	0	0	13	3	0	0
Reed Farm	LAL 9-D	x	20	0	0	0	0	0	0	15	4	4	20	1	6	0	0
Green Country Farms	LAL 10-A	0	20	8	1	0	0	0	0	20	9	0	19	0	0	0	0
Green Country Farms	LAL 10-B	0	20	4	0	0	0	0	1	20	4	0	18	7	0	0	1
David Wofford Farm	LAL 11-A	x	12	0	1	0	1	0	0	9	4	2	0	13	0	0	6
David Wofford Farm	LAL 11-B	x	20	0	1	0	4	0	0	16	14	3	0	20	5	0	9
David Wofford Farm	LAL 11-C	x	18	0	0	0	6	1	1	14	8	3	0	20	0	0	3
David Wofford Farm	LAL 11-D	0	9	0	0	0	0	0	0	4	2	8	0	20	1	1	6

Sampling Information Produced by Conestoga-Rovers Associates

TABLE 1

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ILLINOIS RIVER WATERSHED
SOIL SAMPLING

Farm Name	*Soil Sample*	*Cow Manure Observed in Field*	*Sample Probe in Bag*	*Improper Decontamination Soil On Sample Probe*	*Soil Visible on Gloves*	*Knife Dirty Prior to Sample Collection*	*Cow Manure in Proximity of Sample Location*	*Sampler Driven Through Cow Manure*	*CDM Staff Stepped on Triangle Corner*	*Sampling Knife Used to Scrape Soil Collected From Deeper Than 6 Inches*	*Nitrile/Non-Nitrile Gloved Fingers in Sample Bag*	*Vegetation in 0-2 Inch Sample*	*Vegetation Scraped From Ground Before Sampling Using Sampler Tip*	*Vegetation Not Scraped Prior to Sampling*	*Vegetation Pulled From 0-2 Inch Sample*	*Vegetation Included in 2-4 Inch Sample*	*Vegetation Included in Sample (Depth Not Noted)*
McGarrah Farms	LAL 12-A	x	20	0	0	0	1	0	0	0	1	3	0	5	0	0	4
McGarrah Farms	LAL 12-B	x	20	0	0	0	0	0	0	0	0	7	0	0	4	0	1
McGarrah Farms	LAL 12-C	0	20	0	0	0	0	0	0	0	0	5	2	4	3	0	1
McGarrah Farms	LAL 12-D	x	8	0	0	0	4	1	0	0	0	6	3	0	7	0	0
Collins Farm	LAL 13-A	0	3	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Collins Farm	LAL 13-B	0	1	0	1	0	7	3	0	0	0	2	0	0	1	0	0
Collins Farm	LAL 13-C	x	18	0	2	1	6	0	0	9	2	2	0	0	1	0	6
Collins Farm	LAL 13-D	x	16	1	1	0	0	0	0	1	0	2	0	0	0	0	2
Glen Farm	LAL 14-A	0	3	1	0	0	4	1	0	0	0	1	9	5	20	0	0
Glen Farm	LAL 14-B	0	4	0	0	0	0	0	0	0	0	0	6	6	16	0	0
Glen Farm	LAL 14-C	0	5	0	0	0	0	0	0	0	0	5	3	3	10	1	0
Glen Farm	LAL 14-D	0	4	1	0	0	0	0	0	0	0	5	1	0	6	0	0
2-Saun Farm	LAL 15-A	x	6	0	0	1	11	4	0	0	0	0	0	0	0	0	0
2-Saun Farm	LAL 15-B	x	20	3	0	0	7	2	0	0	0	1	0	0	2	0	0
2-Saun Farm	LAL 15-C	x	8	0	0	0	3	1	0	0	0	1	0	0	2	0	0
2-Saun Farm	LAL 15-D	x	7	0	0	0	0	0	0	0	0	2	0	0	0	0	2
Bill Schwabe Farm	LAL 16-A	0	5	0	0	0	0	0	0	0	0	4	0	0	5	0	4
Bill Schwabe Farm	LAL 16-B	0	4	1	0	0	0	0	0	0	0	16	0	6	4	0	8
Bill Schwabe Farm	LAL 16-C	0	6	0	0	0	0	2	0	0	0	7	0	1	8	0	5
Bill Schwabe Farm	LAL 16-D	0	9	0	0	0	1	0	0	0	0	9	0	1	2	0	5
Woffard Farm (rented)	LAL 17-A	x	16	0	0	0	3	1	0	0	0	5	0	0	3	0	0
Woffard Farm (rented)	LAL 17-B	x	18	4	0	0	3	0	0	0	0	0	0	0	4	0	0
Woffard Farm (rented)	LAL 17-C	x	19	3	1	0	2	0	1	0	0	0	0	0	1	0	1
Woffard Farm (rented)	LAL 17-D	x	20	0	1	0	0	0	0	0	0	0	0	0	4	0	1
Billy Ray Anderson Farm	LAL 18-A	0	20	0	0	0	0	0	10	12	14	11	14	9	7	1	5
Billy Ray Anderson Farm	LAL 18-B	0	20	1	0	0	0	0	17	12	3	14	15	1	2	0	3

TABLE 1

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Farm Name	*Soil Sample*	*Cow Manure Observed in Field*	*Sample Probe in Bag*	*Improper Decontamination Soil On Sample Probe*	*Soil Visible on Gloves*	*Knife Dirty Prior to Sample Collection*	*Cow Manure in Proximity of Sample Location*	*Sampler Driven Through Cow Manure*	*CDM Staff Stepped on Triangle Corner*	*Sampling Knife Used to Scrape Soil Collected From Deeper Than 6 Inches*	*Nitrile/Non-Nitrile Gloved Fingers in Sample Bag*	*Vegetation in 0-2 Inch Sample*	*Vegetation Scraped From Ground Before Sampling Using Sampler Tip*	*Vegetation Not Scraped Prior to Sampling*	*Vegetation Pulled From 0-2 Inch Sample*	*Vegetation Included in 2-4 Inch Sample*	*Vegetation Included in Sample (Depth Not Noted)*
Billy Ray Anderson Farm	LAL 18-C	0	20	0	0	0	0	0	17	12	5	9	7	10	6	0	1
Billy Ray Anderson Farm	LAL 18-D	x	17	0	1	1	0	0	7	14	6	0	5	6	3	0	0
George's Morrison Farm	LAL 19-A	x	15	7	3	4	7	0	7	16	4	5	3	8	3	0	2
George's Morrison Farm	LAL 19-B	x	17	8	0	0	6	0	9	2	0	0	8	1	0	0	0
George's Morrison Farm	LAL 19-C	x	19	6	1	1	4	0	0	11	0	0	11	6	1	0	0
George's Morrison Farm	LAL 19-D	x	19	1	1	1	4	0	0	18	3	0	14	3	1	1	0
Tyson's Old Research Farm	LAL 20-A	x	19	7	0	0	2	0	6	18	2	2	16	0	0	0	0
Tyson's Old Research Farm	LAL 20-B	0	19	0	4	1	0	0	5	17	1	2	17	2	0	0	1
Tyson's Old Research Farm	LAL 20-C	x	20	4	0	0	1	1	1	19	0	0	19	0	3	0	1
Nubbie Farm	LAL 21-A	x	13	0	1	0	1	0	1	17	6	0	5	0	0	0	0
Nubbie Farm	LAL 21-B	x	18	0	1	2	10	1	2	15	11	0	15	0	3	0	0
Nubbie Farm	LAL 21-C	0	20	2	1	0	0	0	3	20	20	2	19	0	3	0	0
Nubbie Farm	LAL 21-D	x	18	0	0	0	1	0	0	18	6	0	4	0	0	0	2
Bill Engleman Farm	LAL 22-A	0	14	0	0	0	0	0	0	19	2	0	4	0	0	0	0
Bill Engleman Farm	LAL 22-B	0	15	0	0	0	0	0	1	19	6	0	2	0	0	0	2
Bill Engleman Farm	LAL 22-C	0	5	0	0	0	0	0	0	16	0	0	0	0	0	0	0
Bill Engleman Farm	LAL 22-D	0	9	0	0	0	0	0	0	20	2	0	1	0	0	0	0
Ricky Reed Farm	LAL 23-A	x	7	1	0	0	6	0	0	20	1	0	5	0	0	0	4
Ricky Reed Farm	LAL 23-B	0	18	0	0	0	0	0	0	19	0	0	0	0	0	0	2
Ricky Reed Farm	LAL 23-C	0	6	0	0	0	0	0	0	16	0	0	0	0	0	0	0
Ricky Reed Farm	LAL 23-D	x	9	1	0	0	0	0	0	18	0	0	2	0	0	0	0

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TABLE 1

LIST OF FIELD ISSUES OBSERVED 2006 - 2007
ILLINOIS RIVER WATERSHED
SOIL SAMPLING

Farm Name	*Soil Sample*	*Post Driver Dropped on Cow Manure*	*Mixing of Sample Depths*	*One Core Collected From Each Sub-Sample Location*	*Two of More Cores Collected From Each Sub-Sample Location*	*Touched Sample With or Without Nitrile Gloves*	*Less Than 6" Recovered, CDM Unclear What Depth Soil is From But Samples Anyway*	*Visible Cow Manure on Sampler Immediately Prior to Sample Collection*
Bill Anderson Section 30	LAL 5-A	2	0	0	20	20	0	0
Julie Anderson-Chancellor Farm	LAL 5-B	0	1	0	20	9	2	0
Julie Anderson-Chancellor Farm	LAL 5-C	0	3	0	20	9	0	0
Julie Anderson-Chancellor Farm	LAL 5-D	0	0	0	20	7	1	0
Anderson Hen Farm # 41	LAL 6-A	0	0	0	20	2	0	0
Anderson Hen Farm # 41	LAL 6-B	0	0	0	20	0	0	0
Anderson Hen Farm # 41	LAL 6-C	0	0	0	20	10	0	0
Anderson Hen Farm # 41	LAL 6-D	0	0	0	20	9	0	0
Pigeon Family Farm	LAL 7-A	0	1	1	19	20	0	0
Pigeon Family Farm	LAL 7-B	1	5	0	20	20	0	0
Pigeon Family Farm	LAL 7-C	0	1	0	20	20	0	0
Pigeon Family Farm	LAL 7-D	0	0	0	20	20	0	0
Ren Butler Farm	LAL 8-A	1	0	0	20	14	0	0
Ren Butler Farm	LAL 8-B	0	0	0	20	20	0	0
Ren Butler Farm	LAL 8-C	0	20	0	20	0	0	0
Ren Butler Farm	LAL 8-D	0	20	0	20	4	0	0
Reed Farm	LAL 9-A	0	2	1	19	7	0	0
Reed Farm	LAL 9-B	0	2	1	19	6	1	1
Reed Farm	LAL 9-C	0	20	20	0	2	0	0
Reed Farm	LAL 9-D	0	0	0	20	20	0	0
Green Country Farms	LAL 10-A	0	0	0	20	4	0	0
Green Country Farms	LAL 10-B	0	0	0	20	11	1	0
David Wofford Farm	LAL 11-A	0	0	0	20	8	0	0
David Wofford Farm	LAL 11-B	1	0	0	20	8	0	0
David Wofford Farm	LAL 11-C	0	0	0	20	7	0	0
David Wofford Farm	LAL 11-D	0	2	0	20	4	1	0

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Farm Name	*Soil Sample*	*Post Driver Dropped on Cow Manure*	*Mixing of Sample Depths*	*One Core Collected From Each Sub-Sample Location*	*Two of More Cores Collected From Each Sub-Sample Location*	*Touched Sample With or Without Nitrile Gloves*	*Less Than 6" Recovered, CDM Unclear What Depth Soil is From But Samples Anyway*	*Visible Cow Manure on Sampler Immediately Prior to Sample Collection*
McGarrah Farms	LAL 12-A	0	0	0	20	1	0	0
McGarrah Farms	LAL 12-B	0	0	20	0	0	0	0
McGarrah Farms	LAL 12-C	0	1	20	0	0	0	0
McGarrah Farms	LAL 12-D	1	1	20	0	0	0	0
Collins Farm	LAL 13-A	0	20	20	0	0	0	0
Collins Farm	LAL 13-B	0	0	20	0	1	0	0
Collins Farm	LAL 13-C	0	2	0	20	4	0	0
Collins Farm	LAL 13-D	0	0	20	0	3	0	0
Glen Farm	LAL 14-A	1	0	0	0	2	0	0
Glen Farm	LAL 14-B	0	0	1	19	0	0	0
Glen Farm	LAL 14-C	1	1	0	20	0	0	0
Glen Farm	LAL 14-D	0	6	20	0	0	0	0
2-Saun Farm	LAL 15-A	0	0	20	0	0	0	0
2-Saun Farm	LAL 15-B	0	0	0	20	0	0	0
2-Saun Farm	LAL 15-C	0	0	20	0	2	0	0
2-Saun Farm	LAL 15-D	0	0	15	5	1	0	0
Bill Schwabe Farm	LAL 16-A	0	0	20	0	0	0	0
Bill Schwabe Farm	LAL 16-B	0	0	0	20	4	0	0
Bill Schwabe Farm	LAL 16-C	0	0	20	0	0	0	0
Bill Schwabe Farm	LAL 16-D	0	1	20	0	0	0	1
Woffard Farm (rented)	LAL 17-A	0	0	20	0	0	0	0
Woffard Farm (rented)	LAL 17-B	0	1	20	0	0	0	0
Woffard Farm (rented)	LAL 17-C	0	0	0	20	3	0	0
Woffard Farm (rented)	LAL 17-D	0	0	20	0	0	0	0
Billy Ray Anderson Farm	LAL 18-A	0	0	0	20	15	0	0
Billy Ray Anderson Farm	LAL 18-B	0	1	20	0	9	0	0

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SOIL SAMPLING

Farm Name	*Soil Sample*	*Post Driver Dropped on Cow Manure*	*Mixing of Sample Depths*	*One Core Collected From Each Sub-Sample Location*	*Two of More Cores Collected From Each Sub-Sample Location*	*Touched Sample With or Without Nitrile Gloves*	*Less Than 6" Recovered, CDM Unclear What Depth Soil is From But Samples Anyway*	*Visible Cow Manure on Sampler Immediately Prior to Sample Collection*
Billy Ray Anderson Farm	LAL 18-C	0	0	20	0	12	0	0
Billy Ray Anderson Farm	LAL 18-D	0	20	20	0	3	0	0
George's Morrison Farm	LAL 19-A	0	12	0	7	6	0	
George's Morrison Farm	LAL 19-B	2	16	0	0	4	0	
George's Morrison Farm	LAL 19-C	0	2	0	0	0	0	
George's Morrison Farm	LAL 19-D	0	5	0	0	1	0	
Tyson's Old Research Farm	LAL 20-A	0	7	1	0	4	0	
Tyson's Old Research Farm	LAL 20-B	0	8	0	1	7	1	
Tyson's Old Research Farm	LAL 20-C	0	2	0	1	3	0	
Nubbie Farm	LAL 21-A	1	1	0	0	5	0	
Nubbie Farm	LAL 21-B	0	1	0	0	14	0	
Nubbie Farm	LAL 21-C	0	2	0	0	15	0	
Nubbie Farm	LAL 21-D	0	0	0	0	0	0	
Bill Engleman Farm	LAL 22-A	0	0	0	0	5	0	
Bill Engleman Farm	LAL 22-B	0	1	0	0	1	1	
Bill Engleman Farm	LAL 22-C	0	0	0	0	0	0	
Bill Engleman Farm	LAL 22-D	0	0	0	0	4	0	
Ricky Reed Farm	LAL 23-A	0	0	0	0	0	0	
Ricky Reed Farm	LAL 23-B	0	0	0	0	0	0	
Ricky Reed Farm	LAL 23-C	0	0	0	0	1	0	
Ricky Reed Farm	LAL 23-D	0	0	0	0	1	0	

TABLE 1

LIST OF FIELD ISSUES OBSERVED 2006 - 2007
ILLINOIS RIVER WATERSHED
WATER SAMPLING

Farm Name	*Water Sample*	*Groundwater Sample Collected Immediately Without Purging*	*Groundwater Sample Collected Directly From Garden Hose*	*Groundwater Sample Collected Directly From Spigot Without Sterilization*	*Pump Used to Sample Spring Discharges Upstream of Spring*	*Cattle in Spring*	*Use of Un-Sterilized Bottles for Bacteria Samples*	*YSI Meter Not Sterilized Prior to Dipping in Spring*	*CDM Using Un-Preserved Bottles*	*CDM Stepped in Spring After Walking Down Path With Cow Manure and Stir up Sediment Upstream of Sample Location*
McGarrah Farms	GW-…-001									
Bill Anderson Section	SW-…-001				x	x	x	x		
Collins Farm	GW-…-002								x	
2-Saun Farm	SW-…-003					x				x
2-Saun Farm	GW-…-004	* purged for 5-10min	x							
Glen Farm	GW-…-005	* purged for 15min		x						
Bill Schwabe Farm	GW-…-006	x								
Bill Schwabe Farm	GW-…-007	x	x							
Bill Schwabe Farm	SW-…-008				x					
Bill Schwabe Farm	SW-…-009					x				

Sampling Information Produced by Conestoga-Rovers Associates

TABLE 1

LIST OF FIELD ISSUES OBSERVED 2006 - 2007
ILLINOIS RIVER WATERSHED
LITTER SAMPLING

Farm Name	*Litter Sample*	*Sample Not Mixed Thoroughly*	*Number of Samples Against Wall*	*Number of Samples Under Feeders*	*Number of Samples Under Water Line*	*Number of Samples in Middle*	*Total Sub-Samples Collected*	*CDM Personnel with Fingers in Sample Container*	*Sub-Samples Collected in Tracks of Cage Handling Machine*	*Sample Dropped on Poultry House Floor was Retrieved and Included in Composite*	*Full Litter Depth Not Recovered in Sub-Samples*	*Part of House Dirt Floor Collected in Composite*	*Two Samples From Same Sub-Sample Location*	*Inconsistent Sub-Sample Due to Trimming of Litter*	*Mix Sample and Fill Jar with Hands*
Pigeon Family Farm	FAC-01	x					22				x				x
Loftin Farm	FAC-02	x	6	4	4	3	17	x			x			x	x
Reed Farm	FAC-03						18		x						
2-Saun Farm	FAC-04	x					18				x				
Glen Farm	FAC-05		4	4	4	4	16		x						
Green Country Farms	FAC-06		7	5	4	2	18			3 times	x	x			
McGarrah Farms	FAC-07	x	6	6	4	2		x	x		x			x	
Bob Schwabe Farm	FAC-08		8	4	3	3	18	x			x	x	x		
Billy Ray Anderson - Sect 30	FAC-09		3	7	6	2	18	x			x				
Julie Anderson-Chancellor	FAC-10		3	7	4	2	16	x							
George's Morrison Farm	FAC-11	x						x			x			x	
Nubbie Farm	FAC-12	x						x	x		x			x	
O'Leary Farm	FAC-13	x						x	x		x			x	
Masters Turkey House	FAC-14	x						x			x			x	
Butler Tyson Green Valley Complex 9	FAC-15	x						x	x		x			x	
Ricky Reed Farm	FAC-16	x						x			x				
Butler Tyson Green Valley Complex 12	FAC-17	x						x			x				

APPENDIX A

CURRICULUM VITAE - JAY CHURCHILL, P. ENG.

JAY A. CHURCHILL, P. Eng.

EDUCATION

B.Sc.(Eng.) University of Guelph, Water Resources Engineering, 1985

Other Courses:

- Conservation Planning, Part 2 (Modules 6-8) USDA-NRCS, October 2006
- Comprehensive Nutrient Management Plan Development Course, Iowa State University, Dept. of Agricultural and Biosystems Engineering, November 2005
- Conservation Planning, Part 1(Modules 1-5) - USDA-NRCS, February 2005
- Michigan/EPA Asbestos Building Inspector, Initial Training Course, Jensen Environmental Training Services, Detroit, Michigan, November 2001
- Conducting Comprehensive Environmental Property Assessments, Department of Engineering Professional Development, University of Wisconsin – Extension, May 1993
- Understanding Remediation, Department of Engineering Professional Development, University of Wisconsin – Extension, March 1990
- 40-hour OSHA HAZWOPER training course (1987) and annual 8-hour refresher courses complying with 29 CFR 1910.120
- CNMP Element Writer Certification for Manure and Wastewater Handling and Storage, Iowa State University, Dept. of Agriculture and Biosystems Engineering, September 2007
- CNMP Element Writer Certification for Land Treatment Practices, Iowa State University, Dept. of Agriculture and Biosystems Engineering, September 2007
- CNMP Element Writer Certification for Nutrient Management - courses in progress

EMPLOYMENT

1993-Present	Project Manager Conestoga-Rovers & Associates
1990-92	Project Coordinator, Conestoga-Rovers & Associates
1986-90	Project Engineer, Conestoga-Rovers & Associates
1985-86	Junior Engineer, Totten Sims Hubicki Associates
1985	Environmental Technician, Ontario Ministry of the Environment

AFFILIATIONS

Association of Professional Engineers of Ontario

CURRENT POSITION WITH CRA

- Project Manager/Senior Engineer:
  - Responsible for management of all aspects of environmental and agricultural engineering projects, including investigations and assessments; environmental and civil construction; remediation, contract management; cost management; and invoicing to Clients

- Provides project management and technical assistance in CRA Agricultural Services Group. Prepares Comprehensive Nutrient Management Plans (CNMPs); prepares work plans for agricultural engineering work, including conducting environmental assessments and identification of agricultural best management practices; conducts environmental assessments; and provides technical oversight of agricultural environmental investigation being conducted by a third party
- Ensures engineering projects are completed in a technically sound manner
- Coordinates effective interaction of various engineering and scientific groups and disciplines, such as engineering, agricultural services, hydrogeology, chemistry, and technical support, to promote successful project completion. Reviews work product of various groups to ensure project goals are achieved
- Arranges for the availability of appropriate personnel and resources
- Responsible for communication with regulatory agencies, client, and the project team

PROFILE OF PROFESSIONAL ACTIVITIES

- *Agricultural Services*
  - managed the completion of environmental assessments and identification of best management practices (BMPs) for surface water and groundwater protection at >200 swine facilities in North Carolina. Developed detailed priority ranking system for BMP implementation, which ensures BMPs will be implemented in an order which achieves maximum environmental benefits in the most cost effective manner. Prepared detailed BMP recommendation report
  - prepared Comprehensive Nutrient Management Plans (CNMP) for several dairy and beef farms in Wisconsin
  - researched and prepared report on Regulatory Foresight for the Biofuel Industry
  - engineering QA oversight of HDPE liner installation for 10-acre agricultural waste storage pond in Oklahoma
  - received CNMP Element Writer certification for Manure and Wastewater Handling and Storage, Iowa State University
  - received CNMP Element Writer certification for Land Treatment Practices, Iowa State University
  - received certification for Conservation Planning, Parts 1 and 2, from United States Department of Agriculture (USDA), Natural Resource Conservation Service (NRCS)
  - through course work and attending training programs, working towards achieving full certification with USDA-NRCS as a Technical Services Provider and certified CNMP Planner
- *Design and Project Management of Remedial Construction*
  - Doepke-Holliday Superfund Site, Johnson County, Kansas (Superfund Site)
    - designed an impermeable multi-layer cap (synthetic liner and drainage net, soil, vegetative cover) over 35-acre site with significant grades
    - managed remedial construction activities associated with impermeable cap construction
    - currently managing long-term groundwater monitoring program, operation and maintenance (O&M) program, and associated USEPA reporting requirements
  - Former PCB Capacitor Manufacturing Plant, Indiana (USEPA and IDEM Site)
    - designed and managed remedial measures for the multi-phase cleanup of PCB-contaminated soils, creek sediments and concrete. Remedial measures included extensive soil excavation (60,000 c.y.); sequenced creek water diversion and sediment excavation; synthetic liner installations; PCB-capacitor segregation and packaging; concrete demolition; extensive

shoring installation; building dismantling and reconstruction; off-site disposal of PCB-contaminated soils and sediments; and surface restoration

- Closure of Numerous Solid Waste Disposal Areas
  - designed remedial measures including excavation of solid wastes and codisposed soil; segregation of wastes from codisposed soil; segregation and off-site disposal or recycling of waste materials by waste type; backfilling of segregated soil; and surface restoration activities
  - managed remedial construction activities
  - Specific examples of key solid waste disposal area closure projects completed include:
    1. Lakeside Memorial Park, Miami, Florida, U.S.A. (100,000 c.y. of solid waste and soil) (1998 – 1999)
    2. Camposanto De Cristo Rescucitado, Ponce, Puerto Rico, U.S.A. (55,000 c.y. of solid waste and soil) (1997 - 1998)
    3. El Senorial Memorial Park, Rio Piedras, Puerto Rico, U.S.A. (5,700 c.y. of solid waste and soil) (1997)
    4. Spring Hill Cemetery, Nashville, Tennessee, U.S.A. (2,100 c.y. of solid waste and soil) (2003)
    5. Semper Concrete, Butler, Pennsylvania, U.S.A. (5,600 c.y. of solid waste and soil) (1998)
    6. Hillcrest Memorial Gardens, Cleveland, Tennessee U.S.A. (12,000 c.y. of solid waste and soil) (2000)
    7. Valley of the Temples Memorial Park, Kaneohe, Oahu, Hawaii (8,700 c.y. of solid waste and soil) (1999)
- Industrial Plant, Buffalo, New York (NYSDEC Site)
  - designed an asphalt cap cover system and vegetated soil cover system over an active industrial site
  - managed remedial construction activities
- Asbestos Abatement (Numerous Sites in Canada)
  - prepared asbestos abatement specifications, some abatement to be completed in conjunction with facility renovation or demolition, and managed abatement projects
  - prepared asbestos management plans (AMPs) for asbestos to remain in place
- Industrial Plant, Wisconsin (WDNR Site)
  - managed remedial activities conducted at a former manufactured gas plant site with coal tar contamination, including groundwater interceptor drain construction; construction of a steel sheet pile groundwater barrier wall; and construction of a groundwater pump and treatment system
- CIW Site, Romulus, Michigan (USEPA Site)
  - managed remedial activities conducted at a former oil recycling facility, including PCB-contaminated oil removal; sludge solidification and removal; tank demolition; and drummed waste repackaging and securement operations

- ***Nature and Extent of Contamination Investigations***
  - Former PCB Capacitor Manufacturing Plant, Indiana (USEPA and IDEM Site)
    - conducted an extensive PCB surface and subsurface soil and sediment sampling program; groundwater and surface water investigations; concrete coring; wipe sampling; and PCB air sampling
  - CIW Site, Romulus, Michigan (USEPA Site)

    - drum sampling in a Level B work environment; tanked liquids sampling at this former oil recycling facility contaminated with PCBs
  - Chemical Manufacturing Site, Charles City, Iowa
    - lagoon sludge sampling
  - Numerous Underground Storage Tank (UST) Sites in the United States and Canada
    - confirmatory soil sampling and groundwater investigations for UST closures

- *Technical Review and Comment/Negotiation of Agency Orders/Consent Decrees/Remedial Actions*
  - Doepke-Holliday Superfund Site, Johnson County, Kansas (Superfund Site)
    - Consent Decree
  - CIW Site, Romulus, Michigan (USEPA Site)
    - CERCLA 106 Order
  - Shavers Farm Site, Walker County, Georgia (USEPA Site)
    - Removal Action completed by USEPA
  - Novak Farm Site, New York (NYSDEC Site)
    - Removal Action completed by NYSDEC

- *Expert Testimony*
  - Doepke-Holliday Superfund Site, Johnson County, Kansas (Superfund Site)
    - participated in mediation sessions with client, remedial contractors, and judge to resolve large remedial construction contract settlement dispute

- *Cost Allocation in Support of Negotiations/Settlements*
  - CIW Site, Romulus, Michigan (USEPA Site)
    - evaluated costs incurred by client for remedial work required to be completed under a CERCLA 106 Order issued by USEPA, and assisted in preparation of client claim for reimbursement from USEPA
  - Shavers Farm Site, Walker County, Georgia (USEPA Site)
    - critically evaluated USEPA expenditures associated with a large Removal Action completed by USEPA for which USEPA was seeking reimbursement from client
  - Novak Farm Site, New York (NYSDEC Site)
    - critically evaluated NYSDEC expenditures associated with a large Removal Action completed by NYSDEC for which NYSDEC was seeking reimbursement from client
  - Large Energy Company (U.S.)
    - conducted a detailed evaluation of actual and potential environmental liabilities associated with in excess of 1,000 active and former energy company facilities

- *Miscellaneous Technical Work*
  - highly skilled in construction contract technical interpretation, enforcement, and administration
  - prepared many contract documents and specifications for various investigative and remedial programs
  - prepared work plans for hazardous waste and solid waste remedial and investigative programs

- prepared detailed engineering reports and environmental sampling and analyses reports for numerous hazardous waste and solid waste remedial construction projects, for submission to government agencies and industry
- prepared detailed cost estimates for remedial programs and environmental monitoring programs, under multiple scenarios and cost expenditure time frames
- prepared Environmental Indicator Determination (Current Human Exposures Under Control, and Migration of Contaminated Groundwater Under Control) documents meeting the requirements of USEPA's RCRA program
- prepared Health and Safety Plans for hazardous waste investigative and remedial programs
- prepared Spill Prevention Control and Countermeasure Plans (SPCCPs) and Stormwater Pollution Prevention Plan (SPPP)
- evaluated chemical composition of soils, groundwater, surface water, and waste materials, based on environmental and regulatory criteria
- designed an explosive gas monitoring system for a sanitary landfill

- ***Key Field Remedial Construction and Related Experience***
  - supervised extensive remedial cleanup activities of PCB-contaminated soils, creek sediments and concrete. Remedial measures included extensive soil and sediment excavation (60,000 c.y.) and off-site disposal; sequenced creek water diversion and sediment excavation; synthetic liner installations; PCB-capacitor segregation and packaging; concrete demolition; extensive shoring installation to accommodate excavation to depth of 18 feet; building dismantling and reconstruction; and surface restoration
  - supervised PCB-transformer decontamination and removal operations; PCB-contaminated soil removal; removal of tanked PCB-contaminated oils; PCB-capacitor packaging and removal; storage tank dismantling; and building demolition
  - provided technical assistance and oversight during drum and contaminated soil excavation activities at a large drummed waste disposal site
  - supervised segregation of hazardous liquid and solid chemicals in a supplied air modified "Level A" work environment within a former metal plating facility
  - coordinated drum securement, sampling, waste classification, and drum removal operations at several sites
  - supervised concrete coring operations inside a PCB-contaminated active plant facility
  - supervised regrading operations and the construction of an impervious clay cover over an industrial landfill
  - collected numerous soil, sediment, surface water, groundwater, concrete core, wipe, sludge, and air samples at numerous hazardous waste sites in the United States and Canada

- ***Other Training and Unique Experience***
  - former CRA internal Quality System (ISO 9001) auditor
  - considerable experience in the installation and testing of synthetic liner systems
  - experience in sheet pile installation for hydraulic containment and structural support
  - experience in angle borings for subsurface soil sampling beneath structures
  - completed Red Cross emergency first aid training and CPR courses