U.S. Environmental Protection Agency

Long Term 2 Enhanced Surface Water Treatment Rule: Cryptosporidium Analytical Method Improvements and Update on Source Water Monitoring Public Meeting

December 7, 2011

1200 Pennsylvania Ave., Washington, DC, USEPA East, Room 1153

On December 7, 2011, The U.S. Environmental Protection Agency's (USEPA) Office of Ground Water and Drinking Water (OGWDW) hosted a public meeting to discuss the *Long Term 2 Enhanced Surface Water Treatment Rule: Cryptosporidium Analytical Method Improvements and Update on Source Water Monitoring.*

This document, prepared by IntelliTech Systems, Inc., summarizes the information presented by USEPA staff and others at the public meeting. Comments and questions from the attendees in the room, from those participating by teleconference, and the responses from the speakers, are also summarized. The agenda and presentation slides were sent to registered participants prior to the meeting for reference during the proceedings. This document generally follows the sequence of activities presented in the agenda.

The presentations are available on USEPA LT2 Rule website, http://water.epa.gov/lawsregs/rulesregs/sdwa/lt2/regulations.cfm, and therefore will not be summarized in detail in this document.

Introduction and Welcome

Dr. Vanessa Speight, the meeting facilitator, welcomed everyone, reviewed the agenda, provided an overview of how the meeting would proceed, and gave instructions on the ground rules for questions and comments. Dr. Speight then introduced Mr. Phil Oshida, Deputy Director of Standards and Risk Management Division USEPA, who welcomed everyone and gave a short discussion on the primary focus of the meeting. Mr. Oshida explained that the meeting was to meet two objectives: (1) to start the six year review process and (2) to meet the recommendation of the Federal Advisory Committee to have public meetings following the first round of monitoring. He continued by saying this was in essence a kickoff meeting to start a discussion to inform the LT2 rule review process. In response to an attendee's question, Mr. Oshida replied that the key purpose of the meeting was to discuss USEPA's monitoring data and methods. He also stated that uncovered finished water reservoirs would be discussed at another meeting to be scheduled during the spring of 2012.

1st Presentation—Overview of LT2 Rule Requirements: Michael Finn, USEPA/OGWDW

Mr. Michael Finn gave a brief overview of the LT2 rule requirements. Following the presentation, a meeting attendee asked for clarification of slide 8 of Mr. Finn's presentation. Mr. Finn explained that slide 8 has two rules encapsulated on one slide: the LT2 Rule and the Stage 2 Disinfectant Byproduct (DBP) Rule. He explained that the intent of the slide was to show that public water systems will not have to make separate and differently timed decisions for treatment installations due to the two different rule implementation paths.

2nd Presentation—LT2 Round 1 *Cryptosporidium* Occurrence: Michael Messner, USEPA/OGWDW

Dr. Michael Messner's presentation summarized LT2 Round 1 *Cryptosporidium* occurrence data from the Data Collection and Tracking System (DCTS), and compared that with the Information Collection Rule (ICR) and ICR Supplemental Survey (SS) data. Dr. Messner's presentation concluded that Round 1 *Cryptosporidium* occurrence was unexpectedly lower than the ICR and SS occurrence statistics.

Dr. Messner explained that he was sharing the data analysis that he had done thus far. Many of the attendees requested further analysis, investigation, and presentations on the following:

- An attendee speculated that the low LT2 Round 1 *Cryptosporidium* occurrence could be due to some attrition of laboratories or because of high occurrence data from the ICR. USEPA indicated that currently there has been no analysis done to date to evaluate the impact of attrition of laboratories on the data.
- An attendee asked how the statistics of slide 13 compare with projections based on the ICR and ICR SS. A request was made for a comparison of all facilities in the DCTS with the number of systems with all nondetects (for *Cryptosporidium*), by percentage to the predicted values and the number of systems that are at or above 0.075 oocysts/L detections.
- An attendee asked if there will be another cost/benefit analysis based on the LT2 *Cryptosporidium* monitoring data, and if so, when will it become available? USEPA indicated they were waiting to review all relevant information before making the decision whether or not to pursue this.
- An attendee asked if the new occurrence data from Round 1 monitoring mean the log removal requirements should be revisited.
- An attendee pointed out that USEPA should consider using the new data to update the Bayesian occurrence modeling. This could be used to estimate the health benefit of Round 2 sampling. If the analysis of LT2 Round 1 *Cryptosporidium* data reveals lower occurrence or more confidence in the results, is the second round of sampling necessary?

- An attendee indicated that the *E. coli* screenings seem to be effective for the schedule 4 groups and asked if it could be assumed it will be equally effective for the other groups. Dr. Messner expressed his belief that a relationship exists between *E. coli* and *Cryptosporidium* even in source waters for large systems, but whether any assumptions based on that belief would be a good strategy is unclear.
- An attendee questioned how the low LT2 Round 1 *Cryptosporidium* occurrence data (informs) the required treatment or required log removal for filtered versus nonfiltered systems. Dr. Messner indicated he would add splitting out the filtered versus nonfilter removal results as a future analysis.
- A teleconference attendee asked if a utility would be able to discount *Cryptosporidium* detected using fluorescence microscopy and differential interference contrast (DIC) microscopy when calculating their bin number if the genotypes, determined by PCR and genotyping, are determined to be not infective to humans. Alternatively, could a utility receive log removal credits for any of the *Cryptosporidium* determined to not be pathogenic to humans? Dr. Messner explained that the rule does not allow for this.

3rd Presentation—Species and Genotypes of *Cryptosporidium*: Ron Fayer, *Agricultural Research Service/USDA*

Dr. Ron Fayer gave a presentation describing the different species and genotypes of *Cryptosporidium*. After Dr. Fayer's presentation, an attendee pointed out that there seemed to be an inconsistency between the apparent ubiquitous nature of *Cryptosporidium* occurring in the environment and the rate at which water systems are finding it in their source water. Multiple questions revolved around identifying the *Cryptosporidium* species that are pathogenic to humans. According to Dr. Fayer, the three most prevalent species pathogenic to humans are *C. parvum*, *C. hominis*, and *C. meleagridis;* although, there are reports of other species that are pathogenic to humans for example; *C. felis, C. ubiquitum,* and *C. muris*. Additionally, Dr. Fayer mentioned that all *Cryptosporidium* cells are very similar when viewed under a microscope and the size difference between them is around 0.4 µm. He also pointed out that there are eight chromosomes in the *Cryptosporidium* genome, three of which are typically used for naming species.

Dr. Fayer noted that the medical community does not always recognize cryptosporidiosis, and it is likely under reported. Also, those that are immunocompromised or malnourished may be especially susceptible to cryptosporidiosis.

4th Presentation—The Importance of Unusual *Cryptosporidium* Species and Genotypes in Human Cryptosporidiosis: Dr. Rachel Chalmers, Director/*Cryptosporidium* Reference Unit/UK Public Health Agencies

Dr. Rachel Chalmers' presentation focused on *Cryptosporidium* species genotype identification and the methods used to identify the species, as well as *Cryptosporidium* epidemiology. Her presentation included a discussion of an outbreak of cryptosporidiosis related to a rabbit getting into a drinking water system's well and contaminating it with *C. cuniculus.*

During the question and answer session, Dr. Chalmers clarified that the rabbit entered through a hole in the treated water storage tank. An attendee questioned why there have not been more cases of cryptosporidiosis in the UK (and other countries) related to the rabbit genotype if they are prevalent in the environment? Dr. Chalmers indicated that there are probably more cases than are being reported, and some cases that are attributed to other genotypes may actually be *C. cuniculus.* Dr. Chalmers stated that 30% of cryptosporidiosis cases have relapses. Additional questions raised by the attendees related to determining exposure and dose. Data were not available to answer them; however, Dr. Chalmers mentioned that starting in 2000, diagnostic laboratories began sending *Cryptosporidium* and stool samples to the UK Public Health Agencies to create a national collection of clinical isolates which would inform exposure.

5th Presentation—The Molecular Detection Toolbox: Applications and Implications on Current and Future National Monitoring Efforts: Dr. Eric Villegas, ORD/USEPA

Dr. Eric Villegas' presentation provided an overview of the current status of molecular based methods for *Cryptosporidium* detection and national monitoring implications. The presentation discussed the development of genotyping; applications of molecular methods for detecting *Cryptosporidium;* strategies to integrate molecular assays with USEPA Method 1623; and, the advantages, limitations, and future considerations of these molecular methods. While current molecular methods, e.g., real-time quantitative (qPCR) have the sensitivity to detect down to a single oocyst, studies conducted by USEPA-ORD scientists showed that the qPCR assay currently does not have the resolution necessary to accurately discriminate samples containing 1, 2, 3, or 5 oocysts.

Success rates of identification and methods to make simplified approaches for typical laboratories with little or no molecular experience were discussed. One researcher noted that for a research program his lab was participating in, the successful genotyping rate for single oocysts is about 50%.

An attendee asked what the likelihood was of naked DNA being carried over post-IMS as it relates to naked DNA on the slide versus whole oocysts detection. Dr. Villegas stated that technology exists to try to remove indigenous DNA and that refining the molecular approach to be more specific to the oocysts itself, not necessarily just the naked DNA, is something worth looking into. There are other researchers who are looking at different systems and trying to address this issue, noted Dr. Villegas.

6th Presentation—LT2 Round 1 *Cryptosporidium* Matrix Spike Recovery: Dr. Michael Messner, OGWDW/USEPA

Dr. Messner presented a summary of LT2 Round 1 *Cryptosporidium* recovery data and compared those data with LT2 projections. After the presentation an attendee asked whether, as part of the Six Year Review Process, USEPA would consider changing lab standards, recovery requirements, or any of the parameters that go into the lab analysis. Dr. Messner indicated that this would come up for discussion after the next presentation.

There was a brief discussion regarding the differences in performance among different certified laboratories and what variables might cause different recoveries, including: different approaches, use of Envirochek® HV Sampling Capsule from Pall Corporation versus the Filta-Max® foam filter from IDEXX, or matrix effects. Were the recoveries comparable for the Envirochek® HV and Filta-Max®? Ms. Carrie Miller commented that DCTS data did not include information on the filter types used, but stated that she knew which laboratories used which filters and could potentially do a comparative analysis.

One of the attendees asked if there had been any analysis with performance weighting based on matrix spike recoveries. Dr. Messner indicated that he has begun to examine the data and at present observed that there are some low recovery laboratories that have assayed many samples, and there are some high recovery laboratories that have assayed many samples, and the mean recovery between the two groups is significantly different. The attendee asked to see this at the next public meeting.

The following questions were also discussed and will likely require further investigation.

- Does the volume of matrix spike samples affect the matrix spike recovery averages for the 10 liter versus 50 liter spikes? One of the attendees indicated it was his experience that it did not.
- Does USEPA have geographical data regarding the matrix sources? Do we see any patterns, in terms of difficult matrices versus relatively easier matrices, in the performance recoveries? Do specific geographic regions have more difficulty recovering the oocysts from their matrix?

- Were data generated by laboratories that did not pass the matrix spike requirements (matrix spike recovery below 13%) added into the database? If so, it may show up as a bias and that data should be removed from the analysis. Dr. Messer stated that his understanding was that there are no corrective action requirements when laboratories have a very low recovery, and there is nothing to keep them from entering the data. He stated that this issue should be reviewed.

7th Presentation—USEPA Method 1623 improvements: Carrie Miller, OGWDW/USEPA

Ms. Carrie Miller presented USEPA Method 1623 and potential improvements, including the use of sodium hexametaphosphate and a bead pellet wash step.

USEPA has been evaluating method modifications to improve performance for laboratories, particularly for those dealing with matrices where recovery is more difficult. Ms. Miller noted that smaller laboratories testing one or a limited number of water matrix types are more able to adjust the method to fit their particular matrix. On the other hand, larger commercial laboratories generally standardize their test for a broad spectrum of water matrices which makes it difficult to adjust the method for a particular water matrix type.

There was a discussion regarding the use of sodium hexametaphosphate to improve recovery, particularly for difficult matrices. For those samples where initial recovery was relatively high, the improvement using sodium hexametaphosphate was generally less pronounced. The data indicated no deleterious effects from the dispersant.

A couple of the attendees questioned the appropriateness of using data from multiple laboratories that have used the flexibility built into method 1623 to modify the procedures. They suggested that this may be skewing either the baseline or results, and they questioned if all of the data should be re-evaluated and the distribution be recalculated. They suggested that this, in turn, could affect how the bins are assigned, especially for those at a bin boundary. This was echoed by a teleconference participant who wanted to know the effect of the new changes for historical data and the future monitoring data and the associated bin classification.

An attendee asked about more stringent requirements for matrix spike and matrix spike duplicates. Ms. Miller responded that it was a logistic and economic decision to do a matrix spike on a percent of the samples.

An attendee questioned what other modifications were considered for USEPA Method 1623 improvement but were discarded, including off-the-slide genotyping. This sparked further discussion of potential issues with molecular genotyping including difficulties in differentiating between species, given that there is 99.5% similarity; and, how and who would maintain and manage such a repository of information. Other issues raised regarding the use of molecular methods were the need for standardization as well as further examination of quality control issues, and quality management.

As a follow-up, an attendee asked how the modifications presented improve the overall accuracy, not just recovery, and asked whether the modifications will better target pathogenic *Cryptosporidium*. How do we know that what is being recovered is being correctly identified by the laboratories when there are now fewer in-person inspections and more remote access by those who certify the laboratories?

Ms. Miller explained how the ongoing proficiency testing and USEPA audits (on-site and remote) are used to assess laboratory capability. She also explained USEPA's training programs for laboratories and how USEPA is working with states and Regions to expand the network of proficient auditors.

Open Questions and Discussion Session

Several questions from one attendee related to how the new data effects binning were posed to Dr. Messner. The questions included:

- How would the new method change the baseline recovery efficiencies?
- Is it important now, for us to re-do the baseline?
- What happens when you change the methodology?
- Do you believe you are changing the recovery, and does that push a number of systems that were sitting within bin boundaries up into higher bins? If so, what might the impacts of that be?
- Do any of the changes we observed in the data, the baseline, the new method approach, etc., have any effect on systems moving between the bins? If so, will a reanalysis of the data readjust that in some way?
- Does anybody get bumped up?

Dr. Messner responded that such modeling could be difficult. It would be important to explore the relationship between recovery and occurrence. It may also be important to include laboratory-specific and matrix-specific effects on recovery. These (matrix and lab effects) may be difficult to separate because some laboratories are dedicated to specific matrices and matrix spike data are limited (typically two matrix spike recoveries per source water).

The attendee followed up with the clarification that the concern is the utilities on the bin edge may get pushed up into a higher bin, but then on the other hand you have shown there is an equal probability that those on the high end will get pushed down into the lower bin. Is there clarity if a utility gets into bin 2 in Round 1, and in Round 2 they are out of bin 2? Do they then get out of bin 2, or once you are in bin 2 or a higher bin are you stuck? Once you get in there you never get out, or, will the second round results set the bin you are in?

Mr. Finn addressed the question stating the rule was not explicit. He explained that while the method modifications and results of Round 2 may have important impacts on what bin a system is in, the final decision will likely be based on answers to questions such as: Is there basis for you to reduce the level of treatment provided when you knew, at the point when you were classified in bin 1, you had some specified level of source water contamination? What happened to the contamination? Did it go way? From a public health and treatment standpoint, it would be hard to go backwards, and there is the anti-backsliding part of the rule we would have to deal with. The impact is largely unknown at this time. Once the data are in, the states with the USEPA's help will decide what the answer is.

A teleconference participant asked: The first presentation showed a slide with the 2nd round of sampling to begin no later than different dates for the water system size. Will there be a timeframe to begin sampling and testing, prior to this date? For example the large systems are supposed to start sampling no later than 2015, but how much earlier are they going to be able to start, and will they be allowed to grandfather in data? They are trying to understand the earliest time a water system can start sampling for Round 2.

Mr. Finn addressed this question saying the way the rule is structured, it explicitly allows for grandfathering in Round 1, but there is no mention of grandfathering for Round 2. The other part of that equation is that in the Microbial/Disinfection Byproducts Federal Advisory Committee's (FAC) Agreement in Principle it is clear that there were six years that passed between rounds.

One attendee noted that he expected to hear more discussion from USEPA about areas that they would consider revising, changing, and modifying for the existing LT2, besides the uncovered reservoir issue. He wants to hear how USEPA is going to incorporate newer methodology into areas of consideration. For example, whether we might utilize genotyping information through some approved sampling plan or through an approved performance based method, for a future request for variance, or for some change in the bin classification in the 2nd round. The attendee also stated that he thinks he has learned that systems have achieved, on average, only a 0.5 log removal of *Cryptosporidium*, without doing anything. So should that be accounted for, as the actual levels are much lower than had been predicted? Should that be considered in bin classifications?

Mr. Finn responded that this would be considered in the Six Year Review and the new information would be incorporated and then these questions would be addressed.

Another attendee summed up several concerns saying that if the method is changed, and there are different results from Round 1 and Round 2, the argument could be made for a Round 3, before changing bins. Is it a case where there is no way to calculate the actual answer because of the changes? And therefore, do we really need to do the Round 2? Can we keep some systems out of a Round 2? Can we use a different trigger, or use a monitoring waiver for systems where results were zero and that are expected to get zeros again? The attendee suggested reconvening the FAC and trying to address all of the issues.

Mr. Carroll responded that changes in binning between Round 1 and Round 2 may occur with or without a new method. He also indicated that the rule did not address the notion of a "Round 3". He explained that, with regard to the method improvements, USEPA is looking at the meeting as a listening session, and an opportunity for the Agency to describe potential to improve the method accuracy at very little additional costs, and with very little additional laboratory time. Ms. Miller pointed out that there is potentially even a time savings when you look at the microscopy being made easier. With regard to the molecular methods, Mr. Carroll explained that his understanding (from talking with OW and ORD microbiologists) is that the molecular approach isn't ready for Round 2, when one considers all that would have to be done to create a laboratory structure/laboratory network to support that.

An attendee suggested one way of dealing with the method change would be to have two matrix spikes. One analysis would be done with the original ingredient solution and one done with the improved method. This would be at a relatively lower cost, particularly when you take into consideration what it might cost a utility if they are kicked up into a higher bin. The attendee also suggested that molecular methods could be incorporated into Round 2 and suggested that only one or two expert laboratories be used for the analysis to eliminate laboratory variability errors. The attendee also pointed out that just because you don't find *Cryptosporidium* today doesn't mean it won't be there in the future.

An attendee wanted to know what the next steps were and when risk and health effects would be discussed. Mr. Oshida indicated that the meeting to be held in the spring of 2012 would address a number of topics, but would focus particularly on LT2 uncovered finished water reservoir requirements. A follow-up question asked how the timing of Round 2 monitoring relates to the Six Year Review process and potential rule changes. Mr. Carroll stated that discussions about next steps and the timing were in progress and no decisions had been made whether or not to require the use of any new or modified method for Round 2 monitoring. .

A commenter asked whether USEPA can unilaterally make changes to USEPA Method 1623 and require that the modified method be used. Mr. Carroll stated that laboratories who wish to use the changes that Ms. Miller described now, (using the sodium hexametaphosphate and the wash step), can do that within the scope of the current 1623, because it is so flexible. Mr. Carroll suggested that requiring use of a modified method would likely require a rule change.

The facilitator, Dr. Speight, asked if there were any lessons learned from the first round of monitoring. There was no particular response, but an AWWA representative in the audience suggested that they could do a survey of the utilities. They could find out what the utilities' experiences were and what the experiences of the laboratories were, and compile the information for USEPA.

Mr. Finn offered anecdotally that some systems don't necessarily have a good understanding of their source water and sources of contamination and are often caught by surprise by results showing contamination. He indicated that he would be concerned about not having Round 2 monitoring for those systems because there is something going on that they don't understand and are therefore not controlling because it is something they are dealing with for a first time. He reiterated that this was anecdotal and there was no hard data.

Dr. Speight rephrased a participant's question about the possibility of more research on the interferences coming from the matrices. Ms. Miller indicated that identifying matrix interferences was useful and there is ongoing work to do so.

A teleconference participant asked via email, "Why monitor source water when the matrix often interferes with the current or modified method used to monitor? Is it not the drinking water for which the PWS is responsible, and therefore, is [it] not the drinking water matrix on which the science should focus now, with regards to the new methods?"

Dr. Villegas indicated that there is additional research data pointing toward monitoring finished water versus source water. There are arguments for both, but some recent data have come from Dr. Huw Smith's research at the Scottish Parasite Diagnostic and Reference Laboratory, where they compared source water and finished water using off-the-slide genotyping for *Cryptosporidium.* The types of organisms detected are quite different. In the source water, the most dominant species detected was *C. andersoni*, which is primarily from cattle and for the most part not really infective. Conversely, the data on their finished water showed the predominant form was *C. ubiquitum*. So the argument now is maybe we should monitor for the actual finished water that we drink. Then the problem is the occurrence, the levels. It's really low, so should we look at not only the interference, but also the volumes? Do we need to sample more than 10 liters? New more robust filtration technology is also available (e.g., hollow fiber filtration) that allows for filtration of larger volumes more efficiently. An attendee cautioned that you can get varying results by sampling different volumes which could have something to do with the filter.

An attendee asked for a date when they could have access to the LT2 Round 1 dataset. USEPA indicated the data would be released at some point but did not give a specific date.

Another attendee expressed strong advocacy for including genotyping in Round 2 and indicated that if it was not included it would be a missed opportunity that would be regretted.

A teleconference attendee wanted to know where he could find the tutorial of the enhancement to the USEPA Method 1623. Ms. Miller of USEPA indicated that the bead pellet wash is in the IMS module located in the training section on the USEPA website, (http://water.epa.gov/lawsregs/rulesregs/sdwa/lt2/regulations.cfm). The Sodium hexametaphosphate addition is expected to be posted in the next 5–6 months.

A teleconference attendee offered the following questions and comments:

- What is the average cost per sample to run USEPA Method 1623 in the United States? It costs us approximately $400 a sample in an USEPA certified lab in Florida.
- How much will it cost, per sample, with the off-the-slide detection method added?
- I'm not sure why the genotyping method is even being considered. Efforts should be made to reduce sampling costs, and not increase it.
- Do not keep USEPA Method 1623, and add on to it. The USEPA is going entirely in the wrong direction.

An attendee stated that the cost for genotyping, assuming the samples are batched, would be about $150 per sample. That is only for samples that test positive by microscopy, which are below 10%, at this point. The cost would even be lower for approximately 25% of those samples because they cannot be typed; therefore, the additional sequence analysis work would not be done. The quantitative PCR approach, while useful for discriminating between *C. parvum* from *C. hominis* species, does not have the resolution to discriminate between one, two, or three oocysts in a sample. The microscopy approach reflected in USEPA Method 1623 is currently the best available option for quantitation at low levels. The molecular genotyping tool can provide additional information of the different types of *Cryptosporidium* species/genotypes that are present. Dr. Villegas presented these findings in his talk earlier in the day. Ms. Miller added that the USEPA did not do a survey of costs but thought that $400 a sample is a pretty good ballpark for the analysis using USEPA Method 1623 (without supplemental genotyping). Eric Villegas noted that prices may decrease since ORD is looking into different methods that use more economical filters, the hollow fiber filters versus the Envirochek® HV.

One attendee mentioned the unique opportunity that LT2 provides with regards to understanding *Cryptosporidium* diversity in the United States and the importance of determining the relative abundance of human infectious species/genotypes that may contaminate surface waters. Thus, the off-the-slide genotyping approach may be of some use to the Agency and water utilities with regards to identifying sources of contamination.

A final caution was expressed by one of the attendees that if there is no opportunity for the laboratories to earn a profit, capability will be lost for both microbiology and chemical testing since those laboratories will either go out of business or pursue another course.

USEPA staff expressed their thanks and noted that more information would be announced in the Federal Register related to the meeting that is slated to be conducted in the spring of 2012.