PORTLAND WATER BUREAU
FROM FOREST TO FAUCET

Randy Leonard, Commissioner
David G. Shaff, Administrator

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September 9, 2011

Mr. David Leland
Program Manager
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P.O. Box 14450
Portland, OR 97293-0450

Dear Mr. Leland:

The Portland Water Bureau has prepared the following responses and accompanying information to address the questions you provided in your August 11, 2011 letter (Attachment CL-1).

As you know, Water Bureau staff had follow-up questions which were discussed with Carrie Gentry by phone and email on August 15th and your responses to the bureau's questions and assumptions were received on August 30th (Attachment CL-2). We have incorporated your clarifications to the attached responses.

Please do not hesitate to contact me with any additional questions or follow-up.

Sincerely,

David G. Shaff
Administrator

Question 1: *Ownership and use of lands within and adjacent to the Bull Run watershed (Figure 2.8).*

A. *We request a larger detailed map with information about specific ownership of and uses of lands within and adjacent to both the hydrologic boundary and the management area boundary; known access including roads and trails with any gates and/or fences; and*
B. *Known potential contamination sources and locations.*[1]
C. *Please also submit information on known use by hikers and pack animals of recreational trails including the Pacific Crest Trail within or adjacent to the area boundaries.*

PWB Responses

Question 1A: Request for larger detailed map.

Enclosed are two large-scale maps that Portland Water Bureau (PWB) prepared in response to the OHA request. While the letter requested a single map, two maps were required in order to show land ownership and land use. The enclosed map titled *Bull Run Watershed Land Ownership* (Attachment 1A-1) shows land ownership, roads, gates, and trails within and adjacent to the Bull Run Watershed Management Unit (BRWMU). The gates that provide access to the Bull Run are named and labeled on the map. This map also includes the three locations within the water supply drainage where sanitary facilities are located as part of the response to question 1B.

The second enclosed large-scale map (Attachment 1A-2), titled *Land Use In and Adjacent to the Bull Run Water Supply*, shows land use within a three-mile buffer of the Bull Run water supply drainage. Land use was classified using a combination of tax lot information, land ownership, and visual inspection of aerial photographs of the area adjacent to the Bull Run water supply boundary. Taxlot GIS data maintained by Metro (RLIS) was clipped to a three-mile buffer around the Bull Run water supply boundary. An additional 2000-foot buffer was added around the area where the Bull Run Closure Area boundary extends beyond a three-mile buffer from the water supply drainage. Aerial photographs (2006, 2008) for this area were inspected and classified by land use based on visual evidence. When multiple land uses were observed, each use was recorded in order of dominance. The classification was simplified to display the dominant land use for each parcel. Forested areas were further classified by land ownership (private vs. government owned) and land management protections (federally-designated wilderness, federally-designated scenic area, and watershed protection lands). All lands associated with the Bull Run watershed protection program that are closed to public entry through a Forest Service closure order, Bureau of Land Management (BLM) closure order, or City code were classified as "watershed protection" lands. The percentage of each land-use classification in the map area is shown in the map legend.

[1] Potential contamination sources was clarified by OHA as meaning potential sources of *Cryptosporidium* in the watershed (See Attachment CL-2).

Question 1B: Known potential Cryptosporidium contamination sources.

Portland's Bull Run water system is protected by legislation and administrative rules that limit human access and control the types of activities that are allowed within the water supply drainage and surrounding management unit. These strong protections serve to eliminate most sources of contamination to the water supply, including microbiological contaminants such as *Cryptosporidium*. With the exception of wildlife, which is the only potentially significant source of *Cryptosporidium* in the Bull Run watershed, all other potential sources of contamination are minor sources that have an extremely low likelihood of introducing contamination to the water supply. The potential sources of contamination located within the Bull Run water supply drainage are explained below.

Wildlife:

As described in the Variance Request, wildlife is the only potentially significant source of *Cryptosporidium* in the Bull Run watershed. To gain a better understanding of the nature of this potential source, Portland developed a scat monitoring program described in Appendix E of the Variance Request. Out of 307 collected samples, only a single sample collected just outside of the water supply drainage tested positive for *Cryptosporidium*, indicating a very low prevalence of *Cryptosporidium* infection in Bull Run wildlife.

Portland has continued its scat monitoring program, and through July 28, 2011 a total of 428 samples have been collected and analyzed. Of these additional 121 samples, one sample collected on June 2, 2011 tested positive for *Cryptosporidium*. This sample, identified as bobcat, was located in the South Fork sub basin of the Bull Run watershed. Genotyping analysis showed the presence of nonhuman-pathogenic *Cryptosporidium* and further DNA sequencing indicated a novel animal-associated genotype. To date, *Cryptosporidium* has been absent from 99.5% of scat samples.

Humans:

Human activity in the Bull Run watershed poses a very minor potential source of *Cryptosporidium* contamination. Authorized human activity is restricted to people working in the watershed and a limited number of visitors on supervised educational tours. All workers and visitors are directed to use the designated sanitary facilities. The main visitor tour bus has a restroom on board and visitors are taken to designated facilities and supervised at all times. There is a minor potential for contamination from unauthorized human intruders. Human intruders are not formally documented but are in general rare and are found on the outskirts of the watershed far from the reservoirs. Bull Run watershed access and security measures are summarized in Appendix G.2 of the Variance Request, pages G-17 to G-18.

Sanitary Facilities:

Three locations within the water supply drainage have sanitary facilities. There are currently a total of four portable toilets and one permanent holding tank within the water supply drainage. The permanent facility is located at Bear Creek House, a historical structure overlooking Dam 1 that is used for lunch stopovers by education tours. Its waste system is a

closed holding tank that is not connected to a leach field. The holding tank level is monitored and the tank is emptied regularly throughout the year. There is no surface flow between the location of the storage tank and the reservoir or streams below. The portable toilets are traded out on a prescribed schedule.

The table below summarizes the sanitary facilities located within the water supply drainage. These are also shown on the attached land ownership map (Attachment 1A-1).

Sanitary facilities within the drinking water supply drainage

Location	Type	Number	Notes
Bear Creek House	Toilets with closed holding tank	1	Tank is emptied regularly
	Portable toilet	1	Located in front of Bear Creek House
Powerhouse #1	Portable toilet	1	Located below Dam #1
Bull Run Lake parking area	Portable toilet	2	Seasonal; removed after summer tour season.

There is an additional portable toilet located on a mobile trailer that may be transported to projects temporarily in and around the water supply drainage. All other Bull Run sanitary facilities are located outside of the water supply drainage and are therefore not shown on the land ownership map. The facilities that are in close proximity, but outside the drainage include the septic system and two portable toilets at Headworks (located downstream of the intake to the drinking water system), and a pit toilet near the Hickman Butte lookout tower.

Domesticated Animals:

Domesticated animals are not authorized within the water supply drainage or the Bull Run Watershed Management Unit (BRWMU). Additionally, domesticated animals do not reside in much of the land surrounding the BRWMU, as wilderness and forested land are the primary land uses adjacent to the BRWMU. Therefore, there is only a minor potential for *Cryptosporidium* contamination from domesticated animals that may rarely enter into the water supply drainage, most likely with human intruders or by wandering from distant private land. Signs or sightings of domesticated animals have been occasionally observed but have not been formally documented.

Question 1C: Information on known use of recreational trails within and around the management unit.

The enclosed land ownership map (Attachment 1A-1) shows the location of recreational trails within and bordering the Bull Run Watershed Management Unit (BRWMU). The Columbia Gorge National Scenic Area, Hatfield Wilderness, and the Lost Lake portion of the Mt. Hood National Forest border the BRWMU on its northern end. Each of these areas contains hiking

trails. An 8.3-mile portion of the Pacific Crest Trail is located within the BRWMU but nearly this entire segment is located outside the water supply drainage. A 1.3-mile segment of the Huckleberry Trail, which links Lost Lake with the Pacific Crest Trail, is located within the BRWMU but is outside the water-supply drainage. A 1.4-mile segment of the Oneonta Creek Trail is located along the BRWMU boundary in the Larch Mountain area.

Water Bureau staff contacted recreation staff on the Mt. Hood National Forest and the Columbia Gorge National Scenic Area to obtain information about usage levels of trails bordering the BRWMU. Attachment 1C-1 documents information obtained from three Forest Service employees who are generally regarded as having the most knowledge about use of trails in this geographic area. No data or estimates are available of the number of person-days of use of the portion of the Pacific Crest Trail located within the BRWMU. Two Forest Service recreation staff that were interviewed for this effort stated that it is very uncommon to see pack animals on this section of the Pacific Crest Trail due to the terrain and slope conditions. Pack animals are not allowed in the Hatfield Wilderness or on Forest Service trails in the Scenic Area.

The Forest Service employee that oversees maintenance of trails in the Scenic Area and the western portion of the Hatfield Wilderness said that there is relatively light use of trails that border the BRWMU in those two areas. He estimated that the Bell Creek Trail receives "a couple of hikers per week" and that the upper portion of Oneonta Creek Trail has about 12 hikers per day.

BLM recently constructed a network of mountain biking trails on BLM land located between Barlow Trail Road and the southern boundary of the BRWMU. These trails are depicted on the Bull Run Watershed Land Ownership map (Attachment 1A-1). At its closest point, the Sandy Ridge mountain bike trail network is located over two miles from the Bull Run water supply drainage boundary. According to Adam Milnor, Recreation Planner with the BLM Salem District, BLM installed traffic counters on the Sandy Ridge mountain bike trail system and it is projected to receive counts of 20,000 to 25,000 in 2011. There is no use of this trail network by pack animals. One of the enclosed attachments is a BLM document titled *Bull Run Watershed Management Unit Trespass Prevention Measures* (Attachment 2-15) that describes the protection measures that BLM has adopted to prevent Bull Run trespass related to development and use of the Sandy Ridge mountain bike trail network.

Question 2: Existing federal, state, and local legal controls on the Bull Run watershed.

We request copies of all the legal control documents currently in effect, including statutes, contracts, agreements, and regulations.

PWB Response

The Bull Run watershed is protected through a variety of federal, state and local legal controls. In addition to statutes, contracts, agreements and regulations currently in effect, two additional categories of controls that provide protection for the Bull Run are: 1) administrative land management plans that are adopted by federal agencies through issuance of an Environmental Impact Statement and Record of Decision, and 2) administrative closure orders that reference specific sections of the Code of Federal Regulations (CFRs). A list of applicable federal, state and local legal controls are provided below. Electronic copies of these documents (or relevant sections) along with additional controls providing protections to the Bull Run watershed are provided as Attachments 2-1 through 2-21.

Federal Controls

- Bull Run Management Act (16 U.S.C. Sec. 482b Note)
- 1990 Mt. Hood National Forest Land and Resource Management Plan (pages Four-295 through Four-317)
- 1994 Northwest Forest Plan
- 1995 BLM Salem District Record of Decision and Resource Management Plan
- 2007 Mt. Hood National Forest Closure Order for the Bull Run Watershed Management Unit -- Closure Order MH-2007-01 (pursuant to 36 CFR 261.50(a), (b) and (e), 36 CFR 261.53(e), 36 CFR 261.54(e), and 36 CFR 261.53(a))
- BLM Temporary Closure Order for the Bull Run Watershed Management Unit -- December 1, 2009 through December 1, 2011 (pursuant to 43 CFR 9268.3(d)(1)(i))
- BLM Permanent Closure Order for the Bull Run Watershed Management Unit -- scheduled to take effect December 2, 2011 (pursuant to 43 U.S.C. 1733(a)), 43 CFR 8360.0-7, and 43 CFR 8364.1)
- 2007 Bull Run Watershed Management Unit Agreement between USDA Forest Service, Mt. Hood National Forest and Portland Water Bureau

State Controls

- ORS 448.295 to ORS 448.325
- State of Oregon Department of Forestry Regulated Closure Proclamations (in Bull Run regulated use area during fire season, pursuant to ORS 477.35 to 477.550)

Local Controls

- Portland City Code Chapter 21.36, Bull Run Watershed Protection
- Section 00203 of Portland Water Bureau contract specifications for construction projects in the Bull Run Watershed Closure Area
- Section 00202 of Portland Water Bureau contract specifications for construction projects

Question 3: Future projected impacts of climate change and reforestation.

We request any analyses that the Water Bureau has commissioned or prepared on the likely future impacts of climate and vegetation changes on the future nature of the Bull Run raw water source.

PWB Response

A staff memorandum providing a more detailed description of Portland Water Bureau climate change efforts is attached (Attachment 3-1) and brief descriptions of the Bureau's major efforts to date are provided below.

The Portland Water Bureau commissioned a study that was completed in 2002 entitled, "The Impacts of Climate Change on Portland's Water Supply: An Investigation of Potential Hydrologic and Management Impacts on the Bull Run System" by Dr. Richard Palmer and Margret Hahn, University of Washington, Dept. of Civil and Environmental Engineering. This is the only commissioned report on climate change for the Bull Run watershed. A full copy is included as Attachment 3-2 and can be found at:
http://cses.washington.edu/db/pubs/abstract111.shtml

As a member of the Water Utility Climate Alliance (WUCA) the Portland Water Bureau has continued to research the impacts of climate change. Through this partnership the bureau has contributed to studies on the science of climate modeling and the application of climate science to decision making about future water resource impacts. The most recent work involves a Pilot Utility Modeling Applications (PUMA) project where Portland is one of four case studies to downscale more recent Global Climate Models to an individual watershed scale to validate and further detail potential impacts on longer time horizons than the 2002 study. The City is working with Dr. Philip Mote at the Climate Decision Support Consortium to develop and implement an Intergovernmental Agreement (IGA) to complete this work by the end of 2012. The primary purpose of this work would be to validate the overall conclusions of the Palmer report and investigate these scenarios further.

The City of Portland did complete and gained approval of a state required Water Management and Conservation Plan (WMCP) in July 2010. This report details the need to further develop the Columbia South Shore Well Field to provide backup for the Bull Run system in light of emergencies or natural events that may impact the availability of the surface system in part due to the potential for future climate change impacts on summer supplies. The availability of the groundwater system provides significant insulation to customers of the Portland water system to the potential impacts of climate change on the hydrology of Bull Run. The WMCP is enclosed as Attachment 3-3 and can be found online at:
http://www.portlandonline.com/water/index.cfm?c=46238.

Question 4: Cryptosporidium testing and data analyses.

We request the following information on this topic:

A. *Recovery rates from Cryptosporidium analyses in historical sampling prior to the LT2 rule.*
B. *Rationale for not using standard ova and parasite concentration and immunofluorescence methods on scat samples.*
C. *Age of scat samples and impact of age on likelihood of finding oocysts.*
D. *How the Poisson distribution formula described in footnote 10, p 3-5 was adapted from Johnson, et. al. 1994.*

PWB Responses

Question 4A: Recovery rates from Cryptosporidium analyses in historical sampling prior to the LT2 rule.

Portland's historical *Cryptosporidium* and *Giardia* recovery rates for the raw water intake are shown in the table below. For LT2 compliance, Portland submitted the matrix spikes collected in 2002 and 2004 in its grandfathered data package. In Portland's continued monthly monitoring through 2009, an additional three matrix spikes were collected. Prior to LT2, matrix spikes were analyzed using EPA Method 1622/23 in 1999 and 2000 for both the ICR Supplemental Survey and AwwaRF project 488.

PWB Historical Matrix Spike Data (Collected at the Raw Water Intake)

Date	Volume (L)	*Crypto* Spike Dose	*Crypto* Recovery	*Giardia* Spike Dose	*Giardia* Recovery	Method	Laboratory	Project / Comments
4/12/1999	10	131.3	30%	N/A	N/A	1622	CH Diagnostics	ICR Supplemental Survey
6/14/1999	10	88.3	45%	N/A	N/A	1622	CH Diagnostics	ICR Supplemental Survey
6/22/1999	94.6*	250	64.3%	252	36.9%	1623	CEC	AwwaRF 488*
8/16/1999	91.5*	250	57.7%	254	47.6%	1623	CEC	AwwaRF 488*
9/13/1999	10.25	154.4	31%	145.6	71%	1623	CH Diagnostics	ICR Supplemental Survey
11/1/1999	68.8*	253	66.8%	253	23.3%	1623	CEC	AwwaRF 488*
11/8/1999	10.5	101.3	20%	98.7	61%	1623	CH Diagnostics	ICR Supplemental Survey
2/14/2000	11	80.6	55%	176.1	66%	1623	CH Diagnostics	ICR Supplemental Survey
2/28/2000	100*	253	87.4%	251	47%	1623	CEC	AwwaRF 488*

Date	Volume (L)	*Crypto* Spike Dose	*Crypto* Recovery	*Giardia* Spike Dose	*Giardia* Recovery	Method	Laboratory	Project / Comments
4/17/2000	100*	252	92.5%	252	37.3%	1623	CEC	AwwaRF 488*
12/17/2002	49	99	20%	99	69%	1623	CEC	per LT2 specifications, grandfathered for LT2 compliance
6/15/2004	50	99	57%	99	55%	1623	CEC	per LT2 specifications, grandfathered for LT2 compliance
2/15/2006	52	99	21%	100	47%	1623	CEC	per LT2 specifications
8/15/2007	50	99	2%	99	41%	1623	CEC	per LT2 specifications
3/18/2009	50	100	41%	101	42%	1623	CEC	per LT2 specifications

*Note: AwwaRF 488 matrix spike data were done by spiking the filters after all the filtration was completed so the spiking data from AwwaRF 488 may not be comparable with data from other projects. The volumes recorded for AwwaRF 488 would have been the volume collected; equivalent volume analyzed may have been less than volume collected, however Clancy Environmental / Tetra Tech (CEC) no longer has these records.

Question 4B: Rationale for not using standard ova and parasite concentration and immunofluorescence methods on scat samples.

PWB developed a scat sampling program as part of the variance sampling plan and study to gain a better understanding of the occurrence of *Cryptosporidium* in wildlife, the only potentially significant source of *Cryptosporidium* in the watershed. In addition, the data was intended to be used to adapt the Pathogen Catchment Budget (PCB) model to the Bull Run watershed, as recommended by the EPA. PCB model developer and microbiologist Dr. Christobel Ferguson recommended the use of immunomagnetic separation (IMS) coupled with immunofluorescent staining for the analysis of wildlife scat samples over standard clinical methods because of the greater sensitivity of the IMS method, which allows the quantification of low concentrations of oocysts that are often typical of wildlife fecal samples.

Research studies indicate that concentrations of *Cryptosporidium* oocysts in wildlife and domestic animal feces can be markedly lower than in symptomatic human cases (Heitman et al. 2002; Cox et al. 2005). Cox et al., for example, reported median oocysts concentrations in positive wildlife and domestic fecal samples ranging from 10^1 to 10^2 oocysts/gram. Given the general short-comings of conventional clinical methods to detect oocysts present in fecal samples at these levels, the use of IMS has been generally adopted by researchers to concentrate oocysts from wildlife and domestic animal fecal samples.

During IMS concentration, oocyst epitopes are bound to magnetizable beads coated with anti-*Cryptosporidium* antibodies. The resulting bead-oocyst complex can be removed from associated particulate material by applying a magnetic force and subsequently disassociated to yield concentrated oocysts. IMS concentration allows for a larger volume of feces to be assayed

and separates oocysts from interfering material in the fecal matter. The use of IMS followed by immunofluorescent staining was shown to detect 10 oocysts/gram, representing a 2-log-unit increase in sensitivity compared to direct fecal smears coupled with immunofluorescent staining of adult bovine feces which had a detection limit of 1000 oocysts/gram (Pereira et a. 1999). Similarly, IMS detection limits reported by Davies et al. (2003) were between 10 and 100 oocysts/gram in fecal samples from various types of animals based on the lowest recoveries from their study.

In contrast, stool samples from symptomatic human cases of *Cryptosporidium* infection generally contain large numbers of oocysts. Therefore, many laboratories process these stool specimens as direct smears that are microscopically evaluated for the presence of oocysts. Increased sensitivity can be achieved by concentrating oocysts from stool specimens using one of several biophysical methods (e.g., centrifugation or flotation) prior to staining, but such methods still lack the sensitivity to detect very low numbers of oocysts. For example, the detection limits for oocyst concentration by salt and sucrose flotation followed by immunofluorescent staining and for fecal smears has been reported to be in the order of 10^3 oocysts/gram and 10^6 oocysts/ml, respectively (Davies et al. 2003). Since the detection limits of these conventional methods is higher than the concentrations that are generally found in wildlife scat, PWB believed that it was prudent to enumerate oocysts using IMS concentration followed by immunofluorescent staining.

A Standard Operating Procedure (SOP) of the method employed by Analytical Services, Inc. (ASI), the lab contracted by PWB to conduct all scat analysis, is provided in Attachment 4B-1. This SOP details the steps employed by ASI to enumerate *Cryptosporidium* and *Giardia* (oo)cysts in Bull Run wildlife fecal samples. Note that ASI has adopted minor modifications recommended by Dr. Ferguson to optimize the recovery of oocysts. This includes the routine use of sodium pyrophosphate to disperse oocysts from feces and the targeted use of diethyl ether to separate interfering fat from fecal samples. As a standard practice, ASI spikes fecal sample with 100 internal calibration oocysts (ColorSeed™) to monitor the performance of each fecal assay. These internal positive controls have shown a wide variety of recovery efficiencies but nonetheless, the sensitivity of the assay remains superior to that of standard clinical assays.

References

Cox, P. et al. 2005. Concentrations of Pathogens and Indicators in Animal Feces in the Sydney Watershed. *Appl. Environ. Microbiol.* 71:5929-5934.

Davies, C. M. et al. 2003. Recovery and Enumeration of *Cryptosporidium parvum* from Animal Fecal Matrices. *Appl. Environ. Microbiol.* 69:2842-2847.

Heitman, T.L., et al. 2002. Prevalence of *Giardia* and *Cryptosporidium* and characterization of *Cryptosporidium* species isolated from wildlife, human, and agricultural sources in the North Saskatchewan River Basin in Alberta, Canada. *Can J Microbiol.* 48: 530-41.

Pereira, M.C. et al. 1999. Comparison of Sensitivity of Immunofluorescent Microscopy to That of a Combination of Immunomagnetic Separation for Detection of *Cryptosporidium Parvum* Oocysts in Adult Bovine Feces. *Appl. Environ. Microbiol.* 65:3236-3239.

Question 4C: Age of scat samples and impact of age on likelihood of finding oocysts.

The hardiness of *Cryptosporidium* oocysts notwithstanding, the initial load of infective oocysts in feces may decline as the deposit ages due to environmental stresses contributing to oocyst inactivation. Several environmental factors are known to influence the viability of oocysts in feces including temperature, pH, ammonia concentration, desiccation, and microbial antagonism (Peng et al. 2008). However, since the *Cryptosporidium* detection method employed by Portland, immunomagnetic separation coupled with immunofluorescence, does not differentiate between viable and inactivated oocysts, infections may be detected even after oocysts are no longer viable. As a fecal deposit ages, there will be a point, dependent on environmental conditions, at which oocysts may degrade, impeding detection by immunofluorescent stains that interact with epitopes on the oocysts wall. As part of Portland's scat monitoring program, specific field and lab protocols were developed to minimize the likelihood that scat age would interfere with the ability to detect *Cryptosporidium* infections in target wildlife.

Several researchers have reported rates of inactivation of *Cryptosporidium* oocysts in feces under different environmental conditions. For example, based on field experiments by Jenkins et al. (1999), 90% of oocysts in fecal piles were reported to degrade between 93 and 142 days (Peng et al. 2008) under two natural temperature regimes. Olson et al. (1999) reported that oocysts could remain infective in feces upwards of 12 weeks (84 days) at low temperatures of –4°C and 4°C, but that degradation accelerated above 25°C. Li et al. (2005) reported higher rates of inactivation based on diurnal oscillations in the internal temperatures of bovine feces exposed to solar radiation on agricultural lands in California. Their results suggest that as internal fecal temperatures reach above 40°C, rapid inactivation occurs at a rate greater than 99.9% per day.[2]

The cited studies address the rate of inactivation, and thus the fraction of infective oocysts that will remain in the fecal matrix as a function of time; and the fraction that is of interest in attempts to quantify public health risk. Even when oocysts are inactivated by environmental stresses, they may still be detected using immunofluorescence assays that employ antibodies raised against oocyst walls. For example, Li et al. were able to report the percentage of oocysts that were intact, partially excysted, or completely empty following one or more 24-hour diurnal temperature cycles in fecal preparations.

To Portland's knowledge, only one study has attempted to determine whether there is an impact on *Cryptosporidium* infection prevalence (e.g., presence/absence) estimates when samples are collected off the ground. Hoar et al. (1999) collected and assayed samples off the ground and directly from the rectum of adult beef cattle to compare prevalence estimated from samples collected in each manner. The prevalence of infection was higher based on rectal fecal samples (6/557; 1.1%) than on ground fecal samples (1/558; 0.2%), but this difference was not statistically significant.

[2] Note that each of the cited studies used a different measure of infectivity, which can contribute to the difference in reported inactivation rates in feces.

Given that *Cryptosporidium* oocysts in feces can degrade as a function of time having a potential to impact the ability to detect infections, Portland worked with David Evans & Associates, Inc. wildlife consultant Phil Rickus and Analytical Services, Inc. (ASI), the lab contracted by PWB to conduct all scat analysis, to develop field and lab protocols to ensure that scat samples were fresh (see Attachment 4C-1). In the field, collected samples must meet strict freshness criteria, the most critical being that the sample must have >50% moisture content. In practice, this means each sample is mechanically tested to ensure that it is still pliable, as would be expected of a fresh deposit.

In the lab, fecal samples are first assayed for *E. coli* prior to being analyzed for *Cryptosporidium* and *Giardia*.[3] The fate of *E. coli* in feces, like *Cryptosporidium* oocysts, is temperature and time dependent. Therefore, adequate concentrations of *E. coli* in a stool were considered a reasonably good indicator of sample freshness for the detection of *Cryptosporidium* oocysts. The sample freshness protocols applied by Portland coupled with the use of a very sensitive assay for detecting oocysts in fecal samples gives a high confidence in the results generated by the scat monitoring program.

References

Hoar, B. R. et al. 1999. Comparison of Fecal Samples Collected per Rectum and Off the Ground for Estimation of Environmental Contamination Attributable to Beef Cattle. *Am. J. Vet. Res.* 60:1352-1356.

Jenkins, M. B. et al. 1999. Use of a Sentinel System for Field Measurement of *Cryptosporidium* parvum Oocyst Inactivation in Soil and Animal Waste. Appl. Environ. Microbiol. 65:1998-2005.

Li, X. et al. 2005. Seasonal Temperature Fluctuations Induces Inactivation of *Cryptosporidium* parvum. Environ Sci Technol. 39:4484-4489.

Olson, M. E. et al. 1999. *Giardia* Cysts and *Cryptosporidium* Oocysts Survival in Soil, Water, and Cattle Feces. *J. Environ. Qual.* 28:1991-1996.

Peng, X. et al. 2008. Evaluation of the Effect of Temperature on the Die-Off Rate for *Cryptosporidium parvum* Oocysts in Water, Soil, and Feces. *Appl. Environ. Microbiol.* 74: 7101-7107.

[3] Note that during the winter season the *E. coli* standard may not be applied because fecal samples are expected to have lower *E. coli* levels when ambient temperatures are low. This approach is further justified by longer survival of *Cryptosporidium* oocysts at lower temperatures, as has been reported by Olson et al. (1999). The *E. coli* standard is also not applied to a few target species for which *E. coli* is not expected to provide meaningful information on sample freshness.

Question 4D: How the Poisson distribution formula described in footnote 10, p 3-5 was adapted from Johnson, et. al. 1994

The recommended use of the Poisson equation and adaptation from Johnson et al. (1994) was provided by Dr. Charles Haas, a recognized expert on quantitative microbial risk assessments. Similar equations based on Dr. Haas' work are used in EPA's modeling of *Cryptosporidium* occurrence as detailed in Appendix B of "Occurrence and Exposure Assessment for the Final Long Term 2 Enhanced Surface Water Treatment Rule".[4] The following text gives an exact description and reasoning behind how the equation quoted in the variance request was adapted.

The formula and description provided in footnote 10, page 3-5 of Portland's Variance Request reads:

> The Poisson distribution is typically used for EPA modeling and risk assessments for sampling of *Cryptosporidium*. The percent confidence values were calculated using the equation below in which μ is the concentration, V is the volume, x is the number of oocysts detected, and α is the percent confidence (adapted from Johnson et al. 1994).
>
> $$\exp(-\mu V)\sum_{j=0}^{x}\frac{(\mu V)^j}{j!}=1-\alpha$$

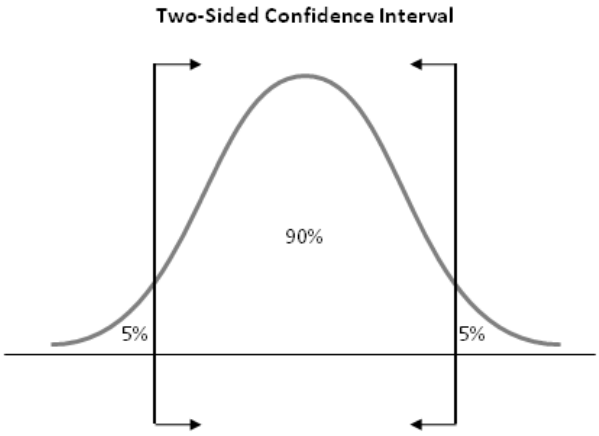
On page 170 of the second edition of Johnson's book "Univariate Discrete Distributions" a section on confidence intervals of the Poisson distribution provides the following description and equations:

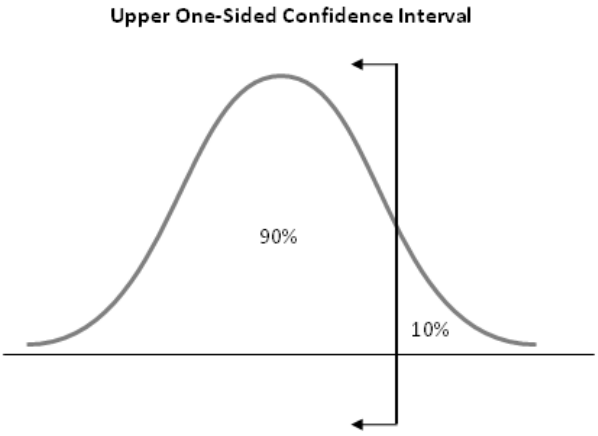
> Since the Poisson distribution is a discrete distribution, it is not possible to construct confidence intervals for θ with an exactly specified confidence coefficient of, say, 100(1 – α)%. *Approximate* 100(1 – α)% confidence limits for θ given an observed value of X, where X has the distribution (4.1), are obtained by solving the equations
>
> $$\exp(-\theta_L)\sum_{j=x}^{\infty}\frac{\theta_L^j}{j!}=\frac{\alpha}{2} \quad (4.61), \qquad \exp(-\theta_U)\sum_{j=0}^{x}\frac{\theta_U^j}{j!}=\frac{\alpha}{2} \quad (4.62),$$
>
> for θ_L, θ_U, respectively, and using the interval (θ_L, θ_U).

[4] USEPA. 2005. Occurrence and Exposure Assessment for the Final Long Term 2 Enhanced Surface Water Treatment Rule. Office of Water, Washington, DC. EPA 815-R-06-002, December 2005.

The equations provided in Johnson et al. are meant for a situation where the confidence level between two different values is desired, a lower bound θ_L and an upper bound θ_U. This is referred to as a two-sided confidence interval. To find the 90% confidence interval, α would equal 0.1 because 100(1 – 0.1) = 90%. Outside of the 90% confidence interval, there is a 5% probability that the value is higher than the interval and a 5% probability that the value is lower than the interval. Thus, in the equations above, α/2 is used to represent this split probability (0.1/2 = 0.05 or 5%).

In the context of the LT2 variance request, only the upper boundary is of interest. This is referred to as a one-sided confidence interval. Specifically, the aim is to show with 90% confidence that the mean concentration is less than 0.000225 oocysts/liter. There is no range being considered, only a single value where the probability of the concentration being lower is desired. Thus only the equation for the upper boundary is utilized and α is no longer divided by 2. The figure below shows a visual representation of the two different types of confidence intervals.





Equations: $\exp(-\theta_L)\sum_{j=x}^{\infty}\frac{\theta_L^j}{j!}=\frac{\alpha}{2}$ $\exp(-\theta_U)\sum_{j=0}^{x}\frac{\theta_U^j}{j!}=\frac{\alpha}{2}$ $\exp(-\theta_U)\sum_{j=0}^{x}\frac{\theta_U^j}{j!}=\alpha$

Additionally, the definition of alpha between the original and adapted equation has been changed. In the original equation, the percent confidence interval is defined as 100(1 – α)%. For the 90% confidence interval, α equals 0.1 since 100(1-0.1) = 90%. In the adapted equation, α instead directly represents the percent confidence. In the case of a 90% confidence interval, α is equal to 0.9. However, the right side of the adapted equation is 1 – α (or 1 – 0.9 = 0.1), so the value used within the calculation is equivalent for both equations, despite a different definition of the variable being used.

$$\exp(-\theta_U)\sum_{j=0}^{x}\frac{\theta_U^j}{j!}=1-\alpha$$

The final adaptation is that θ_U has been replaced with μV. Theta in the original equation represents the parameter of interest. In the case of the LT2 variance application, this value is the expected number of oocysts present in the sampled source water. The term μV is the multiplication of the *Cryptosporidium* concentration (in oocysts/L) and the volume sampled (in liters), which gives the number of oocysts expected to be present in the sampled volume. The variable x is then the number of oocysts actually observed during sampling.

These are the same variable definitions as used by EPA. In Appendix B, page B-4 of the "Occurrence and Exposure Assessment for the Final Long Term 2 Enhanced Surface Water Treatment Rule", the variable λ is used in place of θ within Poisson calculations. Lambda is defined as "the expected count of *Cryptosporidium* in that unit volume of source water". An example is given showing λ being calculated: "With a concentration of 0.1 oocysts per liter and a 3-liter sample, the expected count (λ) is 0.3 (=0.1 * 3)." Thus λ is equivalent to μV.

$$\exp(-\mu V)\sum_{j=0}^{x}\frac{(\mu V)^j}{j!}=1-\alpha$$

In summary, the evolution of the equation incorporating the changes is:

Original Johnson et al. equations $\exp(-\theta_L)\sum_{j=x}^{\infty}\frac{\theta_L^j}{j!}=\frac{\alpha}{2}$, $\exp(-\theta_U)\sum_{j=0}^{x}\frac{\theta_U^j}{j!}=\frac{\alpha}{2}$

Equation only considering the one-side upper bound $\exp(-\theta_U)\sum_{j=0}^{x}\frac{\theta_U^j}{j!}=\alpha$

Equation with definition of α changed $\exp(-\theta_U)\sum_{j=0}^{x}\frac{\theta_U^j}{j!}=1-\alpha$

Equation with θ_U replaced by μV. $\exp(-\mu V)\sum_{j=0}^{x}\frac{(\mu V)^j}{j!}=1-\alpha$