

began in March 1999 and ended in February 2000.

a. *Scope of the Information Collection Rule.* The Information Collection Rule (61 FR 24354, May 14, 1996) (USEPA 1996a) required large PWSs to collect water quality and treatment data related to DBPs and microbial pathogens over an 18-month period. PWSs using surface water or ground water under the direct influence of surface water as sources and serving at least 100,000 people were required to monitor their raw water monthly for *Cryptosporidium*, Giardia, viruses, total coliforms, and *E. coli*. Approximately 350 plants monitored for microbial parameters.

b. *Scope of the ICRSS.* The ICRSS were designed to complement the Information Collection Rule data set with data from systems serving fewer than 100,000 people and by employing an improved analytical method for protozoa (described later). The ICRSS included 47 large systems (serving greater than 100,000 people), 40 medium systems (serving 10,000 to 100,000 people) and 39 small systems (serving fewer than 10,000 people). Medium and large systems conducted 1 year of twice-per-month sampling for *Cryptosporidium*, Giardia, temperature, pH, turbidity, and coliforms. Other water quality measurements were taken once a month. Small systems did not test for protozoa but tested for all other water quality parameters.

### 3. Analytical Methods for Protozoa in the Information Collection Rule and ICRSS

This subsection describes analytical methods for *Cryptosporidium* that were used in the Information Collection Rule and ICRSS. Information on *Cryptosporidium* analytical methods is important for the LT2ESWTR for several reasons: (1) It is relevant to the quality of *Cryptosporidium* occurrence data used to assess risk and economic impact of the LT2ESWTR proposal, (2) it provides a basis for the statistical procedures employed to analyze the occurrence data, and (3) it is used to assess the adequacy of *Cryptosporidium* methods to support source-specific decisions under the LT2ESWTR.

The Information Collection Rule and ICRSS data sets were generated using different analytical methods. The Information Collection Rule Protozoan Method (ICR Method) was used to analyze water samples for *Cryptosporidium* during the Information Collection Rule. For the ICRSS, a similar but improved method, EPA Method 1622 (later 1623), was used for protozoa analyses (samples were analyzed for *Cryptosporidium* using Method 1622 for

the first 4 months; then Method 1623 was implemented so that Giardia concentrations could also be measured).

a. *Information Collection Rule Protozoan Method.* With the Information Collection Rule Method (USEPA 1996b), samples were collected by passing water through a filter, which was then delivered to an EPA-approved Information Collection Rule laboratory for analysis. The laboratory eluted the filter, centrifuged the eluate, and separated *Cryptosporidium* oocysts and Giardia cysts from other debris by density-gradient centrifugation. The oocysts and cysts were then stained and counted. Differential interference contrast (DIC) microscopy was used to examine internal structures.

The Information Collection Rule Method provided a quantitative measurement of *Cryptosporidium* oocysts and Giardia cysts, but it is believed to have generally undercounted the actual occurrence (modeling, described later, adjusted for undercounting). This undercounting was due to low volumes analyzed and low method recovery. The volume analyzed directly influences the sensitivity of the analytical method and the Information Collection Rule Method did not require a specific volume analyzed. As a result, sample volumes analyzed during the Information Collection Rule varied widely, depending on the water matrix and analyst discretion, with a median volume analyzed of only 3 L.

Method recovery characterizes the likelihood that an oocyst present in the original sample will be counted. Loss of organisms may occur at any step of the analytical process, including filtration, elution, concentration of the eluate, and purification of the concentrate. To assess the performance of the Information Collection Rule Method, EPA implemented the Information Collection Rule Laboratory Spiking Program. This program involved collection of duplicate samples on two dates from 70 plants. On each occasion, one of the duplicate samples was spiked with a known quantity of Giardia cysts and *Cryptosporidium* oocysts (the quantity was unknown to the laboratory performing the analysis), and both samples were processed according to the method. Recovery of spiked *Cryptosporidium* oocysts ranged from 0% to 65% with a mean of 12% and a standard deviation nearly equal to the mean (relative standard deviation (RSD) approximately 100%) (Scheller *et al.* 2002).

b. *Method 1622 and Method 1623.* EPA developed Method 1622 (detects *Cryptosporidium*) and 1623 (detects

*Cryptosporidium* and Giardia) to achieve higher recovery rates and lower inter- and intra-laboratory variability than previous methods. These methods incorporate improvements in the concentration, separation, staining, and microscope examination procedures. Specific improvements include the use of more effective filters, immunomagnetic separation (IMS) to separate the oocysts and cysts from extraneous materials present in the water sample, and the addition of 4, 6-diamidino-2-phenylindole (DAPI) stain for microscopic analysis. The performance of these methods was tested through single-laboratory studies and validated through multiple-laboratory validation (round robin) studies.

The per-sample volume analyzed for *Cryptosporidium* during the ICRSS was larger than in the Information Collection Rule, due to a requirement that laboratories analyze a minimum of 10 L or 2 mL of packed pellet with Methods 1622/23 (details in section IV.K). To assess method recovery, matrix spike samples were analyzed on five sampling events for each plant. The protozoa laboratory spiked the additional sample with a known quantity of *Cryptosporidium* oocysts and Giardia cysts (the quantity was unknown to the laboratory performing the analysis) and filtered and analyzed both samples using Methods 1622/23. Recovery in the ICRSS matrix spike study averaged 43% for *Cryptosporidium* with an RSD of 47% (Connell *et al.* 2000). Thus, mean *Cryptosporidium* recovery with Methods 1622/23 under the ICRSS was more than 3.5 times higher than mean recovery in the Information Collection Rule lab spiking program and relative standard deviation was reduced by more than half.

Although Methods 1622 and 1623 have several advantages over the Information Collection Rule method, they also have some of the same limitations. These methods do not determine whether a cyst or oocyst is viable and infectious, and both methods require a skilled microscopist and several hours of sample preparation and analyses.

### 4. *Cryptosporidium* Occurrence Results from the Information Collection Rule and ICRSS

This section describes *Cryptosporidium* monitoring results from the Information Collection Rule and ICRSS. The focus of this discussion is the national distribution of mean *Cryptosporidium* occurrence levels in the sources of filtered and unfiltered plants.