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Legionnaires' Disease Bacterium in Power Plant Cooling Systems: Phase I Final Report

Prepared by
Oak Ridge National Laboratory
University of Tennessee
Savannah River Laboratory

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Prepared by

OAK RIDGE NATIONAL LABORATORY
Environmental Sciences Division
P.O. Box X
Oak Ridge, Tennessee 37830

Authors
S. W. Christensen
J. A. Solomon
S. B. Gough

Subcontractors

UNIVERSITY OF TENNESSEE
Knoxville, Tennessee 37916

Author
R. L. Tyndall

SAVANNAH RIVER LABORATORY
Aiken, South Carolina 29801

Author
C. B. Fliermans

Prepared for

Electric Power Research Institute
3412 Hillview Avenue
Palo Alto, California 94304

EPRI Project Manager
J. S. Mattice

Ecological Studies Program
Energy Analysis and Environment Division

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Prepared by
Oak Ridge National Laboratory
University of Tennessee
Savannah River Laboratory

ABSTRACT

As Phase I of a two-phase study, a survey was undertaken of the distribution, density, viability, and infectivity of Legionnaires' Disease Bacteria (*Legionella*) in power plant cooling systems. Water samples were collected during each of the four seasons at various locations within each of nine power plants and from ambient waters at each site. Measurements of a number of physical and chemical characteristics were made, and *Legionella* profiles (density, viability, and infectivity for guinea pigs) were obtained. *Legionella* were detected in nearly all samples. Water from closed-cycle cooling systems frequently had lower densities of *Legionella* than the ambient water. Nonetheless, infectious *Legionella*, as defined by their isolation from inoculated guinea pigs, were significantly more likely to be found in samples from the plant-exposed water of closed-cycle plants than in samples from once-through plants or in ambient samples. A new species (*L. oakridgensis*) was initially isolated from two of the sites, and it has since been found to have a widespread distribution. Two other organisms found to cause illness in guinea pigs may also be new species. Phase II of the project involves investigating possible cause/effect relationships between physicochemical variables and *Legionella*. This work may contribute toward eventual control techniques for this pathogen.

EPRI PERSPECTIVE

PROJECT DESCRIPTION

This interim report for RP1909-1 describes results of a survey of power plant cooling waters for Legionnaire's Disease Bacteria (LDB). Four times during the year at each plant, water samples were collected near the intake, where water was unaffected by plant operation, as well as inside the plant and in the discharge plume. The nine power plants sampled included a range of geographical locations, cooling system designs, and water qualities. Water quality characteristics and LDB density and viability were determined for each sample, and selected samples were injected into guinea pigs to determine sample infectivity. Various correlation techniques were used to examine presumptive relationships between sample characteristics. These results represent the final output of Phase I of the project and will form the basis in Phase II for investigation of causal relationships between these characteristics.

PROJECT OBJECTIVES

The overall objective of this project is to define the relationship between LDB and power plant cooling systems. Interim objectives are (1) to investigate the presence and viability of LDB and infectivity (to guinea pigs) of water samples collected seasonally at a series of typical power plants; (2) to establish causal relationships between water quality and plant operational characteristics and LDB presence, viability, and infectivity; and (3) to determine LDB characteristics in aerosol samples collected in and near power plants during normal operating conditions and downtime periods.

PROJECT RESULTS

Effects of power plant operation on water sample LDB variables appear to depend on the characteristic (e.g., density and infectivity) chosen for comparison, type of cooling system, and season of the year. Conclusions based on analysis of Phase I results can be summarized as follows:

- Viability of LDB in replicate water samples was not consistent.

- Viability tended to be lower in summer and fall but was not different in ambient and plant-affected water samples.

- In spring, LDB density was lower in plant-affected waters at closed-cycle plants, but in other seasons and at all open-cycle plants, densities were equivalent in ambient and plant-affected waters.

- Infectious samples were found in all seasons in the plant-affected waters but only in summer, fall, and winter in ambient waters.

- A higher proportion of infectious samples was found in plant-affected waters of closed-cycle plants than in ambient waters; no such differences were found at open-cycle plants.

- Sample infectivity could not be related to LDB density, viability, or combinations of the two variables.

- Several methods of statistical analysis indicated a number of physiochemical variables that appear related to LDB density and infectivity, but cause-effect relationships cannot be established from these data.

A new species of LDB (Legionella oakridgensis) was identified during this study. The clinical importance of this species is uncertain, but it was the third most prevalent Legionella organism isolated from samples that caused infection in the guinea pigs.

The above conclusions should be considered preliminary until confirmed by Phase II studies.

Jack S. Mattice, Project Manager
Energy Analysis and Environment Division

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SUMMARY

Legionnaires' Disease Bacteria (Legionella) are a component of the normal aquatic community that, when aerosolized, can be pathogenic to man. Studies on the source of infection of various outbreaks of Legionella at times have implicated cooling towers used in air conditioning systems. Surveys have established that the organisms are ubiquitous, occurring in natural waters as well as in cooling tower systems of all sizes and configurations. The Electric Power Research Institute (EPRI) judged it desirable to support a study of the distribution, abundance, and infectivity of Legionella in power plant cooling systems as a step toward the possible development of control techniques. This report presents the results from the first phase of this project.

Water samples were collected during each of the four seasons at various locations within each of nine power plants and from ambient waters at each site. Measurements of temperature, dissolved oxygen, pH, conductivity, alkalinity, phosphate, nitrate, ammonia, and inorganic and total dissolved carbon were obtained for each sample. In addition, the samples were concentrated 500-fold by centrifugation and processed to determine the density of Legionella using direct fluorescent antibody staining. Viability, defined as possession of a functional electron transport apparatus, was estimated using a tetrazolium dye. Infectivity of selected samples was also determined by intraperitoneal inoculation of guinea pigs and subsequent isolation of the bacterium on agar. Data were entered into a computer data base and analyzed statistically.

Legionella were detected in nearly all samples, whether from ambient (source) water or from plant-exposed water. In addition, infectious Legionella were found fairly frequently in both ambient and plant-exposed water. The Knoxville serogroup of L. pneumophila was the most prevalent subgroup, followed by the Los Angeles serogroup of L. pneumophila and the newly discovered species, L. oakridgensis.

Cell densities of Legionella were significantly greater during the spring in ambient waters supplying the closed-cycle plants in our study than those in ambient waters supplying the once-through plants. The reason for this is not understood, but latitudinal differences and the types of water bodies associated with the use of closed-cycle cooling may be relevant. During the spring and summer, cell concentrations were significantly ($P < 0.05$) reduced at closed-cycle plants in the plant-exposed water compared to those in the ambient water.

The viability of Legionella populations was significantly greater in ambient water of once-through plants than in that of closed-cycle plants during both spring and summer. Changes in viability with power plant passage occurred in closed-cycle plants, but the changes were not consistent in direction.

The density of viable Legionella cells was calculated as the product of the total cell density and the proportion of viable cells. As was true for total cell densities, densities of viable cells were significantly greater in the spring in ambient waters at the closed-cycle sites than those in ambient waters at the once-through sites, and viable cell densities were significantly reduced during the spring and summer in plant-exposed water from closed-cycle plants.

Pure cultures of Legionella isolated from tissues of inoculated animals were considered presumptively infectious. Infectivity was not confirmed by reinoculation of guinea pigs. For this and other reasons, it is not possible to directly relate infectivity in this study to human risk. Infectious Legionella were isolated from ambient waters in all seasons except spring and from plant-exposed water during all seasons. Although infectivity appeared to be lower in the spring than in the other seasons, this trend was not statistically significant. Infectious Legionella were significantly more likely to be found ($P < 0.01$) in samples from the plant-exposed water of closed-cycle plants than in samples from once-through plants or in ambient samples. In addition, the property of infectivity seemed to be associated more with some power plants than with others. Contrary to expectation, infectivity could not be related to Legionella density or viability, or to combinations of those two characteristics in a straightforward manner. Analyses indicated a number of physical and chemical variables that may be related to Legionella abundance, viability, and infectivity, but cause/effect relationships cannot be established from the present data.

A new species (Legionella oakridgensis) was initially isolated from two of the sites and has since been found to have widespread distribution. The clinical importance of this species has not yet been determined. Two other organisms apparently causing illness in guinea pigs may also be new species. Personnel at the Centers for Disease Control (CDC) have been unable to classify one of these microbes as belonging to any known genus.

The results indicate that the current practice of using densities of Legionella greater than 10^8/L as the sole "trigger" for instituting control measures may not be appropriate for all systems, because density alone is an unsatisfactory predictor of infectivity. The factors affecting the infectivity of Legionella should be studied in more controlled settings to enable cause/effect relationships to be established. Phase II of the continuing EPRI project has been modified somewhat from the original plan to focus on identifying such cause/effect relationships. Results may contribute to eventual control techniques for this pathogen.

Section 1

INTRODUCTION

Preliminary screening studies conducted under the Oak Ridge National Laboratory (ORNL) Exploratory Studies Program and a pilot study sponsored by the Electric Power Research Institute (EPRI) showed that artificial heating of lakes by power plants may facilitate the emergence, propagation, and dissemination of free-living microorganisms pathogenic to man (Stevens et al. 1977; Tyndall et al. 1978, 1979; Fliermans et al. 1979). In addition, recent data indicate the association of Legionnaires' Disease Bacteria (Legionella) with cooling systems (Fraser 1980, Grace et al. 1981). Studies on the source of infection of various outbreaks of Legionnaires' Disease have at times implicated cooling towers (e.g., Deubner and Gilliam 1977, CDC 1978).

Until the 1976 outbreak of fatal pulmonary disease at a "Legionnaires'" convention in Philadelphia, it was thought that all major groups of human biological pathogens had been characterized. The subsequent isolation of Legionella dispelled this misconception. The etiological agents of Legionnaires' Disease are gram-negative bacilli (McDade et al. 1977) unrelated to other known bacteria, as demonstrated with techniques of DNA homology, guanine-cytosine ratios, gas-liquid chromatography (Moss et al. 1977), and immunofluorescence (Cherry et al. 1978). All examined specimens are gram-negative, weakly oxidase-positive, catalase-positive, rod-shaped to filamentous bacteria (Brenner et al. 1979, Chandler et al. 1978). The organism has been classified as a new genus, Legionella, and the type species is Legionella pneumophila McDade (Brenner et al. 1979).

Direct fluorescent antibody (FA) staining provides a valuable technique for detecting various strains of Legionella in both clinical and environmental samples. McKinney et al. (1979) prepared antisera in rabbits against numerous strains of Legionella. These antisera were conjugated to fluorescein isothiocyanate (FITC) and were used to stain a wide variety of Legionella isolates. The majority of L. pneumophila isolates are antigenically

distinguished by this process into six groups called serogroups. These have been designated: serogroup 1 (as represented by the Knoxville 1 isolate), serogroup 2 (Togus 1), serogroup 3 (Bloomington 2), serogroup 4 (Los Angeles 2), serogroup 5 (Dallas 1E), and serogroup 6 (Chicago 2).

In addition to L. pneumophila, six other species have been identified. Legionella pneumophila (especially four of its serogroups) has been considered of major importance clinically. The clinical importance of a new species, which was recently discovered by this team (see the Results section), has yet to be determined. The present literature on Legionnaires' Disease reflects the fact that virtually all of the research to date has been conducted on the diagnostic, clinical, immunologic, taxonomic, physiologic, and growth aspects of the bacterium. Lattimer and Ormsbee (1981) provide a review of much of this work. One of the major shortcomings of this extensive research has been the lack of work on the definition of the ecological niche of the etiological agent (Legionella itself), its physiological ecology, and its relationship to other microorganisms.

Energy production is associated with temperature increases in cooling waters. In addition, there is an increasing trend to use high-temperature closed-cycle cooling lakes and towers. These considerations, coupled with the observation that microbial pathogens may proliferate in cooling waters, indicated that further studies were needed to evaluate the extent and options for control of this potential problem. During the spring of 1980, ORNL submitted a proposal to EPRI to fund research on Legionella for a three-year period. Subsequent discussions by the staff of both institutions resulted in agreement on the scope of work for the project, and ORNL received initial funding. Phase I of the project consisted of screening studies to (a) characterize the distribution, abundance, viability, and virulence of Legionella in power plant cooling systems and associated ambient waters, and (b) study the relationships among Legionella characteristics and environmental parameters. Phase II, which was designed in part based on the outcome of Phase I, involves studies in the laboratory and in field experiments using enclosure chambers to investigate suspected causal relationships between environmental factors and Legionella characteristics. This report presents the results of Phase I.

Section 2

MATERIALS AND METHODS

The goals of Phase I of the study were (a) to characterize Legionella profiles (abundance, viability, and virulence) in power plant cooling systems and associated ambient waters, and (b) to study the relationships among Legionella profiles and environmental factors. In this section, the procedures by which these goals were accomplished are presented in relation to the power plant sites, the sampling procedures, and the analysis of data. The power plant sites were selected before actual sampling began, while sampling and the refinement of statistical techniques for analyzing the data proceeded concurrently.

POWER PLANT SITES

Site Selection

Candidate sites were chosen, using the INFORUM data base (Hannon 1978) and other sources, on the basis of geography, cooling system type, system reliability (when known), type of water body utilized, and amount and quality of available background ecological data. These candidate facilities included northern and southern locales, but an attempt was made to include only north-midwestern and southeastern plants, due to sampling logistics. The final selection of nine sites was done by EPRI in consultation with the participating utilities. Approximately one-half of the sites are "northern," with the remainder considered "southern," although no sites are sampled below approximately 34°N latitude (e.g., the latitude of Atlanta, Georgia). Sites were chosen to include a wide range of power plant configurations, consistent with the purpose of the study, and were not based on any prior knowledge of Legionella distributions. The names of the sites have remained confidential and are not disclosed in this document; sites are referred to by letter codes A through I. It was felt that the early publicity which occurred due to the dramatic nature of the discovery of Legionella might otherwise result in unfair discrimination against the sites sampled.

distinguished by this process into six groups called serogroups. These have been designated: serogroup 1 (as represented by the Knoxville 1 isolate), serogroup 2 (Togus 1), serogroup 3 (Bloomington 2), serogroup 4 (Los Angeles 2), serogroup 5 (Dallas 1E), and serogroup 6 (Chicago 2).

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Power Plant Operating Characteristics

The plants chosen for sampling can be conveniently separated into three categories: once-through, variable-mode, and closed-cycle. Sites A through D have once-through plants located on reservoirs or lakes; none has cooling towers (Table 2-1). Sites A through C are southern (i.e., below the Mason-Dixon line, or below approximately 40° north latitude), while site D is northern. Sites E and F have variable-mode plants with mechanical-draft towers. They are capable ofvarying their operating characteristics over a wide range, from once-through without towers to recirculating (closed-cycle) with towers. Site E is located on a southern reservoir, while F is a northern site on a river. Except in the two instances noted in Table 2-1, it was possible to identify the plants at these sites as operating essentially in either a once-through or a closed-cycle mode during the sampling in our study. Sites G, H, and I are all northern, with closed-cycle plants.

Information on biocide treatment (usually chlorination) was obtained from plant records and/or plant personnel at the time of sampling. Chlorination practices varied among these plants from no chlorination (plants A and B) to daily chlorination (plant G). In general, sample collections were arranged to avoid periods of actual chlorination of the water being sampled. Because chlorine levels in the water were not measured, chlorination was categorized relative to the sampling dates, entered into the data base (see Appendix Table A-4), and utilized as appropriate in the statistical analyses. Two other operation-related parameters which varied were generating levels and use of cooling towers. Variation in generating levels was reflected in the temperature change across the condenser; sample temperature and two temperature-change indices were included in the analyses. Variation in use of towers occurred only at plants E (plant was shut down in summer sampling and towers were bypassed in winter sampling) and I (towers were bypassed except for summer sampling). Use of towers was included in the data base, but because of the confounding of cooling towers with closed-cycle operation, the mode of operation (once-through vs closed-cycle) was used as the more relevant distinguishing variable.

SAMPLING PROCEDURES

Sample Collection

Sampling was conducted during each of the four seasons (spring, summer, fall, and winter) at each power plant (Table 2-2). A mobile laboratory from the Microbial Ecology Laboratory at Savannah River Laboratory (SRL; Fig. 2-1) was used at some

Table 2-1

COOLING SYSTEM CHARACTERISTICS, LOCATION, AND OPERATING MODES OF POWER PLANTS IN THE PHASE I STUDY

Plant Code	Cooling System Characteristics	Geographic Location	Operating Modes by Season[a]			
			Spring	Summer	Fall	Winter
A	Once-through, reservoir, no towers	Southern	O	O	O	O
B	Once-through, reservoir, no towers	Southern	O	O	O	O
C	Once-through, reservoir, no towers	Southern	O	O	O	O
D	Once-through, lake, no towers	Northern	O	O	O	O
E	Variable mode, reservoir, mechanical-draft towers	Southern	O	N	O	O
F	Variable mode, river, mechanical-draft towers	Northern	C	M	C	C
G	Closed-cycle, river, natural-draft towers	Northern	C	C	C	C
H	Closed-cycle, river, mechanical-draft towers	Northern	C	C	C	C
I	Closed-cycle, river, mechanical-draft towers, cooling pond	Northern	C	C	C	C

[a]Key to operating modes: C = closed-cycle, M = mixed-mode, N = not operating, O = once-through.

Table 2-2

SAMPLING DATES[a] FOR THE PHASE I STUDY

Plant code	Spring	Summer	Fall	Winter
A	3/11/81	7/13/81	10/19/81	1/11/82
B	3/11/81	7/14/81	10/20/81	1/12/82
C	3/12/81	8/04/81	10/21/81	1/28/82
D	4/22/81	7/22/81	10/09/81	12/30/81
E	3/13/81	8/11/81	10/22/81	1/27/82
F	4/20/81	7/20/81	10/07/81	12/23/81
G	3/26/81	7/31/81	9/21/81	2/05/82
H	4/20/81	7/20/81	10/06/81	12/22/81
I	4/21/81	7/21/81	10/08/81	12/24/81

[a]Dates are in the format month/day/year.

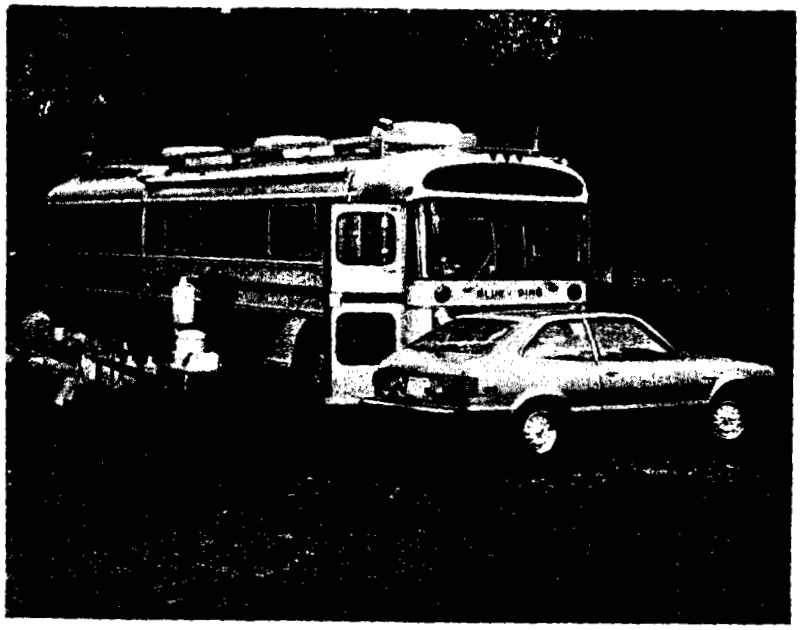


Figure 2-1. Mobile laboratory during sample processing.

of the sites. This facility is capable of processing samples; that is, samples can be concentrated by centrifugation, reagents can be added at the site, and some chemical analyses can be performed. Use of the mobile laboratory was practical when the sampling sites were not widely separated. For the northern sites (D, F, G, H, and I) it was not feasible to use the mobile laboratory. These sites were sampled by driving or flying to the areas and sending unconcentrated (fresh) samples back to SRL by air such that they were concentrated and processed within approximately 24 h.

In view of the array of sites to be sampled and the schedule for sampling them, it was determined that eight water samples per site could be accommodated for each sampling period. A particular unit at each site, expected to be in operation for the one-year period, was chosen for repeated (seasonal) sampling. During a particular visit, however, an alternate unit was used if necessary to avoid chlorination concurrent with sampling. Typical sample collection locations are shown schematically in Fig. 2-2 for each of the three power plant types in the study: once-through, variable-mode, and closed-cycle. To provide a measure of the variation in the data, three replicates were taken from each of the intake and outfall areas. Except in the summer, the three intake and three outfall samples were each taken at a single location. In the summer, one sample of each type was taken at the usual location (solid circles in Fig. 2-2), while the other two of each type were collected at different locations (broken circles in Fig. 2-2).

Intake samples at the once-through plants were taken from the surface of the ambient (source) water, after the skimmer wall (if any) but before the intake screens. Both of the variable-mode plants in the study (plants E and F) have skimmer walls with subsurface openings for the intake water. Intake samples therewere collected from surface waters outside of the skimmer wall to prevent possiblecontamination with recirculating water. At plant E, these samples were collected immediately adjacent to the skimmer wall, while at plant F they were collected about fifty yards in front of the wall. At two of the three closed-cycle plants, intake samples were collected from the makeup water after it had been pumped from the source water.

Outfall samples at once-through plants were taken from the discharge canal or from the receiving water body, close to the point of discharge. At variable-mode

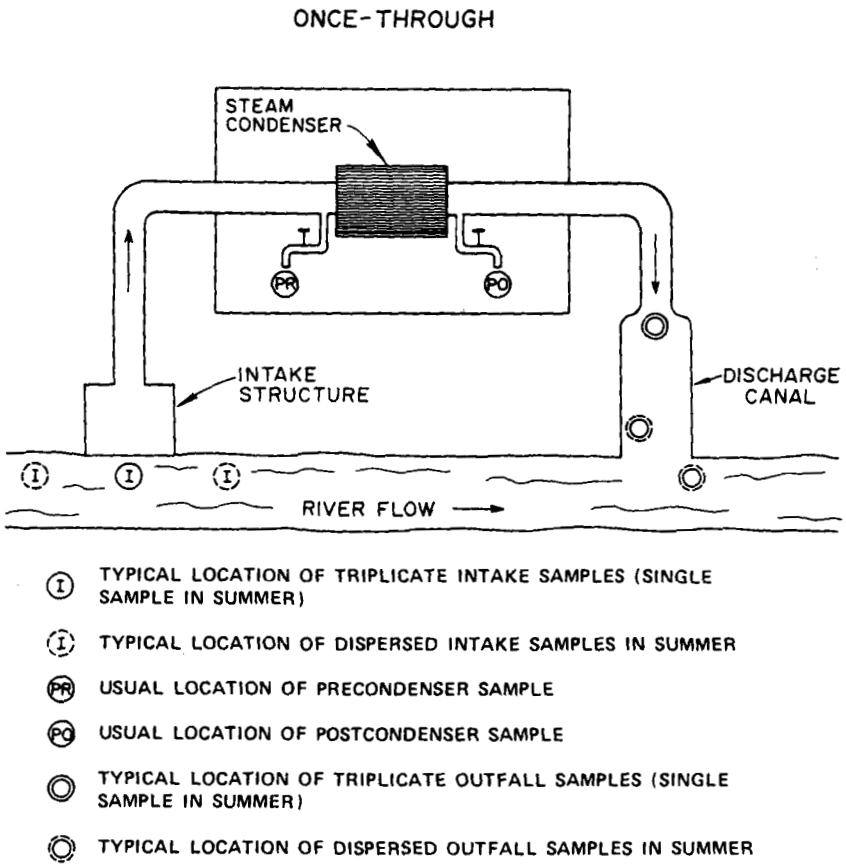
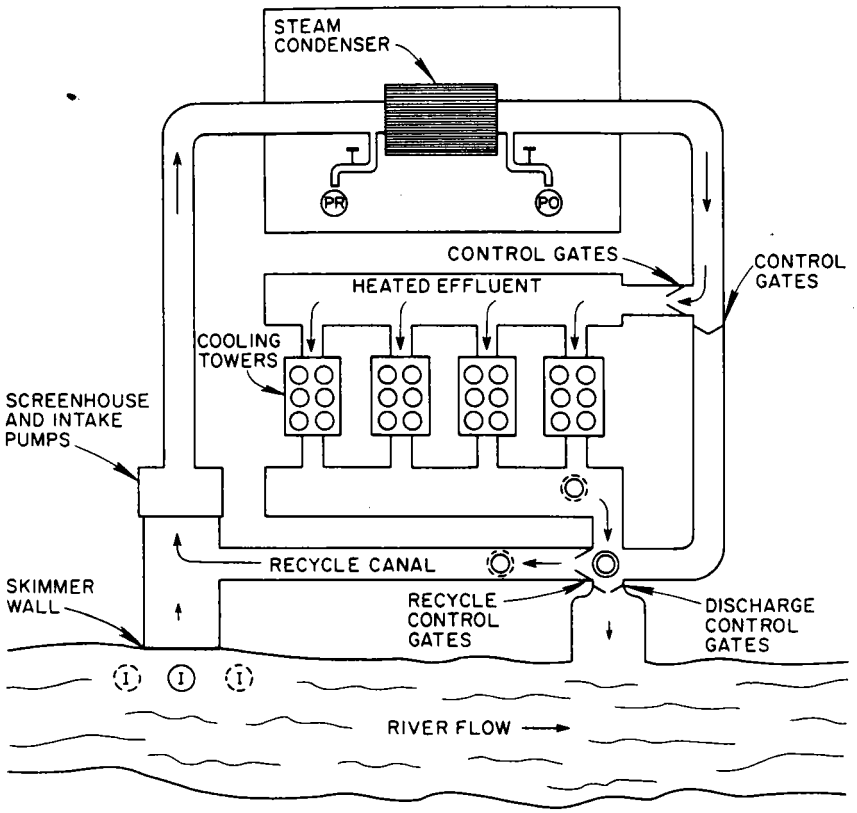


Figure 2-2. Schematic diagram showing typical sample collection locations for each of the three power plant types in the study.

VARIABLE-MODE



CLOSED-CYCLE

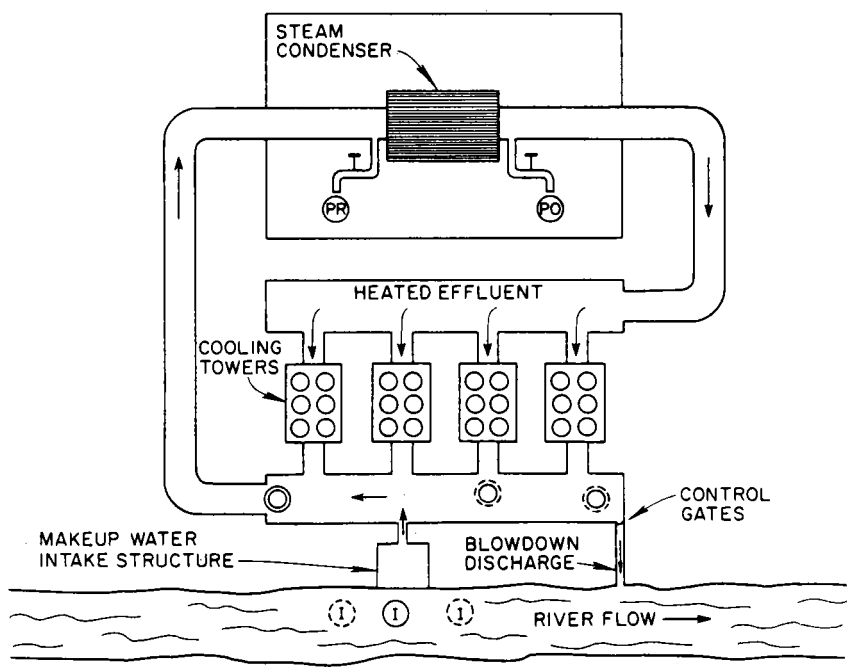


Figure 2-2. (Continued)

and closed-cycle plants the outfall samples were taken before the point of discharge and represent conditions within the circulating water system.

The other two types of water samples taken at each facility were pre- and postcondenser samples. Due to access restrictions at most plants, these samples and the corresponding temperature measurements were nearly always taken by power plant personnel rather than by SRL or ORNL workers. Pre- and postcondenser samples were usually collected from spigots on or near the water boxes. At a few plants these spigots were not available. In such cases, precondenser samples were collected in pumphouses near the plant, and postcondenser samples at the point of entry to the cooling tower system.

Plant operators were queried before and during sampling about recent biocide use. Samples were generally not taken for some time after biocide application unless such application was frequent. For each sampling, operational conditions and any unusual plant occurrences or aberrant environmental conditions were noted. Twenty-liter grab samples were collected, and temperature, pH, conductivity, and dissolved oxygen were normally determined simultaneously using a Hydrolab Surveyor Multiprobe Analyzer (Hydrolab Corp., Austin, Texas). Eight liters of the sample were then concentrated 500-fold for subsequent pathogen analysis (Fig. 2-3); the remainder was available for further analysis of water chemistry.

Sample Treatment

Physicochemical Parameters. As mentioned above, temperature, pH, conductivity, and dissolved oxygen were usually measured immediately upon sample collection. On the same day, the aliquots reserved for chemical analysis were processed in the mobile laboratory using standard methods (APHA 1980) to determine ammonia, nitrate, phosphate, alkalinity, total dissolved carbon, and inorganic carbon (Appendix Tables A-3 and A-4; organic carbon was obtained by difference). For sites where the mobile laboratory was not employed, temperature, pH, conductivity, and dissolved oxygen were still measured with the multiprobe analyzer at the time of sample collection. Aliquots of the sample were then treated as described below to preserve them for subsequent analysis following receipt of the samples at SRL.

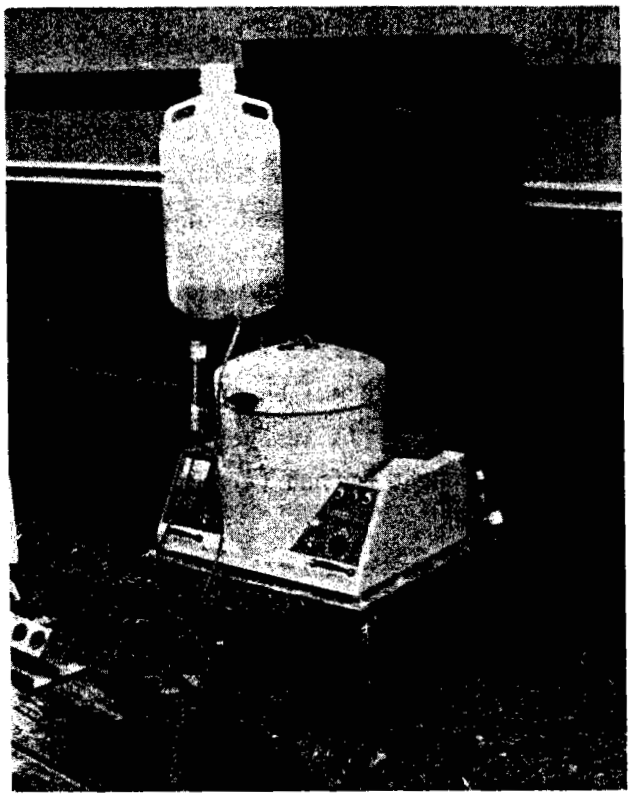


Figure 2-3. Continuous-flow centrifugation of sample for pathogen analysis, with 8-L sample bottle.

Calibration of the Hydrolab Surveyer Multiprobe Analyzer was performed at each plant site. An internal calibration is sufficient for temperature and conductivity. The pH probe was calibrated using a series of buffered solutions. The membrane-covered dissolved oxygen electrode was calibrated using air-saturated water at known temperature and barometric pressure.

One liter of sample was preserved for ammonium nitrogen and nitrate nitrogen analysis by addition of 40 mg of $HgCl_2$ before shipping. Samples were filtered through 0.45-µm glass fiber filters before analysis. Ammonium nitrogen was measured by direct nesslerization on filtered samples pretreated with zinc sulfate and alkali to remove the turbidity reaction with the nessler reagent. Rochelle salt solution was used to stabilize the reaction before the colorimetric analysis. Nitrate nitrogen was determined by cadmium reduction of the nitrate to nitrite and subsequent measurement of the amount of nitrite present in the sample by colorimetric techniques.

A second 1-L aliquot of sample was preserved for analysis for orthophosphate by acidification with 1 mL of concentrated HCl before shipping. The sample was filtered through a 0.45-µm glass fiber filter prior to orthophosphate determination using the ascorbic acid method (a colorimetric technique).

A third 1-L aliquot of sample was preserved for carbon analysis by acidification with concentrated HCl to a pH of 1.5 to 2.0 before shipping. The sample was filtered through a 0.45-µm glass fiber filter prior to analysis. Total carbon was determined by heating a subsample to 900°C and measuring the CO_2 evolved in an infrared analyzer. Inorganic carbon was determined for a second subsample by measuring the CO_2 evolved after treating the sample on quartz chips with phosphoric acid at 150°C. Organic carbon was obtained by difference.

A final aliquot was preserved for determination of alkalinity by shipping the sample in an 8-L polyethylene bottle completely filled and tightly capped. Whole water samples were processed by titration with a normalized acid. Alkalinity is a measurement of the buffering capacity of the water and usually reflects the carbonate, bicarbonate, and hydroxide content of the sample.

Pathogen Parameters. The bulk of the sample (8 L), consisting of the portion not reserved for the chemical analysis, was treated with 2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT). This dye indicates organisms

capable of respiration (referred to in this report as "viable") by staining individual bacteria which have an active electron transport system (Fliermans et al. 1981a, Packard 1971). Each sample was then concentrated 500-fold at 12,000 rpm by continuous-flow centrifugation (Figs. 2-3 and 2-4). At ORNL, the concentrates were further processed for Legionella population density and viability determinations (Appendix Table A-3), and some samples were further tested for infectivity (Appendix Table A-5) according to the procedures detailed below.

To determine densities by the direct fluorescent antibody technique (DFA), which estimates the number of Legionella organisms per unit volume, a 0.010-mL subsample of the concentrate was pipetted into 6-mm-diam wells on toxoplasmosis slides (Cel-Line Associates, Minotola, New Jersey; Fig. 2-5). These slides were air-dried, heat-fixed, and treated with a polyvalent antiserum containing fluorescent antibodies against four serogroups of Legionella pneumophila (Knoxville 1, Togus 1, Bloomington 2, and Los Angeles 1). This antiserum was prepared by the Centers for Disease Control as previously described (Cherry et al. 1978). Samples were viewed by epifluorescence microscopy at 1000X using either a Zeiss Universal or a Nikon Labophot microscope, and the number of Legionella, as shown by fluorescing cells with the appropriate morphology, was determined in 100 fields. These counts were then used to estimate the number of organisms present per unit volume of original sample.

These same prepared toxoplasmosis wells were then viewed with a combination of epifluorescence and bright field microscopy (50-100 fields) to determine the number of fluorescing organisms containing formazan crystals. This count, based on electron transport system activity, was used to estimate the proportion of viable organisms.

Selected samples were used for animal testing. Infectivity was assessed by determining isolation of Legionella from injected guinea pigs. Uncompromised guinea pigs (Abyssinia, Hartley, or American breed, 2 to 3 months old) whose baseline body temperatures had been established were injected intraperitoneally with 2 mL of the concentrated sample and were observed over a 10-d period for fever and overt illness (lethargy, ruffled fur, watery eyes, etc.). Animals having a temperature rise of 0.6°C or more for two consecutive days or those with a lower temperature rise but also showing any overt symptoms during the first 10 d after injection were sacrificed and necropsied. Peritoneal swabs and organ

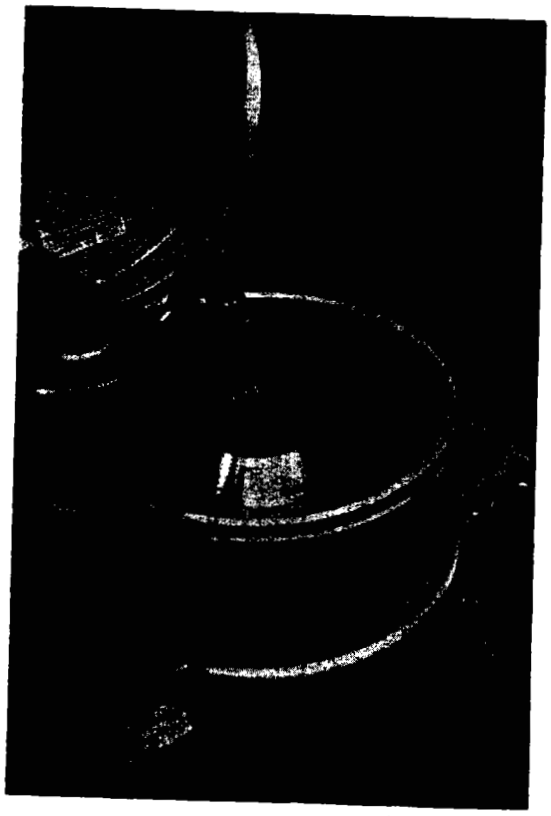


Figure 2-4. Continuous-flow centrifuge, shown in the open position.

Figure 2-5. Concentrated subsample being pipetted into well of toxoplasmosis slide.

tissues were cultured on charcoal yeast extract agar (CYE) at 35°C. Colonies typical of Legionella were then transferred to slants of CYE, and at times colonies were plated onto other substrates. Legionella isolates grow only on CYE agar or on a similar medium, and then only if supplemented with cysteine and iron (Feeley et al. 1978). The resultant Legionella isolates were then identified with respect to species and/or serogroups by the FA technique. Legionella-like organisms not typeable by the FA technique were sent to CDC for further analysis.

Pure cultures of Legionella isolated from tissues of inoculated animals were considered presumptively infectious. Infectivity was not confirmed by reinoculation of guinea pigs. For this and other reasons, it is not possible to directly relate infectivity in this study to human risk.

STATISTICAL METHODS

All numerical data collected were stored in a computer data base using the Statistical Analysis System (SAS; Helwig and Council 1979). Data reduction as well as many statistical analyses and graphical procedures were performed using SAS. The BMDP system (Dixon 1981) was relied upon for the stepwise logistic regression procedure (program LR).

The goal of statistical analysis was twofold: (1) document any existing trends in Legionella population characteristics with respect to the power plant environment and (2) if possible, relate those trends to causative factors. Toward this end, the data were partitioned into two sets of variables, the response variables (those describing population characteristics) and the explanatory variables (those possibly causing the observed changes).

Response Variables

The three types of measurements made of Legionella per se (density, viability, and infectivity) served as the response (or dependent) variables. Cell density, also called abundance or concentration, was measured in cells per milliliter. The logarithm_{10} of cell density was used in the parametric statistical analyses, where assumptions of normality of the errors and homogeneity of variance are made.

The proportion of respiring cells (here called viability) was expressed as a percentage of the total number of cells counted. When fewer than five cells were actually observed from a sample (corresponding to less than 14 cells/mL), the

estimation of proportion viable was judged unreliable and was omitted from the analysis. Prior to parametric statistical analysis, an arcsin transformation ($\sin^{-1}\sqrt{p}$) was applied to the proportion viable.

The product of cell density and viability yields the density of viable cells, an intuitively meaningful variable. After applying a logarithmic transformation, this variable was investigated in the same manner as were density and viability.

The response variable infectivity was not determined for all samples collected, due both to the cost of the procedure and to the extensive effort and facilities required. At the beginning of the study it was deemed appropriate to test for infectivity mainly when cell densities exceeded a level of 10^3 cells/mL. This policy was reconsidered after the first sampling season, so that during subsequent seasons a minimum of one sample from each location (four per site) was tested for infectivity. A total of 143 samples were injected, representing 122 different site-location-season combinations.

Because injection of one sample involved the inoculation of two guinea pigs followed by a judgment of illness, autopsy/necropsy, and isolation and identification of microorganisms, the labeling of a sample as "infectious" or "noninfectious" was not necessarily unambiguous. The following procedure was used for this determination. The outcome of the injection for each animal was classified as shown in Table 2-3. Two guinea pigs rather than one were used to lessen the impact of animal to animal variability. The sample from which both animals were injected was called "infectious" if either animal was classified "1" (Table 2-3). If both animals were classified "4" or "5" the sample was deleted (with respect to analyses of infectivity) as uninterpretable. Otherwise the sample was called "noninfectious" (see Table 2-4).

Since the variable infectivity could take on only two possible values, parametric statistical techniques were not appropriate. Thus, no transformations of this response variable were necessary.

Explanatory Variables

The primary explanatory (predictor or independent) variables investigated were the season of the year, the operating mode of the plant, and whether the sample

Table 2-3

CLASSIFICATION OF INDIVIDUAL GUINEA PIG RESPONSES TO INJECTION OF CONCENTRATED ENVIRONMENTAL SAMPLE

Classification	Descriptive Criteria
1	Legionellosis symptoms followed by autopsy. *Legionella* isolated from tissues and identified as to species/serogroups.
2	No symptoms. No autopsy.
3	Symptoms followed by autopsy. No organisms isolated.
4	Symptoms followed by autopsy. Contamination of isolation plates made identification and isolation of *Legionella*, if present, impossible.
5	Toxic reaction. Animal died before any Legionellosis symptoms could have developed.

Table 2-4

CRITERIA FOR DESIGNATING A SAMPLE "INFECTIOUS"

Outcome of Guinea Pig Injections		Classification[a] of Sample
Animal 1[a]	Animal 2[b]	
1	1	+
1	2	+
1	3	+
1	4	+
1	5	+
2	2	-
2	3	-
2	4	-
2	5	-
3	3	-
3	4	-
3	5	-
4	4	Delete[c]
4	5	Delete[c]
5	5	Delete[c]

[a]+ = infectious, - = noninfectious.

[b]See Table 2-3 for interpretation of number.

[c]These samples were deleted (with respect to analyses of infectivity) as uninterpretable.

had undergone plant passage. In addition, the physical/chemical variables measured with each sample served as explanatory variables in the regression analyses discussed later.

Season. In spring and fall, the two transitional seasons, the latitudinal differences between the northern and southern sites were expected to influence the data. To minimize this influence, the southern plants were sampled first in the spring and last in fall (see Table 2-2 for dates). Mean temperatures for the two geographic areas are given in Table 2-5.

Operating Mode. The operating mode classifications used in the analyses are given in Table 2-1. Although plants E and F were capable of considerable variation in operating mode, comparison of water temperatures at the various sampling locations, coupled with information from plant personnel, allowed classification of plant E as being effectively once-through and plant F as being predominantly closed-cycle. The two aberrant cases, plants E and F in summer (Table 2-1), were not used in analyses involving operating mode as a variable.

Plant Passage. Four locations at each plant site were sampled regularly: intake, precondenser, postcondenser, and outfall. Samples from these locations were classified as representing either "ambient" (source) or "plant-exposed" conditions. The intake water samples were collected from areas where little or no plant-circulated water should be present, and they were assumed not to have been significantly affected by power plant operation. All intake samples were called ambient, as were the precondenser samples from once-through plants. All postcondenser and outfall samples, and the precondenser samples from closed-cycle plants, were called plant-exposed. This variation in classification of precondenser samples results from the fact that closed-cycle plants recirculate their cooling water, so that most of the water in the precondenser water box would already have passed through the condenser tubes and cooling towers one or more times. Inferences about changes with plant passage, therefore, were based on comparisons between ambient and plant-exposed samples.

Physical/Chemical Measurements. Eleven physical/chemical variables were routinely obtained for each sample taken: water temperature, pH, dissolved oxygen, conductivity, alkalinity, phosphate, nitrate nitrogen, ammonium nitrogen, total carbon, inorganic carbon, and organic carbon. In addition, several other variables were derived from these.

Table 2-5

MEAN TEMPERATURE (°C) AND STANDARD DEVIATION OF AMBIENT (SOURCE) WATER FOR THE NORTHERN AND SOUTHERN PLANTS DURING EACH SEASON

Season	Location of Plant[a]	
	North	South
Spring	9.3 (2.6)[b]	11.1 (1.0)
Summer	23.6 (3.7)	26.9 (4.0)
Fall	13.2 (4.1)	18.9 (1.3)
Winter	2.9 (1.8)	5.6 (2.3)

[a]Northern plants included plants D, F, G, H, and I. Southern plants were A, B, C, and E.

[b]1 standard deviation.

Water temperature was the actual temperature of the sample at the time it was taken. The temperature of the water in the sample, however, may have changed drastically only minutes prior to taking the measurement (e.g., in the post-condenser water box). A variable designated growth temperature was therefore created to describe temperature conditions under which the organisms would likely have been growing for a substantial time period. For all intake samples, the individual sample temperature was used as the growth temperature. For the remaining samples at once-through plants, the mean intake sample temperature was used. For outfall samples at closed-cycle plants, the individual sample temperature was used as the growth temperature. Pre- and postcondenser samples at closed-cycle plants were assigned the mean of the outfall temperatures (representing cooling tower basin conditions). Adjustments were occasionally made in this protocol to allow for specialized conditions. For example, at plant I all plant-exposed samples were assigned the mean of the outfall temperature and the precondenser temperature as the growth temperature, because considerable temperature decrease occurs in the cooling pond at this plant.

Shock temperature estimated the thermal shock to which the sample had been exposed. This was assigned a zero value for all ambient samples. For all plant-exposed samples, it consisted of the difference between post- and precondenser samples. Shock temperature was therefore greater than zero for all postcondenser and outfall samples, as well as for precondenser samples in closed-cycle plants.

Transformations were made on other physical/chemical variables in an effort to simulate the actual relationship between that factor and the response variable. Thus, the logarithms of conductivity, phosphate, alkalinity, nitrate, and ammonia were sometimes used, as was the square of pH.

Statistical Procedures

To detect differences in cell density, viability, and density of viable cells as a function of operating mode, season, and plant passage, analysis of variance was used. Variation attributable to each individual plant was removed by using plant as a blocking variable. Duncan's multiple range test was used to rank the means. Because analyses were performed on transformed data, any means reported from

these analyses are based on the transformed variables. To investigate density and viability differences between ambient and plant-exposed waters in individual plants, a three-way ANOVA with all interactions was first employed. The error mean square from this analysis was then used to estimate the variance in the Least Significant Difference (LSD) test (Sokol and Rohlf 1969) for each individual plant during each season.

The nature of the variable "infectivity" was different from that of density and viability in that the latter are continuous variables and infectivity can take on only two values: infectious or noninfectious. For this reason different statistical methods were required to analyze the infectivity data. Comparisons among seasons, plant operating modes, and locations were made with contingency table tests. Here, the observed frequencies of infectious and noninfectious samples from various categories (e.g., seasons) were compared with frequencies expected under the null hypothesis that the presence of infectivity is independent of those categories.

An attempt was made to examine differences in the prevalence of infectivity before and after power plant passage so that an increase or a decrease in infectivity associated with power plant passage could be directly addressed. To summarize the results for each plant/season combination, a new variable - the proportion infectious - was created for each location. The proportion infectious (p) was defined as

$$p = \frac{\text{number of infectious samples at location } i}{\text{total number of samples injected (with interpretable results) at location } i},$$

where location i = ambient water or plant-exposed water at one power plant (A, B, ..., I) during one season (spring, summer, fall, winter). An interpretable result includes all positive or negative results and excludes contaminated and toxic reactions.

The difference between the p's for the ambient water and those for the plant-exposed water at a given plant was calculated for each season and classified as an increase, a decrease, or no change (see Appendix Tables A-1 and A-2). A contingency table was used to analyze these data.

Potential relationships between the variables cell density, viability, and density of viable cells were investigated using both graphical methods and correlation analyses of the transformed variables. To detect any relationship between these variables and infectivity, Student's t-test was used, first on the data set as a whole and then by pairing the means of the infectious and noninfectious samples by season and plant (i.e., a paired t-test). Some analyses were reexamined using nonparametric methods to test the robustness of conclusions, given modest violations of the assumption of normality.

The second goal of data analysis was the detection of relationships between the measured physicochemical variables (temperature, pH, phosphate, etc.) and the Legionella profile variables (cell density, viability, density of viable cells, and infectivity). Two specific types of multiple regression were used for this purpose: multiple linear regression to "predict" the continuous variables and logistic regression to "predict" infectivity.

A stepwise procedure was used initially to screen the 14 measured or derived independent variables. The primary regression diagnostics used to select the best model from the backward, stepwise, and maximum R^2 stepwise procedures were Mallow's Cp statistic and R^2 values for each model as well as the use of data subsetting to investigate stability of the results.

Two nonparametric techniques (Spearman correlation and, for infectivity, the Wilcoxon sign-rank test) were used along with the regression techniques as confirmatory analyses. The multivariate techniques of discriminant analysis and principal components analysis were also used as investigative tools and to verify stability of results achieved by the regression techniques. In addition, principal components analysis was useful in revealing the underlying data structure and possibly some of the patterns of multicollinearity.

In presenting and discussing results, the term "significant" is used to denote statistical significance at the 0.05 level unless a different level of significance is stated.

Section 3

RESULTS

The results of the Phase I research are reported in five subsections. The first subsection is concerned with the qualitative distribution of the serogroups of Legionella among the power plant sites in this study. Second, the quantitative patterns of Legionella density, viability, and infectivity, particularly with respect to power plant site, cooling mode, and season, are examined. The relationships among the variables in this study (e.g., between infectivity and the density of viable cells; between total cell density and physicochemical variables, etc.) are examined in the third subsection. Fourth, the discovery of a new species of Legionella is described. Finally, the finding of several untypeable pathogenic Legionella (bacteria that cannot be assigned to any of the existing species) and of another unidentifiable pathogenic organism is discussed.

DISTRIBUTION OF SPECIES AND SEROGROUPS OF INFECTIOUS Legionella-LIKE ORGANISMS

An analysis of the different Legionella species and/or serogroups isolated in the course of this study reveals that the Knoxville serogroup of L. pneumophila is the most prevalent. This Legionella was isolated from a total of 19 samples from six of the nine plant sites. It is interesting in this regard that the Knoxville serogroup of L. pneumophila is also the most prevalent Legionella isolated from clinical samples (personal communication, Dr. H. Wilkinson, CDC). Surprisingly, the second and third most prevalent Legionella isolates were the Los Angeles serogroup of L. pneumophila and the newly discovered species L. oakridgensis (see later subsection). Los Angeles serogroup of L. pneumophila was isolated from 15 samples and was found at seven of the nine sites. L. oakridgensis was isolated from 13 samples and from three of the nine test sites. The role of L. oakridgensis in human disease has yet to be determined.

Plants A, B, C, and D are all once-through facilities. Analysis of results from these individual test sites shows that both ambient and plant-exposed water from site A yielded infectious Knoxville serogroup of L. pneumophila and L. gormanii (Tables 3-1 and 3-2). In addition, the Los Angeles serogroup of L. pneumophila

Table 3-1

TYPES OF PATHOGENS ISOLATED FROM AMBIENT WATER SAMPLES[a]

Site	Spring	Summer	Fall	Winter
A		K	K,GO,LA	
B				
C		K	"VIBRIO"	K
D			LA,CH	
E			OR[b]	
F	c	c	LA,J19	
G	c	K,CH		BOZ
H		c	LA	LA
I		d		LA

[a]Key to symbols:

Legionella pneumophila serogroups:
CH = Chicago
K = Knoxville
LA = Los Angeles

Other Legionella species:
BOZ = Legionella bozemanii
GO = Legionella gormanii
J19 = Species of Legionella not typeable with antiserum prepared against known species and/or serogroups of Legionella, but reacting with antiserum made against a "J19" (Jamestown) strain of Legionella isolated from a power plant following an outbreak of Pontiac Fever in workers cleaning condenser tubes.
OR = Legionella oakridgensis

Other organisms:
"VIBRIO" = Vibrio-like organism, at present unidentifiable by the Centers for Disease Control (CDC).

[b]Isolated from precondenser water box samples during once-through operation. Because this plant has not always operated in a once-through mode, it is possible that the L. oakridgensis isolate reflects colonization during a previous period of closed-cycle operation.

[c]One or more samples were injected. Samples were either toxic to the guinea pigs or resulted in contaminated plates upon subsequent plating of tissue; therefore, Legionella could not have been isolated if present.

[d]No samples were injected.

Table 3-2

TYPES OF PATHOGENS ISOLATED FROM PLANT-EXPOSED WATER SAMPLES[a]

Site	Spring	Summer	Fall	Winter
A		K	CH	GO
B				
C		CH	"VIBRIO"	
D		LA		LA,L?
E	K	[b]		LA
F	OR	K	K,BL,LA,OR	K,CH,OR
G	K,OR,BL,LA	CH,OR	K,OR	K,CH,OR
H	[c]	LA	LA,CH	LA
I	K	K,CH	K,TO	

[a]Key to symbols:

Legionella pneumophila serogroups:
BL = Bloomington
CH = Chicago
K = Knoxville
LA = Los Angeles
TO = Togus

Other Legionella species:
GO = Legionella gormanii
L? = Species of Legionella not typeable with antiserum prepared against known species and/or serogroups of Legionella.
OR = Legionella oakridgensis

Other organisms:
"VIBRIO" = Vibrio-like organism, at present unidentifiable by the Centers for Disease Control (CDC).

[b]Plant was not operating; samples were not injected.

[c]One or more samples were injected. Samples were either toxic to the guinea pigs or resulted in contaminated plates upon subsequent plating of tissue; therefore, Legionella could not have been isolated if present.

was isolated from ambient waters and the Chicago serogroup was isolated from plant-exposed water at this site. Infectious Legionella were not detected in either ambient or heated waters from site B. The Knoxville serogroup of L. pneumophila was isolated from ambient water at site C, while the plant-exposed waters contained both the Knoxville and Chicago serogroups of L. pneumophila. The unknown, vibrio-like (i.e., weakly gram-negative, comma-shaped bacteria capable of growth at 25 to 37°C) organism was isolated from both types of water (see final subsection). Ambient waters at site D yielded Los Angeles and Chicago serogroups of L. pneumophila, while plant-exposed waters contained, in addition to the Los Angeles serogroup, an unknown species of Legionella (see final subsection).

Plants E and F are capable of variable-mode operation. Legionella oakridgensis was isolated from a precondenser sample at site E, while the plant-exposed waters yielded the Knoxville and Los Angeles serogroups. At site F, ambient water samples yielded the Los Angeles serogroup of L. pneumophila. In addition, an unnamed species apparently identical to the "Jamestown 19" isolate (see final subsection) was found. Plant-exposed waters at this site yielded Los Angeles, Knoxville, Bloomington, and Chicago serogroups as well as L. oakridgensis.

Plants G, H, and I are all closed-cycle facilities. Ambient water from site G yielded L. bozemanii and the Knoxville and Chicago serogroups of L. pneumophila, whereas the Chicago, Knoxville, Los Angeles, and Bloomington serogroups of L. pneumophila as well as L. oakridgensis were isolated from the plant-exposed waters. The Los Angeles serogroup of L. pneumophila was the only infectious Legionella isolated from ambient water at sites H and I. The Los Angeles and Chicago serogroups of L. pneumophila were isolated from site H while the Knoxville, Chicago, and Togus serogroups were isolated from the plant-exposed waters at site I.

PATTERNS OF DENSITY, VIABILITY, AND INFECTIVITY

In this subsection, analyses of variance and Least Significant Difference (LSD) tests are used to examine the density and viability of Legionella in relation to the main categorical variables of this study: cooling mode (once-through vs closed-cycle), sample location (ambient vs plant-exposed water), and season of the year. Contingency tables are used to perform similar analyses for infectivity.

Cell Density

Patterns in Ambient Waters. Total cell densities of *Legionella* in ambient (source) waters differed between seasons and between the two types of plants. Overall, the highest cell densities in source water were found in the colder seasons (spring and winter), with summer and fall having significantly lower concentrations, as shown below.

Cell Densities in Ambient Waters

Season	Mean	Rank*
Spring	592	a
Winter	381	a
Summer	161	b
Fall	44	c

*Means with the same rank are not significantly different from one another ($P < 0.05$).

Examination of these densities at the two types of plants reveals that the closed-cycle sites are primarily responsible for these seasonal differences. The following table shows that there is a significant difference in the spring between cell densities in the ambient waters at closed-cycle sites compared with ambient waters at once-through sites.

Cell Densities in Ambient Waters

Mode	Spring	Summer	Fall	Winter
Once-through	116 *	116	44	311
Closed-cycle	7795	289	46	549

*An asterisk between two numbers indicates that they are significantly different from one another ($P < 0.05$).

In spring, the ambient locations at closed-cycle sites had cell densities roughly one to two orders of magnitude higher than did the corresponding ambient location sat once-through sites with the exception of plant E; these closed-cycle ambient concentrations were the highest cell densities found during the study. The source of this difference in ambient waters between the two plant types is not understood and confounds interpretation of operating mode differences. Although the once-through plants in this study are located primarily in the south, plant D is in the north. Thus the difference is not due solely to differences in latitude. Another possible explanation, which could not be ruled out, is the possible preferential siting of closed-cycle plants on water bodies which have a set of features in common (e.g., size or distance from human population centers).

Patterns Reflecting Plant Passage

The effects of plant passage are shown in Fig. 3-1 and Table 3-3 and 3-4. Cell densities were significantly lower in plant-exposed water than in ambient water of closed-cycle sites as a whole, during both spring and summer (Table 3-3). This effect was not true of most of the once-through plants. The difference (Table 3-4) in the spring at plant E, a variable-mode plant operated in once-through configuration, may be related to the fact that the intake samples there were collected from the surface water outside of but adjacent to a skimmer wall which blocked entry of surface water above a depth of 1.4 m. Similar possible artifacts of sample location at other sites are minimal or nonexistent. In general, plant-exposed waters from once-through sites could not be expected to have population densities different than ambient samples, because insufficient time would have elapsed between any plant impact and collection of the sample (i.e., water collected at the outfall would have been exposed to any temperature shock only a few minutes earlier). In the recirculating water of a closed-cycle plant, however, the same cell population would be retained after any impact and could in fact reflect cell density changes brought on by passage through the power plant condenser or by other attributes of the closed-cycle system.

Table 3-4 shows, using asterisks, the results of the Least Significant Difference (LSD) test for individual power plants by season. Eight significant ($P < 0.05$) decreases in density between ambient and plant-exposed water were found, all at plants operating in a closed-cycle mode except for plant E, discussed above.

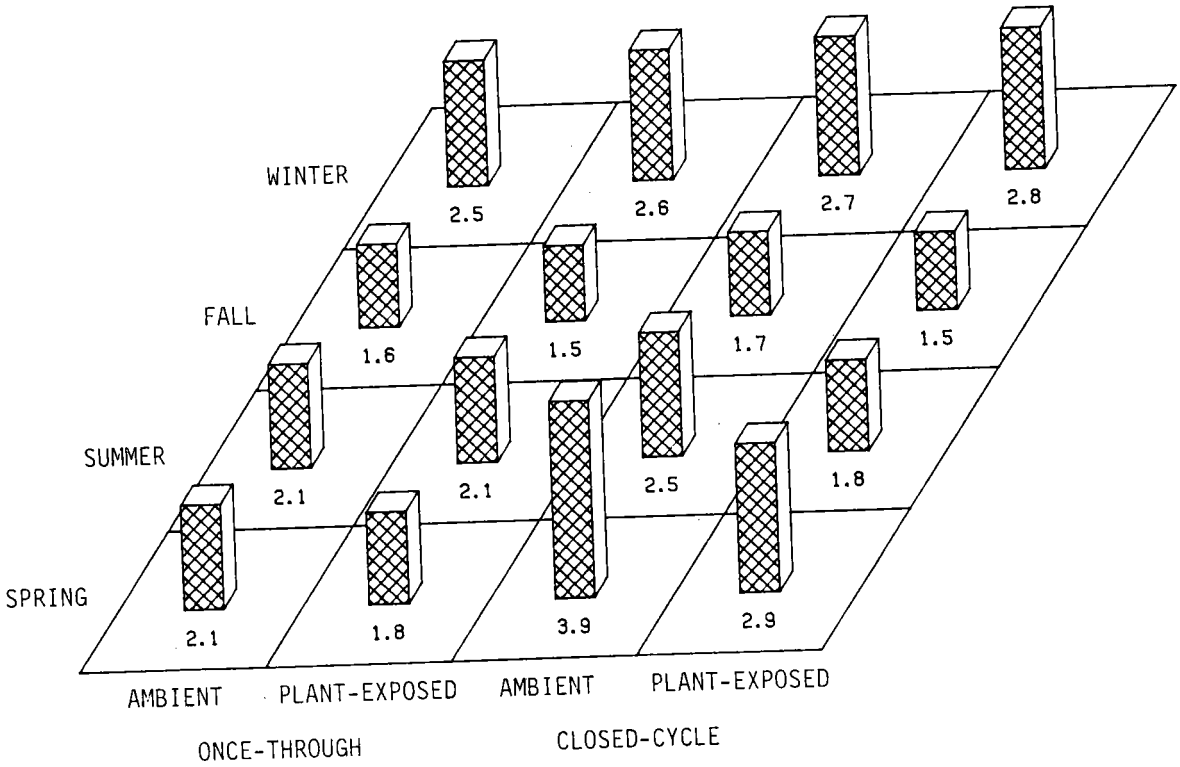


Figure 3-1. Mean *Legionella* cell densities [$\log_{10}$ (number of cells/mL)] by season, type of cooling system, and location of sample.

Table 3-3

COMPARISON OF MEAN CELL DENSITIES[a] OF *Legionella* (NUMBER OF CELLS/mL) BEFORE AND AFTER PLANT PASSAGE FOR THE TWO OPERATING MODES

Season	Operating Mode	Location[b]		
		Ambient		Plant-exposed
Spring	Once-through	116		67
		*		*
	Closed-cycle	7795	-----*-----	884
Summer	Once-through	116		118
	Closed-cycle	289	-----*-----	65
Fall	Once-through	44		32
	Closed-cycle	46		35
Winter	Once-through	311		390
	Closed-cycle	549		647

[a]An analysis of variance with season, operating mode and location as main effects and specific plant as a blocking variable was used to derive the mean square error for later use in Duncan's multiple range test.

[b]An asterisk between two numbers indicates that these two means are significantly different from one another ($P < 0.05$).

Table 3-4

MEAN CELL DENSITIES OF *Legionella* (NUMBER OF CELLS/mL)[a]

Season	Sample Location	Plants Operating in Once-through Mode					Plants Operating in Closed-cycle Mode			
		A	B	C	D	E	F	G	H	I
Spring	Ambient	35	48	22	227	15,733 *	3,967 *	8,767	10,133 *	11,333 *
	Plant-exposed	33	35	28	233	343	508	19,400	300	248
Summer	Ambient	520	140	71	56	300[b]	263[c]	183	650 *	327 *
	Plant-exposed	235	225	105	128	[b]	4,081[c]	368	146	95
Fall	Ambient	63	80	33	21	93	40	45	49	372 *
	Plant-exposed	70	59	45	22	42	36	112	30	55
Winter	Ambient	665	185	320	114	1,205	1,360	957	4,450 *	45
	Plant-exposed	337	352	710	158	1,070	2,050	1,640	315	56

[a]Mean cell densities separated by an asterisk represent a significant ($P < 0.05$) decrease with passage through a power plant.

[b]Plant was shut down.

[c]Mixed operating mode.

Viability

Differences between the source waters of once-through versus closed-cycle plants were observed for viability levels (Fig. 3-2 and Table 3-5) as was the case with the cell densities discussed earlier. Here, once-through plant sites showed significantly higher viability levels in source water compared with those of closed-cycle plants during both spring and summer.

A change in the proportion of viable cells concomitant with passage through power plants was indicated (Table 3-5) in both spring and summer in closed-cycle plants. In spring an increase was indicated, while in summer the proportion viable decreased. Inspection of such changes in individual plants (Table 3-6) revealed that plants I and probably F were largely responsible for the spring increase, while no individual plants contributed disproportionately to the summer decrease.

Density of Viable Cells

The product of density and proportion of viable cells yields the density of viable cells, an intuitively meaningful combination of these variables.

Patterns in Ambient Waters. As the following table shows, the ambient waters at closed-cycle sites had significantly higher densities of viable cells during the two cold seasons than during the warmer seasons.

Viable Cell Densities at Closed-cycle Sites

Season	Mean	Rank*
Spring	645	a
Winter	243	a
Summer	14	b
Fall	7	b

*Means with the same ranking letter are not significantly different from one another ($P < 0.05$).

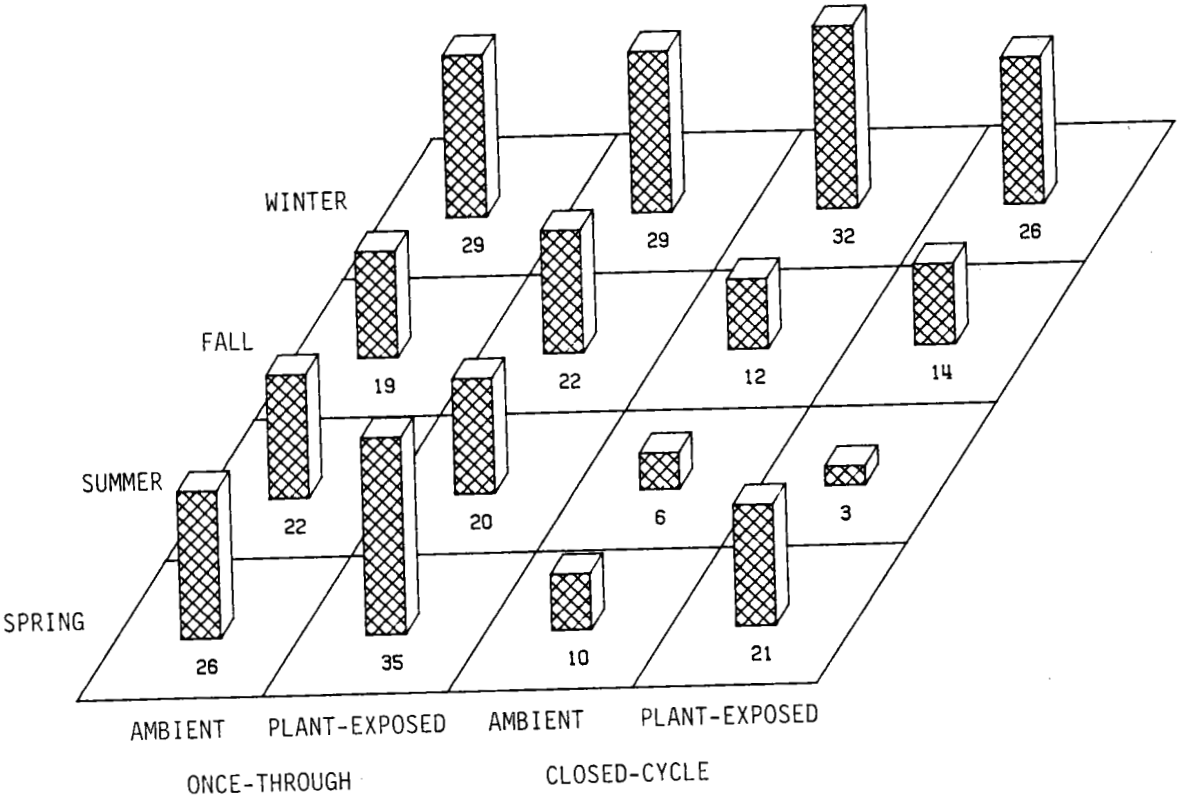


Figure 3-2. Mean Legionella viability (percent) by season, type of cooling system, and location of sample.

Table 3-5

COMPARISON OF MEAN VIABILITY LEVELS[a] OF *Legionella* (PERCENT OF TOTAL CELL NUMBER ALIVE) BEFORE AND AFTER PLANT PASSAGE FOR THE TWO OPERATING MODES

Season	Operating Mode	Location[b]	
		Ambient	Plant-exposed
Spring	Once-through	26	35
		*	
	Closed-cycle	10 -----*-----	21
Summer	Once-through	22	20
		*	*
	Closed-cycle	6 -----*-----	3
Fall	Once-through	19	22
	Closed-cycle	12	14
Winter	Once-through	29	29
	Closed-cycle	32	26

[a]An analysis of variance with season, operating mode, and location as main effects and specific plant as a blocking variable was used to derive the mean square error for later use in Duncan's multiple range test.

[b]An asterisk between two numbers indicates that these two means are significantly different from one another ($P < 0.05$).

Table 3-6

MEAN VIABILITIES OF *Legionella*
(PERCENT OF TOTAL CELL NUMBER ALIVE, ESTIMATED BY THE INT METHOD)[a]

Season	Sample Location	Plants Operating in Once-through Mode					Plants Operating in Closed-cycle Mode			
		A	B	C	D	E	F	G	H	I
Spring	Ambient	25	47	25	27	22	12	28	4	5
	Plant-exposed	30	49	45*	29	30	28	23	7	37*
Summer	Ambient	29	30	27	11	12[b]	27[c]	10	2	13
	Plant-exposed	29	21	20	16	[b]	24[c]	7	3	5
Fall	Ambient	29	20	25	14	17	15	8	30	13
	Plant-exposed	36	28	16	18	21	17	9	30	16
Winter	Ambient	37	37	15	23	40	27	42	32	31
	Plant-exposed	29	23	27	31	41	15	40	31	13

[a]Means separated by an asterisk represent a significant ($P < 0.05$) increase with passage through a power plant.

[b]Plant was shut down.

[c]Mixed operating mode.

While ambient waters at once-through sites (see below) had higher densities of viable cells in winter than during the three remaining seasons, these values were far below those found in ambient waters in winter at closed-cycle sites.

Viable Cell Densities at Once-through Sites		
Season	Mean	Rank*
Winter	83	a
Spring	21	b
Summer	20	b
Fall	7	b

*Means with the same ranking letter are not significantly different from one another ($P < 0.05$).

As shown in Table 3-7, the densities of viable cells were significantly higher during the spring in ambient waters supplying the closed-cycle plants than those in ambient waters supplying the once-through plants.

Patterns Reflecting Plant Passage: The effect of plant passage on the number of viable cells varied with operating mode (Fig. 3-3 and Table 3-7). No differences were seen in once-through plants, while a dignificant decrease was noted in closed-cycle plants during the spring and summer. Significant changes with plant passage in individual plants are shown in Table 3-8. Of the five decreases with power plant passage, four were found in closed-cycle plants. The exception, previously discussed in connection with total cell densities, was plant E in the spring.

In summary, the density of viable cells seems to be a meaningful combination of the two variables, cell density and percent viability. Higher such densities are found during the cold months than during the warm months, particularly in closed-cycle source waters. A decrease in viable cell density is associated with closed-cycle plant passage in spring and summer.

Table 3-7

COMPARISON OF MEAN DENSITIES OF VIABLE *Legionella* CELLS[a] (NUMBER OF CELLS/mL) BEFORE AND AFTER PLANT PASSAGE FOR THE TWO OPERATING MODES

Season	Operating Mode	Location[b]	
		Ambient	Plant-exposed
Spring	Once-through	21	17
		*	*
	Closed-cycle	645 -----*-----	162
Summer	Once-through	20	32
			*
	Closed-cycle	14 -----*-----	2
Fall	Once-through	7	8
	Closed-cycle	7	6
Winter	Once-through	83	103
	Closed-cycle	243	126

[a]An analysis of variance with season, operating mode and location as main effects and specific plant as a blocking variable was used to derive the mean square error for later use in Duncan's multiple range test.

[b]An asterisk between two numbers indicates that these two means are significantly different from one another ($P < 0.05$).

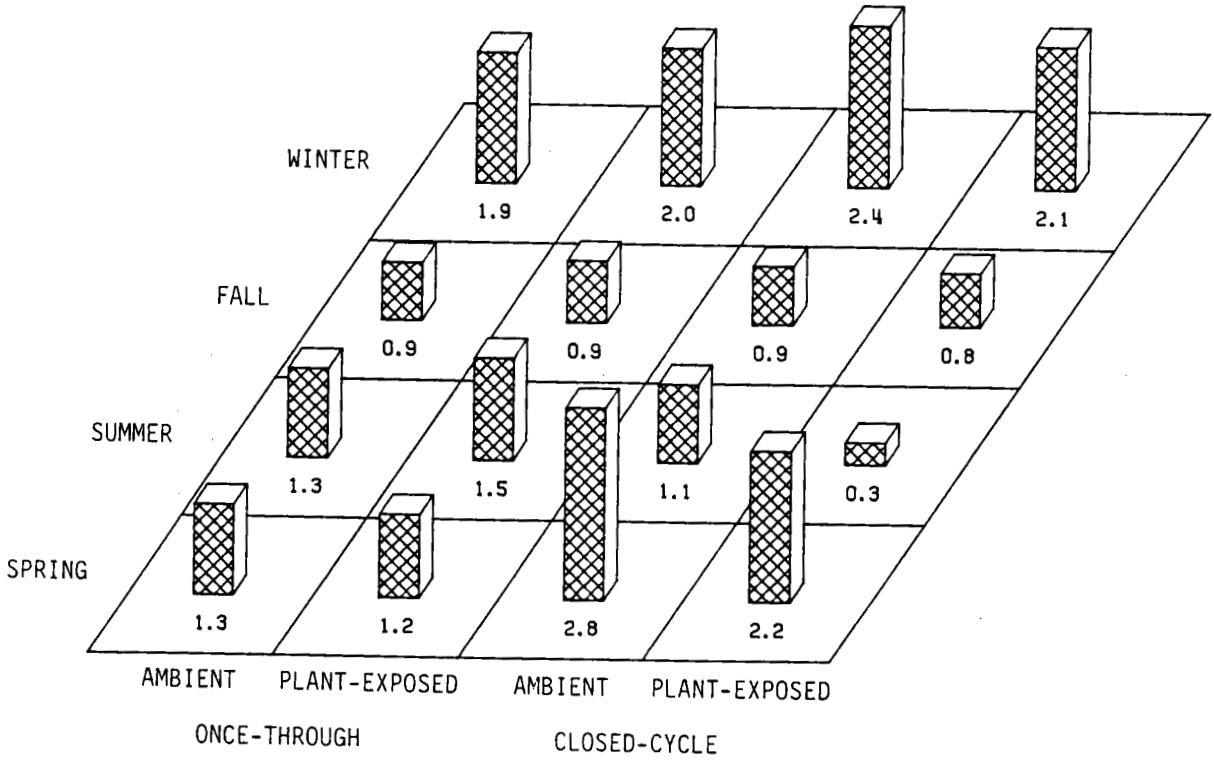


Figure 3-3. Mean density of viable Legionella [$\log_{10}$ (number of viable cells/mL)] by season, type of cooling system, and location of sample.

Table 3-8

MEAN DENSITIES OF VIABLE *Legionella* CELLS (NUMBER OF VIABLE CELLS/mL)
(DENSITY OF ALL *Legionella* TIMES VIABILITY)[a]

Season	Sample Location	Plants Operating in Once-through Mode					Plants Operating in Closed-cycle Mode			
		A	B	C	D	E	F	G	H	I
Spring	Ambient	10	30	5	56	2,951 *	523	2,610	402 *	510
	Plant-exposed	9	18	15	67	102	143	4,902	20	99
Summer	Ambient	107	46	19	6	34[b]	74[c]	16	4	43 *
	Plant-exposed	68	56	25	28	[b]	777[c]	41	4	7
Fall	Ambient	18	17	9	3	9	7	4 *	23	56 *
	Plant-exposed	23	19	7	5	9	15	9	10	9
Winter	Ambient	225	61	46	27	494	305	433	1,483 *	19
	Plant-exposed	94	78	182	61	431	293	674	101	7

[a]Mean cell densities separated by an asterisk represent a significant ($P < 0.05$) change with passage through a power plant.

[b]Plant was shut down.

[c]Mixed operating mode.

Infectivity

Infectivity of a sample, used as a measure of virulence, was determined by injection of a standard quantity of the concentrated sample into each of two guinea pigs. If Legionella were isolated from either of the two guinea pigs, after appearance of typical Legionellosis symptoms, then that sample was classified "infectious" (see "Response Variables" in Section 2 for a detailed description of the protocol for interpreting infectivity results). Initially, only samples with more than 10^3 Legionella cells/mL (by FA determination) were injected. After examination of the spring data, the decision was made to inject at least one sample from each sampling location, regardless of the Legionella density. For this reason replication is somewhat uneven in the spring period, with better coverage in the other three seasons (Appendix Table A-5).

Figure 3-4 shows the occurrence of infectious samples by season, type of cooling system, and location of sample. Of the 143 samples tested for infectivity, 51 were infectious, 70 were noninfectious, and 22 were unsuccessful tests (infectivity could not be determined due to interference from contaminants or toxic reactions). Such interference was most acute in the spring, with 28% of the tested samples having inconclusive results for this reason. Although the absolute number of toxic or contaminated samples is not very great, their occurrence was sometimes crucial. For example, toxicity was found in all three intake samples at plant F in spring, leaving no way to estimate the presence of infectivity in ambient water and thus an inability to determine whether the high levels of infectivity in plant-exposed water were due to plant passage or were present in the incoming water.

Spring had the lowest infectivity rate, with 32% of the samples tested (9 out of 28) yielding positive results (Table 3-9). The other three seasons had somewhat higher infectivity rates and were similar to each other: 48, 43, and 45% of samples yielded positive results in the summer, fall, and winter, respectively. These differences, including spring, are not significantly different from each other.

Examination of infectivity levels at individual plant sites revealed that site G, a northern closed-cycle plant, had the highest levels of infectivity, with nearly four-fifths (15 out of 19) of all the tested samples throughout the entire year yielding positive results (see Table 3-9). Plant F had the second highest

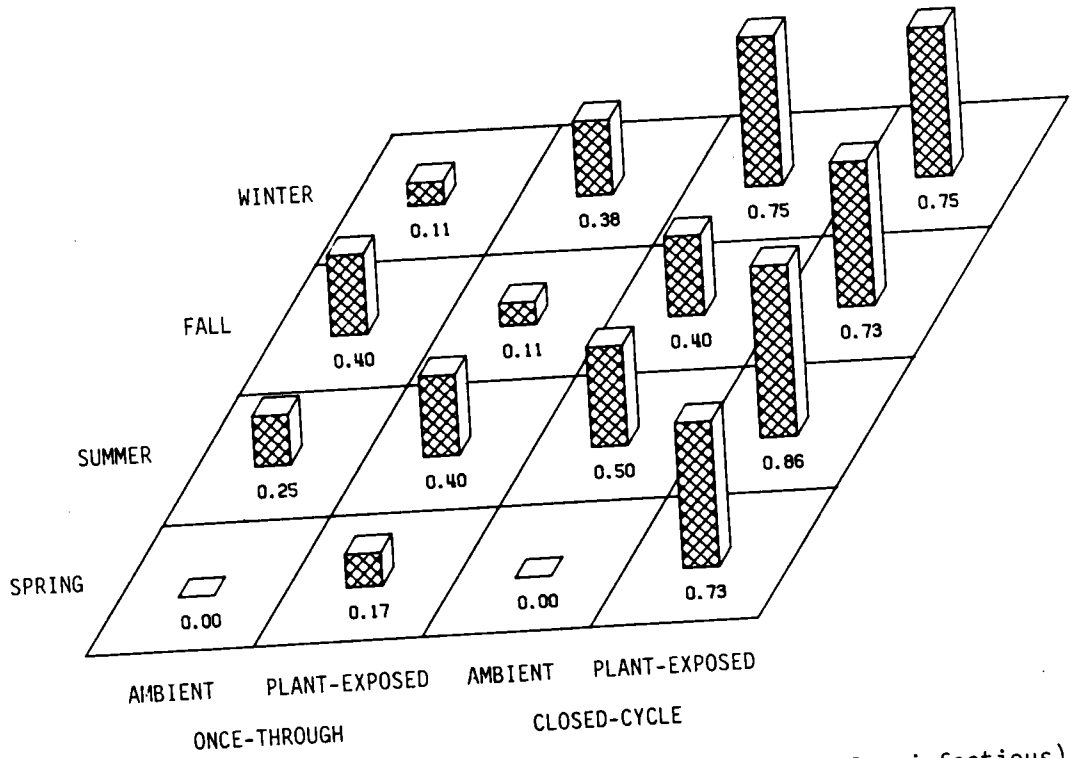


Figure 3-4. Mean Legionella infectivity (proportion of samples infectious) by season, type of cooling system, and location of sample.

Table 3-9

NUMBER OF SAMPLES (INTAKE, PRECONDENSER, POSTCONDENSER, AND OUTFALL COMBINED) YIELDING POSITIVE RESULTS ON INFECTIVITY TESTING/NUMBER OF SAMPLES TESTED WITH INTERPRETABLE RESULTS

Plant	Season				Annual Percent Infectious
	Spring	Summer	Fall	Winter	
A	0/2	2/5	3/3	1/4	43
B	0/2	0/5	0/4	0/3	0
C	0/2	3/4	0/4	1/4	29
D	0/3	1/4	1/4	1/4	20
E	1/3	0/1[a]	1/4	1/2	30
F	2/5	1/1[b]	3/4	2/3	62
G	5/5	4/5	2/5	4/4	79
H	0/3	1/2	3/4	2/2	55
I	1/3	2/2	2/3	1/3	55
Totals	9/28	14/29	15/35	13/29	42
	(32%)	(48%)	(43%)	(45%)	

[a]Plant was shut down.

[b]Mixed operating mode.

overall level of infectivity with 62% (8 out of 13) of successfully tested samples producing positive results. As mentioned above, toxicity unfortunately proved to be a problem here. Plant B, a southern once-through plant, produced no infectious samples. Thus, there is an association of infectious Legionella with certain power plants; infectivity is not distributed evenly among them ($P < 0.01$).

There was more infectivity associated with all closed-cycle power plant sites (i.e., ambient and plant-exposed samples combined) than with once-through power plant sites ($P < 0.01$; see Table 3-10A): 64% of the samples from closed-cycle sites yielded infectious results compared with 24% at the once-through sites. The data were examined in more detail to detect patterns of distribution of infectivity at these two plant types. It was found that a disproportionately large number of the plant-exposed samples from closed-cycle plants were infectious ($P < 0.01$; see Table 3-10B and Fig. 3-5). Further comparisons of infectivity of plant-exposed water from closed-cycle plants to infectivity of water from other sources showed significant differences in all cases. In particular, plant-exposed water from closed-cycle sites was significantly more infectious than plant-exposed water from once-through sites ($P < 0.01$; see Table 3-10C) or ambient water at closed-cycle sites ($P < 0.05$; see Table 3-10D). The latter result indicates that some attribute of the closed-cycle systems tends to cause an increase in infectivity over ambient conditions. A different analysis, based on the change in proportion infectious with plant passage (i.e., $p_E - p_A$ see Appendix Tables A-1 and A-2) was unable to show a statistically significant difference in infectivity between ambient and plant-exposed water in closed-cycle plants compared with once-through plants (Table 3-10E). An interfering factor in this analysis was that toxicity and/or contamination frequently made it impossible to determine changes in infectivity. This phenomenon, which was particularly pronounced for ambient samples at closed-cycle sites (Appendix Table A-2), reduced the power of this test.

The analyses in Table 3-10 were repeated using a different protocol for judging infectivity. Rather than relying on the isolation of Legionella from at least one febrile guinea pig as the sole basis for judging a sample "infectious," a less restrictive criterion was used. Cases in which a guinea pig developed fever but where no organisms could be isolated, which were normally classified noninfectious (with respect to Legionella), were now classified infectious under the presumption that Legionella is often difficult to isolate and could have been responsible for the symptoms. This resulted in the shifting of 20 samples from

Table 3-10

CONTINGENCY TABLE ANALYSES OF INFECTIVITY TEST RESULTS

Analysis

A. Infectivity at once-through and closed-cycle sites: ambient and plant-exposed samples combined.

Operating Mode	Test Result: Positive	Test Result: Negative
Once-through	16	50
Closed-cycle	34	19

$X^2 = 19.22$**

B. Infectivity at once-through and closed-cycle sites: ambient and plant-exposed samples treated separately.

Operating Mode	Sample Location	Test Result (Chi-squared): Positive	Test Result (Chi-squared): Negative
Once-through	Ambient	7 (3.4)	26 (2.5)
	Plant-exposed	9 (1.7)	24 (1.2)
Closed-cycle	Ambient	6 (0.1)	10 (0.1)
	Plant-exposed	28 (10.0)	9 (7.2)

$X^2 = 26.15$**

C. Infectivity of plant-exposed water at once-through and closed-cycle sites.

Operating Mode	Test Result: Positive	Test Result: Negative
Once-through	9	24
Closed-cycle	28	9

$X^2 = 16.40$**

D. Infectivity of ambient and plant-exposed water at closed-cycle sites.

Sample Location	Test Result: Positive	Test Result: Negative
Ambient	6	10
Plant-exposed	28	9

$X^2 = 7.08$*

E. Effect of plant passage on infectivity.

Operating Mode	Change in Infectivity with Plant Passage: Decrease, No Change	Change in Infectivity with Plant Passage: Increase
Once-through	13	6
Closed-cycle	4	6

$X^2 = 2.18$

*Statistically significant ($P < 0.05$) association between the factors tested.

**Statistically significant ($P < 0.01$) association between the factors tested.

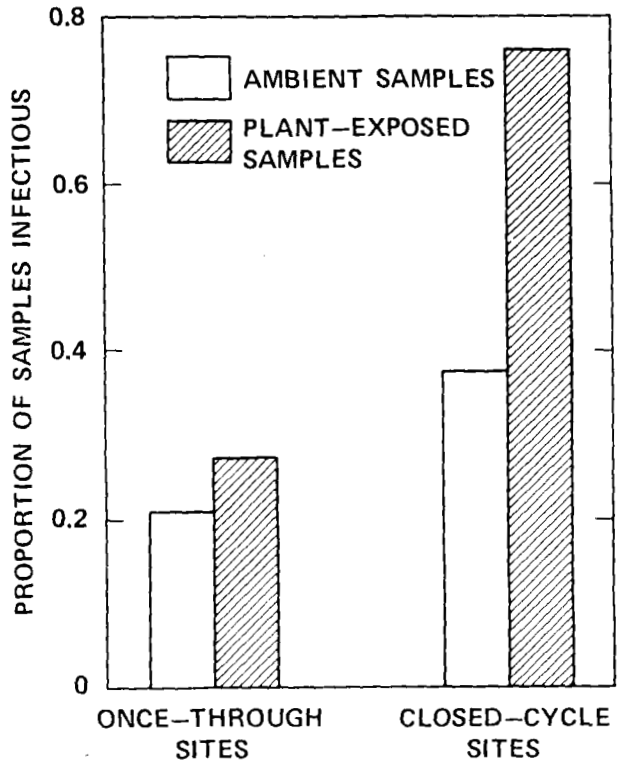


Figure 3-5. Mean Legionella infectivity (proportion of samples infectious) by type of cooling system and location of sample.

the "noninfectious" to the "infectious" category. Results of the analyses were the same with respect to both level of significance and direction of effect.

In summary, infectious Legionella were isolated from ambient waters in all seasons except spring and from plant-exposed water during all seasons. Infectious Legionella were most likely to be isolated from the plant-exposed water of a closed-cycle plant. In addition, infectious Legionella was associated more with some plants (F and G) than with others (B). Relationships between infectivity and the other variables will be discussed in following subsections.

RELATIONSHIPS AMONG VARIABLES

In this subsection, a variety of statistical techniques are applied to investigate the relationships among the variables included in the study. First, selected plausible interrelationships among the Legionella profile variables (cell density, viability, and infectivity) are examined using correlations, the Student's t-test, and the Wilcoxon two-sample test. The second and final subsection deals with the problem of identifying relationships between the physicochemical variables and the Legionella profile variables, using multiple linear regression (sometimes transformed into analysis of covariance by inclusion of categorical variables, such as plant or season, as predictors), logistic regression, and discriminant analysis.

Interrelationships Among Profile Variables

The possibility of interrelationships among Legionella density, viability, and infectivity was examined by first forming hypotheses about relationships which seemed reasonable and then testing those hypotheses.

Relationship Between Viability and Density. Correlation analysis was used to test for any consistent relationship between viability and density. Analyses were done for all data and by individual seasons, using transformed variables. The critical values for significance in these individual tests were adjusted, using Bonferroni's method of calculating simultaneous confidence levels (Harris 1975), to take into account the multiple comparisons being made. No correlations were significant at the 0.05 level, but during spring there was a negative correlation between density and viability ($r = -0.28$; $P < 0.08$).

To further investigate this relationship, the analysis was repeated for two subsets of the original full data set: all ambient water samples and plant-exposed samples from closed-cycle plants only. For the closed-cycle plant-exposed samples a significant correlation ($r = +0.34$) existed between density and viability.

Relationship Between Infectivity and Other Profile Variables. Here, the hypotheses to be tested were that the infectivity of *Legionella* for guinea pigs is a function of the density (number per mL) of *Legionella*, of the viability (proportion alive) of *Legionella*, or of the product of density and viability (i.e., the density of live *Legionella* cells). Student's t-test was used to test these hypotheses. Only those infectious samples in which one of the four serogroups included in the polyvalent antiserum (Knoxville 1, Togus 1, Bloomington 2, and Los Angeles 1) was isolated from guinea pig tissues were included in the analysis, because the measurements of *Legionella* density and viability include only those four serogroups (Table 3-11). No significant differences in density, viability, or the concentration of viable cells were detected between the infectious and the noninfectious groups of samples.

Relationships Between Profile Variables and Physicochemical Variables

Separate analyses were performed using density, viability, the density of live cells, and infectivity as dependent variables, with the various physicochemical variables measured for each sample as explanatory (predictor) variables. The purpose of these analyses was to seek possible causal relationships between environmental factors and *Legionella* characteristics. Suspected relationships could then be investigated under more controlled laboratory conditions during Phase II of the project.

Cell Density. Two statistical techniques were applied to investigate possible effects of physicochemical variables on *Legionella* density: Spearman correlation (a nonparametric test on the correlation of the ranks of the variables) and multiple linear regression. Analyses were performed on the complete data set and on a subset of the data including only closed-cycle plant-exposed samples. The results are summarized in Table 3-12. Not surprisingly, the variables indicated as being statistically significant in the various analyses are not always the same. This instability in the results is one of a number of reasons for being cautious in the interpretation of these analyses.

Table 3-11

MEAN VALUES OF *Legionella* PROFILE VARIABLES IN INFECTIOUS AND NONINFECTIOUS SAMPLES

Profile Variable	Infectious Samples Mean (standard error)	Noninfectious Samples Mean (standard error)
Density (cells/mL)	1156 (743)	1125 (357)
Log of density	2.21 (0.17)	2.23 (0.09)
Viability (proportion alive)	0.23 (0.03)	0.21 (0.02)
Arc sin of viability	0.47 (0.05)	0.44 (0.03)
Density x viability (Density of viable cells)	215 (117)	140 (60)
Log of (density x viability)	1.49 (0.22)	1.39 (0.11)

Table 3-12

RELATIONSHIP BETWEEN CELL DENSITY[a] OF *Legionella* AND PHYSICOCHEMICAL VARIABLES[b] AS INDICATED BY SPEARMAN CORRELATION ANALYSIS AND STEPWISE MULTIPLE LINEAR REGRESSION ANALYSIS

	Correlation[c]		Regression[d]	
Variable	All Data	Closed-cycle, Plant-exposed	All Data	Closed-cycle, Plant-exposed
Growth temperature	-0.15	-0.34		
Shock temperature		+0.29	-	
Conductivity			-	
pH		+0.34		
PO_4		-0.27	-	
NH_4	+0.29			+
Inorganic carbon			+	+
Organic carbon		-0.35	+	
Chlorination			+	
Alkalinity		-0.38	-	
NO_3	+0.26	+0.29	+	
Dissolved oxygen	+0.14			
Total carbon		-0.27		
R^2 (variance explained, %)			68	77

[a]As the transformed value, $\log_{10}$ (number of cells/mL).

[b]Variables included were growth temperature, shock temperature, conductivity (as log), alkalinity, pH (as 2nd power), dissolved oxygen, nitrate, phosphate (as log), ammonia, inorganic carbon, organic carbon, total carbon, and chlorination within 2 d. Plant was always included in the regressions to remove among-plant variance.

[c]A value indicates that the Spearman correlation coefficient was significant at the 0.10 level or better. A blank indicates no significant correlation.

[d]An entry indicates that the variable was included in the "best" model predicting cell density. The sign is the sign of the coefficient for that variable.

With respect to the correlation analysis, it is important to realize that a significant correlation between two variables does not imply a cause-effect relationship. Although there may be a plausible biological basis for such a relationship, a statistically significant correlation may simply reflect the effect of other variables not included in the analysis. At best, correlation should be considered suggestive of a causal relationship. Multiple regression is a technique that reduces some of the uncertainty arising from a simple correlation analysis by including a number of explanatory variables that may operate together to influence the response variable. This technique also can lead to erroneous conclusions, however, particularly when it is used to analyze field data, as in our study (McFadden 1963, Draper and Smith 1981). In such data, the researcher must accept for the predictor variables those values that happen to occur together, rather than predetermined values. Caution is needed in interpreting multiple regression analyses using this kind of data.

The analyses summarized in Table 3-12 are, however, suggestive of relationships between Legionella density and those variables that appear repeatedly and in a consistent manner. In particular, higher levels of inorganic carbon and nitrate may increase Legionella abundance, while higher growth temperatures, alkalinity, and phosphate may have a negative effect. Among the correlation analyses, none of the coefficients reveals a particularly strong relationship with total cell density.

Viability. The analysis for Legionella viability (Table 3-13) parallels that for density. Regression analysis, using the entire data set, reveals that none of the variables included explains a significant portion of the variance. When only the closed-cycle plant-exposed samples are considered, yielding a much more uniform but smaller data set, ammonia appears to have a negative effect on viability. Among the correlations, again none of the variables appears to have a noticeably strong relationship with viability.

Density of Viable Cells. Table 3-14 presents a summary of the results of the parallel analyses for the estimated concentration of viable cells. In the regression using all of the data, the three carbon parameters appear to be important in explaining the variance in density of viable cells. Also, high conductivity and shock temperature seem to reduce the concentration of viable cells, while higher nitrate levels increase it. Some of the correlation coefficients (i.e., pH, shock temperature, alkalinity, and total and organic

Table 3-13

RELATIONSHIP BETWEEN VIABILITY LEVELS[a] OF *Legionella* AND PHYSICOCHEMICAL VARIABLES[b] AS INDICATED BY SPEARMAN CORRELATION ANALYSIS AND STEPWISE MULTIPLE LINEAR REGRESSION ANALYSIS

Variable	Correlation[c]		Regression[d]	
	All Data	Closed-cycle, Plant-exposed	All Data	Closed-cycle, Plant-exposed
NH_4				-
Organic carbon	-0.29	-0.25		
Growth temperature	-0.26			
Shock temperature		+0.32		
Alkalinity	-0.29			
pH		+0.25		
Dissolved oxygen	+0.18			
Total carbon	-0.31	-0.22		
Chlorination	-0.16			
PO_4	+0.15	-0.28		
Inorganic carbon	-0.20	+0.24		
Conductivity	-0.27			
R^2 (variance explained, %)			-	52

[a]As the transformed value, $\sin^{-1}(\sqrt{INT})$.

[b]Variables included were growth temperature, shock temperature, conductivity (as log), alkalinity, pH (as 2nd power), dissolved oxygen, nitrate, phosphate (as log), ammonia, inorganic carbon, organic carbon, total carbon, and chlorination within 2 d. Plant was always included in the regressions to remove among-plant variance.

[c]A value indicates that the Spearman correlation coefficient was significant at the 0.10 level or better. A blank indicates no significant correlation.

[d]An entry indicates that the variable was included in the "best" model in predicting viability. The sign is the sign of the coefficient for that variable.

Table 3-14

RELATIONSHIP BETWEEN THE CONCENTRATION OF VIABLE *Legionella* CELLS[a] AND PHYSICOCHEMICAL VARIABLES[b] AS INDICATED BY SPEARMAN CORRELATION ANALYSIS AND STEPWISE MULTIPLE LINEAR REGRESSION ANALYSIS

Variable	Correlation[c]		Regression[d]	
	All Data	Closed-cycle, Plant-exposed	All Data	Closed-cycle, Plant-exposed
Conductivity			-	-
pH		+0.42		
Dissolved oxygen	+0.29	+0.25		
PO_4		-0.32		
NH_4	+0.21	-0.25		
Inorganic carbon			+	
Chlorination			+	
NO_3	+0.30	+0.23	+	
Growth temperature	-0.28	-0.32		
Organic carbon		-0.46	+	-
Total carbon		-0.41	-	
Alkalinity	-0.19	-0.44		
Shock temperature		+0.43	-	
R^2 (variance explained, %)			69	72

[a]As the transformed variable, $\log_{10}$ (number of viable cells/mL).

[b]Variables included were growth temperature, shock temperature, conductivity (as log), alkalinity, pH (as 2nd power), dissolved oxygen, nitrate, phosphate (as log), ammonia, inorganic carbon, organic carbon, total carbon, and chlorination within 2 d. Plant was always included in the regressions to remove among-plant variance.

[c]A value indicates the Spearman correlation coefficient was significant at the 0.10 level or better. A blank indicates no significant correlations.

[d]An entry indicates that the variable was included in the "best" model predicting the density of viable cells. The sign is the sign of the coefficient for that variable.

carbon) are higher here than with total cell density or viability, indicating again that the density of viable cells may be the most meaningful of the three measures. As in the previous analyses, these relationships may or may not be causal.

Infectivity. Table 3-15 contains a summary of results obtained by applying three statistical techniques to the data for infectivity. Because infectivity is a categorical (i.e., yes or no) variable, different methods of analysis are used. The Wilcoxon rank-sum test is employed to determine whether the values of each physical or chemical variable differ between those samples classified as infectious and those samples classified as noninfectious. For the same reason, logistic regression is used, rather than multiple linear regression, to relate infectivity to the physicochemical variables. Stepwise discriminant analysis is also utilized. This is similar in some respects to logistic regression in that it uses many predictor variables to classify observations into separate categories.

As in previous analyses for the other *Legionella* profile variables, a considerable amount of "variable switching" occurs (i.e., depending on the type of analysis or the data set used, different variables are identified as "significant"). Conductivity is the most consistently selected variable; higher infectivity appears to be associated with greater conductivity. A negative relationship between infectivity and dissolved oxygen is also suggested. In addition, higher values of organic and inorganic carbon as well as growth temperature seem to be associated with higher infectivity. As is true for the other profile variables, identifying reliable cause/effect relationships between infectivity and physical and chemical variables will require the sort of controlled experiments planned for Phase II of this project.

Using the function fitted by logistic regression, it is possible to correctly classify (i.e., "predict") the infectivity of 77% of the samples in the total data set; using discriminant analysis, 78% are correctly classified. Further work with this technique, when additional environmental and infectivity data are obtained, should lead to an improved understanding of, and ability to predict, *Legionella* infectivity.

Table 3-15

RELATIONSHIP BETWEEN INFECTIVITY OF *Legionella* AND PHYSICOCHEMICAL VARIABLES[a] AS INDICATED BY THE WILCOXON RANK-SUM TEST, STEPWISE MULTIPLE LOGISTIC REGRESSION, AND STEPWISE DISCRIMINANT ANALYSIS

Variable	Wilcoxon[b] Rank Sum Test[c]		Logistic Regression[d]		Discriminant Analysis[e]	
	All Data	Closed-cyle, Plant-exposed	All Data	Closed-cycle, Plant-exposed[f]	All Data	Closed-cycle, Plant-exposed
Conductivity	+		+		+	+
pH					+	
NH_4	+					
Total carbon	+	-	-			
Shock temperature	+					-
NO_3	+					
Dissolved oxygen	-					-
PO_4						-
Growth temperature	+					+
Alkalinity		+				
Chlorination	+					
Inorganic carbon	+				+	
Organic carbon	+	-	+		+	
Overall percent classified correctly			77		78	92

[a]Physicochemical variables included were growth temperature, shock temperature, conductivity (as log), alkalinity, pH (as 2nd power), dissolved oxygen, nitrate, phosphate (as log), ammonia, chlorination within 2 d, and organic and inorganic carbon.

[b]This is equivalent to the Mann-Whitney U test, a nonparametric equivalent to the Student's t-test.

[c]The sign represents the direction of a significant ($P < 0.10$) difference between the infectious and noninfectious samples (i.e., a positive sign implies that the virulent samples had a higher mean score on this variable).

[d]The sign represents the sign of the coefficient in the best model selected by the stepwise procedure.

[e]The sign represents the weighting provided by the coefficients of the linearized discriminant functions (i.e., a positive sign indicates that higher values on this variable are associated with the virulent classification).

[f]This analysis could not be performed due to insufficient sample size.

A NEW SPECIES OF Legionella

Water sample concentrates obtained during the spring sampling from the cooling tower basins and water boxes at site G and inoculated into American breed guinea pigs resulted in temperature increases of $\geq$ 1.0°C within 3 to 6 d after inoculation. The guinea pigs were sacrificed 5 to 6 d after inoculation and tissues were plated on CYE agar. Late appearing (>5 d) colonies from plated spleen tissue had cultural and microscopic characteristics of Legionella and were not reactive on FA analysis with known Legionella antisera (Table 3-16). The pre- and postcondenser water box samples yielded isolates OR15 and OR12, respectively. The OR10, 16, 18, and 19 isolates were obtained from inoculation of cooling tower waters (Table 3-16).

Two separate samples of cooling tower water concentrates from site F in the spring also yielded Legionella isolates not typeable with known Legionella antisera (isolates OR4, 6, 23, 24, 30). Guinea pigs inoculated with the samples showed temperature rises of 0.8 to 1.4°C and were sacrificed the fourth or fifth day after injection. Legionella-like bacteria, untypeable with any of the conjugates (Table 3-16), were isolated from both spleen and liver tissues plated on CYE agar.

None of the isolates from either site could be grown on blood or brain-heart infusion agar. Growth on yeast extract agar was variable. In general, all of the isolates initially grew poorly on CYE agar plates. The gram-staining characteristics of the isolates were similar to those seen with other species of Legionella. Weakly gram-negative rods of varying lengths were apparent on microscopic examination of all the isolates (Table 3-16).

All 11 isolates were weakly catalase-positive. Conjugated antibodies [obtained through the courtesy of the reagent branch, Centers for Disease Control (CDC)] specific for the six serogroups of L. pneumophila and six other known Legionella species did not react with the 11 Oak Ridge (OR) isolates. Conversely, conjugate prepared against the OR10 isolate (obtained through the courtesy of W. B. Cherry, CDC) reacted maximally with all 11 OR isolates (Table 3-16). Detailed studies of the OR isolates by Orrison et al. (in press) at the CDC have confirmed these isolates as a new species of Legionella, now named Legionella oakridgensis.

Table 3-16

CHARACTERISTICS OF OAK RIDGE ISOLATES OF *Legionella*

Characteristic	Isolates										
	OR4	OR6	OR10	OR12	OR15	OR16	OR18	OR19	OR23	OR24	OR30
Isolation site	F	F	G	G	G	G	G	G	F	F	F
Growth on:											
CYE	+	+	+	+	+	+	+	+	+	+	+
BHI	-	-	-	-	-	-	-	-	-	-	-
Blood agar	-	-	-	-	-	-	-	-	-	-	-
Gram stain[a]	Gr.-	Gr.-	Gr.-	Gr.-	Gr.-	Gr.-	Gr.-	Gr.-	Gr.-	Gr.-	Gr.-
Catalase	+	+	+	+	+	+	+	+	+	+	+
Reaction with conjugates against:											
L. pneumophila[b]	-	-	-	-	-	-	-	-	-	-	-
L. bozemanii	-	-	-	-	-	-	-	-	-	-	-
L. gormanii	-	-	-	-	-	-	-	-	-	-	-
L. micdadei	-	-	-	-	-	-	-	-	-	-	-
L. dumoffii	-	-	-	-	-	-	-	-	-	-	-
L. longbeachae	-	-	-	-	-	-	-	-	-	-	-
L. jordanis[c]	-	-	-	-	-	-	-	-	-	-	-
OR10 isolate	+	+	+	+	+	+	+	+	+	+	+

[a]Gr.- = gram-negative rods with morphology suggestive of *Legionella*.

[b]Serogroups 1 to 6.

[c]Determined by Orrison et al. (in press).

This new species was isolated from these same two sites (F and G) in other seasons. In addition, it was isolated from site E in the fall. Analyses of plant-exposed and ambient water concentrates from various locations in the continental United States show a wide distribution and variable concentration of the Oak Ridge species of Legionella, not unlike those of the four major serogroups of L. pneumophila combined (Tyndall 1982a; Tyndall et al., submitted).

ADDITIONAL POSSIBLE NEW SPECIES AND OTHER PATHOGENIC MICROORGANISMS

In addition to L. oakridgensis, other Legionella and another currently unidentified microbial pathogen were isolated during the course of this study.

A Legionella species not reactive with antisera against known species of Legionella was isolated from site F. Analysis of this isolate by personnel at CDC showed it to be identical to a previously isolated but currently unnamed new species (Jamestown 19). This species is of particular interest to the present study because it was originally isolated by Gorman and Feely (CDC) from a power plant site at which plant personnel contracted the nonpneumonic form of Legionellosis (Pontiac Fever) while cleaning condenser tubes (Fraser et al. 1979).

Yet another untypeable Legionella was isolated from a wintertime discharge sample at plant D. This isolate is being examined by CDC investigators to determine if it too is a new Legionella species.

In addition to the Legionella isolates, another microbial pathogen has been isolated from fall samples of intake and discharge water at plant C. The guinea pigs inoculated with discharge samples developed high fevers within 3 d after inoculation and, on autopsy, pure cultures of the vibrio-like (i.e., weakly gram-negative, comma-shaped bacteria capable of growth at 25-37°C) organism grew from the plated tissue. Personnel at CDC have examined this isolate and have been unable to classify the microbe as belonging to any known genus.

Section 4

DISCUSSION

DISTRIBUTION OF SPECIES AND SEROGROUPS OF INFECTIOUS *Legionella*

Legionella pneumophila was the causative agent of the Legionnaires' Disease outbreak in Philadelphia in 1976. This species of *Legionella* was also subsequently implicated in similar outbreaks of pulmonary disease prior to and after the Philadelphia episode. Indeed, *L. pneumophila* is the cause of a majority of clinically diagnosed cases of Legionellosis. This bacterium, along with several other *Legionella* species, appears to be a part of the natural aquatic environment and is known to be capable of surviving wide ranges of physical and chemical conditions (Fliermans et al. 1981b).

It is not unexpected that results of the Phase I EPRI study have shown *L. pneumophila* to be the predominant *Legionella* isolate. *Legionella pneumophila* serogroups were widely distributed in all systems. Infectious *L. pneumophila* was detected in all but one of nine cooling systems. The prevalence of infectious *L. pneumophila* in thermally altered waters is not surprising in view of the observation that source water for the power plants yielded infectious *L. pneumophila* from seven of the nine test sites.

In contrast to *L. pneumophila*, the newly discovered *L. oakridgensis* has yet to be isolated from clinical specimens but was the second most prevalent species isolated in the Phase I study. While FA analysis of various ambient and thermally altered waters indicates a wide distribution of this *Legionella*, the isolation of infectious *L. oakridgensis* was more site-specific. Twelve of thirteen isolates were obtained from only two of the nine sites.

Legionella oakridgensis was not isolated from sites totally devoid of thermal additions. The apparent site dependency for the presence of infectious *L. oakridgensis* may in part explain why it has yet to be detected in clinical material and why it may be important in future studies delineating the impact of this *Legionella* species on human health.

PATTERNS OF DENSITY, VIABILITY, AND INFECTIVITY

Cell densities of Legionella in water samples were estimated by the DFA technique, using a polyvalent antiserum reactive against four serogroups of L. pneumophila. It is likely that other serogroups and species of Legionella were present in the samples but were not stained and therefore were not enumerated. A different possibility is that the counts include non-Legionella cells because of cross-staining by the DFA reagents. Although this is known to be possible, it is expected to occur infrequently if at all (Fliermans et al. 1981b).

Cell densities of Legionella in ambient waters were significantly greater in the cold seasons (winter and spring) than those in the warm seasons. In addition, densities were significantly greater during the spring in ambient waters of closed-cycle plants in our study than those in ambient waters of the once-through plants. The reason for this is not understood, but latitudinal differences and the types of water bodies associated with the use of closed-cycle cooling may be relevant. During the spring and summer, cell concentrations were significantly reduced in the plant-exposed water at closed-cycle plants compared to those in the ambient water. An analysis of individual plants showed that in seven of the fifteen sampling events at closed-cycle plants, significant density decreases with plant passage were found. The single significant change in density found in the 19 once-through sampling events (plant E in the spring) may have been an artifact of the location of the intake sampling.

The finding that significant changes in density are, for the most part, restricted to plants operating in a closed-cycle mode is not surprising. A once-through plant passes water through the condenser at a very high rate. It is difficult to conceive of a biological process proceeding rapidly enough in the condenser tubes to make a substantial change in the density of microorganisms in once-through cooling water (with the exception of purposeful anti-biofouling procedures), even though attachment and sloughing of microbes, including bacteria, occur continuously (Battaglia et al. 1981). Any population-level effects of once-through passage on cell density, due for example to thermal shock, would not be evident until well after the water had been returned to the environment. Closed-cycle cooling systems, on the other hand, provide:

- a markedly changed set of growth conditions due to concentration of dissolved materials, and

- repetition of thermal shock as well as continued thermal stress due to recirculation.

The analysis of the Legionella cell density data indicates that closed-cycle operation creates conditions that often markedly decrease Legionella population densities.

Estimates of the viability (proportion alive) of Legionella populations using the INT method yielded highly variable results. Measurements from replicate samples sometimes differed greatly, and some samples with viability estimated as zero caused Legionellosis in guinea pigs. Analysis of variance indicated that the viability of Legionella populations was greater in source water of once-through sites compared with that of closed-cycle sites in spring and summer. The result of primary interest from the viability data is that no consistent significant difference with plant passage could be found. As a result, there is little basis in our study for concluding that power plant operation increases or decreases the viability of Legionella.

Attempts to relate the viability of Legionella to population density showed a tendency for smaller natural populations of Legionella in the spring to be more viable than larger natural populations. In general, there is reason to expect that the relationship between density and viability will vary, depending on whether the population is in a healthy, increasing state or a stressed, declining state. This in turn should depend in large part on nutrient conditions and the degree of physical and chemical stress imposed by the environment. Phase II of this project, which will include both laboratory experiments and field chamber studies, should clarify these interrelationships.

The density of viable Legionella cells was calculated as the product of the total cell density and the proportion of viable cells. Patterns for the densities of viable Legionella cells were generally similar to those found for total Legionella cell densities. The largest viable cell densities were found at closed-cycle sites in the spring. In addition, the densities of viable cells were significantly greater in the spring in ambient waters at the closed-cycle sites than those in ambient waters at the once-through sites, and viable cell densities were significantly reduced in plant-exposed water from closed-cycle plants during the spring and summer. Like the trends for the density of total Legionella, the reasons underlying these patterns are not understood.

The infectivity of Legionella for guinea pigs is inferred from isolation of the bacteria after intraperitoneal injection of a water sample concentrate. Pure cultures of Legionella isolated from tissues of inoculated animals are considered presumptively infectious. Infectivity was not confirmed by reinoculation of guinea pigs. Infectious Legionella were isolated from ambient waters in all seasons except spring and from plant-exposed water during all seasons. Although infectivity appeared to be lower in the spring than in the other seasons, this trend was not statistically significant. The property of infectivity was, however, associated more with some plants than with others. Infectious Legionella were significantly more likely to be found in samples from the plant-exposed water of closed-cycle plants than in samples from once-through plants or in ambient samples. When the association between infectivity and various measures of Legionella density and viability was probed, no significant relationships were found.

In view of these results, it is instructive to consider what the guinea pig tests for infectivity mean. The intraperitoneal injection (i.e., into the abdominal cavity) contained the Legionella concentrated from approximately 1 L of water. Subsequent isolation of Legionella and absence of other bacteria on culture plates smeared with tissues of sacrified sick guinea pigs is considered presumptive evidence that the Legionella caused the illness. While this is an accepted clinical procedure, and necessary if virulence is to be assessed given the existing constraints and in the absence of a more practical technique, it is not possible to directly relate the results to human risk. This is so both because infectivity was not confirmed by reinoculation of guinea pigs and because the quantity of material, the route of exposure, and the size and species of the host animal all differ greatly.

Nonetheless, the finding that water from closed-cycle systems tends to be more infectious for guinea pigs than the corresponding ambient water implies that, given an exposure pathway, there is potentially greater risk to humans from these waters. Because the infectivity for guinea pigs cannot be quantitatively related to human risk, these data cannot in themselves determine whether the potential risk is realized or not. The fact that aerosolization of water in some closed-cycle cooling systems could provide an exposure pathway to humans suggests that the factors underlying infectivity are worthy of further study and that the search for effective Legionella control measures (Tyndall 1982b, England et al. 1982) should continue. In addition, the finding that Legionella density was not

a predictor of infectivity in this study indicates that the current practice (Fliermans et al. 1982) of using densities of Legionella greater than 10^8/L as the sole "trigger" for instituting control measures may not be appropriate for all systems.

RELATIONSHIPS BETWEEN PROFILE VARIABLES AND PHYSICOCHEMICAL VARIABLES

One major goal of this EPRI project is to gain an understanding of the factors that influence the abundance, viability, and infectivity of Legionella. Ideally, this will involve establishing cause-and-effect relationships between factors important to Legionella and the Legionella profile variables (density, viability, and infectivity). It will then be more feasible to pursue alternative techniques for control of the organism.

During the analysis of the first Phase I sampling (the data collected in the spring), it became evident that understanding the ecology of Legionella in relation to power plant cooling systems would be more difficult than originally anticipated. Density of Legionella was useless as a predictor of infectivity. Furthermore, no clear relationships could be found between the Legionella profile variables and the physicochemical variables measured in the study. As more data became available from the summer and fall samplings, these early difficulties persisted.

Once it became obvious that straightforward relationships would not be found, biostatistical effort was devoted to the application of conventional statistical methods and of some less conventional methods to analyze the data. The techniques of multiple linear regression, analysis of covariance, nonparametric correlation, logistic regression, and discriminant analysis have been successful in identifying physical and chemical factors which may relate to Legionella density, viability, and infectivity. At the same time, because it was realized that firm cause-and-effect relationships would likely not be found in the Phase I study, the plans for Phase II of the research were modified. More emphasis in the early stages of Phase II is being placed on controlled laboratory experiments designed to identify factors associated with the density, viability, and virulence of Legionella and on studies of isolated populations held in membrane chambers placed in field situations. Appropriate statistical techniques will be used on the more structured Phase II data to attempt to confirm some of these

possible relationships as real, while also examining the role of more complex factors such as dissolved organic material (Tison et al. 1980).

THE NEW SPECIES OF *Legionella*

While the guinea pig inoculations and subsequent plating of injected tissues on CYE agar are techniques designed for isolating *L. pneumophila*, the investigators also realize that other *Legionella*-like pathogens may be present in the test samples. Consequently, atypical colonies on the CYE agar test plates are often examined and characterized, particularly in cases where no typical *Legionella* colonies are present.

One product of this effort was the isolation of *L. oakridgensis*. Two unusual characteristics were apparent on the initial isolation of these *Legionella*-like bacteria. First, the colonies did not appear until four or more days after plating of the tissue on CYE agar. Second, the bacteria, although presumptively *Legionella*, did not cross-react with antisera prepared against known *Legionella* species (Table 3-16).

Some guinea pigs from which *L. oakridgensis* was isolated also yielded *L. pneumophila*. Thus, the elevated fever and other signs of illness (i.e., lethargy, ruffled fur, etc.) in these animals could have been due to infection with *L. pneumophila* or *L. oakridgensis* or both. The two guinea pigs inoculated with water concentrates from site F in the spring, however, yielded only *L. oakridgensis*. These pigs were febrile and showed overt signs of illness. This indicated that *L. oakridgensis* was pathogenic. Subsequent studies by Orrison and Cherry at CDC confirmed the pathogenicity of *L. oakridgensis*. It will be of interest to determine whether evidence of human infection with *L. oakridgensis* can be found.

While the major goal of this ongoing study is aimed at delineating those ecological variables important in the propagation of infectious *Legionella*, the isolation of the new species is illustrative of the value of environmental information as input to clinical studies. For example, many clinical specimens are treated with formalin prior to analysis, obliterating any possibility of isolating new species of pathogens. Thus environmental material serves not only as a source of clinical infection but also as a reservoir from which the isolation of previously undiscovered pathogens of clinical importance is possible.

OTHER UNIDENTIFIED PATHOGENIC MICROORGANISMS

As discussed in the Results section, two other possibly new Legionella species, in addition to the new L. oakridgensis, were isolated from sick guinea pigs after sample injection. Also, an unclassifiable, vibrio-like microbe was isolated. The implications of these findings are germane to the study of Legionella and other environmental pathogens as a whole, as well as being of interest to studies of pathogens in power plant cooling systems per se. The Jamestown 19 isolate originated in a sample taken from the intake of plant F. The unidentified vibrio-like organism was isolated from both the intake and the discharge water of a once-through plant (plant C). The third unusual isolate, which may be a new species of Legionella, was isolated only from the discharge of plant D, another once-through facility. Because two out of three of these unusual isolates were found in ambient waters, and because we uniformly failed to find differences in Legionella profiles between ambient and plant-exposed water for once-through facilities, it is reasonable to conclude that the power plant cooling systems should not be considered the cause of these unusual findings. Rather, these and similar unknown organisms are most likely normal aquatic fauna.

IMPLICATIONS OF THE PHASE I STUDY

The results of Phase I of this EPRI project do have implications for the study of power plant cooling systems. Pathogenic organisms such as Legionella occupy selected ecological niches in both natural and man-made habitats. These habitats appear to be primarily aquatic rather than terrestrial. The power plant worker and the populus at large also occupy selected niches. Our studies have centered on the elucidation of pathogenic microorganisms in power plant cooling systems for reasons which define the practical significance of this project: the clear importance of determining whether niches for these pathogens and man, especially the power plant worker, overlap substantively and, if so, the implications for the prudent management of the natural and particularly the man-made habitats where such overlap occurs. It appears from Phase I of this study that Legionella and power plant personnel can on occasion occupy the same ecological niche for at least some portion of the time. Infectious Legionella do exist, at least at some times and sites, in the cooling waters. In closed-cycle systems the potential for selection and aerosolization of pathogenic organisms exists. The significance of these findings will be addressed in subsequent research so that a proper perspective between occupational exposure and natural environmental exposure can be established.

Section 5

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Section 6

GLOSSARY

For the reader's convenience, the following specialized terms are briefly defined here in the context of their use in this report.

Antibody: Any of various serum globulins normally present or produced in response to infection or administration of suitable antigens that combine specifically with antigens and neutralize toxins, agglutinate bacteria or cells, and precipitate soluble antigens.

Antigens: Substances that, when injected into animals, stimulate formation of proteins called antibodies that will interact uniquely with the specific antigen.

Febrile: Of or relating to fever; malaised by fever.

Intraperitoneal injection: Injection through skin and peritoneum into the abdominal cavity.

Peritoneum: The smooth transparent serous membrane that lines the cavity of the abdomen of a mammal. It is reflected inward over the abdominal and pelvic viscera.

Polyvalent: Relating to those antibodies capable of interacting with more than one toxin, antigen, or kind of microorganism.

Serogroup: A group of Legionella isolates belonging to the same species yet containing common antigens serologically distinct from other isolates of the same species.

Serologic: Pertaining to tests or reactions using serum.

Serum: The liquid portion of blood remaining after removal of the blood cells and clotting components.

Uncompromised: A term indicating that the health of the test animal is not impaired by stress, disease, inadequate diet, or other factors that would weaken it.

APPENDIX TABLES

Table A-1

INFECTIVITY RESULTS BY POWER PLANT AND SEASON: ONCE-THROUGH OPERATION

Plant	Season	Ambient Waters: Total Number of Samples Injected[a]	Ambient Waters: Proportion Infectious (p_A)	Plant-exposed Waters: Total Number of Samples Injected[a]	Plant-exposed Waters: Proportion Infectious (p_E)	Change in Infectivity[b] (p_E-p_A)
A	Spring	1	0	1	0	0
	Summer	2	0.50	3	0.33	-0.17
	Fall	2	1.00	1	1.00	0
	Winter	2	0	2	0.50	+0.50
B	Spring	1	0	1	0	0
	Summer	2	0	3	0	0
	Fall	2	0	2	0	0
	Winter	2	0	1	0	0
C	Spring	1	0	1	0	0
	Summer	2	0.50	2	1.00	+0.50
	Fall	2	0	2	0	0
	Winter	2	0.50	2	0	-0.50
D	Spring	2	0	1	0	0
	Summer	2	0	2	0.50	+0.50
	Fall	2	0.50	2	0	-0.50
	Winter	2	0	2	0.50	+0.50
E	Spring	1	0	2	0.50	+0.50
	Fall	2	0.50	2	0	-0.50
	Winter	1	0	1	1.00	+1.00

[a]Samples that proved contaminated or toxic were excluded.

[b]A positive number indicates higher infectivity in plant-exposed water than in ambient water; a negative number indicates the converse.

Table A-2

INFECTIVITY RESULTS BY POWER PLANT AND SEASON: CLOSED-CYCLE OPERATION

Plant	Season	Ambient Waters: Total Number of Samples Injected[a]	Ambient Waters: Proportion Infectious (p_A)	Plant-exposed Waters: Total Number of Samples Injected[a]	Plant-exposed Waters: Proportion Infectious (p_E)	Change in Infectivity[b] (p_E-p_A)
F	Spring	c	c	5	0.40	c
	Fall	1	1.00	3	0.67	-0.33
	Winter	1	0	2	1.00	+1.00
G	Spring	c	c	5	1.00	c
	Summer	2	0.50	3	1.00	+0.50
	Fall	1	0	4	0.50	+0.50
	Winter	1	1.00	3	1.00	0
H	Spring	3	0	c	c	c
	Summer	c	c	2	0.50	c
	Fall	2	0.50	2	1.00	+0.50
	Winter	1	1.00	1	1.00	0
I	Spring	2	0	1	1.00	+1.00
	Summer	0	•	2	1.00	•
	Fall	1	0	2	1.00	+1.00
	Winter	1	1.00	2	0	-1.00

[a]Samples that proved contaminated or toxic were excluded.

[b]A positive number indicates higher infectivity in plant-exposed water than in ambient water; a negative number indicates the converse.

[c]All injected samples proved contaminated or toxic. Infectivity could not be assessed.

Table A-3

PHASE I DATA: PROFILE, PHYSICAL, AND SOME CHEMICAL PARAMETERS

Plant	Season	Sample Location	Legionella Density (per mL)[a]	Viability (%)	Temperature (°C) Sample	Growth	Shock	pH	NH_4 (mg/L)	NO_3 (mg/L)	PO_4 (mg/L)
A	Spring	Intake	19	0	11.4	11.4	0.0	8.4	0.16	.	.
		Intake	38	20	11.4	11.4	0.0	8.4	0.13	.	32.80
		Intake	32	40	11.4	11.4	0.0	8.4	0.14	.	32.40
		Precondenser	53	40	11.1	11.4	0.0	8.4	0.18	.	32.90
		Postcondenser	54	14	21.1	11.4	10.0	7.2	0.15	.	22.40
		Outfall	37	40	22.4	11.4	10.0	7.3	0.05	.	22.70
		Outfall	21	67	22.4	11.4	10.0	7.3	0.06	.	25.00
		Outfall	18	0	22.4	11.4	10.0	7.3	0.06	.	28.40
A	Summer	Intake	320	17	30.4	30.4	0.0	6.2	0.46	0.05	0.38
		Intake	290	48	30.5	30.5	0.0	6.2	0.45	0.03	0.37
		Intake	270	42	30.5	30.5	0.0	6.5	0.42	0.03	0.40
		Precondenser	1200	10	30.6	30.5	0.0	6.1	0.48	0.03	0.28
		Postcondenser	220	31	39.4	30.5	8.8	6.7	0.50	0.04	0.65
		Outfall	170	36	38.2	30.5	8.8	6.6	0.52	0.04	0.40
		Outfall	300	28	39.9	30.5	8.8	6.7	0.50	0.03	0.38
		Outfall	250	23	38.8	30.5	8.8	6.8	0.53	0.03	0.40
A	Fall	Intake	83	25	18.6	18.6	0.0	6.5	0.38	0.08	0.33
		Intake	69	28	18.6	18.6	0.0	6.6	0.34	0.08	0.37
		Precondenser	38	33	18.9	18.6	0.0	6.6	0.47	1.30	0.44
		Postcondenser	70	50	32.2	18.6	13.3	6.4	1.32	2.50	0.50
		Outfall	42	46	28.2	18.6	13.3	6.4	0.38	0.08	0.33
		Outfall	93	14	28.2	18.6	13.3	6.4	0.38	0.08	0.33
		Outfall	74	33	28.2	18.6	13.3	6.4	0.38	0.08	0.30
A	Winter	Intake	950	44	5.0	5.0	0.0	5.9	0.60	2.10	0.58
		Intake	870	15	5.0	5.0	0.0	6.0	0.60	2.10	0.58
		Intake	540	37	5.0	5.0	0.0	5.9	0.60	2.10	0.58
		Precondenser	300	50	5.2	5.0	0.0	5.9	1.14	2.30	0.58
		Postcondenser	580	21	17.4	5.0	12.2	6.2	0.68	2.10	0.58
		Outfall	170	25	20.2	5.0	12.2	6.2	0.68	2.80	0.65
		Outfall	350	35	20.2	5.0	12.2	6.2	0.68	2.80	0.65
		Outfall	250	35	20.2	5.0	12.2	6.2	0.68	2.80	0.65

Table A-3 (continued)

Plant	Season	Sample Location	Legionella Density (per mL)[a]	Viability (%)	Temperature (°C) Sample	Growth	Shock	pH	NH_4 (mg/L)	NO_3 (mg/L)	PO_4 (mg/L)
B	Spring	Intake	45	40	10.4	10.4	0.0	9.5	0.02	.	3.00
		Intake	86	87	10.4	10.4	0.0	9.5	0.02	.	2.80
		Intake	43	62	10.4	10.4	0.0	9.5	0.03	.	3.09
		Precondenser	19	0	12.0	10.4	0.0	9.5	0.05	.	2.90
		Postcondenser	35	50	20.0	10.4	8.0	7.7	0.03	.	2.90
		Outfall	42	67	17.2	10.4	8.0	7.8	0.03	.	3.57
		Outfall	38	40	17.2	10.4	8.0	7.8	0.03	.	3.90
		Outfall	26	40	17.2	10.4	8.0	7.8	0.09	.	3.69
B	Summer	Intake	180	53	22.7	22.7	0.0	6.3	0.42	0.03	0.45
		Intake	99	29	22.7	22.7	0.0	6.2	0.35	0.03	0.35
		Intake	170	30	23.1	23.1	0.0	6.2	0.42	0.03	0.33
		Precondenser	110	9	22.5	22.8	0.0	6.3	0.35	0.03	0.32
		Postcondenser	120	11	31.5	22.8	9.0	6.2	0.40	0.03	0.40
		Outfall	80	12	29.5	22.8	9.0	6.1	0.35	0.03	0.38
		Outfall	320	41	28.5	22.8	9.0	6.1	0.38	0.03	0.38
		Outfall	380	18	30.4	22.8	9.0	6.3	0.32	0.03	0.32
B	Fall	Intake	83	33	20.9	20.9	0.0	6.6	0.32	0.80	0.26
		Intake	77	0	20.9	20.9	0.0	6.5	0.30	0.80	0.28
		Intake	51	17	20.9	20.9	0.0	6.6	0.29	0.70	0.27
		Precondenser	110	30	21.5	20.9	0.0	6.5	0.33	0.90	0.33
		Postcondenser	130	18	24.8	20.9	3.3	6.5	0.30	0.80	0.28
		Outfall	22	36	21.7	20.9	3.3	6.5	0.29	0.80	0.33
		Outfall	1	.	21.7	20.9	3.3	6.5	0.28	0.70	0.28
		Outfall	83	31	21.9	20.9	3.3	6.5	0.28	0.70	0.28
B	Winter	Intake	140	50	9.0	9.0	0.0	6.7	0.37	0.90	0.43
		Intake	200	33	9.0	9.0	0.0	6.7	0.37	0.90	0.43
		Intake	150	55	9.0	9.0	0.0	6.7	0.37	0.90	0.43
		Precondenser	250	10	9.8	9.0	0.0	6.7	0.48	0.80	0.50
		Postcondenser	500	10	22.3	9.0	12.5	6.4	0.39	0.80	0.43
		Outfall	310	27	22.3	9.0	12.5	6.4	0.38	0.90	0.38
		Outfall	220	17	22.3	9.0	12.5	6.3	0.38	0.90	0.38
		Outfall	380	37	22.3	9.0	12.5	6.3	0.38	0.90	0.38

Table A-3 (continued)

Plant	Season	Sample Location	Legionella Density (per mL)[a]	Viability (%)	Temperature (°C) Sample	Growth	Shock	pH	NH_4 (mg/L)	NO_3 (mg/L)	PO_4 (mg/L)
C	Spring	Intake	19	50	10.0	10.0	0.0	9.5	0.04	.	1.0
		Intake	6	.	10.0	10.0	0.0	9.5	0.04	.	1.0
		Intake	19	25	10.0	10.0	0.0	9.5	0.04	.	1.0
		Precondenser	42	0	9.8	10.0	0.0	9.7	0.05	.	0.8
		Postcondenser	16	33	16.5	10.0	6.7	9.0	0.03	.	0.9
		Outfall	45	0	18.8	10.0	6.7	8.0	0.05	.	1.0
		Outfall	13	.	18.8	10.0	6.7	8.0	0.06	.	1.0
		Outfall	38	100	18.8	10.0	6.7	8.0	0.07	.	1.0
C	Summer	Intake	74	39	29.6	29.6	0.0	8.4	0.90	0.30	0.2
		Intake	48	33	29.6	29.6	0.0	8.0	0.90	0.30	0.2
		Intake	100	25	29.6	29.6	0.0	8.4	0.90	0.31	0.2
		Precondenser	61	11	32.8	29.6	0.0	8.2	1.00	0.30	0.1
		Postcondenser	83	8	38.9	29.6	6.1	7.0	0.75	0.30	0.2
		Outfall	83	23	36.5	29.6	6.1	6.9	0.80	0.29	0.2
		Outfall	83	12	36.7	29.6	6.1	7.1	0.90	0.29	0.2
		Outfall	170	37	37.0	29.6	6.1	7.1	0.85	0.26	0.2
C	Fall	Intake	22	14	17.5	17.5	0.0	7.6	0.32	0.08	0.2
		Intake	35	27	17.5	17.5	0.0	7.5	0.32	0.08	0.2
		Intake	25	25	17.4	17.4	0.0	7.6	0.32	0.08	0.2
		Precondenser	48	33	18.3	17.5	0.0	7.6	0.35	0.09	0.9
		Postcondenser	38	25	24.4	17.5	6.1	7.2	0.32	0.08	0.2
		Outfall	32	10	25.1	17.5	6.1	7.2	0.32	0.08	0.2
		Outfall	86	15	25.2	17.5	6.1	7.2	0.32	0.08	0.2
		Outfall	22	14	25.1	17.5	6.1	7.2	0.32	0.08	0.2
C	Winter	Intake	270	16	3.5	3.5	0.0	6.8	0.35	1.20	0.4
		Intake	130	19	3.5	3.5	0.0	6.8	0.35	1.20	0.4
		Intake	460	13	3.5	3.5	0.0	6.8	0.35	1.20	0.4
		Precondenser	420	13	3.3	3.5	0.0	6.8	0.37	0.08	0.3
		Postcondenser	590	40	14.4	3.5	11.1	6.5	0.35	0.09	0.3
		Outfall	870	6	10.4	3.5	11.1	6.5	0.35	1.20	0.3
		Outfall	660	38	10.4	3.5	11.1	6.5	0.35	1.10	0.3
		Outfall	720	26	10.4	3.5	11.1	6.5	0.35	1.10	0.3

Table A-3 (continued)

Plant	Season	Sample Location	Legionella Density (per mL)[a]	Viability (%)	Temperature (°C) Sample	Growth	Shock	pH	NH_4 (mg/L)	NO_3 (mg/L)	PO_4 (mg/L)
D	Spring	Intake	450	20	8.6	8.6	0.0	8.2	0.48	1.00	0.21
		Intake	160	31	8.6	8.6	0.0	8.2	0.45	1.20	0.28
		Intake	99	32	8.6	8.6	0.0	8.2	0.33	1.00	0.37
		Precondenser	200	27	9.4	8.6	0.0	8.2	0.58	1.10	0.27
		Outfall	310	21	13.0	8.6	5.6	8.4	0.60	1.10	0.25
		Outfall	230	39	13.0	8.6	5.6	8.4	0.61	1.20	0.22
		Outfall	160	28	13.0	8.6	5.6	8.4	0.33	1.60	0.25
D	Summer	Intake	40	0	18.7	18.7	0.0	8.3	0.10	1.60	0.21
		Intake	64	13	18.7	18.7	0.0	8.3	0.00	0.40	0.25
		Intake	100	12	18.7	18.7	0.0	8.3	0.09	0.75	0.20
		Precondenser	20	20	19.0	18.7	0.0	8.2	0.09	0.75	0.24
		Postcondenser	1	.	22.0	18.7	3.0	8.2	0.09	0.90	0.22
		Outfall	140	11	22.7	18.7	3.0	8.2	0.09	0.60	0.22
		Outfall	210	17	22.7	18.7	3.0	8.2	0.10	1.20	0.18
		Outfall	160	20	22.7	18.7	3.0	8.2	0.09	2.00	0.24
D	Fall	Intake	35	9	13.2	13.2	0.0	8.0	0.41	1.30	0.30
		Intake	13	.	13.2	13.2	0.0	8.0	0.39	1.20	0.29
		Intake	22	14	13.2	13.2	0.0	8.0	0.41	1.20	0.32
		Precondenser	15	20	13.2	13.2	0.0	8.0	0.39	1.20	0.33
		Postcondenser	17	33	17.2	13.2	4.0	8.0	0.33	1.30	0.33
		Outfall	16	0	17.2	13.2	4.0	8.0	0.39	1.20	0.28
		Outfall	51	19	17.2	13.2	4.0	8.0	0.38	1.30	0.28
		Outfall	3	.	17.2	13.2	4.0	8.0	0.65	2.40	0.33
D	Winter	Intake	110	20	4.4	4.4	0.0	8.2	0.37	1.10	0.39
		Intake	140	14	4.4	4.4	0.0	8.2	0.36	1.20	0.39
		Intake	26	25	4.4	4.4	0.0	8.2	0.36	1.00	0.37
		Precondenser	180	33	4.4	4.4	0.0	8.2	0.37	1.00	0.46
		Postcondenser	96	12	11.2	4.4	6.8	8.0	0.37	1.20	0.38
		Outfall	220	50	11.2	4.4	6.8	8.0	0.33	0.80	0.35

Table A-3 (continued)

Plant	Season	Sample Location	Legionella Density (per mL)[a]	Viability (%)	Temperature (°C) Sample	Growth	Shock	pH	NH_4 (mg/L)	NO_3 (mg/L)	PO_4 (mg/L)
E	Spring	Intake	23000	16	12.7	12.7	0.0	10.6	0.39	.	35.00
		Intake	9400	44	12.7	12.7	0.0	10.6	0.33	.	11.20
		Intake	14800	7	12.7	12.7	0.0	10.6	0.36	.	14.70
		Postcondenser	480	29	28.0	12.7	15.3	8.8	0.41	.	24.00
		Outfall	380	24	21.2	12.7	15.3	8.9	0.33	.	22.90
		Outfall	230	18	21.2	12.7	15.3	8.9	0.35	.	31.00
		Outfall	280	48	21.2	12.7	15.3	8.9	0.42	.	39.50
E	Summer	Intake	250	9	22.8	22.8	0.0	8.9	0.45	0.03	11.20
		Intake	160	16	22.8	22.8	0.0	8.9	0.45	0.03	11.50
		Intake	490	11	22.8	22.8	0.0	8.9	0.45	0.03	12.00
		Outfall	170	19	23.1	.	.	8.5	0.32	0.03	25.75
		Outfall	330	9	23.1	.	.	8.5	0.32	0.03	25.75
		Outfall	220	4	23.1	.	.	8.5	0.35	0.03	27.00
E	Fall	Intake	64	35	18.4	18.4	0.0	7.4	0.45	0.08	0.37
		Intake	29	22	18.3	18.3	0.0	7.3	0.43	0.08	0.39
		Intake	29	11	18.3	18.3	0.0	7.4	0.45	0.08	0.36
		Precondenser	250	1	18.3	18.3	0.0	7.3	0.48	0.08	0.36
		Postcondenser	29	11	28.9	18.3	10.6	7.9	0.48	0.09	0.35
		Outfall	29	22	24.6	18.3	10.6	7.9	0.43	0.08	0.34
		Outfall	67	19	24.6	18.3	10.6	7.9	0.45	0.08	0.37
		Outfall	42	31	24.5	18.3	10.6	7.9	0.44	0.08	0.36
E	Winter	Intake	630	35	4.3	4.3	0.0	7.0	1.10	3.10	0.64
		Intake	1900	43	4.3	4.3	0.0	7.0	1.10	3.10	0.64
		Intake	1800	41	4.3	4.3	0.0	7.0	1.10	3.10	0.64
		Precondenser	490	41	6.2	4.3	0.0	7.0	1.28	4.40	0.78
		Postcondenser	160	47	24.7	4.3	18.5	7.0	1.18	3.50	0.78
		Outfall	120	36	11.9	4.3	18.5	7.0	1.18	3.10	0.68
		Outfall	2100	43	11.9	4.3	18.5	7.0	1.18	3.10	0.68
		Outfall	1900	37	11.9	4.3	18.5	7.0	1.18	3.10	0.68

Table A-3 (continued)

Plant	Season	Sample Location	Legionella Density (per mL)[a]	Viability (%)	Temperature (°C) Sample	Growth	Shock	pH	NH_4 (mg/L)	NO_3 (mg/L)	PO_4 (mg/L)
F	Spring	Intake	5400	18	11.2	11.2	0.0	8.9	0.62	1.90	0.24
		Intake	3800	10	11.2	11.2	0.0	8.9	0.65	1.80	0.23
		Intake	2700	8	11.2	11.2	0.0	8.9	0.67	1.90	0.24
		Precondenser	410	29	23.0	27.4	20.0	8.9	0.59	1.50	0.48
		Postcondenser	540	18	43.0	27.4	20.0	8.7	0.58	2.00	0.39
		Outfall	460	38	27.4	27.4	20.0	8.7	0.72	1.90	0.39
		Outfall	560	33	27.4	27.4	20.0	8.7	0.80	2.20	0.48
		Outfall	570	24	27.4	27.4	20.0	8.7	0.65	1.70	0.40
F	Summer	Intake	350	27	29.7	29.7	0.0	8.2	0.80	1.80	0.49
		Intake	280	35	29.7	29.7	0.0	8.2	0.66	1.80	0.48
		Intake	160	18	29.7	29.7	0.0	8.2	0.65	1.90	0.42
		Precondenser	20000	19	29.0	.	.	8.2	0.68	1.80	0.58
		Postcondenser	240	20	43.0	.	.	8.2	0.66	1.50	0.51
		Outfall	20	40	27.6	.	.	8.3	0.68	1.60	0.57
		Outfall	80	15	27.6	.	.	8.3	0.68	1.70	0.58
		Outfall	64	25	27.6	.	.	8.3	0.69	1.80	0.55
F	Fall	Intake	1	.	12.1	12.1	0.0	7.9	0.94	1.40	0.55
		Intake	74	4	12.1	12.1	0.0	7.9	0.96	1.30	0.52
		Intake	44	27	12.1	12.1	0.0	7.9	0.94	1.40	0.48
		Precondenser	6	.	23.0	27.7	16.0	7.9	0.79	1.90	0.64
		Postcondenser	130	18	39.0	27.7	16.0	8.3	0.79	1.90	0.57
		Outfall	38	17	27.7	27.7	16.0	8.2	0.68	1.80	0.57
		Outfall	6	.	27.7	27.7	16.0	8.2	0.68	1.90	0.57
		Outfall	1	.	27.7	27.7	16.0	8.2	0.68	1.80	0.60
F	Winter	Intake	680	50	0.0	0.0	0.0	8.0	0.88	2.30	0.46
		Intake	1300	12	0.0	0.0	0.0	7.9	0.59	2.30	0.46
		Intake	2100	20	0.0	0.0	0.0	7.9	0.64	2.20	0.61
		Postcondenser	1300	17	40.0	27.8	19.9	8.4	0.57	2.00	0.46
		Outfall	2800	13	27.8	27.8	19.9	8.4	0.63	2.30	0.48

Table A-3 (continued)

Plant	Season	Sample Location	Legionella Density (per mL)[a]	Viability (%)	Temperature (°C) Sample	Growth	Shock	pH	NH_4 (mg/L)	NO_3 (mg/L)	PO_4 (mg/L)
G	Spring	Intake	11000	32	4.4	4.4	0.0	8.8	0.36	2.50	0.05
		Intake	8600	40	4.4	4.4	0.0	8.8	0.40	2.70	0.06
		Intake	6700	13	4.4	4.4	0.0	8.8	0.38	2.70	0.05
		Precondenser	21000	20	17.8	17.8	16.1	8.3	0.39	3.80	0.98
		Postcondenser	15000	11	33.9	17.8	16.1	8.0	0.41	3.90	0.08
		Outfall	22000	33	17.8	17.8	16.1	8.6	0.42	3.90	0.08
		Outfall	23000	44	17.8	17.8	16.1	8.6	0.49	4.00	0.09
		Outfall	16000	8	17.8	17.8	16.1	8.6	0.41	3.80	0.11
G	Summer	Intake	270	5	23.3	23.3	0.0	7.4	0.42	0.90	0.26
		Intake	99	13	23.3	23.3	0.0	7.2	0.61	1.20	0.20
		Intake	180	12	23.3	23.3	0.0	7.1	0.60	1.20	0.23
		Precondenser	67	10	28.9	28.9	17.2	7.9	0.46	1.90	0.27
		Postcondenser	1	.	46.1	28.9	17.2	7.7	0.48	1.90	0.29
		Outfall	32	10	28.9	28.9	17.2	7.9	0.52	2.00	0.24
		Outfall	1700	9	24.4	24.4	0.0	7.7	0.38	1.10	0.22
		Outfall	40	0	28.9	28.9	17.2	7.8	0.57	2.20	0.30
G	Fall	Intake	58	22	21.0	21.0	0.0	6.6	.	.	.
		Intake	29	0	21.0	21.0	0.0	6.5	.	.	.
		Intake	48	0	21.0	21.0	0.0	6.6	.	.	.
		Precondenser	120	3	26.0	24.0	16.2	6.6	.	.	.
		Postcondenser	130	5	42.2	24.0	16.2	6.9	.	.	.
		Outfall	54	18	24.0	24.0	16.2	6.9	.	.	.
		Outfall	96	10	24.0	24.0	16.2	6.9	.	.	.
		Outfall	160	10	24.0	24.0	16.2	6.9	.	.	.
G	Winter	Intake	1100	57	4.4	4.4	0.0	7.1	0.48	2.00	0.40
		Intake	1100	47	4.4	4.4	0.0	7.1	0.48	1.90	0.35
		Intake	670	23	4.4	4.4	0.0	7.1	0.48	1.90	0.40
		Precondenser	1200	52	18.9	18.9	15.0	7.3	0.55	2.90	0.48
		Postcondenser	1300	14	33.9	18.9	15.0	8.0	0.58	3.20	0.37
		Outfall	1800	29	18.9	18.9	15.0	7.1	0.62	3.20	0.45
		Outfall	2100	44	18.9	18.9	15.0	7.1	0.61	3.10	0.43
		Outfall	1800	62	18.9	18.9	15.0	7.1	0.61	3.10	0.40

Table A-3 (continued)

Plant	Season	Sample Location	Legionella Density (per mL)[a]	Viability (%)	Temperature (°C) Sample	Growth	Shock	pH	NH_4 (mg/L)	NO_3 (mg/L)	PO_4 (mg/L)
H	Spring	Intake	10000	5	10.8	10.8	0.0	8.7	0.63	0.75	0.10
		Intake	9400	4	10.8	10.8	0.0	8.7	0.65	0.80	0.12
		Intake	11000	3	10.8	10.8	0.0	8.7	0.65	0.40	0.11
		Precondenser	480	8	22.2	20.6	13.3	8.7	2.60	5.50	1.25
		Postcondenser	240	11	35.5	20.6	13.3	8.0	2.50	4.00	0.76
		Outfall	410	3	20.6	20.6	13.3	8.0	2.60	6.10	1.95
		Outfall	150	6	20.6	20.6	13.3	8.0	2.60	6.10	1.35
		Outfall	220	6	20.6	20.6	13.3	8.0	2.70	6.00	1.22
H	Summer	Intake	100	4	24.2	24.2	0.0	7.6	0.68	0.23	0.48
		Intake	870	0	24.2	24.2	0.0	7.6	0.68	0.15	0.38
		Intake	980	0	24.2	24.2	0.0	7.6	0.66	0.23	0.52
		Precondenser	290	4	29.0	27.7	12.0	7.3	2.40	3.50	1.85
		Postcondenser	320	0	41.0	27.7	12.0	7.2	2.40	4.33	1.55
		Outfall	16	0	27.7	27.7	12.0	7.2	2.30	3.80	1.75
		Outfall	60	0	27.7	27.7	12.0	7.2	2.40	3.60	3.20
		Outfall	44	9	27.7	27.7	12.0	7.2	2.40	4.00	1.80
H	Fall	Intake	99	33	9.4	9.4	0.0	7.8	0.45	0.90	0.30
		Intake	3	.	9.4	9.4	0.0	7.8	0.08	0.90	0.29
		Intake	46	27	9.4	9.4	0.0	7.8	0.45	0.80	0.26
		Precondenser	49	28	24.0	21.8	13.0	7.8	1.20	4.50	1.25
		Postcondenser	41	25	37.0	21.8	13.0	7.6	1.22	3.20	0.88
		Outfall	22	66	21.8	21.8	13.0	7.6	1.22	2.90	1.02
		Outfall	10	.	21.8	21.8	13.0	7.6	1.24	3.30	0.88
		Outfall	29	0	21.8	21.8	13.0	7.6	1.21	3.10	0.96
H	Winter	Intake	5100	41	3.6	3.6	0.0	7.6	0.53	1.10	0.41
		Intake	3800	23	3.6	3.6	0.0	7.6	0.57	1.10	0.39
		Postcondenser	490	33	35.1	18.6	13.8	7.6	1.72	4.40	0.55
		Outfall	140	29	18.6	18.6	13.8	7.6	1.83	4.40	0.98

Table A-3 (continued)

Plant	Season	Sample Location	Legionella Density (per mL)[a]	Viability (%)	Temperature (°C) Sample	Growth	Shock	pH	NH_4 (mg/L)	NO_3 (mg/L)	PO_4 (mg/L)
I	Spring	Intake	14000	2	11.5	11.5	0.0	8.5	0.65	1.00	0.17
		Intake	9000	9	11.5	11.5	0.0	8.5	0.63	0.75	0.15
		Intake	11000	4	11.5	11.5	0.0	8.5	0.59	0.80	0.16
		Precondenser	350	47	22.2	28.4	13.4	8.5	0.51	1.40	0.16
		Postcondenser	220	44	35.6	28.4	13.4	8.6	0.65	1.20	0.15
		Outfall	360	42	34.6	28.4	13.4	8.6	0.55	1.20	0.13
		Outfall	140	16	34.6	28.4	13.4	8.6	0.53	1.30	0.13
		Outfall	170	34	34.6	28.4	13.4	8.6	0.51	1.10	0.13
I	Summer	Intake	330	16	23.7	23.7	0.0	7.7	0.57	1.20	0.44
		Intake	280	9	23.7	23.7	0.0	7.7	0.58	1.10	0.43
		Intake	370	14	23.7	23.7	0.0	7.7	0.57	1.60	0.57
		Precondenser	24	0	27.8	26.2	9.4	7.7	0.73	0.04	0.34
		Postcondenser	56	0	37.2	26.2	9.4	7.4	0.55	0.04	0.34
		Outfall	160	5	24.7	26.2	9.4	7.8	0.59	1.60	0.55
		Outfall	160	10	24.7	26.2	9.4	7.8	0.55	1.80	0.58
		Outfall	76	11	24.7	26.2	9.4	7.8	0.52	1.00	0.30
I	Fall	Intake	320	10	10.4	10.4	0.0	8.2	0.45	1.10	0.29
		Intake	96	13	10.4	10.4	0.0	8.2	0.48	0.90	0.29
		Intake	700	17	10.4	10.4	0.0	8.2	0.42	0.90	0.30
		Postcondenser	54	0	29.1	23.2	11.7	6.8	0.39	0.70	0.28
		Outfall	45	29	29.1	23.2	11.7	6.8	0.49	0.70	0.34
		Outfall	64	20	29.1	23.2	11.7	6.8	0.42	0.80	0.27
		Outfall	55	16	29.1	23.2	11.7	6.8	0.42	0.60	0.32
I	Winter	Intake	13	.	2.0	2.0	0.0	8.4	0.56	0.88	0.38
		Intake	67	31	2.0	2.0	0.0	8.4	0.56	0.88	0.30
		Intake	54	32	2.0	2.0	0.0	8.4	0.58	0.79	0.38
		Postcondenser	61	0	23.4	15.8	13.4	8.6	0.43	1.02	0.41
		Outfall	51	25	21.6	15.8	13.4	8.6	0.42	1.00	0.51

[a]The value 1 indicates that Legionella density in the sample was below the limit of detection.

Table A-4

PHASE I DATA: ADDITIONAL CHEMICAL PARAMETERS

Plant	Season	Sample Location	Total Carbon (mg/L)			Dissolved Oxygen (mg/L)	Alkalinity (mg $CaCo_3$/L)	Conductivity (µS/cm)	Chlorination
			Total	Organic	Inorganic				
A	Spring	Intake	6.2	3.1	3.1	10.84	9.2	103	0
		Intake	5.8	2.8	3.0	10.84	9.2	103	0
		Intake	6.2	2.4	3.8	10.84	9.2	103	0
		Precondenser	6.7	3.6	3.1	10.51	.	110	0
		Postcondenser	7.5	4.8	2.7	9.44	.	92	0
		Outfall	5.4	2.8	2.6	9.52	10.3	89	0
		Outfall	5.5	2.4	3.1	9.52	10.3	89	0
		Outfall	8.2	5.3	2.9	9.52	10.3	89	0
A	Summer	Intake	10.8	7.9	2.9	8.24	13.8	68	0
		Intake	8.0	4.9	3.1	8.30	21.2	69	0
		Intake	8.0	5.0	3.0	8.31	15.6	78	0
		Precondenser	11.7	8.5	3.2	8.29	14.7	63	0
		Postcondenser	8.7	5.7	3.0	6.90	20.2	82	0
		Outfall	8.3	5.2	3.1	7.20	16.6	75	0
		Outfall	9.1	5.8	3.3	6.95	16.6	81	0
		Outfall	10.6	7.6	3.0	7.20	18.9	80	0
A	Fall	Intake	5.7	2.0	3.7	8.60	13.3	93	0
		Intake	5.0	1.5	3.5	8.60	16.6	93	0
		Precondenser	7.4	4.3	3.1	8.60	12.0	93	0
		Postcondenser	9.0	5.1	3.9	7.20	18.4	87	0
		Outfall	5.2	1.6	3.6	7.75	15.2	88	0
		Outfall	5.3	1.7	3.6	7.74	15.6	88	0
		Outfall	5.1	1.5	3.6	7.75	16.1	88	0
A	Winter	Intake	9.7	6.6	3.1	13.70	17.9	78	0
		Intake	9.7	6.6	3.1	13.70	17.9	78	0
		Intake	9.7	6.6	3.1	13.70	17.9	78	0
		Precondenser	7.9	4.3	3.6	13.70	18.4	78	0
		Postcondenser	8.7	5.3	3.4	12.30	17.0	77	0
		Outfall	7.7	4.6	3.1	12.30	16.6	77	0
		Outfall	7.7	4.6	3.1	12.30	16.6	77	0
		Outfall	7.7	4.6	3.1	12.30	16.6	77	0

Table A-4 (continued)

Plant	Season	Sample Location	Total Carbon (mg/L) Total	Organic	Inorganic	Dissolved Oxygen (mg/L)	Alkalinity (mg $CaCo_3$/L)	Conductivity (µS/cm)	Chlorination
B	Spring	Intake	3.5	2.1	1.4	10.26	10.5	53	0
		Intake	2.9	1.4	1.5	10.26	10.5	53	0
		Intake	3.1	1.7	1.4	10.26	10.5	53	0
		Precondenser	2.3	1.1	1.2	10.04	.	44	0
		Postcondenser	7.5	6.4	1.1	9.52	.	43	0
		Outfall	6.7	5.7	1.0	9.64	12.9	43	0
		Outfall	3.5	2.1	1.4	9.64	12.9	43	0
		Outfall	2.9	1.4	1.5	9.64	12.9	43	0
B	Summer	Intake	5.3	3.6	1.7	5.95	17.5	30	0
		Intake	5.5	3.4	2.1	6.01	13.3	30	0
		Intake	5.8	3.7	2.1	6.10	19.8	29	0
		Precondenser	8.0	6.2	1.8	5.98	16.6	32	0
		Postcondenser	5.5	3.8	1.7	7.19	16.6	30	0
		Outfall	7.8	5.9	1.9	5.79	19.3	29	0
		Outfall	5.3	3.5	1.8	6.08	17.9	29	0
		Outfall	4.8	3.0	1.8	7.10	18.9	29	0
B	Fall	Intake	3.1	1.3	1.8	7.66	16.1	30	0
		Intake	2.8	1.0	1.8	7.67	14.3	30	0
		Intake	2.7	0.8	1.9	7.70	16.6	30	0
		Precondenser	4.8	3.0	1.8	7.67	15.6	30	0
		Postcondenser	2.9	1.1	1.8	7.40	15.2	30	0
		Outfall	3.8	1.7	2.1	7.88	11.0	30	0
		Outfall	2.7	0.9	1.8	7.88	10.6	30	0
		Outfall	2.5	0.6	1.9	7.70	11.5	30	0
B	Winter	Intake	3.4	1.5	1.9	11.85	40.0	29	0
		Intake	3.4	1.5	1.9	11.85	40.0	29	0
		Intake	3.4	1.5	1.9	11.85	40.0	29	0
		Precondenser	5.8	4.0	1.8	11.85	21.2	29	0
		Postcondenser	3.3	1.4	1.9	11.10	36.3	28	0
		Outfall	2.6	0.6	2.0	11.10	20.7	28	0
		Outfall	2.6	0.6	2.0	11.15	20.7	28	0
		Outfall	2.6	0.6	2.0	11.10	20.7	28	0

Table A-4 (continued)

Plant	Season	Sample Location	Total Carbon (mg/L) Total	Organic	Inorganic	Dissolved Oxygen (mg/L)	Alkalinity (mg $CaCo_3$/L)	Conductivity (µS/cm)	Chlorination
C	Spring	Intake	7.2	2.6	4.6	11.43	27.6	127	0
		Intake	11.2	6.8	4.4	11.43	27.6	127	0
		Intake	7.9	3.4	4.5	11.43	27.6	127	0
		Precondenser	11.6	6.9	4.7	11.83	15.2	120	0
		Postcondenser	7.2	2.6	4.6	11.20	18.4	124	3
		Outfall	6.9	2.1	4.8	10.90	28.5	124	3
		Outfall	7.6	2.4	5.2	10.90	28.5	124	3
		Outfall	7.1	2.3	4.8	10.90	28.5	124	3
C	Summer	Intake	9.3	7.5	3.8	9.61	191.8	131	0
		Intake	13.4	6.0	7.4	9.30	197.3	134	0
		Intake	10.0	6.1	3.9	8.91	192.7	127	0
		Precondenser	13.7	7.5	6.2	9.30	197.8	135	0
		Postcondenser	9.3	5.3	4.0	7.20	191.4	124	3
		Outfall	9.7	3.7	4.0	7.50	184.9	123	3
		Outfall	9.5	6.6	2.9	7.50	181.7	120	3
		Outfall	10.1	5.8	4.3	7.66	190.9	123	3
C	Fall	Intake	22.3	0.6	21.7	9.10	120.1	264	0
		Intake	23.0	0.4	22.6	9.05	118.7	264	0
		Intake	22.8	1.2	21.6	9.06	116.8	264	0
		Precondenser	23.7	3.0	20.7	9.05	116.8	264	0
		Postcondenser	23.1	1.9	21.2	9.42	120.1	269	3
		Outfall	24.0	1.2	22.8	9.30	122.4	268	3
		Outfall	25.5	3.9	21.6	9.30	133.4	268	3
		Outfall	23.1	0.9	22.2	9.28	133.4	268	3
C	Winter	Intake	3.0	1.3	1.7	14.98	12.4	65	0
		Intake	3.0	1.3	1.7	14.98	12.4	65	0
		Intake	3.0	1.3	1.7	14.98	12.4	65	0
		Precondenser	5.6	4.0	1.6	14.98	6.9	65	0
		Postcondenser	3.2	1.6	1.6	14.30	10.1	65	3
		Outfall	2.7	1.0	1.7	14.30	11.5	65	3
		Outfall	2.7	1.0	1.7	14.30	11.5	65	3
		Outfall	2.7	1.0	1.7	14.30	11.5	65	3

Table A-4 (continued)

Plant	Season	Sample Location	Total Carbon (mg/L) Total	Organic	Inorganic	Dissolved Oxygen (mg/L)	Alkalinity (mg $CaCo_3$/L)	Conductivity (µS/cm)	Chlorination
D	Spring	Intake	74.0	44.0	30.0	10.94	220.8	353	0
		Intake	82.0	54.0	28.0	10.94	125.6	353	0
		Intake	72.0	46.0	26.0	10.94	124.7	353	0
		Precondenser	71.0	40.0	31.0	11.23	.	354	0
		Outfall	73.0	45.0	28.0	11.06	127.9	354	3
		Outfall	70.0	43.0	27.0	11.06	118.7	354	3
		Outfall	72.0	43.0	29.0	11.06	132.9	354	3
D	Summer	Intake	48.1	23.0	25.1	9.02	207.5	273	0
		Intake	42.6	18.6	24.0	9.02	207.9	273	0
		Intake	45.2	19.6	25.6	9.02	209.3	273	0
		Precondenser	40.3	17.2	23.1	9.37	206.1	273	0
		Postcondenser	43.4	18.5	24.9	8.70	206.5	286	3
		Outfall	41.3	17.5	23.8	8.75	207.0	286	3
		Outfall	46.6	22.1	24.5	8.75	187.7	286	3
		Outfall	45.7	19.9	25.8	8.75	211.1	286	3
D	Fall	Intake	40.0	7.0	33.0	10.65	110.9	138	0
		Intake	44.0	11.0	33.0	10.65	107.2	138	0
		Intake	41.0	9.0	32.0	10.65	105.8	138	0
		Precondenser	34.0	18.0	16.0	10.65	114.5	138	0
		Postcondenser	41.0	25.0	16.0	10.37	109.0	299	3
		Outfall	38.0	22.0	16.0	10.37	106.3	299	3
		Outfall	41.0	25.0	16.0	10.37	107.2	299	3
		Outfall	46.0	30.0	16.0	10.37	101.7	299	3
D	Winter	Intake	30.0	5.0	25.4	11.15	104.9	136	0
		Intake	27.0	2.0	24.8	11.17	96.6	136	0
		Intake	27.0	2.0	24.6	11.00	101.2	136	0
		Precondenser	28.0	3.0	25.1	11.15	98.0	136	0
		Postcondenser	27.0	3.0	23.7	10.84	98.4	301	3
		Outfall	29.0	2.0	26.8	10.80	99.8	300	3

Table A-4 (continued)

Plant	Season	Sample Location	Total Carbon (mg/L) Total	Organic	Inorganic	Dissolved Oxygen (mg/L)	Alkalinity (mg $CaCo_3$/L)	Conductivity (µS/cm)	Chlorination
E	Spring	Intake	19.0	3.6	15.4	11.97	25.8	188	0
		Intake	6.8	3.3	3.5	11.97	25.8	188	0
		Intake	6.2	3.1	3.1	11.97	25.8	188	0
		Postcondenser	15.3	3.2	12.1	9.08	47.4	179	1
		Outfall	18.3	7.4	10.9	9.24	34.5	179	1
		Outfall	18.7	7.3	11.4	9.24	34.5	179	1
		Outfall	18.9	7.1	11.8	9.24	34.5	179	1
E	Summer	Intake	22.8	3.1	19.7	9.50	26.8	182	0
		Intake	20.3	8.3	12.0	9.50	26.8	183	0
		Intake	23.7	12.0	11.7	9.50	26.0	182	0
		Outfall	18.4	14.8	3.6	8.25	36.3	179	3
		Outfall	12.1	7.0	5.1	8.25	36.4	178	3
		Outfall	19.0	7.7	11.3	8.25	37.9	179	3
E	Fall	Intake	19.3	4.3	15.0	8.62	79.6	204	0
		Intake	16.7	3.7	13.0	8.63	74.1	205	0
		Intake	19.7	5.2	14.5	8.62	73.1	204	0
		Precondenser	19.7	6.1	13.6	8.63	76.4	204	0
		Postcondenser	17.1	3.8	13.3	8.10	71.8	206	1
		Outfall	17.7	3.7	14.0	8.80	70.8	206	1
		Outfall	18.3	4.5	13.8	8.81	73.6	206	1
		Outfall	17.7	3.3	14.4	8.81	75.4	206	1
E	Winter	Intake	15.6	6.7	8.9	14.78	38.6	120	0
		Intake	15.6	6.7	8.9	14.78	38.6	120	0
		Intake	15.6	6.7	8.9	14.78	38.6	120	0
		Precondenser	17.4	9.0	8.4	14.78	39.6	120	0
		Postcondenser	16.3	6.7	9.6	13.19	37.7	121	1
		Outfall	14.7	6.3	8.4	13.19	33.1	121	1
		Outfall	14.7	6.3	8.4	13.19	33.1	121	1
		Outfall	14.7	6.3	8.4	13.19	33.1	121	1

Table A-4 (continued)

Plant	Season	Sample Location	Total Carbon (mg/L) Total	Organic	Inorganic	Dissolved Oxygen (mg/L)	Alkalinity (mg $CaCo_3$/L)	Conductivity (µS/cm)	Chlorination
F	Spring	Intake	84.0	55.0	29.0	13.65	141.7	387	0
		Intake	84.0	53.0	31.0	13.65	148.1	387	0
		Intake	86.0	53.0	33.0	13.65	.	387	0
		Precondenser	106.0	72.0	34.0	13.70	.	391	1
		Postcondenser	100.0	65.0	35.0	7.35	.	465	1
		Outfall	98.0	63.0	35.0	7.20	162.8	462	1
		Outfall	100.0	63.0	37.0	7.20	.	462	1
		Outfall	100.0	62.0	38.0	7.20	160.1	462	1
F	Summer	Intake	136.0	98.0	38.0	7.12	311.0	438	0
		Intake	129.0	91.0	38.0	7.12	314.2	438	0
		Intake	127.0	92.0	35.0	7.12	297.6	438	0
		Precondenser	123.0	88.0	35.0	7.00	301.8	441	1
		Postcondenser	132.0	93.0	39.0	6.30	299.0	422	1
		Outfall	138.0	101.0	37.0	8.99	331.2	419	1
		Outfall	126.0	92.0	34.0	8.99	321.5	419	1
		Outfall	126.0	91.0	35.0	8.99	331.7	419	1
F	Fall	Intake	66.0	44.0	22.0	10.06	150.0	389	0
		Intake	66.0	44.0	22.0	10.06	152.7	389	0
		Intake	69.0	45.0	24.0	10.06	152.3	389	0
		Precondenser	75.0	52.0	23.0	10.06	154.6	389	1
		Postcondenser	70.0	42.0	28.0	6.77	154.6	427	1
		Outfall	69.0	41.0	28.0	7.97	157.3	419	1
		Outfall	66.0	39.0	27.0	7.97	160.1	419	1
		Outfall	69.0	41.0	28.0	7.97	157.8	419	1
F	Winter	Intake	49.0	12.0	37.0	12.09	150.9	395	0
		Intake	49.0	11.0	38.4	12.07	132.0	395	0
		Intake	50.0	9.0	40.8	12.33	172.0	395	0
		Postcondenser	52.0	9.0	43.4	9.09	163.8	492	1
		Outfall	56.0	10.0	45.9	9.10	177.1	492	1

Table A-4 (continued)

Plant	Season	Sample Location	Total Carbon (mg/L) Total	Organic	Inorganic	Dissolved Oxygen (mg/L)	Alkalinity (mg $CaCo_3$/L)	Conductivity (µS/cm)	Chlorination
G	Spring	Intake	6.5	3.0	3.5	.	14.7	.	0
		Intake	7.6	4.0	3.6	.	14.7	.	0
		Intake	12.1	8.6	3.5	.	14.7	.	0
		Precondenser	12.8	8.0	4.8	.	14.3	.	3
		Postcondenser	10.1	5.2	4.9	.	20.9	.	3
		Outfall	9.7	4.8	4.9	.	21.6	.	3
		Outfall	9.8	4.7	5.1	.	21.6	.	3
		Outfall	10.0	5.0	5.0	.	21.6	.	3
G	Summer	Intake	17.4	9.6	7.8	.	67.2	.	0
		Intake	15.5	9.9	5.6	.	50.1	.	0
		Intake	15.2	9.6	5.6	.	52.0	.	0
		Precondenser	35.4	21.5	13.9	.	118.2	.	3
		Postcondenser	32.3	18.7	13.6	.	115.9	.	3
		Outfall	31.4	19.2	12.2	.	108.6	.	3
		Outfall	18.8	9.9	8.9	.	76.4	.	3
		Outfall	30.5	16.3	14.2	.	60.7	.	3
G	Fall	Intake	.	.	.	.	.	.	0
		Intake	.	.	.	.	.	.	0
		Intake	.	.	.	.	.	.	0
		Precondenser	.	.	.	.	.	.	3
		Postcondenser	.	.	.	.	.	.	3
		Outfall	.	.	.	.	.	.	3
		Outfall	.	.	.	.	.	.	3
		Outfall	.	.	.	.	.	.	3
G	Winter	Intake	9.4	3.6	5.8	8.90	25.8	77	0
		Intake	9.3	3.7	5.6	8.90	25.3	77	0
		Intake	10.0	4.1	5.9	8.90	27.6	77	0
		Precondenser	17.8	7.1	10.7	9.00	44.2	185	3
		Postcondenser	14.4	5.0	9.4	8.75	42.8	200	3
		Outfall	20.5	9.1	11.4	8.85	46.9	170	3
		Outfall	15.9	5.3	10.6	8.85	49.2	170	3
		Outfall	14.1	4.7	9.4	8.85	47.4	170	3

Table A-4 (continued)

Plant	Season	Sample Location	Total Carbon (mg/L) Total	Organic	Inorganic	Dissolved Oxygen (mg/L)	Alkalinity (mg $CaCo_3$/L)	Conductivity (µS/cm)	Chlorination
H	Spring	Intake	97.0	67.0	30.0	11.60	150.9	310	0
		Intake	91.0	59.0	32.0	11.60	146.7	310	0
		Intake	90.0	58.0	32.0	11.60	134.8	310	0
		Precondenser	138.0	133.0	5.4	7.85	149.2	320	3
		Postcondenser	134.0	127.0	7.4	6.31	47.1	2580	3
		Outfall	130.0	121.0	9.0	8.25	43.2	2580	3
		Outfall	130.0	124.0	6.0	8.25	46.0	2580	3
		Outfall	132.0	126.0	6.0	8.25	45.1	2580	3
H	Summer	Intake	79.0	46.0	33.0	7.22	278.3	297	0
		Intake	72.0	39.0	33.0	7.22	282.4	297	0
		Intake	80.0	45.0	35.0	7.22	274.2	297	0
		Precondenser	143.0	133.0	10.0	7.28	114.1	317	3
		Postcondenser	129.0	119.0	10.0	6.44	153.6	1720	3
		Outfall	128.0	119.0	9.0	7.32	104.0	1720	3
		Outfall	141.0	131.0	10.0	7.32	113.6	1720	3
		Outfall	126.0	118.0	8.0	7.32	109.5	1720	3
H	Fall	Intake	71.0	46.0	25.0	10.95	146.3	301	0
		Intake	69.0	43.0	26.0	10.95	149.0	301	0
		Intake	68.0	43.0	25.0	10.95	163.3	301	0
		Precondenser	96.0	75.0	21.0	10.95	66.7	301	3
		Postcondenser	93.0	67.0	26.0	7.20	69.0	1745	3
		Outfall	95.0	72.0	23.0	9.15	81.0	1745	3
		Outfall	97.0	72.0	25.0	9.15	76.4	1745	3
		Outfall	97.0	72.0	25.0	9.15	76.8	1745	3
H	Winter	Intake	51.0	11.0	40.4	14.91	152.3	411	0
		Intake	47.0	12.0	35.0	14.91	147.7	411	0
		Postcondenser	77.0	51.0	25.9	8.30	90.2	1832	3
		Outfall	76.0	51.0	24.7	8.30	93.8	1832	3

Table A-4 (continued)

Plant	Season	Sample Location	Total Carbon (mg/L) Total	Organic	Inorganic	Dissolved Oxygen (mg/L)	Alkalinity (mg $CaCo_3$/L)	Conductivity (µS/cm)	Chlorination
I	Spring	Intake	81.0	50.0	31.0	10.40	125.1	316	0
		Intake	78.0	48.0	30.0	10.40	126.0	316	0
		Intake	79.0	49.0	30.0	10.40	122.4	316	0
		Precondenser	100.0	65.0	35.0	8.50	.	400	2
		Postcondenser	92.0	58.0	34.0	7.11	.	430	2
		Outfall	93.0	59.0	34.0	7.25	137.1	428	2
		Outfall	96.0	63.0	33.0	7.25	138.0	428	2
		Outfall	93.0	63.0	30.0	7.25	138.5	428	2
I	Summer	Intake	52.0	24.0	28.0	5.61	244.7	310	0
		Intake	61.0	28.0	33.0	5.61	236.4	310	0
		Intake	52.0	24.0	28.0	5.61	243.8	310	0
		Precondenser	73.0	47.0	26.0	8.10	259.0	391	1
		Postcondenser	69.0	44.0	25.0	6.00	238.3	265	1
		Outfall	53.0	26.0	27.0	7.15	201.5	278	1
		Outfall	55.0	28.0	27.0	7.15	201.9	278	1
		Outfall	50.0	22.0	28.0	7.15	199.6	278	1
I	Fall	Intake	47.0	13.0	34.0	10.16	104.9	124	0
		Intake	45.0	11.0	34.0	10.16	93.8	124	0
		Intake	48.0	14.0	34.0	10.16	97.1	124	0
		Postcondenser	60.0	26.0	34.0	8.19	125.1	412	2
		Outfall	59.0	26.0	33.0	8.23	130.6	410	2
		Outfall	59.0	26.0	33.0	8.23	120.1	410	2
		Outfall	59.0	23.0	36.0	8.23	123.7	410	2
I	Winter	Intake	47.0	12.0	35.0	11.51	195.9	316	0
		Intake	46.0	10.0	36.0	11.40	191.0	315	0
		Intake	47.0	11.0	36.0	11.40	201.5	315	0
		Postcondenser	60.0	16.0	34.0	9.00	180.0	435	1
		Outfall	63.0	19.0	34.0	9.10	193.7	430	1

Key to chlorination:

0 = No chlorination.
1 = Very infrequent chlorination; no chlorination within 30 d prior to sampling.
2 = Chlorination within 30 d but not within 2 d prior to sampling.
3 = Chlorination within 2 d prior to sampling.

Table A-5

PHASE I DATA: RESULTS OF SAMPLE INJECTIONS

Plant	Season	Sample Location	Infectivity[a]	Serotypes Isolated
A	Spring	Precondenser	-	
		Postcondenser	-	
A	Summer	Intake	+	Knox
		Precondenser	-	
		Postcondenser	-	
		Outfall	-	
		Outfall	+	Knox
A	Fall	Intake	+	Knox, *L. gormanii*
		Precondenser	+	LA
		Postcondenser	T	
		Outfall	+	Chic
A	Winter	Intake	-	
		Precondenser	-	
		Postcondenser	-	
		Outfall	+	*L. gormanii*
B	Spring	Precondenser	-	
		Postcondenser	-	
B	Summer	Intake	-	
		Precondenser	-	
		Postcondenser	-	
		Outfall	-	
		Outfall	-	
B	Fall	Intake	-	
		Precondenser	-	
		Postcondenser	-	
		Outfall	-	
B	Winter	Intake	-	
		Precondenser	-	
		Postcondenser	-	
		Outfall	T	
C	Spring	Precondenser	-	
		Postcondenser	-	
C	Summer	Intake	+	Knox
		Precondenser	-	
		Postcondenser	+	Chic
		Outfall	+	Chic
C	Fall	Intake	-	(Vibrio-like)
		Precondenser	-	
		Postcondenser	-	
		Outfall	-	(Vibrio-like)
C	Winter	Intake	-	
		Precondenser	+	Knox
		Postcondenser	-	
		Outfall	-	

Table A-5 (continued)

Plant	Season	Sample Location	Infectivity[a]	Serotypes Isolated
D	Spring	Intake	-	
		Precondenser	-	
		Outfall	-	
D	Summer	Intake	-	
		Precondenser	-	
		Postcondenser	-	
		Outfall	+	LA
D	Fall	Intake	-	
		Precondenser	+	Chic, LA
		Postcondenser	-	
		Outfall	-	
D	Winter	Intake	-	
		Precondenser	-	
		Postcondenser	+	LA, New sp?
		Outfall	-	
E	Spring	Intake	C,T	
		Intake	C,T	
		Intake	-	
		Postcondenser	-	
		Outfall	+	Knox
E	Summer[b]	Intake	-	
		Outfall	-	
E	Fall	Intake	-	
		Precondenser	+	OR10
		Postcondenser	-	
		Outfall	-	
E	Winter	Intake	-	
		Precondenser	C,T	
		Postcondenser	+	LA
		Outfall	T	
F	Spring	Intake	T	
		Intake	T	
		Intake	T	
		Precondenser	-	
		Postcondenser	-	
		Outfall	+	OR10
		Outfall	+	OR10
		Outfall	-	
F	Summer[c]	Intake	C	
		Precondenser	C	
		Postcondenser	C	
		Outfall	+	Knox

Table A-5 (continued)

Plant	Season	Sample Location	Infectivity[a]	Serotypes Isolated
F	Fall	Intake	+	LA, New sp.?
		Precondenser	-	
		Postcondenser	+	Knox, Bloom
		Outfall	+	LA, OR10
F	Winter	Intake	-	
		Postcondenser	+	Knox, Chic
		Outfall	+	OR10
G	Spring	Intake	C,T	
		Precondenser	+	Bloom, OR10
		Postcondenser	+	Knox, OR10
		Outfall	+	OR10
		Outfall	+	Knox, OR10
		Outfall	+	LA, Knox
G	Summer	Intake	+	Knox, Chic
		Intake	-	
		Precondenser	+	
		Postcondenser	C,T	
		Outfall	+	OR10
		Outfall	+	Chic
G	Fall	Intake	-	
		Precondenser	-	
		Postcondenser	-	
		Outfall	+	Knox, OR10
		Outfall	+	OR10
G	Winter	Intake	+	L. bozemanii
		Precondenser	+	OR10
		Postcondenser	+	Knox
		Outfall	+	Chic
H	Spring	Intake	-	
		Intake	-	
		Intake	-	
		Precondenser	C	
		Postcondenser	T	
		Outfall	T	
H	Summer	Intake	C	
		Precondenser	-	
		Postcondenser	+	LA
H	Fall	Intake	-	
		Intake	+	LA
		Precondenser	T	
		Postcondenser	+	LA
		Outfall	+	Chic, LA

Table A-5 (continued)

Plant	Season	Sample Location	Infectivity[a]	Serotypes Isolated
H	Winter	Intake	+	LA
		Postcondenser	C,T	
		Outfall	+	LA
I	Spring	Intake	-	
		Intake	-	
		Precondenser	+	Knox
		Postcondenser	C,T	
		Outfall	T	
I	Summer	Precondenser	+	Knox, Chic
		Postcondenser	+	Knox
I	Fall	Intake	-	
		Postcondenser	+	Togus
		Outfall	+	Knox
I	Winter	Intake	+	LA
		Postcondenser	-	
		Outfall	-	

[a]Key to infectivity:

+ = Sample infectious.
- = Sample noninfectious.
C = Sample contaminated, *Legionella* infectiousness could not be determined.
T = Sample toxic, infectiousness could not be determined.

[b]Plant was shut down. These results were not included in the statistical analyses.

[c]Plant was operating in mixed mode. These results were not included in the statistical analyses.

EPRI

EPRI EA-3153
RP1909-1
Interim Report
June 1983

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Contractors: Oak Ridge National Laboratory, University of Tennessee, Savannah River Laboratory

This report discusses Phase I of a two-phase study on Legionnaires' Disease Bacteria. This half of the study presents the findings of a survey of the distribution, density, viability, and infectivity of *Legionella* in power plant cooling systems. Findings from nine power plants are presented. In addition, a species identified at two sites is discussed as well as the identification of two other organisms found to cause illness in guinea pigs. 118 pp.

EPRI Project Manager: J. S. Mattice

Cross-References:
1. EPRI EA-3153 2. RP1909-1 3. Ecological Studies Program 4. *Legionella*

ELECTRIC POWER RESEARCH INSTITUTE
Post Office Box 10412, Palo Alto, CA 94303 415-855-2000

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This report discusses Phase I of a two-phase study on Legionnaires' Disease Bacteria. This half of the study presents the findings of a survey of the distribution, density, viability, and infectivity of *Legionella* in power plant cooling systems. Findings from nine power plants are presented. In addition, a species identified at two sites is discussed as well as the identification of two other organisms found to cause illness in guinea pigs. 118 pp.

EPRI Project Manager: J. S. Mattice

Cross-References:
1. EPRI EA-3153 2. RP1909-1 3. Ecological Studies Program 4. *Legionella*

ECOLOGICAL STUDIES PROGRAM

EPRI

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LEGIONELLA

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