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Presence of Pathogenic Microorganisms in Power Plant Cooling Waters
Final Report for October 1, 1981, to June 30, 1983

R. L. Tyndall

ENVIRONMENTAL SCIENCES DIVISION
Publication No. 2208

Prepared for
Paul Hayes, Project Representative
Environmental Effects Research Branch
U.S. Nuclear Regulatory Commission
Under Interagency Agreement DOE-40-550-75

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PRESENCE OF PATHOGENIC MICROORGANISMS IN POWER PLANT COOLING WATERS

FINAL REPORT FOR OCTOBER 1, 1981, to JUNE 30, 1983

R. L. Tyndall*

ENVIRONMENTAL SCIENCES DIVISION
Publication No. 2208

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Task: Pathogenic Microorganisms in Closed-Cycle Cooling Systems

OAK RIDGE NATIONAL LABORATORY
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SUMMARY

Air was sampled at the point of discharge and at short distances downwind and upwind from industrial and power plant cooling towers. Both high-volume electrostatic and impinger type samplers were used. Concentrates of the air samples were analyzed for Legionnaires' Disease Bacteria (LDB). In some cases, the samples were also tested for the presence of free-living amoebae. The concentrations of LDB in the air samples were well below the minimal infectious dose for guinea pigs and precluded testing of the samples for infectious LDB. Results of LDB analysis were related to the meteorological conditions at the time of sampling. Generally, the concentrations of LDB in the air at the discharge of the cooling towers were 1×10^{-6} to 1×10^{-7} of that found in comparable volumes of tower basin water. During periods of high humidity and wind speed, LDB was detected in a few downwind samples and one upwind sample. One site with extensive construction and excavation activity had higher LDB concentrations in air samples relative to other sites. Nonpathogenic *Naegleria* were present in one of two air samples taken in the mist at the base of a natural-draft cooling tower.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. The concentration of Legionnaires' Disease Bacteria (LDB) in air discharged from cooling towers was generally 1×10^{-6} to 1×10^{-7} that found in comparable volumes of basin water.

2. The proximity of air samples to source waters containing LDB during periods of high wind speeds may explain the presence of LDB in some upwind and downwind air samples.

3. Air samples of about 1.5×10^5 to 2.0×10^5 L were considered an adequate test sample relative to the approximately 3×10^3 L inhaled by individuals during an 8-h period.

4. Free-living amoebae such as *Naegleria* can be detected in cooling tower mists such as those generated at the base of natural-draft cooling towers.

Recommendations

1. Even though concentrations of LDB in air were determined solely by the fluorescent antibody technique on a limited number of samples, the number of LDB in discharged air and the dilution expected from the Gaussian distribution of the plume downwind from the cooling towers did not indicate a need for further reduction of LDB concentrations outside the test plant environs.

2. Based on a limited number of samples, the data indicate that industrial hygiene measures may be appropriate for in-plant personnel where exposure to higher concentrations of LDB or pathogenic amoebae could occur during maintenance of cooling water systems.

ABSTRACT

TYNDALL, R. L. 1983. Presence of pathogenic microorganisms in power plant cooling waters: Final Report for October 1, 1981, to June 30, 1983. ORNL/TM-3364; NUREG/CR-8809. Oak Ridge National Laboratory, Oak Ridge, Tennessee. 29 pp.

The content of Legionnaires' Disease Bacteria (LDB) in air discharged from industrial and power plant cooling towers was compared with the concentrations found in the cooling-tower basin water. Concentrates of the power plant air samples were also tested for the presence of free-living amoebae. The content of LDB in basin water was generally 1×10^6 to 1×10^7 times greater than that found in comparable volumes of discharge air. The content of LDB in air samples correlates with other sampling data that indicate that the amount of water discharged in cooling-tower plumes is generally one part of water by volume to 1×10^7 to 1×10^8 parts of air. Nonpathogenic *Naegleria* were isolated from one air sample taken in the mist generated at the base of a natural-draft cooling tower.

INTRODUCTION

Legionnaires' Disease initially attained notoriety after an outbreak in 1976 in which a number of fatalities occurred in Philadelphia. The causative agent of Legionnaires' Disease (LD) (i.e., *Legionella pneumophila*) and other *Legionella* species (LDB) are normal components of the aquatic microflora (Tison et al. 1980; Tyndall 1982a; Fliermans et al. 1981). To date, eight different species of *Legionella* have been described (Brenner et al. 1979; Brenner et al. 1980; Cherry et al. 1982; England et al. 1980; Hebert et al. 1980; McKinney et al. 1980; McKinney et al. 1981; Morris et al. 1980; Orrison et al. 1983). *Legionella pneumophila* can cause epidemics of pneumonia (LD) or a non-pneumonic combination of fever, headache, and myalgia (Pontiac fever). Aerosolization of contaminated water appears to be a prime method of epidemic spread of the organism. Common-source outbreaks of LD have occurred in a pattern that suggests airborne spread of aerosol nuclei such as that generated by air-conditioning cooling towers. Indeed, studies of the source of infection of some LD outbreaks at times have implicated cooling towers associated with air-conditioning systems as the dispersal vehicles (Dondero et al. 1980; Deubner and Gilliam 1977). Known outbreaks have occurred in hospitals, hotels, a university student center, and the New York garment district (Dondero et al. 1980; Cordes et al. 1980; Politi et al. 1979).

Previous studies of electric power plants focused on the relationship of thermal discharges and the presence of pathogenic free-living amoebae and LDB (Tyndall et al. 1981a; Tyndall 1982b; Tyndall et al. 1981b). Pathogenic amoebae were present in thermal discharges of many northern plants but were not detected in source waters. However, pathogenic amoebae have been found in ambient southern waters. LDB was found in both source water and cooling waters of electric power plants. This is not unexpected because the ubiquity of LDB in most, if not all, natural surface waters has been amply demonstrated (Tison et al. 1980; Tyndall 1982a; Fliermans et al. 1981; Christensen et al. 1983).

These studies have confirmed that LDB is a normal component of the aquatic microflora. As an aquatic bacterium, LDB poses little threat to public health because the organism cannot infect unless inhaled. However, the introduction of cooling-tower technology and concomitant aerosolization of thermally altered water has altered the usually benign aquatic state of LDB. Studies of large industrial towers using wastewater showed that a variety of bacteria were aerosolized and discharged via the tower plume including *Escherichia*, *Shigella*, *Yersinia*, and *Serratia* (Adams and Lewis 1979).

Little information is available, however, on the discharge of LDB in the plumes of industrial cooling towers such as those of electric power plants. Because LDB is found in most ambient surface waters and many tap waters, the organism will be introduced into cooling towers and, indeed, has been demonstrated to exist in most cooling towers that have

been examined. The concentrations of *Legionella* in cooling-tower waters generally range from 10^4 to 10^6 per liter of water (Tyndall 1982a; Tyndall 1982b).

To determine if and to what degree LDB is discharged from large industrial cooling towers, including those of electric power plants, air sampling was initiated. Samples were taken at the point of discharge and at short distances downwind and upwind from the towers. Concentrations of LDB in air samples were then compared to those found in the basin water and related to meterological conditions at the time of sampling.

MATERIALS AND METHODS

Sites

Three industrial air-conditioning cooling towers in the Oak Ridge, Tennessee, area were studied. The tower at site A is a mechanical-draft tower (Fig. 1). The tower has fiberglass fill and a circulation flow rate of 6.0×10^3 gal (2.27×10^4 L) per minute. The circulating water is treated twice weekly with a combination of quaternary amines and tributyltin.

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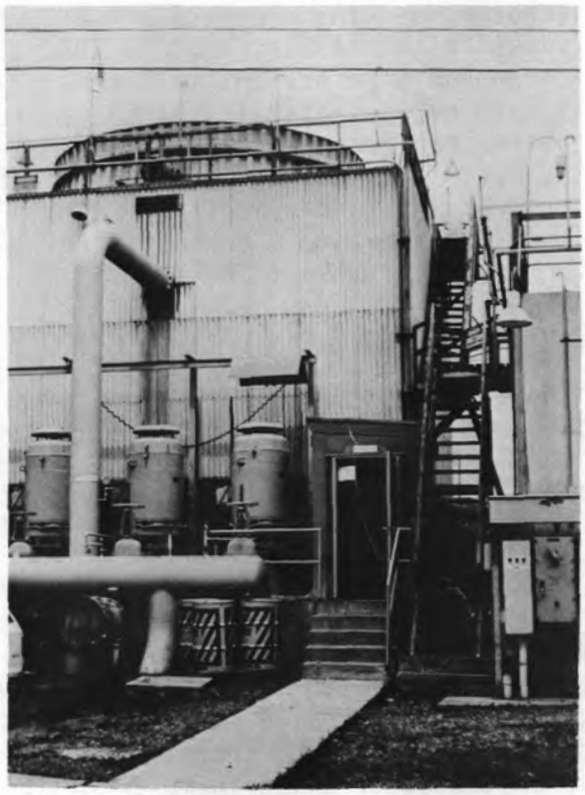

Fig. 1. Mechanical-draft cooling tower at site A.

The tower at site B (Fig. 2) is also a mechanical-draft design having asbestos fill and an all-metal squirrel-cage blower for air movement, rather than a fan as found in most mechanical-draft towers. Water was circulated at a rate of 400 gal (1.51×10^3 L) per minute. Biocide treatment consisted of twice weekly applications of quaternary amines and aliphatic amines.

A large mechanical-draft tower at site C (Fig. 3) had a circulation rate of 3.0×10^3 to 1.6×10^4 gal (1.14×10^4 to 6.06×10^4 L) of water per minute. The tower had redwood fill and was treated twice weekly with quaternary amines and aliphatic amines. Potable water was used for makeup water at tower A through C. Drift eliminators were used on all of the towers.

Three power plant cooling towers were also studied. The tower at site D (Fig. 4) was a natural-draft tower having an asbestos fill. The water is circulated at a rate of approximately 5.0×10^5 gal (1.89×10^6 L) per minute and is treated twice daily with 1 μg/mL of chlorine. The tower at site E (Fig. 5) was also a natural-draft type having asbestos fill and a circulation rate of 2.5×10^5 gal (9.46×10^5 L) per minute and treated twice daily with 2 μg/mL of chlorine. The mechanical-draft towers at power plant site F (Fig. 6) contained fiberglass fill material and received no biocide treatment. The power plant at site F had five separate towers, each having from 11 to 14 fans per tower. Water was circulated through each tower at a rate of 3.0×10^5 gal (1.14×10^6 L) per minute. Source water for the tower at site D was from a freshwater lake and at sites E and F was from rivers.

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Fig. 2. Mechanical-draft cooling tower at site B.

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Fig. 3. Mechanical-draft cooling tower at site C.

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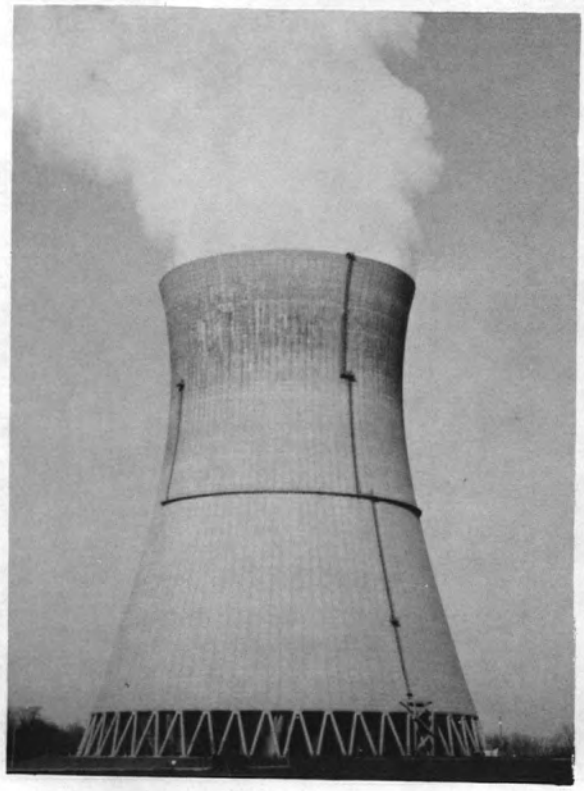

Fig. 4. Natural-draft cooling tower at site D.

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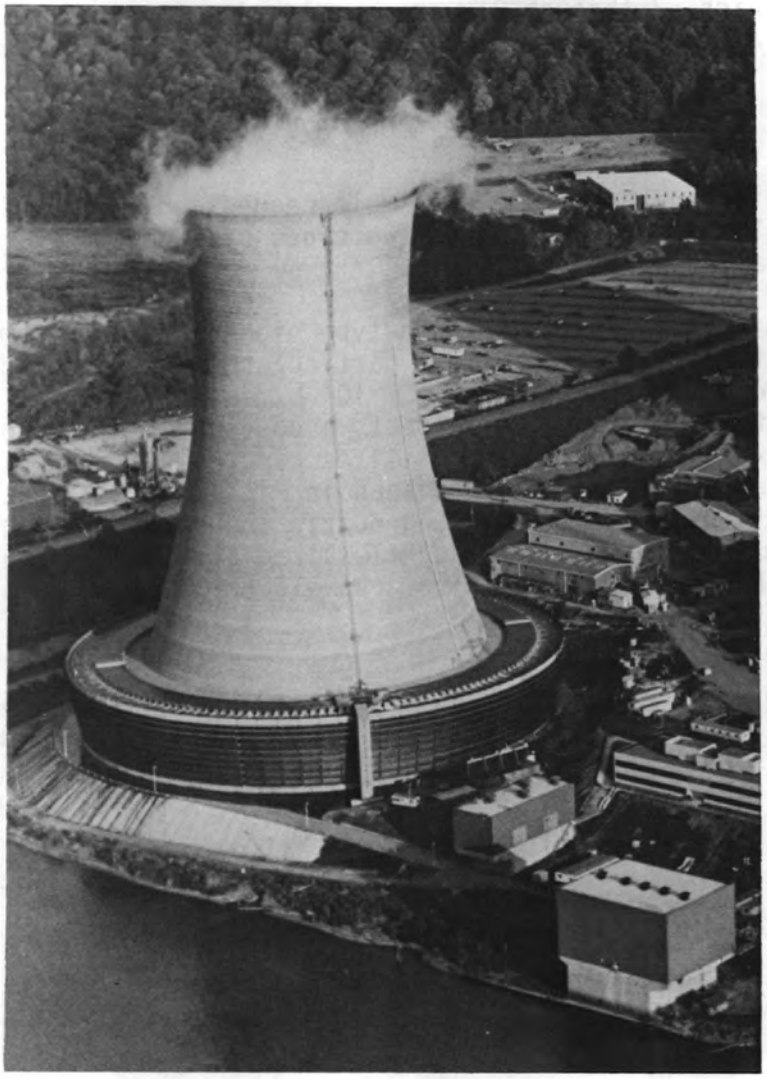

Fig. 5. Natural-draft cooling tower at site E.

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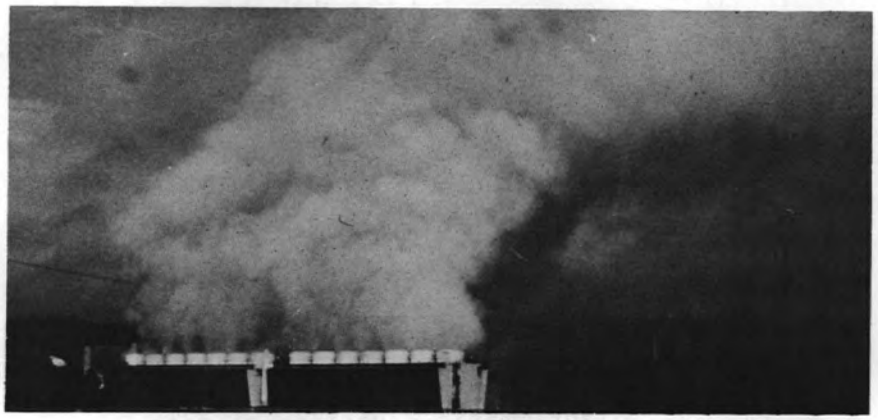

Fig. 6. Mechanical-draft cooling tower at site F.

Drift eliminators were used in all of the power plant towers. Towers at all sites (Figs. 7 through 9) were sampled in cooperation with the environmental control specialist at each facility.

Samplers

Depending on the volume of sample needed and the physical constraints of the sampling location, one of two types of samplers (high-volume or impinger) was used.

The Sci-Med Model M-3A Litton-type high-volume air sampler (Figs. 10 and 11) is designed to collect particulate matter continuously from a large sample of air (maximum of 1×10^3 L/min) and deposit it into a small amount of liquid. This effects a concentration factor on the order of 5×10^5. The sampler utilizes electrostatic precipitation to separate particles from air. As seen in Fig. 10, the aerosol is drawn into the unit through a converging nozzle and passes through the center of a high-voltage plate. It then flows radially between this plate and a lower, rotating collection disc. An electric potential of 15 kV is

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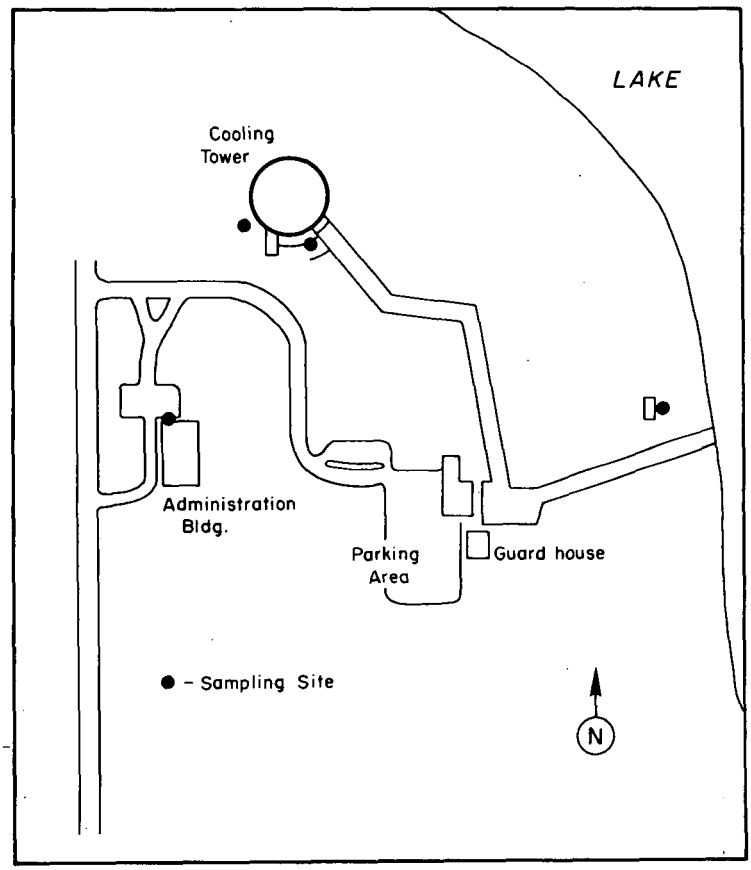

Fig. 7. Diagrammatic depiction of site D.

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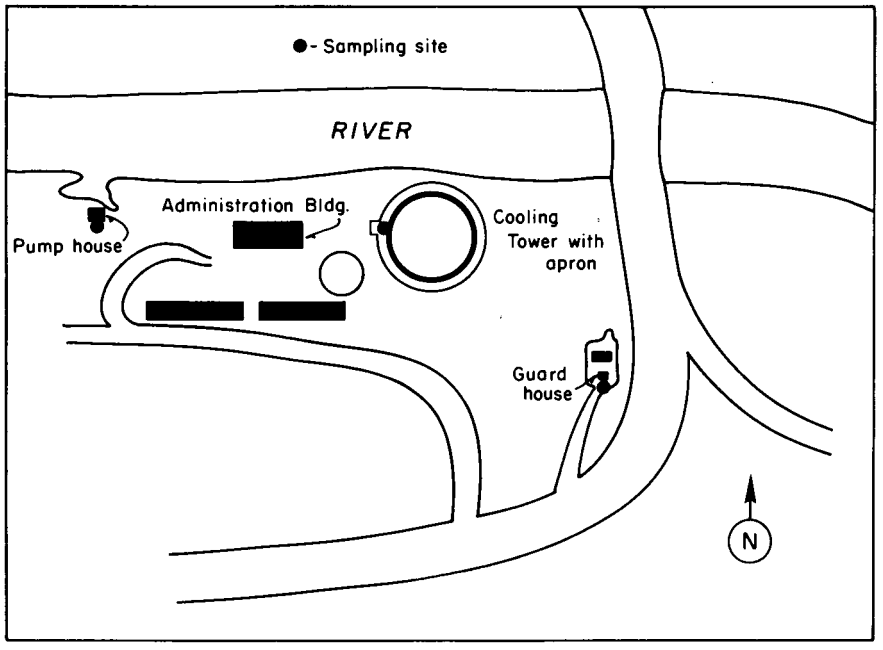

Fig. 8. Diagrammatic depiction of site E.

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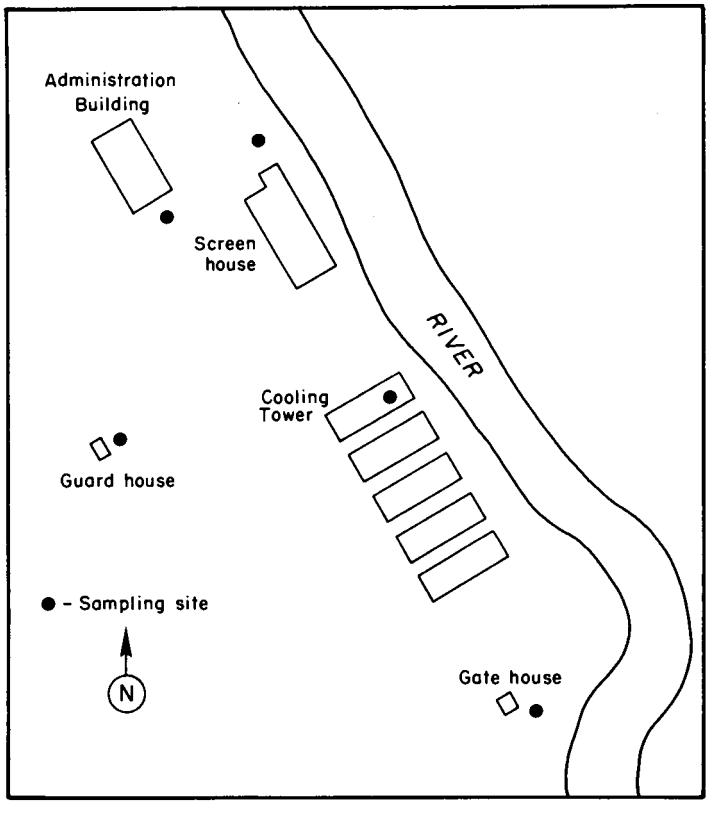

Fig. 9. Diagrammatic depiction of site F.

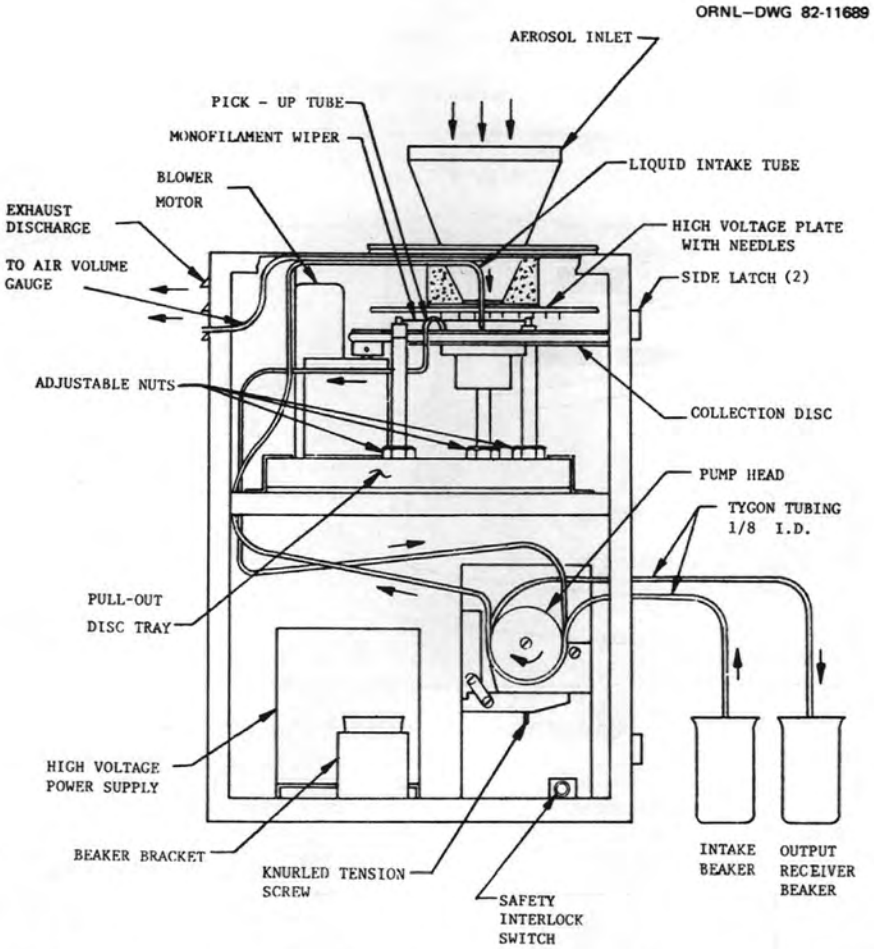

Fig. 10. Diagrammatic view of the high-volume air sampler.

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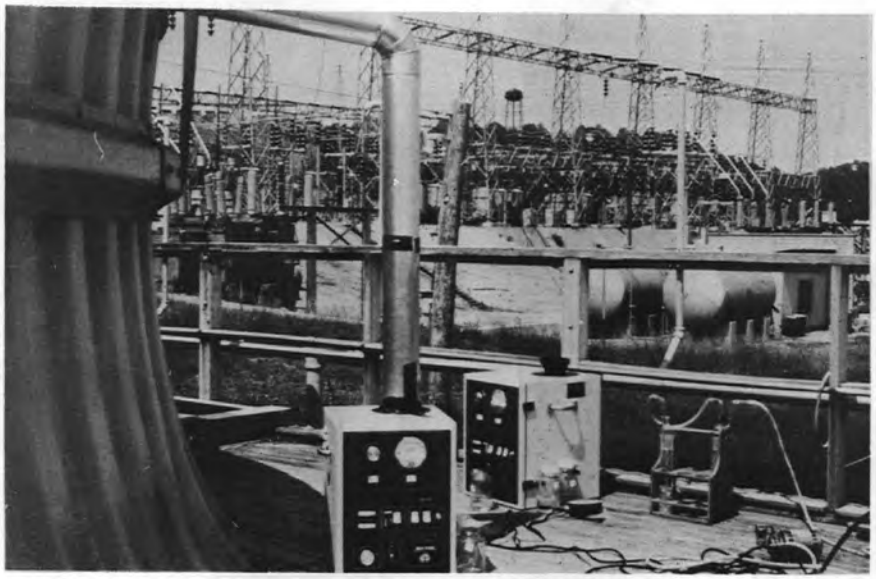

Fig. 11. High-volume sampler showing extension used for sampling air at the point of discharge.

maintained across the space between the plate and the disc and creates two effects: (1) a corona is emitted from a ring of 60 needles located concentric to the air inlet so that particles (including bacteria) exposed to air ions created from the corona acquire an electric charge and (2) the electric field provides the driving force to precipitate charged particles onto the lower disc. Liquid is pumped onto the center of the collection disc and, because of the centrifugal force, forms a thin moving film over the entire disc surface. A monofilament wiper is stretched across the disc to ensure an even distribution of liquid on the disc. The liquid at the edge of the disc is then removed by a pick-up tube and is pumped to a receiver located outside the sampler for analysis of *Legionella* content. This sampler has been successfully used in isolating micrococci, streptococci, and other bacteria from cooling tower aerosols (Adams and Lewis 1979). When used in the current study, the samplers were adjusted to collect 800 L of air per minute, according to data supplied by the manufacturer. At this sampling rate, the collection efficiency is 70% or higher for particles as small as 0.1 μm in diameter. LDB are generally 0.5 to 0.7 μm wide and from 2.0 to 20.0 μm long. Prior to each sampling period, the sampler tubing was decontaminated with 70% ethanol and given two 30- to 60-min rinses with distilled water. The last rinse was collected and analyzed to ensure the absence of LDB prior to sampling. Without these precautions LDB could contaminate the sampler tubing and the subsequent air sample. Air samples were collected for 3 to 4 h during which time the approximately 1.4×10^5 to 1.9×10^5 L of air was sampled. When air was sampled at the point of discharge from the mechanical-draft cooling towers at site A, B, and C, the sampling rate was adjusted to approximately the velocity of the discharged air. The velocity of air discharged from the mechanical-draft cooling towers at site F was greater than the velocity of intake air of the sampler.

The Greenberg-Smith impinger air sampler is a simple vacuum-driven device capable of processing approximately 25 L of air per min (Fig. 12). The "inhaled" air is passed through sterile filtered water such that the bacteria remain in the water phase. A second reservoir is connected in tandem with the first; any bacteria inadvertently carried through the first collector will be collected in the second. The samplers were run for 3 to 4 h, processing approximately 4.5×10^3 to 6.0×10^3 L of air. After sampling, the two 150-mL water reservoirs were combined and analyzed for *Legionella*. The flow rate of the air sampler was calibrated by means of a rotameter. According to the manufacturer, efficiency of particle collection for the impinger samplers was approximately 80%. After each use of the impinger, reservoirs were throughly cleaned. Immediately before use, the reservoirs were rinsed with sterile distilled water. The rinse water was analyzed to confirm the absence of LDB.

Legionella Analysis

At test sites, both air and water samples were collected. After collection, the samples were brought to the laboratory for analysis.

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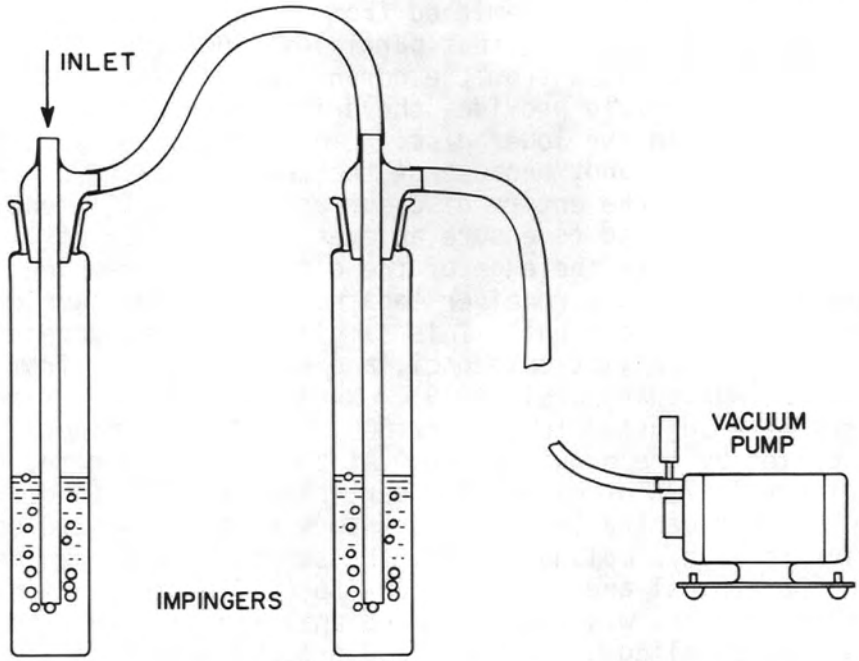

Fig. 12. Diagrammatic representation of an impinger sampler.

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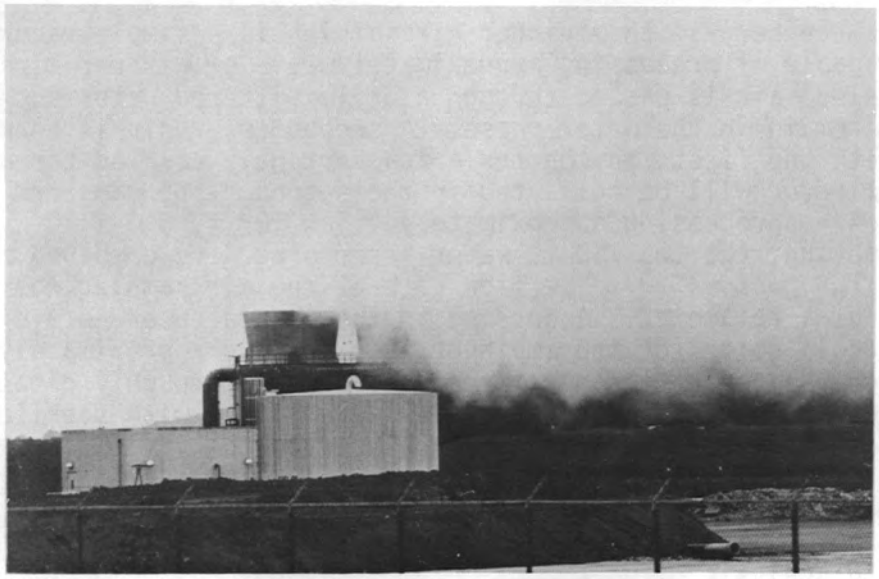

Fig. 13. Plume from cooling towers remaining at ground level, similar to conditions found at plant F during the September 1981 sampling period.

Water was centrifuged (Sorvall SS3) at 4000 *g* for 45 min at room temperature to concentrate suspended microorganisms. Aliquots of the centrifugates were examined by the direct fluorescent antibody test (DFA) using antisera specific for LDB. Subsamples (0.010 mL) were pipetted into predesignated 7-mm diameter wells on toxoplasmosis slides (Cel-Line Associates, Minotala, New Jersey). Negative controls were fluorescein-conjugated sera prepared from the preimmunization sera of rabbits later immunized with the Knoxville strain (serogroup 1) of *L. pneumophila*. Test and control antisera were supplied by the Centers for Disease Control (Atlanta, Georgia). Samples were viewed by epifluorescence microscopy. Fluorescing cells having morphological characteristics of *L. pneumophila* were counted in 50 fields at 1000X magnification. The number of LDB detected by each of three polyvalent antisera was determined, and the total was normalized to the number per 100 L of unconcentrated sample. The three polyvalent antisera detect the serotypes of *L. pneumophila*, *L. gormanii*, *L. dumoffii*, *L. micdadei*, *L. bozemanii*, and *L. longbeachae*.

Animal Studies

The low numbers of LDB present in the air sample concentrates precluded the usual method of infectivity testing by intraperitoneal inoculation of guinea pigs. Testing of air samples for concentrations and serotypic profiles of LDB left relatively little material for animal testing. Attempts were made to inoculate guinea pigs intranasally with some samples in hopes that this route of inoculation would be more sensitive to infection with low numbers of LDB. In such tests, Hartley or American breeds of guinea pigs were anesthetized with Nembutal. Approximately 0.25 mL of air sample concentrate was placed on the nares and subsequently inhaled by the anesthesitized animals. Rectal temperatures were taken daily and animals showing temperature rise and signs of disease such as lethargy, ruffled fur, etc., were sacrificed and lung tissue was removed. The tissue was tested for LDB by fluorescent microscopy using specific anti-LDB antisera and by plating on charcoal-yeast extract (CYE) agar plates.

Identification of Amoebae

Aliquots of air sample concentrates from the power plant sites were also placed on nonnutrient agar plates seeded with *E. coli*. Plates were incubated at 37 and 44°C and observed daily for outgrowth of amoebae. *Naegleria* were identified in the samples by (1) their ability to grow at a temperature of 44°C, (2) their ability to flagellate, (3) their trophozoite and cyst morphology, and (4) their capacity to produce acute encephalitis in mice.

The flagellation tests were performed at 37°C. After harvesting, a drop of trophozoites suspended in sterile distilled water was placed on a coverslip and inverted on a cavity slide (hanging-drop technique). The hanging drops were examined under a phase-contrast microscope for the presence of flagellates after 30 min and 1, 2, and 3 h. Morphologic

characteristics typical of the *Naegleria* trophozoite and cyst (i.e., eruptive-like formation of the pseudopodia and smooth cyst wall, respectively) were taken into consideration for identifying amoebae belonging to the genus *Naegleria*.

To determine pathogenicity, amoebae growing at 45°C were harvested from the plates, washed, suspended in sterile distilled water, and intranasally instilled in weanling mice.

RESULTS

The results of this study show that air discharged from the cooling towers contain minimal numbers of *Legionella*. As seen in Tables 1 through 7, the concentration of *Legionella* per 100 L of air generally ranged from less than one to several hundred, whereas the cooling tower water generally had *Legionella* concentrations 1×10^6 to 1×10^7 times that found in the air.

The concentration of LDB per 100 L of air collected with high-volume samplers at the discharge of the two large, mechanical-draft industrial cooling towers ranged from 1.7 to 51 (Tables 1 and 3 and Figs. 1 and 3). The concentration of LDB in the corresponding cooling waters from the tower basins ranged from approximately 8×10^6 to 8×10^9 per 100 L. Thus, the ratio of LDB per unit volume of air at the point of discharge was generally only 1×10^{-6} to 1×10^{-8} that found in the water.

The concentration of LDB in 100 L of air sampled at the point of discharge from a third, smaller mechanical-draft cooling tower was approximately 300 relative to about 1×10^9/100 L of water (Table 2). As opposed to towers at sites A and C, the draft in the smaller cooling tower (site B, Fig. 2) is generated by a rotary blower rather than by a large fan. As in the towers at sites A and C, the ratio of LDB in air is only about 1×10^{-7} that found in the basin water.

Like the industrial test towers, the towers at power plant F are also of the mechanical-draft type. The concentration of LDB in air samples taken at the point of discharge was approximately 3 to 6 per 100 L of air. No LDB could be detected in the downwind air samples at power plant F in the July 1982 samples. The bacteria could be detected in the downwind air samples in September 1981 when the relative humidity was approximately 96% and the plume could be seen to reach ground level in the area of the sampler. LDB was also detected in one of the downwind air samples and one upwind sample in the October 1982 samples. As in the case of the mechanical-draft industrial cooling towers, the content of LDB in air samples was only about 1×10^{-7} or 1×10^{-8} that found in the basin water (Table 6 and Fig. 6).

The large natural-draft cooling towers at power plants D and E (Figs. 4 and 5) could not be sampled at the point of discharge from the towers. The tower at plant E, however, did have an apron of turbulent heated water surrounding the tower such that the mist emanating from the

Table 1. Concentration of LDB at industrial site A

Sample type and location	Type of air sampler	LDB/100 L of air or water
	5/6/82	
Air, base of tower	LVAS[a]	2.5×10^0
Air, top of tower proximal to discharge	LVAS	1.1×10^1
Water, basin	NA[b]	9.9×10^8
	5/14/82	
Air, top of tower, proximal to discharge	LVAS	8.0×10^0
	IMP[c]	<1.0[d]
Air, top of tower discharge	LVAS	3.1×10^1
Water, basin	NA	8.0×10^9
	5/18/82	
Air, top of tower proximal to discharge	LVAS	3.5×10^0
	IMP	<1.0[d]
Air, top of tower, discharge[e]	LVAS	5.1×10^1
	5/20/82	
Air, top of tower proximal to discharge	LVAS	<1.0[d]
	IMP	<1.0[d]
Air, top of tower, discharge[e]	LVAS	7.0×10^0
	5/21/82	
Air, top of tower	LVAS	3.3×10^1
Air, top of tower, proximal to discharge	LVAS	1.5×10^2
	IMP	<1.0[d]
Air, top of tower, proximal to discharge	LVAS	2.0×10^0
Air, top of tower, discharge		6.7×10^0
Water, basin	NA	8.0×10^6

[a]LVAS = Large-volume air sampler (Litton-type).

[b]NA = Not applicable.

[c]IMP = Impinger air sampler.

[d]<1.0 = below the limits of detection.

[e]Pipe extended from top of LVAS into the discharge from the tower.

Table 2. Concentration of LDB at industrial site B for August 18, 1982

Sample type and location	Type of air sampler	LDB/100 L of air or water
Air, mist at tower discharge	IMP[a]	3.2×10^2
Air, base of tower	IMP	3.4×10^1
Air, downwind (∿50 m)	LVAS[b]	4.8×10^0
Water, basin	NA[c]	8.8×10^8

[a]IMP = Impinger air sampler.

[b]LVAS = Large-volume air sampler (Litton-type).

[c]NA = Not applicable.

Table 3. Concentration of LDB at industrial site C

Sample type and location	Type of air sampler	LDB/100 L of air or water
	4/15/82	
Top of tower	IMP	3.2×10^1
	4/21/82	
Air, base of tower	LVAS[a]	<1.0[b]
Air, top of tower, discharge[c]	LVAS	1.7×10^0
Water, basin	NA[d]	5.1×10^7

[a]LVAS = Large-volume air sampler (Litton-type).

[b]<1.0 = below the limits of detection.

[c]Pipe extended from top of LVAS into the discharge from the tower.

[d]NA = Not applicable.

Table 4. Concentration of LDB at power plant site D

Sample type and location	Type of air sampler	LDB/100 L of air or water
	11/8/82	
Air, upwind (∿2000 m)	LVAS[a]	LA[b]
Air, downwind (∿15 m)	IMP[c]	<1.0[d]
Mist, base of tower (∿7 m)	LVAS	8.1×10^0
	11/9/82	
Air, downwind (∿2000 m)	LVAS	1.5×10^1
Mist, base of tower	IMP	<1.0[d]
Mist, base of tower	LVAS	3.7×10^0
Water, basin	NA[e]	4.0×10^6
	4/16/83	
Air, upwind (∿2000 m)	IMP	<1.0[d]
Air, downwind (∿2000 m)	IMP	<1.0[d]
Air, base of tower (∿7 m)	LVAS	<1.0[d]
Water	NA	3.4×10^8

[a]LVAS = Large-volume air sampler (Litton-type).

[b]LA = Lab accident. Analysis of rinse water from sampler indicates that the tubing was contaminated with LDB.

[c]IMP = Impinger air sampler.

[d]<1.0 = below limits of detection.

[e]NA = not applicable.

water could be sampled. The air samples from this mist in 1982 contained LDB at concentrations ranging from 100 to several thousand per 100 L of air (Table 5). Most of the downwind air samples at site E also contained LDB in concentrations ranging from approximately 10 to several thousand per 100 L of air. Surprisingly, some upwind samples at site E in 1982 also contained LDB, ranging from approximately 2 to 500 LDB per 100 L of air. As opposed to the mechanical-draft towers, the concentrations of LDB in air relative to comparable volumes of basin water were higher at site E in the 1982 samples. The air samples generally contained 1×10^{-4} to 1×10^{-6} the concentration of LDB found in tower basin water (Table 5). In the 1983 samples, the concentrations of LDB in air samples were reduced to levels comparable to those at other test sites.

Table 5. Concentration of LDB at power plant site E

Sample type and location	Type of air sampler	LDB/100 L of air or water
	8/10/82	
Air, upwind (∿500 m)	LVAS[a]	4.8×10^2
Air, downwind (∿500 m)	LVAS	5.8×10^3
Air, tower apron[b]	IMP[c]	1.9×10^3
Water, basin		5.5×10^7
	8/11/82	
Air, upwind (∿500 m)	LVAS	2.4×10^2
Air, downwind (∿500 m)	LVAS	2.1×10^3
Air, tower apron	IMP	5.5×10^3
Water, basin	NA[d]	1.2×10^7
	9/20/82	
Air, upwind (∿500 m)	LVAS	2.0×10^0
Air, downwind (∿500 m)	LVAS	1.4×10^2
Air, tower apron	IMP	1.0×10^2
Water, basin	NA	5.3×10^6
	9/21/82	
Air, upwind (∿500 m)	LVAS	2.0×10^0
Air, downwind (∿500 m)	LVAS	8.0×10^0
Air, tower apron	IMP	1.4×10^2
Water, basin	NA	9.3×10^6
	5/9/83	
Air, upwind (∿900 m)	LVAS	18×10^0
Air, downwind (∿500 m)	LVAS	12×10^0
Air, tower apron	IMP	<1.0[e]
Water, apron	NA	5.1×10^8
Water, basin	NA	2.4×10^8

[a]LVAS = Large-volume air sampler (Litton-type).

[b]An apron of heated water with associated mist was located partway up the side of the tower.

[c]IMP = Impinger air sampler.

[d]NA = not applicable.

[e]<1.0 = below the limits of detection.

Table 6. Concentration of LDB at power plant site F

Sample type and location	Type of air sampler	LDB/100 L of air or water
	9/17/81	
Downwind (∿500 m)	IMP[a]	8.1×10^0
Basin water	NA[b]	1.6×10^8
	7/19/82	
Upwind (∿1000 m)	LVAS[c]	<1.0[d]
Downwind (∿1000 m)	LVAS	<1.0[d]
Downwind (∿500 m)	IMP	<1.0[d]
Makeup water	NA	1.9×10^8
	7/20/82	
Upwind (∿1000 m)	LVAS	<1.0[d]
Downwind (∿1000 m)	LVAS	<1.0[d]
Downwind (∿500 m)	IMP	<1.0[d]
Basin water	NA	1.5×10^8
	10/20/82	
Air, top of tower, discharge	LVAS[c]	2.8×10^0
Air, downwind (∿1000 m)	IMP	1.3×10^1
Air, upwind (∿1000 m)	LVAS	<1.0[d]
	10/21/82	
Air, top of tower, discharge	LVAS	6.1×10^0
Air, downwind (∿1000 m)	LVAS	<1.0[d]
Air, upwind (∿1000 m)	IMP	3.4×10^0
Water, makeup	NA	1.0×10^8
Water, basin	NA	1.2×10^8

[a]IMP = Impinger air sampler.

[b]NA = Not applicable.

[c]LVAS = Large-volume air sampler (Litton-type).

[d]Pipes extended from top of LVAS into the discharge from the tower.

Table 7. Average[a] concentration of *Legionella* in air and water samples from all locations

Site	Average concentration[b] of *Legionella* for all dates sampled				
	Basin water	Air			
		Tower discharge	Tower basin	Downwind	Upwind
A	5.0×10^8	1.7×10^1	*c*	*c*	*c*
B	8.8×10^8	3.2×10^2	3.4×10^1	4.8×10^0	*c*
C	5.1×10^7	1.7×10^0	$<1.0 \times 10^0$	*c*	*c*
D	4.0×10^6	*c*	6.0×10^6	8.0×10^0	<1.0[d]
E	1.8×10^7	*c*	1.9×10^2[e]	2.0×10^2	1.2×10^2
F	1.5×10^8	4.5×10^0	*c*	3.0×10^0	0.8×10^0

[a]Towers at sites B and C only sampled one time.

[b]Concentration per 100 L of air or water.

[c]Not tested.

[d]<1.0 = below limit of detection.

[e]Mist emanating from an apron of heated water on the side of the natural-draft tower about 100 ft from the base of the tower.

An unscheduled outage at site D occurred coincident with the initiation of the November 1982 sampling. Although water circulation through the natural-draft tower continued during the outage and the mist formation at the base of the tower was continuously generated, the heated visible plume discharged from the top of the tower rapidly dissipated. Although LDB could not be detected in the downwind sample on the first sampling day, some LDB was detected in the mist at the base of the tower (Table 4 and Fig. 4). Sampling on the second day of the outage was carried out in a light rainfall and 20 MPH winds. Samples taken under these conditions showed some LDB in both tower mist and downwind samples. Concentrations of LDB in these samples was 3 and 15 LDB per 100 L of air, respectively, compared to 4×10^6 LDB per 100 L of tower water. Little if any LDB could be detected in the 1983 air samples at site D.

Attempts to culture LDB directly from air sample concentrates by plating on CYE agar were unsuccessful because the agar plates were quickly overgrown by bacteria other than LDB. Similarly, intranasal inoculation of guinea pigs with air sample concentrates also resulted in infection of lung tissues with bacteria other than LDB (Table 8).

Of the air sample concentrates from the power plants, only mist samples from site D in 1982 were positive for free-living amoebae. Amoebic outgrowth was evident on plates seeded with the mist sample.

Table 8. Results of intranasal inoculation of guinea pigs with air sample concentrates

Site	Sample type	Approx. No. of LDB inoculated	Days before sacrifice[a]	Analysis of lung tissue	
				CYE[b]	FA[c]
NA[d]	*L. pneumophila* (serotype L)	1×10^6	7	+ LDB	+
B	Discharge air	8×10^0	7	+ non-LDB[e] - LDB	-
B	Air-mist proximal to discharge	4×10^1	7	-	-
A	Discharge air	2×10^1	4	+ non-LDB - LDB	-
A	Basin water	1×10^5	4	+ non-LDB - LDB	-

[a]Number of days between inoculation and sacrifice of test animals.

[b]Results of culturing lung tissue on CYE agar plates.

[c]Analysis of lung tissue by fluorescent antibody test using specific anti-*Legionella* antisera.

[d]NA = Not applicable. Culture of *L. pneumophila* inoculated intranasally into guinea pigs to demonstrate subsequent presence and isolatability from lung tissue.

[e]+ = positive; - = negative.

The trophozoite, cyst morphology, and ability to grow at 44°C indicated the presence of *Naegleria*. A positive flagellation test confirmed the amoebae as *Naegleria*. Intranasal inoculation of mice with the *Naegleria* did not produce encephalitis, indicating the isolate was not the pathogenic *N. fowleri*.

DISCUSSION

It is interesting that several of the air samples having the highest concentrations of *Legionella* were those in which the samplers were placed in mist rising from heated water rather than from air being rapidly discharged from fan-driven mechanical-draft towers. Such was the case with the industrial tower at site B. In contrast to the other industrial towers, air is discharged from this tower with minimal force by means of a squirrel-cage blower. Similarly, relatively high concentrations of *Legionella* were found in samples taken from the cooling-tower apron of the power plant at site E from samplers also placed in the mist emanating from turbulent heated waters.

Why some of the other air samples taken at site E also had higher *Legionella* concentrations than other sites is unclear. Considering the low wind speed, the sampling of upwind air may have been too close to the tower to serve as a proper control. Nevertheless, the concentrations of *Legionella* in the downwind samples, as well as some upwind samples, are orders of magnitude higher than those in air samples taken at other sites. Perhaps the mist emanating from the tower apron could have contributed to the concentration of LDB in the air samples. Another possible explanation is that, of all the test sites, only site E had extensive construction in progress. This is of interest because it has been suggested that some outbreaks of Legionnaires' Disease might be associated with excavation (Thacker et al. 1978). That excavation contributed to the increased concentrations of LDB is indicated by the decrease in LDB in air samples collected in 1983, when excavation was no longer in progress (Table 5).

Alternatively, *Legionella* concentrations in air samples taken from the discharge of the mechanical-draft tower at site F may be somewhat diminished because of increased air velocity past the orifice of the sampler. Unlike the sampling of the industrial towers, the velocity of air discharged from the power plant tower was greater than the intake velocity of the sampler.

Meterological effects (Tables 9 and 10) could have accounted for detection of LDB in some samples. The LDB detected in the downwind impinger sampler in the September 1981 tests at plant F were taken when the humidity was high and the plume was obviously touching the ground similar to that seen in Fig. 13. Also, the control sample at the October 21 sampling at plant F was taken in high winds within 100 m downwind of the river when white caps were visible and aerosols were undoubtedly being generated. Thus, LDB in the source river water could have been aerosolized and inhaled by the control, upwind air samplers.

With the exception of site E, the concentration of LDB found in 100 L of air discharged from cooling towers was approximately 1×10^{-6} to 1×10^{-7} that found in 100 L of basin water. Evidence that this ratio of LDB in air relative to basin water reflects the amount of water in the aerosolized discharge is indicated by the work of Shofner et al. (1975; Shofner 1983). Sampling of various industrial cooling towers showed that the weight of water in air discharged from cooling towers ranged from 10^{-3} g/m^3 to 10^{-1} g/m^3. Thus, relative to the density of water (i.e., 10^6 g/m^3), the amount of water in the tower discharge averaged approximately 1 part in 1×10^8 parts of air. These experimental results may explain the relatively low concentrations of LDB detected in 100 L of air discharged from cooling towers in the current study.

The relative scarcity of LDB in the air samples made determinations of viability and infectivity difficult. Plating of the air sample concentrates on CYE agar with or without antibiotics resulted in overgrowth of the agar plates by predominately gram-negative bacteria other than *Legionella*. Similarly, intranasal inoculation of guinea pigs also

Table 9. Meterological conditions at power plant sites D and E

Site	Date	Average temperature (°C)	Conditions during sampling period		
			Relative humidity (%)	Wind[a] speed (m/s)	Wind direction
D	11/8/82	11.1	65.0	5.8	West
D	11/9/82	6.7	91.0	8.4	East
D	4/16/83	23.9	60.0	4.1	Southwest
E	8/10/82	21.6	62.5	2.5	Northwest
E	8/11/82	19.1	71.2	1.2	West
E	9/20/82	19.7	37.2	3.0	Southwest
E	9/21/82	13.6	91.6	1.4	West
E	5/9/83	8.9	26.2	6.8	North
E	5/10/83	12.8	22.0	4.1	Northwest

[a]75 m above the ground.

Table 10. Meterological conditions at power plant F

Date	Average temperature (°C)	Conditioning during sampling period		
		Relative humidity (%)	Wind speed (m/s)	Wind direction
9/17/81	17.6	96.1	1.4	Southeast
7/19/82	29.4	44.2	1.6	North
7/20/82	25.2	68.5	2.3	Northwest
10/20/82	17.4	53	4.1	South
10/21/82	10.8	39	5.9	Northwest

ORNL PHOTO 3617-83

Fig. 14. Mist at base of cooling tower at site D from which *Naegleria* were isolated.

resulted in gross pathology of the exposed lung tissue, which upon plating on CYE agar yielded gram-negative bacteria such as *Pseudomonas* and *Proteus* (Table 8). Because of these difficulties, determination of viability and infectivity of the LDB was not possible and concentration of LDB could only be determined by FA analysis.

Although the amount of LDB per 100 L of discharged air is small, the total LDB discharged per hour in large cooling towers is considerable. For instance, each of the five large mechanical towers at power plant F discharges approximately 1.5×10^6 ft^3/min. If 100 L of air from these contains only five LDB, an hour's discharge would approximate 13×10^7 LDB per operating tower. Conversely, the dilution effects by the Gaussian distribution of the discharged air and the deleterious effects of sunlight can be expected to substantially dilute and reduce the content of viable LDB in discharged air (ONRR-DSE 1976). In addition, available data relating to dilution of cooling water discharge indicates an approximate hundredfold dilution of particles in air 1×10^3 m vs 1×10^4 m downwind from the cooling towers such that the content of viable LDB per unit volume of air beyond the plant environs would be greatly diminished relative to that about the towers (ONRR-DSE 1976).

There is currently insufficient evidence to relate levels of LDB in air with risk of human infection. The amount of inhaled LDB necessary for causing human infection is unknown but would depend in part on the virulence of the LDB population and the susceptibility of the host.

As evidenced in the LD outbreak in Pontiac, Michigan, guinea pigs have been shown susceptible to LDB when placed in an environment in which humans have been infected (Glick et al. 1978). Studies with healthy guinea pigs indicate the 50% lethal dose of highly virulent LDB via inhalation is approximately 1.4×10^5 organisms (Berendt et al. 1980). If the susceptibility of humans is similar to guinea pigs and proportional to the weight of the host, then approximately 1.4×10^7 virulent LDB would be a 50% lethal dose for humans. Breathing of approximately 350 L/h of air containing 5 LDB/100 L of air would require an exposure of approximately 8×10^5 h to inhale 1.4×10^7 organisms. Even if the concentrations of LDB were 500/100 L of air, an exposure of approximately 8×10^3 h would be required to inhale 1.4×10^7 LDB. Although extrapolation of infectivity from guinea pigs to humans is fraught with difficulties, it is useful for the sake of discussion inasmuch as appropriate human data is unavailable.

Analysis of human sera for significant levels of antibodies to *Legionella* that could be used as indicators of past infection has been carried out on various populations. Some of these studies indicated that individuals involved with cooling-tower maintenance had no obvious increase in anti-*Legionella* antibodies relative to the control groups not directly involved with cooling towers (Goldman and Marr 1980). Similarly, wastewater treatment workers exposed to bacterial aerosols did not exhibit markedly altered levels of anti-*Legionella* antibodies compared to control populations (Clark et al. 1980).

Contrasted with this relative lack of evidence for human infection by exposure to dilute aerosols is the overt LD that has occurred in workers cleaning power plant cooling systems or condenser tubes (Deubner and Gilliam 1977). Also, several outbreaks were associated with entrainment of cooling-tower discharges in relatively closed areas (Dondero et al. 1980; Glick et al. 1978). It has been observed by Fliermans (1983) that LDB can be found in high concentrations in association with some aquatic microphytes and macrophytes. The LDB apparently feeds upon such biota. In addition, free-living amoebae can also support the growth of high concentrations of LDB (Tyndall and Domingue 1982). Thus, as the relatively low concentrations of LDB in cooling-tower discharges reflect a proportion of the higher concentration found free in the basin water, such concentrations in water probably reflect the even larger concentrations of LDB associated with various biota. Therefore, the highest concentration in the LDB gradient would be expected in association with other cooling-tower biota and the least, in discharged air. Thus, the greater contact an individual has with the most concentrated LDB populations, particularly if these become aerosolized, the more likely it becomes that infection may result.

Consequently, aerosols produced by cleaning of biota from cooling-system components might create a greater exposure to LDB than aerosols from cooling-tower discharge. The limited data from the current study, together with serologic and limited epidemiologic data, support this supposition and suggest that if there are problems relative to the association of LDB with power plant cooling waters, they may be more germane to the area of industrial hygiene rather than public health.

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The content of Legionnaires' Disease Bacteria (LDB) in air discharged from industrial and power plant cooling towers was compared with the concentrations found in the cooling-tower basin water. Concentrates of the power plant air samples were also tested for the presence of free-living amoebae. The content of LDB in basin water was generally 1×10^6 to 10×10^6 times greater than that found in comparable volumes of discharge air. The content of LDB in air samples correlates with other sampling data that indicate that the amount of water discharged in cooling-tower plumes is generally one part of water by volume to 10×10^6 to 100×10^6 parts of air. Nonpathogenic Naegleria were isolated from one air sample taken in the mist generated at the base of a natural-draft cooling tower.

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