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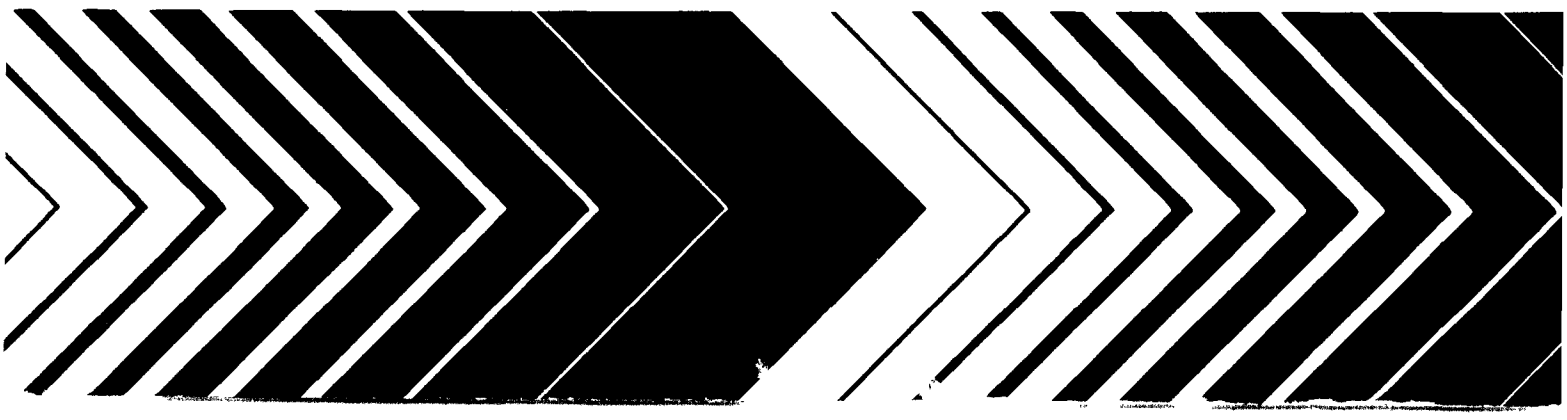
The Evaluation of Microbiological Aerosols Associated with the Application of Wastewater to Land

Pleasanton, California



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THE EVALUATION OF MICROBIOLOGICAL AEROSOLS ASSOCIATED WITH THE APPLICATION OF WASTEWATER TO LAND: PLEASANTON, CALIFORNIA

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FOREWORD

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. To that end, the Environmental Protection Research Division of the U.S. Army Medical Bioengineering Research and Development Laboratory conducts comprehensive basic and applied research in support of the Surgeon General's responsibilities in environmental protection to include air, land, and water pollution control and disposal of hazardous wastes and pesticides, and in occupational health associated with exposure to chemicals. The primary mission of the Health Effects Research Laboratory is to provide a sound health effects data base in support of the regulatory activities of the U.S. Environmental Protection Agency. HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report describes a joint research effort by the two laboratories. An in-depth microbiological evaluation was made at a site where treated municipal wastewater was spray irrigated. Special emphasis was given to microbial transport by aerosols.

ABSTRACT

The purpose of this study was to determine the extent that individuals near spray irrigation sites are exposed to microorganisms in wastewater aerosols. This report reviews a monitoring effort of a spray irrigation site utilizing unchlorinated secondarily-treated wastewater from biofiltration treatment processes. Objectives included an in-depth pathogen screen of wastewater, establishing the relationship between pathogen levels and traditional indicator organisms, monitoring microorganisms in air within 600 meters of the spray source, and development/validation of a microbiological dispersion model for predicting aerosol pathogen concentrations. Effluent was monitored for microbiological, chemical, and physical characteristics and extensive microorganism and dye aerosol samples were collected (77 aerosol runs). Enteroviruses were detected in air, but at a very low density. Conclusions: There is considerable underestimation of aerosol pathogen levels when using traditional indicators to predict human exposures. A microbiological dispersion model may be used with minimal monitoring to estimate exposure. There is little correlation between wastewater levels of traditional indicators and pathogens. Aerosols containing microorganisms are generated by spray irrigation of wastewater; they do survive aerosolization and can be transported to nearby populations. Until dose-response relationships are developed, neither the levels of aerosolized microorganisms that constitute a hazard nor the degree of required wastewater disinfection can be specified.

This report was submitted in fulfillment of Contract DAMD 17-75-C-5072 by Southwest Research Corporation under the sponsorship of the U.S. Environmental Protection Agency and the U.S. Army. This report covers a period from June 1975 to March 1978.

EXECUTIVE SUMMARY

Application to land is perhaps the oldest method of disposing of wastewater, and an attractive modern alternative. The advantages include re-use of the water, avoiding contamination of bodies of surface water, and return of nutrients in the wastewater to the soil. The most frequent means of application is spray irrigation, which can be applied to a wide variety of land sites where the topography of the land would make other methods of irrigation infeasible.

Aerosols containing pathogenic microorganisms are created during wastewater spraying and the organisms can be transported to populated areas by prevailing winds. The principal purposes of this program were to determine to what extent individuals living near sites practicing spray irrigation are exposed to these microorganisms and to gain insight into the potential health effects. Gastro-intestinal and respiratory illnesses such as dysentery, typhoid fever, and infectious hepatitis might be spread by spraying poorly-treated, undisinfected wastewater.

The study was conducted at a spray irrigation site associated with a sewage treatment plant located in Pleasanton, California. Here, treatment plant effluent was utilized to irrigate grazing lands. The wastewater is secondarily treated but not chlorinated by a process called contact biofiltration, and approximately 1.4 million gallons per day are sprayed onto the fields. The program was designed with three potential phases, Phase I was to be a site characterization, Phase II to be extensive aerosol monitoring effort, and Phase III an epidemiology study of the exposed population. This report covers the Phase II monitoring effort, which was conducted over the period from May 1976 to April 1977.

There were two distinct efforts performed in Phase II. The sewage treatment plant receives a large input from the Alameda County Fair during a month-long period each summer and the sewage effluent at this time is not typical of the remainder of the year. The decision was made not to monitor during this period, but to monitor both before and after the fair. The two efforts were conducted with differing sets of objectives, and these were designated as Pre-Fair and Post-Fair.

The principal objectives of the Pre-Fair study were to perform an in-depth pathogen screen of the wastewater, to establish the relationship in wastewater between pathogen levels and levels of the traditional indicator organisms (total and fecal coliform and standard bacterial plate count), to determine microorganism levels in air within 100 meters of the spray source, and to begin the assessment of factors thought to affect the levels of pathogenic organisms collected in aerosol samples, including aerosolization efficiency, pathogen survival upon becoming airborne (impact), and microbiological die-off with time (viability decay). These factors were to be used to begin development of a predictive model of pathogen concentration to estimate the degree of exposure of the nearby populations.

The objectives of the Post-Fair study were primarily oriented toward the development and validation of a predictive model. To accomplish this goal, air sampling was to be conducted to 600 meters downwind, the factors affecting microbiological aerosols were to be identified and quantified over a wide range of meteorological conditions, and sufficient aerosol runs were to be completed to permit model development. The model developed from the Pre-Fair and Post-Fair data was then to be validated using data from studies at Fort Huachuca and Deer Creek and some Pleasanton data not usable in model development.

To accomplish these objectives, it was necessary to monitor the effluent for its chemical and physical characteristics as well as microbiological constituents and to obtain large volumes of the wastewater for pathogen screening. Extensive aerosol samples were to be collected downwind and upwind of the spray line to determine the concentration of both traditional microorganism groups and of pathogens, and to compare

these concentrations with the expected levels based on the spray rate and the microorganism levels in the effluent. Additionally, samples were taken after the injection of dye into the wastewater to allow estimation of the proportion of the sprayed effluent that became aerosolized.

Routine monitoring of the wastewater for chemical, physical, and microbiological parameters was accomplished during Pre-Fair by taking a composite sample from the aeration basin during the hours of spraying. Chemical and physical analyses included total and free chlorine, pH, total organic carbon (TOC), total solids, and total suspended solids (TSS). In addition, one-half of the composite samples were tested for biochemical oxygen demand (BOD), chemical oxygen demand (COD), total phosphorus, hardness, and the nitrogen series, (nitrite, nitrate, ammonia, and organic nitrogen). Microbiological analyses run on all wastewater samples included total and fecal coliform, standard bacterial plate count, coliphage, and assays for selected pathogens, (*Klebsiella, Pseudomonas,* fecal streptococci, *Clostridium perfringens,* and 3- and 5-day enteroviruses).

To perform the wastewater pathogen screen, eight large-volume (20L) samples of effluent were taken at intervals throughout the Pre-Fair period and at the beginning of the Post-Fair period. These were sent to the UTSA-CART laboratory in San Antonio for a semi-quantitative screen to determine those microorganisms appearing with frequency in the effluent and to assist in selection of organisms for routine assay of wastewater and aerosol samples.

In the Post-Fair study routine chemical/physical analyses of the wastewater included pH, TOC, TSS and conductivity. Three samples were collected over the Post-Fair period for more detailed analyses, which included BOD, COD, total phosphorus, and the nitrogen series. Microbiological analyses conducted on all wastewater and aerosol samples included total coliform, coliphage, standard bacterial plate count, and selected pathogens. The pathogens sought in the Post-Fair period were limited to fecal streptococci and mycobacteria. The wastewater analyses were conducted on a composite sample taken in conjunction with each microbiological aerosol run.

Microbiological aerosol monitoring during both the Pre- and Post-Fair studies for microbiological aerosols was conducted using large-volume (1000 l/min) = 1 m^3/min) electrostatic precipitator samplers. These samplers were selected because the large volume of air sampled over a 30-minute period increases the sensitivity for the microbiological assay. Twenty-one successful aerosol runs were made during the Pre-Fair study and an additional 29 in the Post-Fair study. The collection and transfer medium selected was brain-heart infusion broth with 0.1 percent Tween 80®, which was shown to be adequate for sample concentration and for preservation of the microorganisms. The samples were analyzed for the same microbiological parameters as the wastewater, with the exception of one run for which the collecting fluids from all samplers were pooled for conduct of a pathogen screen.

A minimum of eight samplers was specified for each run and these were deployed along predesignated configurations to obtain the necessary information to perform the mathematical modeling. The distances of the samplers from the spray line were selected based upon sampling protocols and prevailing meteorological conditions. One sampler was used at a remote, upwind location to ascertain background concentrations of the organisms sought.

All-glass impingers were used to collect the aerosols from the dye runs, to determine the wastewater aerosolization efficiency of the sprinklers. Seven dye aerosol runs were conducted in Pre-Fair and an additional ten in Post-Fair.

In the Pre-Fair study, it was determined that virus levels in air consistently fell below the detection limit of the method and that special procedures would be required to obtain the necessary sensitivity. Two special virus runs were conducted in the Post-Fair study which increased the sensitivity and allowed estimation of impact factors for the enteroviruses. These two runs were conducted with all available samplers operating

close to the spray line under meteorological conditions expected to result in high virus aerosol concentrations. The sampler collection medium was changed every 30 minutes and the samplers run for a total of about three hours. The collecting fluids were pooled and concentrated for analysis so that the results were based upon a total of over 5000 m^3 of air.

An explicit model for predicting downwind concentrations of pathogens was developed by expanding more general mathematical dispersion models. The model adds factors for microorganism impact, viability decay, and aerosolization efficiency, to the standard diffusion model estimate of pathogen concentration based on source strength. The distributions of aerosolization efficiency and the impact and decay values for each organism were determined and these were used to allow evaluation of the model using monitoring data from other sites.

The study was supported by an extensive quality assurance program conducted primarily during the Pre-Fair portion of Phase II. Chemical, physical, and microbiological methods used were subjected to accuracy and precision studies, and alternative laboratories were used, where feasible, to verify the results. Certain aerosol runs were made in both Pre- and Post-Fair to allow the determination of the precision of the microbiological assay procedures and the estimation of any sampler collection efficiency bias.

The Phase II study yielded several important conclusions. From the wastewater monitoring, the conclusion was reached that wastewater quality as measured by chemical and physical parameters was unrelated to the generation or transport of microbiological aerosols. In addition, little correlation could be found in the wastewater between levels of total coliform, fecal coliform, standard bacterial plate count, and coliphage (the traditional indicator organisms), with the levels of the pathogens which they are intended to indicate.

Results obtained from the aerosol studies indicate that use of the traditional indicator organisms to predict human population exposure results in extreme underestimation of pathogen levels. The pathogens studied survived the wastewater aerosolization process much better than did the indicator organisms. Based upon the results of this study, fecal streptococci may be an appropriate indicator due to ease of assay, levels routinely seen in wastewater, and the similarity of their hardiness upon impact and viability decay rate to those of the pathogenic organisms of interest.

Large-volume samplers of the type used in this study are most useful for obtaining the sensitivity required for assay for bacteria in aerosol samples, especially at background and far downwind locations. Sampling and analyses for enteroviruses in wastewater aerosols requires even greater volumes of air and only a special effort such as that performed here can be expected to provide the necessary sensitivity to allow their detection.

The dispersion model developed in this study was validated. It was shown to produce satisfactory results when used to predict aerosol concentrations at three sites. Most of the predicted results fell within a factor of five of the measured concentrations when non-chlorinated effluent was being sprayed. The use of such a model with minimal monitoring is a viable alternative to extensive aerosol monitoring, and is significantly less costly.

The overall conclusion of Phase II of this program is that microbiological wastewater aerosols are generated by spray irrigation, do survive aerosolization, and can be transported to nearby populations. The most reliable means of reducing a potential health hazard from pathogenic aerosols is by disinfecting the wastewater before spraying. Until the necessary dose-response relationships are developed, neither the level of aerosolized microorganisms that constitute a hazard nor the degree of required disinfection can be specified.

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I. SUMMARY AND CONCLUSIONS

A. Wastewater

1. Throughout the Pre-Fair and Post-Fair portions of this study there were no abrupt changes in wastewater quality which appeared to have an adverse effect on the results of the study. In general, the wastewater effluent applied was of relatively consistent day-to-day quality. No significant changes were observed during the conduct of a single aerosol run.

2. In general the quality of the irrigated wastewater was typical of an undisinfected, secondarily treated domestic wastewater. Mean values during the Pre-Fair studies were: BOD-18.7 mg/L, COD-99.5 mg/L, TOC-33.0 mg/L, pH 8.4, hardness 235.2 mg/L, TSS033.0 mg/L, total phosphorus-5.6 mg/L, and nitrite, nitrate, ammonia and organic nitrogen-0.15 mg/L, 0.06 mg/L, 23.9 mg/L, and 5.6 mg/L, respectively. However, there were periods when the wastewater quality decreased to that of a poor quality undisinfected secondarily treated domestic wastewater.

3. Neither the wastewater quality nor the slight changes in the wastewater quality as measured by traditional chemical/physical parameters appeared to have an impact on the generation or transport of microbiological aerosols in this study.

4. A strong relationship was observed among TOC, COD, and BOD in determining the overall quality of wastewater at the site. The significance level of the correlation between TOC and BOD was 0.006, and for the other pairs was less than 0.001.

5. Pathogenic bacteria and viruses were found consistently in the effluent samples, and coliphage were found in all effluent samples. A wide range of levels of these microbial components was found. Concentration levels routinely varied by one order of magnitude and variation often approached two orders of magnitude.

B. Methodology

1. The quantitative evaluation of microbiological wastewater aerosols during field studies requires high-volume sampling equipment, competent personnel and extensive laboratory and logistical resources.

2. Studies conducted on the aerosol collection media, the temperature at which the samples are shipped, and the total time from collection to analysis were examined in detail in the laboratory. The results led to the design of adequate methods for sampling and analysis such that pathogenic organisms were found consistently.

3. Some difficulties were encountered in contamination of the high-volume aerosol samplers between aerosol runs. This problem appeared primarily in the standard bacterial plate count and *Pseudomonas* assays. Special care must be taken to adequately decontaminate high-volume aerosol samplers between aerosol runs.

4. The microbiological aerosol data varied substantially in quality and informational content. Accordingly, a suitable aerosol data weighting procedure was employed, according to consistent rules, in conducting the aerosol factor analyses.

5. There is substantial imprecision using the methods employed in this study for measuring microbiological concentrations in aerosol samples. The aerosol measurement coefficients of variation were 17% for dye, 50% for total coliform and standard bacterial plate count, 58% for fecal coliform and *Pseudomonas*, 60% for *Clostridium perfringens*, 73% for coliphage, 74% for *Klebsiella*, 77% for fecal streptococci, and 81% for mycobacteria. While the microbiological aerosol variation due to field sampling sources is considerable, even more variation was caused by analytical sources such as sample processing, shipping, and laboratory procedures. Relatively little of the analytical variability is reflected in replicated analyses, which is the usual manner of reporting analytical variation.

6. A special study of respiratory viruses in wastewater found confirmed viruses in five of the forty roller tubes cultured. Typing disclosed that four of the five tubes contained ECHO virus 6, while the other viral isolate could not be identified. Echoviruses 6 may occur as either a respiratory-tract virus or as an enteric virus. The failure to isolate purely respiratory viruses in the Pleasanton wastewater confirmed our suspicion that the likelihood of finding respiratory viruses in wastewater is very small.

7. Reliable enterovirus aerosol concentrations can be detected by the methods employed in the two special virus aerosol runs conducted at Pleasanton. The measured enterovirus aerosol concentrations obtained, 0.011 pfu/m^3 and 0.017 pfu/m^3, were 1-1/2 orders of magnitude higher than was expected based on the measured wastewater concentrations during these runs.

8. At sites with aerosol source strengths similar to the Pleasanton site and with sampling and assay methods currently available, it is generally not advisable to conduct microbiological aerosol sampling at distances beyond 200 meters from a wastewater aerosol source.

9. In the quality assurance aerosol runs for systematic sampler differences, it was concluded that after correcting for the air flow rates, there was no systematic bias in microbiological collection efficiency among the high-volume samplers evaluated.

10. An acceptable state-of-the-art procedure extending previous models [1,2,3,4] has been developed for the estimation of microorganism aerosol concentrations in wastewater aerosol downwind from a spray irrigation site. The microbiological dispersion model developed permits the prediction of downwind aerosol concentrations of specific pathogen and indicator microorganism groups emanating from sprayed wastewater aerosols. This multiplicative model, $P = D \bullet E \bullet I \bullet e^{\lambda a}$, incorporates a diffusion factor D for which any applicable standard atmospheric dispersion model can be used; a wastewater aerosolization efficiency factor E that depends upon atmospheric and operating conditions; and microbiological impact and viability decay (die-off) factors I and $e^{\lambda a}$ which depend upon the microorganism group and atmospheric conditions. If allowance is made for the imprecise nature of microbiological aerosol data, this multiplicative model appears adequate to represent microbiological dispersion.

11. Prediction by the microbiological dispersion model of the pathogenic microorganism concentrations from wastewater aerosol sources to which downwind workers and nearby residents are exposed is the most promising method of determining their level of pathogen exposure. However, the model has been validated only to downwind distances of about 100 meters.

C. Aerosol and Wastewater Microbiology

1. Prior to selecting the test organisms for a study of this nature (or for the microbiological monitoring at a spray irrigation site) it is essential that several site specific screens of a wide variety of organisms (both pathogens and indicators) be conducted.

2. Over the ranges of the effluent microorganism concentrations obtained during sampling at Pleasanton (from one order of magnitude for coliphage and total coliform to well over two orders of magnitude for *Pseudomonas* and *Clostridium perfringens*), there are only the most tenuous of relationships between some pathogenic organisms and some indicator organisms. For all practical purposes the use of indicator organisms as a measure of specific pathogen levels in wastewater is invalid.

3. The geometric mean aerosol concentrations obtained at 50 meters downwind of the wetted spray area were:

standard bacterial plate count	460/m^3
total coliform	2.4 MFC/m^3
fecal coliform	0.37 MFC/m^3
coliphage	0.38 PFU/m^3

fecal streptococci	0.61 CFU/m^3
Pseudomonas	34.CFU/m^3
Klebsiella	<5. CFU/m^3
Clostridium perfringens	0.9 CFU/m^3
mycobacteria	0.8 CFU/m^3
enteroviruses (3 and 5 day)	0.014 PFU/m^3

Individual aerosol measurements frequently differed by more than an order of magnitude from these mean values.

4. Limited particle size data obtained with two-stage Andersen samplers showed a substantial portion in the respirable range. The median percent respirable particle values downwind of the spray line were 44 percent for total count and 74 percent for total coliform. In general, there was a higher percentage of respirable particles at close downwind distances (5 to 25 meters), than at background and farther downwind distances. These meager data is in general agreement with more thorough particle size studies performed at other sites[5,6,7]. Particle size was not considered in the mathematical modeling.

5. The use of most of the traditional organisms for monitoring wastewater aerosols results in a gross underestimation of pathogen levels. Total coliform, fecal coliform, coliphage, and standard bacterial plate count, which are commonly used as indicators of wastewater pathogens, do not survive wastewater aerosolization nearly as well as do the pathogens studied.

6. One of the better indicators for wastewater aerosol monitoring may well be fecal streptococci due to the relative ease of the assay, the levels found in the wastewater, its relative hardiness during aerosolization, and its relatively low viability decay rate. However, an apparent problem was the occasional presence of fecal streptococci in aerosols due to non-wastewater sources.

7. Although *Klebsiella* was relatively prevalent in the wastewater, it was far less prevalent in the wastewater aerosol. It appears that *Klebsiella* die off rapidly during the aerosolization process. This finding is in contrast to data seen in the literature which consistently report *Klebsiella* as the predominant pathogen found in the air near spray irrigation sites and near sewage plants. More confirmation steps were used in this study than in earlier studies. If the confirmation steps had been stopped at the point used by other investigators, more values would have been reported as *Klebsiella* when, in fact, they were primarily other organisms of the mucoid type.

8. There was no significant difference in the coliform or coliphage concentration in corresponding effluent samples taken from a spray head during the aerosol runs and from the effluent composite samples at the pond pump. The standard bacterial plate count, however, was significantly higher in the spray field samples. The correlations of the spray field and pond composite microorganism concentrations were generally significant, but not adequate for prediction.

9. The median aerosolization efficiency E obtained for the Rainbird® impact sprayers over 17 dye runs during Phase II at Pleasanton was 0.33%. There was over an order of magnitude of variation in aerosolization efficiency estimates from the tenth percentile (0.09%) to the ninetieth percentile (1.8%). Eighty percent of this variation in aerosolization efficiency at Pleasanton appears to result from changes in meteorological conditions (air temperature, wind velocity, and solar radiation) that affect the evaporative capability of the air.

10. The median impact factor estimates I for the microorganism groups studied were 0.13 for fecal coliform (13% survive aerosol impact), 0.16 for total coliform, 0.21 for standard bacterial plate count, 0.34 for coliphage, 0.89 for mycobacteria, 1.2 for *Clostridium perfringens*, 1.7 for fecal streptococci, 14 for *Pseudomonas*, ca. 10 for three-day enteroviruses (mostly polioviruses), and ca. 40 for all (3-day and 5-day) enteroviruses. Most individual impact factor estimates were quite imprecise, reflecting the imprecision of the microbi-

ological aerosol concentration measurements. Since the middle range of impact factor values (fortieth to sixtieth percentiles) for each microorganism group were quite consistent, they are considered to be characteristic of the microorganism groups' typical survival through aerosol impact.

11. As indicated by impact factors exceeding 1.0, the enteroviruses and some hardy bacterial pathogens are frequently found in wastewater aerosols at higher concentrations than could be expected based on their wastewater concentrations. The survival, masking, and mechanical splitting hypotheses discussed later may collectively explain this phenomenon.

12. The indicator microorganism groups, especially total coliform and fecal coliform, experience more consistent and rapid die-off with aerosol age than do the pathogenic bacteria evaluated. The viability decay rates λ for total coliform and fecal coliform were more rapid, more reliable, and more frequently detectable than those of the other microorganism groups. The median viability decay rates were $-0.032s^{-1}$ for total coliform and $-0.023s^{-1}$ for fecal coliform. Viability decay was less rapid for coliphage, *Clostridium perfringens*, and standard bacterial plate count and its effect could only be ascertained within 100 meters on about half the runs. Viability decay could seldom be ascertained for fecal streptococci, mycobacteria, and *Pseudomonas*. No attempt was made to determine the viability decay of enteroviruses.

13. The range of impact factor estimates for each microorganism group was broad, generally covering two orders of magnitude from the tenth percentile to the ninetieth percentile. The detectable viability decay rates of each microorganism group also covered a wide range. Preliminary analyses suggest ambient conditions such as low relative humidity, high wind velocity, and a large temperature differential between wastewater and air may reduce the initial survival, i.e. produce low impact factor values I. Viability decay may be more rapid with high solar radiation, high temperatures, and middle or low relative humidity. Research is needed to quantify the relationships of impact factor variation and viability decay rate variation to the ambient atmospheric conditions for the specific microorganism groups.

14. The accuracy and precision of microbiological dispersion model predictions have, in general, been validated to 100 meters downwind of spray sources of unchlorinated wastewater aerosols. Most model predictions (e.g., 77 percent for standard bacterial plate count, 71 percent for total coliform, and 80 percent for coliphage) were within a factor of five of the net measured aerosol concentrations evaluated. Considering the imprecision and cost of measuring microorganism aerosol concentrations from spray irrigation by field sampling, using predictions of the microbiological dispersion model supplemented with minimal field sampling does appear to be a preferable alternative to extensive field sampling when the sprayed wastewater does not contain residual chlorine.

D. General

Results of this study indicate that pathogenic organisms do survive as aerosols from the spray irrigation of wastewater and can be transported downwind into nearby populated areas. Until such time as more information is developed on the infectious dose for a particular organism, effective control of pathogens must be accomplished prior to spray irrigation to minimize this risk. The most reliable means of reducing potential health hazards from pathogenic aerosols is by disinfecting the wastewater before spraying. Until the necessary dose-response relationships are developed neither the level of aerosolized microorganisms that constitute a hazard nor the degree of required disinfection can be specified.

II. RECOMMENDATIONS

1. Dose-response relationships need to be developed for the pathogenic microorganisms prevalent in wastewater aerosols. The available techniques include epidemiological studies of human populations, sentry animal studies, laboratory animal studies, clinical studies, and/or professional judgment. Epidemiological studies may be the best technique. The evaluation of spray irrigation as a viable means of disposing of wastewater will remain incomplete until such dose-response relationships can be established.

2. A reliable procedure is needed for selected values of the model parameters I and λ when predicting microorganism aerosol concentrations using the microbiological dispersion model. There are preliminary indications in our data supported by considerable published data that the I and λ values for a microorganism group depend highly upon ambient atmospheric conditions. A careful multivariate analysis of the existing values of I, λ, and the meteorological conditions for each Pleasanton run should be conducted. The resulting relationships of microorganism I and λ values as a function of ambient meteorological conditions would provide a substantive basis for their selection in model prediction applications.

3. To assess the predictive capacity of the microbiological dispersion model, a thorough evaluation of the model should be conducted through a limited field sampling program using the Pleasanton study methods at other sites. The enhanced model to be evaluated should incorporate the I and λ selection procedure discussed in the preceding recommendation. The model evaluation conducted herein needs to be extended because the field and sampling methods differed from the Pleasanton methods, pathogens were not assayed at sites other than Pleasanton, and no adequate I and λ parameter selection procedure existed.

III. STUDY DESCRIPTION

A. Statement of the Problem

Application of wastewater to land is perhaps the oldest method of disposing of wastes by man. The more recent trend has been to discharge treated and untreated wastes into streams, lakes, and oceans. During the past several years, there has been increased interest in applying treated wastewater to land as an alternative to discharging into surface waters, in order to avoid the contamination of these bodies of water. The Environmental Protection Agency (EPA) has in recent years required that applicants for federal construction grants (Section 201) for wastewater treatment facilities show in their request that they have considered the application of wastewater to land as an alternative.[8] More recently, the EPA has announced plans to press vigorously for the recycling of wastewater via land application.[9] There are several advantages to applying treated wastewater to land, including re-use of the water, avoiding contamination of surface waters, and return of nutrients to the soil. Many small communities primarily in arid regions, have long employed wastewater land-application systems as a means of water conservation and waste disposal. During the past several years, a large metropolitan area, Muskegon County (Michigan), has completed a project involving biological treatment, storage lagoons, disinfection, and spray irrigation for disposal of wastewater from a population of more than 170,000.

Land application of wastewater can be accomplished by several methods, which can be categorized as overland flow, rapid infiltration, and slow infiltration. Spray irrigation is perhaps the most popular method of wastewater application because it can be applied to a wide variety of land sites and the irrigation apparatus can be moved readily from one location to another. For many municipalities, both small- and medium-sized, spray irrigation is the most attractive means for land application of treated wastewater. Recreational areas such as parks, golf courses, and highway right-of-ways, can be irrigated. Irrigation of land sites immediately adjacent to the waste treatment facility for growing of grass cover is also an economically and environmentally attractive use of the wastewater. The applicability of spray irrigation to a much larger segment of waste treatment facilities will necessitate that these spray sites be located adjacent to the facilities and, therefore, probably near populated areas. Certainly, application of wastewater to recreational areas by spray irrigation has a high potential for contact between the wastewater aerosol and individuals, both the spray applicators and surrounding populations.

A number of investigators have raised questions regarding the health and hygienic aspects of application of wastewater to land, especially by spray application. These investigators include Wellings, *et al.*,[10] Sorber and Guter,[11] Parson, *et al.*,[12] Katzenelson and Teltch,[13] and Elliott and Ellis[14].

Wellings, *et al.*, cautioned the utilization of spray irrigation of wastewater because of the many unknowns regarding the fate of pathogenic bacteria and especially viruses. These authors' studies have been primarily directed at possible contamination of ground water by viruses following spray application of treated wastewater. Sorber and Guter examined the literature regarding the health aspects of land application of wastewater by spray irrigation and concluded that there was a probability of humans inhaling pathogenic aerosols near a spray irrigation site. They recommended that research be performed to determine the viability of human pathogenic microorganisms present in biological aerosols from spray irrigation of wastewater. They also suggested that there be a determination of the type of spray distribution system, nozzles, and associated operating pressures necessary to minimize the health hazards from biological aerosol formations.

Earlier work by investigators such as Ledbetter and Randall[15] showed that activated sludge treatment facilities operations were sources of aerosolized bacteria, some of which might be pathogenic. These bacterial microorganisms were from aerosolized wastewater in the aeration basins present in the treatment facilities. It is, therefore, quite probable, as pointed out by Sorber and Guter, that spray application of treated wastewater

with various types of spray irrigation equipment and with different spacing and type of nozzles would significantly influence the quantities of aerosols formed. The aerosols generated by the sprinklers might contain pathogenic bacteria and viruses, and they might consist of particles in the size range of 0.01 to 50 microns.[16]

From the data seen in the literature, it appears that spray irrigation of treated wastewater will result in formation of aerosols which may contain pathogenic microorganisms. Inhalation of pathogenic microorganism may lead to infection. This is a major source of the public health concern for this method of wastewater disposal and the reason for conducting this research. From limited data, it appears that some of these organisms will remain viable for extended periods of time and may be carried by winds for considerable distances from the spray irrigation sites.

An environmental monitoring effort was performed by Katzenelson and Teltch near spray irrigation fields in Israel. The effluent sprayed on these fields was from partially-treated undisinfected municipal wastewater and levels of coliform bacteria in the effluent were approximately the same as those seen in raw wastewater present in the United States. Coliforms were measured in aerosols and a portion of these aerosol samples was further examined for the presence of *Salmonella*. They found elevated levels of coliform bacteria 300 meters downwind from the spray irrigation site. In one of the aerosol samples collected at 600 meters downwind, they found an isolate of *Salmonella*. They calculated that an individual 100 meters downwind from the wastewater sprinkler line would inhale approximately 36 coliform bacteria in a period of 10 minutes. These authors concluded from these preliminary results that there may be a relatively neglected potential danger to agricultural workers and neighboring settlements from the use of spray irrigation wastewater. They stated that proper disinfection of wastewater for irrigation purposes may be the most effective means of reducing this risk.

Sorber, *et al.*,[17] conducted an extensive environmental monitoring effort at a field site in Arizona where chlorinated secondary municipal effluent was used to irrigate a golf course. These authors performed field testing of both chlorinated and unchlorinated effluent to enumerate bacteria present in the effluent, to determine the fraction of wastewater entering the aerosol state, and to determine the survival in aerosols of total aerobic, indicator, and selected pathogenic bacteria. *Klebsiella* was the most commonly found pathogen, with fecal streptococci found in some samples. When chlorinated effluent (versus unchlorinated effluent) was sprayed, much lower levels of pathogenic bacteria were found in the aerosol samples, although the reduction was less than expected from comparison of pre- to post-chlorinated wastewater bacteria levels. Elevated levels of bacterial aerosols were measured out to 200 meters downwind of the spray line. It was estimated that bacterial levels would be present above background up to 500 to 1800 meters for unchlorinated wastewater, depending on meteorological conditions. A prediction model was developed in the course of this study for estimating concentrations of microbiological constituents downwind of the spray line. Components of the model included meteorological and other measurements taken downwind as well as the concentration of the microorganism in the effluent. In the discussion on microbial aerosols from the spray irrigation of wastewater, these authors point out that the factors of prime interest are: (1) the biological aerosol concentration at any distance from the source, (2) the buffer zone required to reduce such aerosols to near background levels, and (3) the effect of terminal disinfection or other polishing wastewater treatments on aerosol strength. The aerosol levels of viable microorganisms are dependent upon the levels of these organisms in the wastewater, the proportion of wastewater that is aerosolized, the volume of water sprayed per unit time, the aerosol decay rate and the atmospheric stability, wind speed, and other meteorological parameters. These authors defined aerosolization efficiency as the proportion of wastewater that becomes divided into droplets sufficiently small to remain in an airborne state. They found a statistically significant increase in the mass median diameter of viable bacteria-bearing particles with distances downwind of the spray irrigation sites. They postulated that this may indicate a lower rate of decay for bacteria associated with the larger particle size material. An alter-

native mechanism suggested was that the droplets receive pre-existing clumps or aggregates of many bacteria cells which tend to form larger solid nuclei. There was no indication that the particle size distribution for a viable airborne bacteria recovered from chlorinated effluent aerosols differed from those recovered in experiments without chlorination. They suggested that terminal disinfection is probably a more practicable and cost effective means of limiting the problems with pathogenic aerosols than are buffer zones.

Little information is available concerning the possible health effects following exposure to the low levels of bacteria and possible viruses that are present in ambient air near spray irrigation facilities. It is known that the inhalation of specified quantities of pathogenic bacteria or viruses can infect persons, but epidemiological data concerning a definable health effect following exposure to aerosols from a wastewater treatment facility are quite limited. Clark, *et al.* [18] reviewed the literature concerning the possible health effects of persons exposed to municipal wastewater via physical and aerosol routes of exposure. The review covered microbial aerosols from activated sludge, trickling filter, and spray irrigation of wastewater. They found that no correlation had been made between specific microorganism levels and the incidence of selected diseases. They reported on a 3-year prospective epidemiology-serology study to be performed in their laboratory involving sewage maintenance workers and an appropriate control group. This study has been expanded to include sewage plant operators and appropriate controls for study of effects of the exposure to aerosols from wastewater plant operations. Overall, the prospective study is aimed at determining the possible health effects from exposure to relatively high levels of wastewater via physical and oral ingestion of wastewater for the sewage workers and to inhalation of aerosols for the plant operators. These studies include collection of symptomatology data, blood, urine, throat swabs, and stools for examination for viral, bacterial, and parasitic isolation, as well as a detailed serological analysis. The results of this study should provide indications of possible health effects at relatively high levels of exposure to adult male populations.

A retrospective epidemiological study of communicable diseases associated with spray irrigation of wastewater was performed near settlements in Israel by Katzenelson, *et al.*[19]. These authors examined the incidence of enteric communicable diseases in 77 kibbutzim (agricultural settlements) practicing spray irrigation with partially-treated, nondisinfected wastewater. These data were compared with that from 130 kibbutzim not practicing spray irrigation. They found that the incidence of Shigellosis, Salmonellosis, typhoid fever, and infectious hepatitis were two to four times higher than for those communities not practicing spray irrigation. They also noted that for the months in which there was no spray irrigation, i.e., the winter months, there were no differences between the study populations for these enteric diseases. The study populations (positive) lived from 100 to 3000 meters from the spray irrigation field. This retrospective study provides some epidemiological evidence for an increased risk for enteric communicable diseases among populations living near sites spray irrigating with municipal wastewater. The study does not identify the transmission route, that is, via physical or aerosol exposure, nor does it directly relate spray irrigation with these elevated diseases. The operators of the spray irrigation facilities live in these kibbutzim; thus, one pathway might be via physical exposure to the wastewater irrigation site workers and via the clothes of the irrigation site workers when they return home. These authors point out the need for caution in the utilization of poorly-treated wastewater via spray irrigation near residential areas. A follow-up study is underway.

A prospective environmental epidemiological study by Carnow, *et al.*, at the University of Illinois (EPA Grant R-805003-01) is nearing completion. This environmental epidemiological study was performed near an existing activated sludge treatment facility located in Skokie, Illinois. This effort has similarities to the study (to be discussed below) conducted by Southwest Research Institute in the type of monitoring performed. However, a major difference is that it was located near an existing wastewater treatment facility, whereas the study conducted by Southwest Research was designed for a new plant with a before-operational monitoring effort and a monitoring effort after the plant was in operation. The objective of Skokie study was to collect

health effects data relative to the operation of the plant and to examine these data with regards to health effects related to the distance the population lives from the plant. Environmental monitoring was performed and an assessment of the bacterial microorganisms present around this plant was made. This study had 269 households living from about 600- to 800-meters from the center of the wastewater treatment plant.

An environmental monitoring and a prospective epidemiological study was performed by Johnson, *et al.*[20] of Southwest Research Institute for a new activated sludge treatment plant located near Chicago, Illinois. The purpose of this study was to identify possible health effects which might be attributed to the operation of an activated sludge wastewater treatment plant. The program was to involve three independent modes of investigation of a new activated sludge treatment plant prior to its operation and after its initial operation:

- Environmental monitoring in the vicinity of the site to determine the source and transport of indicator and pathogenic microorganisms and of trace metals.
- A health survey of a cross section of the households located within a 5-km radius of the plant aeration basin to examine the incidence of respiratory and intestinal diseases and symptoms.
- Analysis of clinical specimens from more than 200 participants residing within 3.5 km of the plant to determine viral antibody titers, to isolate pathogenic bacteria, viruses, and parasites, and to measure trace metal concentrations.

Each of the above was designed to determine whether the data followed a pattern that might implicate the wastewater treatment plant as a health hazard. The results showed that for the trace metals studied (cadmium, lead, mercury, and zinc), only mercury appeared to be elevated as a result of the wastewater treatment plant and that mercury elevation was not transported away from the plant. Also, the levels of these trace metals were not elevated in the soil and water samples collected in neighborhood residential areas relative to background levels. The plant did appear to be a source of indicator bacteria, coliphage, pathogenic bacteria, and enteroviruses emanating from its aeration basins. However, the levels of these microbial agents in air, soil, and water samples in the neighboring residential areas were not distinguishable from background levels. From the patterns observed in the household health survey, there was an increased incidence of skin disease and in the symptoms of nausea, vomiting, general weakness, diarrhea, and pain in chest on deep breathing among residents living close and in prevalent downwind directions from the wastewater treatment plant. Viral antibody tests and attempted isolation of many pathogenic bacteria, parasites, and viruses, however, yielded no evidence of an adverse wastewater treatment plant effect. The findings overall obtained in this study did not detect a public health hazard for populations living beyond 400 meters of this well-operated wastewater treatment plant. The lack of sufficient participants living close to the wastewater treatment plant precluded an assessment of the possible hazard near the plant. The study does confirm results seen in previous studies of wastewater treatment plants in that the plant is a source of pathogenic bacteria and possible viruses; however, levels above background were not obtained at distances beyond approximately 300 meters. Of the pathogenic bacteria monitored in aerosol samples (fecal streptococci, *Salmonella, Shigella, Pseudomonas*, and *Klebsiella*), only *Pseudomonas*, fecal streptococci, and *Klebsiella* were found. *Pseudomonas* was present in virtually all aerosol samples, while fecal streptococci and *Klebsiella* were found in only a few. One aerosol sample taken 300 meters downwind was positive for enteroviruses (poliovirus type III).

A recent review by Akin, *et al.*,[21] of the health hazards associated with the treatment and disposal of wastewater effluents and sludge, reports that in the absence of adequate epidemiological data to evaluate the potential health hazard from pathogens applied to soil, the monitoring for the occurrence of the pathogens in the environment must be the primary public health measure. These authors also pointed out the possible health hazards of parasites in the application of treated wastewater and sludge to land. In particular, the protozoan of greatest health interest in the past several years has been *Giardia lamblia*. Cysts from this protozoan

are able to survive many of the wastewater and sludge treatment processes and only a small number of cysts are necessary to infect humans. However, because of their size, it is questionable whether the cysts can travel as aerosols for large distances.

From the review of the literature on environmental monitoring, it is apparent that treated wastewater can contain significant quantities of microorganisms and pathogenic bacteria and viruses. It also appears that various processes involved in wastewater treatment facilities can create aerosols of these wastewaters containing viable microorganisms. Spray irrigation appears to offer the greatest potential for aerosolization for bacterial organisms. Some evidence is indicated that bacteria are present in aerosols from these sites. Bacterial organisms are present in air around spray irrigation facilities at distances up to at least 300 meters downwind and model projections indicate that these aerosols could be present above background out to several kilometers. The studies also indicate that various meteorological conditions can have a significant impact on the viability of these organisms in air following aerosolization. In general, high wind conditions, high humidity, and low solar radiation tend to enhance the viability of bacterial aerosols.

B. Study Background

This research effort was jointly funded by the U.S. Army Medical Research and Development Command, Fort Detrick, Maryland, and the Health Effects Research Laboratory of the Environmental Protection Agency, Cincinnati, Ohio. Each of these governmental organizations needs definitive data regarding the possible health effects associated with spray irrigation of municipal wastewater. The Army has a number of installations throughout the United States and foreign countries where disposal of municipal wastes is required. Application of treated wastewater to land, especially via spray irrigation, is an attractive means of final disposal and serves as an alternative to discharge into a watercourse. This permits the reuse of the wastewater for irrigation purposes and avoids the discharge of wastes into rivers, lakes, and oceans. As stated earlier, the Environmental Protection Agency has been an advocate of land application of treated wastewater as an alternative to discharging into surface waters. During recent times, the EPA has announced that it intends to press vigorously for the use of construction grant funds for wastewater treatment plant facilities to be directed at application of treated wastewater to land. The funds obligated for construction of new wastewater facilities in the United States are in the billions of dollars and the EPA has both the authority and the responsibility to insure that these facilities are constructed to effectively treat wastewater in the most economical and cost-effective means possible. In addition, the EPA must insure that the health of people in communities near these facilities is protected and that surface and ground waters are not significantly contaminated by chemical or biological pollutants. As discussed earlier, spray application of wastewater is one of the most attractive land application methods from an engineering standpoint. It does, however, offer the possibility that aerosols, formed during spraying operations, can be transported to nearby human populations and that some portion of the population will be infected by wastewater-associated pathogenic microorganisms. The initial objective of this study was to collect information regarding the types and quantities of microbial organisms emanating from a spray irrigation facility and to study these concentrations downwind of the spray facility into a populated area. A potential extension of this effort would examine the health status of a population adjacent to the spray fields as compared with a suitable control population. The initial monitoring study was designed such that sufficient numbers of samples would be collected and analyzed, along with a sound quality control program so that statistically valid data would be obtained. The overall study would provide both the U.S. Army and the EPA with much of the data necessary to provide design criteria for construction and performance of spray irrigation facilities. This is extremely important since it is apparent that the utilization of spray irrigation of treated municipal wastewater could have numerous advantages over conventional wastewater treatment for a wide section of the United States.

This project was performed in three phases. At the completion of each phase, an analysis of the data

was made and a report prepared, with the final design of the next phase of study dependent upon the results of the previous phase. Thus, there was considerable flexibility during the conduct of this effort to adjust the program's emphasis on the basis of gained knowledge.

Phase I was designed to select a suitable site for the conduct of the study and to develop the optimum methods for sampling and analysis of wastewater and aerosol samples for various types of bacteria, viruses, and chemical constituents. Phase II was designed to perform extensive environmental monitoring of the selected spray site to cover a period of some eight months. This phase would accurately measure the quantities and types of pathogens and other constituents under a variety of meteorological conditions and a range of source strengths of these materials in the wastewater. Phase II was divided into two parts: one conducted prior to a county fair held near the selected site, labeled "Pre-Fair", and the second performed afterward, labeled "Post-Fair". In general, the objectives of the Pre-Fair portions of Phase II were aimed at characterization of the aerosols for bacterial and viral microorganisms within 100 meters of the spray fields. Environmental monitoring during the Post-Fair phase emphasized sampling and analysis of selected pathogenic microorganisms, with aerosol monitoring conducted at distances up to 600 meters from the spray fields, extending into the populated areas. Phase III of this program is an optional phase to be directed at examining the potential human health effects of the spray irrigation facility. The study would examine a population living near the spray irrigation facility and compare their health status with a suitable control population. A decision to conduct Phase III has not been made but will follow examination of the data obtained during Phases I and II. The findings for the Phase I study are summarized in the Phase I report published in December 1975.[22]

C. Phase I Summary

Following a telephone survey of known wastewater spray irrigation facilities in the United States and an on-site survey of two locations, the wastewater land treatment system in Pleasanton, California, was selected for evaluation. The site selection criteria are listed in the Phase I report, but one of the important requirements, and one of the most difficult to satisfy, was that a suitable population had to live within one mile of the spray irrigation facility. The Pleasanton, California site met most of the desired criteria, including an adequate study and control population.

The Phase I results indicated that some of the initially-selected methods for sampling, sample transport, and analysis were inadequate to accurately determine the levels of pathogenic bacteria and viruses in both effluents and aerosols. Measurement of indicator organisms and other chemical constituents showed that the wastewater at this site was of a quality to be expected of a plant not practicing disinfection.

It was apparent from the preliminary aerosol sampling conducted during Phase I that the micrometeorology of the site would complicate the overall air sampling protocol and the interpretation of the data. No significant daily or hourly changes were seen in the chemical or biological constituents measured in the effluent, apparently due to the utilization of two aeration ponds at the end of the treatment plant that appeared to dampen cyclic changes which might have occurred through the plant. This made the environmental monitoring study easier since a rather uniform quality of wastewater was being sprayed.

One of the important findings of Phase I was that high-volume aerosol samplers were essential for measurement of pathogenic microorganisms present in the air downwind from the spray irrigation site. Samplers such as the Andersen and AGI, although perhaps having similar sampling efficiencies, would not sample sufficient quantities of air to provide the necessary sensitivity. It was concluded that aerosols generated from the adjacent secondary wastewater treatment plant itself should not complicate the study of aerosols from the spray irrigation fields because the head work, aeration chamber, and trickling filter were all covered to control odors. There was a possibility that the aeration ponds, also adjacent to the spray fields, could generate aerosols and confound the field study results.

D. Phase II Objectives and Design

1. Phase II Objectives

As stated above, Phase II was divided into two subtasks, Pre-Fair and Post-Fair. The Pre-Fair monitoring effort was performed so it would be completed prior to the beginning of a large county fair held in Pleasanton, California in the summer months. During the fair, a large influx of wastewater from animal holding facilities produced a typical wastewater effluent and no monitoring was to be performed during the fair or immediately afterwards. The Post-Fair monitoring effort was conducted after the effluent had returned to more typical characterization.

The objectives of Phase II are listed below for both Pre-Fair and Post-Fair.

a. Pre-Fair Objectives

The following is a list of the primary objectives to be accomplished during the Pre-Fair activities of Phase II.

Microbial Aerosol Runs

- begin evaluation of factors affecting microbiological aerosol levels within 100 meters of the spray source.

Dye Runs

- determine the aerosolization efficiency range of the sprinkler irrigation machinery used at the Pleasanton site.

Effluent Samples

- determine in-depth pathogen screen
- assess validity of using the common measures of wastewater microbiological quality (standard bacterial plate count, total coliforms, fecal coliforms) as indicators of the pathogen levels of the effluent.
- examine relationships between the microbiological and chemical water quality constituents.

Quality Assurance

- determine accuracy and precision of laboratory analyses
- determine if there are systematic differences in high-volume sampler collection efficiency.

b. Post-Fair Objectives

- identify and quantify the factors affecting microbiological aerosol levels over a wide range of meteorological conditions.
- develop a general microbiological dispersion/die-off model for appropriate microorganism groups that can be applied at other wastewater spray irrigation sites.
- predict the downwind microbiological aerosol concentration in the residential areas adjacent to the spray fields, relative to background levels.

2. Phase II Design

The types of samples obtained in the Phase II study (Pre- and Post-Fair) are summarized in Table III. D-I.

Table III.D-1.
SUMMARY OF PHASE II DESIGN

Wastewater Samples	Pre-Fair					Post-Fair		
	Daily Composites	Microbiological Run Composites	Dye Run Grabs	500 ml Grabs	Large Volume (20l) Grabs	Microbiological Run Composites	Dye Run Grabs	Large Volume (20l Grabs)
Chemical Analyses								
Total Chlorine	X			X				
Free Chlorine	X			X				
pH	X					X		
Total Organic Carbon (TOC)	X					X		
Total Solids	X							
Total Suspended Solids (TSS)	X					X		
Biochemical Oxygen Demand (BOD)	X*					X		
Chemical Oxygen Demand (COD)	X*					X*		
Total Phosphorus	X*					X*		
Nitrite Nitrogen	X*					X*		
Nitrate Nitrogen	X*					X*		
Ammonia Nitrogen	X*					X*		
Organic Nitrogen	X*					X*		
Conductivity						X		
Rhodamine Dye			X				X	
Microbiological Analyses								
Standard Bacterial Plate Count	X	X			X	X		X
Total Coliform	X	X			X	X		X
Fecal Coliform	X	X			X			
Coliphage	X	X			X	X		X
Fecal Streptococci		X			X	X		X
Pseudomonas		X			X			
Klebsiella		X			X			
Clostridium perfringens		X			X			
Mycobacteria					X	X		X
3-day enterovirus		X			X			
5-day enterovirus		X			X			
Pathogen screen					X			X

Table III.D-1 (cont'd)

Aerosol Samples	Pre-Fair				Post-Fair			
	Microbiological	Dye	Quality† Assurance	Large‡ Volume	Microbioloigcal	Dye	Quality Assurance	Special Virus
Microbiological Analyses								
Standard Bacterial Plate Count	X		5	X	X		X	
Total Coliform	X		5	X	X		X	
Fecal Coliform	X		5	X				
Coliphage	X		2	X	X		X	
Fecal Streptococci	X		2		X			
Pesudomonas	X		2					
Klebsiella	X		2					
Clostridium perfringens	X		2					
Mycobacteria					X		X	
3-day enterovirus	X							X
5-day enterovirus	X							X
Pathogen screen				X				
Rhodamine Dye Analyses		X				X		

* not run on all samples

† various analyses, number of runs indicated

‡ pooled collection fluid from all samplers

E. Participating Organizations and Principal Personnel

The research effort documented in this report has been conducted by Southwest Research Institute with the significant support and contributions of a number of other organizations. The following is a listing of participating organizations:

1. Southwest Research Institute (SwRI)
   San Antonio and Houston, Texas
2. The University of Texas at San Antonio (UTSA)
3. The Pacific Environmental Laboratory (PEL)
   San Francisco, California
4. Naval Biosciences Laboratory (NBL)
   Oakland, California
5. Environmental Quality Analysts (EQA)
   San Francisco, California
6. Dugway Proving Ground
   Dugway, Utah
7. Manpower, Incorporated
   Hayward, California
8. Sunol Sewage Treatment Plant
   Pleasanton, California
9. H. E. Cramer, Incorporated
   Salt Lake City, Utah

The study site is located at the Sunol Plant, listed above, and a detailed description of the site and the principal personnel supporting this study at the site is presented in the following section of this report. Details regarding area of responsibility and principal participating personnel for organizations other than the Sunol Plant are presented in Table III.E-1. The organization directing the study is Southwest Research Institute and all other organizations listed are acting in support of SwRI in this study. All personnel listed are permanent professional staff at the various institutions, with the exception of those listed for Manpower, Incorporated. The personnel listed for Manpower, Incorporated are temporary employees hired for the field surveys. The field survey team, though temporary staff rather than permanent professional staff, exhibit the same high degree of professionalism as the permanent staff.

Table III.E-1.
PARTICIPATING ORGANIZATIONS AND PRINCIPAL PERSONNEL

Organization	Responsibility of Organization	Responsibility Participants	Primary Responsibility of Participant
SwRI	Project direction and final results	D. E. Johnson, Ph.D.	Project director
		J. W. Register, Jr.	Direction of field activities
		D. E. Camann	Statistician
		R. E. Thomas	Statistician
		R. J. Prevost	Subcontracting and reports
		J. L. Gulinson	Meteorology
		J. M. Taylor	Air sampling
		H. J. Harding	Air sampling
		J. Salinas	Air sampling
		J. Trevino	Air sampling
		J. Paulk	Air sampling
UTSA	Analysis of coliphage pathogenic bacteria viruses	B. P. Sagik, Ph.D.	Direction of analysis
		C. A. Sorber, Ph.D.	Direction of analysis
		M. N. Guentzel, Ph.D.	Analytical methods
		B. E. Moore	Virology
PEL	Chemical analysis, indicator microbial parameters	T. Nakamura	Supervising chemist
		N. Harper	Bacteriologist
NBL	Consultant organization	M. A. Chatigny	Consultants in aerobiology
		H. Wolochow, Ph.D.	Consultants in aerobiology
EQA	Alternate laboratory used in quality assurance study	J. Tyler	Laboratory director
Dugway	Meteorological support	C. Spendlove, Ph.D.	Aerosol sampling
		E. Rengers	Meteorology
		J. Scudiri	Meteorology
Manpower, Inc.	Temporary employment firm used to hire field survey team	T. Rooney	Chemical technician
		P. Anderson	Air sampling
		J. DeNicola	Air sampling
		G. Langlois	Air sampling
		D. Lewis	Air sampling
		R. Purdie	Air sampling
		R. Stover	Air sampling
		B. Pruett	Air sampling
		D. Gaines	Air sampling
		J. Graham	Air sampling
		N. Houlding	Air sampling
		C. March	Air sampling
		G. Murdock	Air sampling
		E. Sternstein	Air sampling
		B. McLeod	Air sampling
		M. Sturgis	Air sampling
		L. Harrison	Air sampling
		R. Menzimer	Air sampling
		D. Wheaton	Air sampling
		J. Synder	Air sampling
		M. Mitchell	Air sampling
		M. Krause	Equipment maintenance and air sampling
H. E. Cramer, Inc.	Aerosol modeling and additional statistical support	K. Dumbauld	Direction of aerosol modeling
		A. Anderson	Aerosol modeling

IV. STUDY SITE

A. Site Description

1. Sunol Sewage Treatment Plant

As documented in the Phase I report, a water reclamation plant in the City of Pleasanton, California, was selected as the study site: the City of Pleasanton Sunol Sewage Treatment Plant.

A schematic of the study area is shown in Figure IV. A-1. A population with middle-class socioeconomic characteristics is located within one mile to the east/southeast of the plant. This population is located in a recently completed subdivision off Mission Drive. Mission Drive runs east-west, and the street begins on Sunol opposite the treatment plant. The prevailing winds in this area are from the southwest to northwest quadrant; thus, this inhabited area would be downwind of the spray fields. There is a population in this subdivision to conduct an epidemiological study, and there are also suitable control populations in Pleasanton with middle-class socioeconomic characteristics located more than 2000 meters from the spray fields. The people in the subdivision near the treatment plant live from 600 to 1000 meters southeast of the edge of the spray fields.

The Pleasanton water reclamation plant is under the control of the city government of Pleasanton, California. City personnel involved in the study are as follows:

City Manager	Clayton E. Brown
Assistant City Manager	Alan B. Campbell
Director of Public Works Field Services	H. Arnold Eaton
Public Works Field Superintendent	Arthur N. Monaco
Plant Foreman	John Wayneberg
Treatment Plant Operator	George Oxsen
Laboratory Analysis	Jerry Taylor

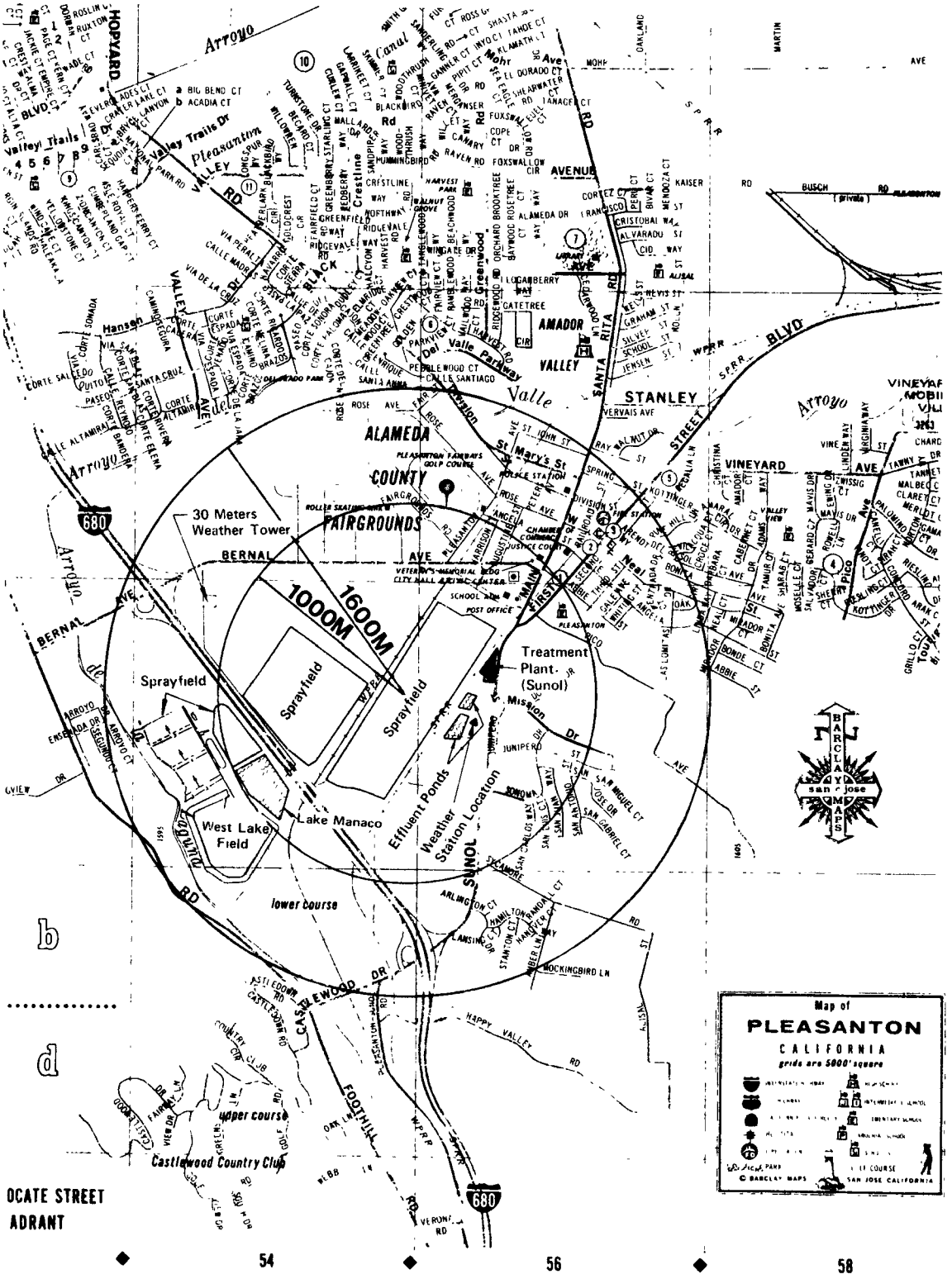
Arthur Monaco is directly responsible for the operation of the water reclamation plant, including the spray irrigation system. The city had no immediate (within one year) plans to change the operation of this treatment plant with respect to the spray irrigation of wastewater. The plant was modified just prior to the Pre-Fair study by the addition of an activated biofilter process (ABF) following the trickling filter to enhance the treatment system's biochemical oxygen demand (BOD) removal efficiency. The spray operation is nearly a "break-even" cost operation for the city because of the income it derives from the leasing of the irrigated pastures to cattlemen. The beef cattle which graze on the grass (44% alta tall fescue, 33% Ariki perennial rye, and 23% Potomac Grass Orchard) appear to grow well without supplemental food. The grazing of dairy cattle on the spray fields is prohibited. Beef cattle must wait seven days before grazing on fields that have been spray irrigated. The cattle are moved ahead of the sprayers and the normal rotation of the spayers through the fields ensures that a sufficient period of time has elapsed.

An average of 1.4 million gallons per day (MGD) of sewage is treated by trickling filtration and is stored in aeration ponds with a total retention volume of three million gallons. Approximately 600 gal./min. is recycled during irrigation from pond number 2 outlet to pond number 1 inlet to promote further oxidation. Pumping into the irrigation system from pond number 2 begins daily between 8 and 9 A.M. - and continues for a period of 16 to 18 hours depending on the early morning level of the pond, anticipated inflow, and precipitation. For optimum operation, the pond level is kept between 2.4 and 3.0 ft., with the most desirable level being 2.6 to 2.7 ft. The objective is to spray daily until about one-half of the wastewater present in the two ponds has been sprayed.

There are four major industrial waste sources in the area:

- Cheese Factory: The cheese factory waste probably has the greatest effect on the overall BOD input to the plant. Data obtained by the Kennedy Engineers, Incorporated for the City of Pleasanton indicate that the

Figure IV.A-1.
SCHEMATIC OF STUDY SITE



BOD level discharge waste from the cheese factory is approximately ten times that of normal domestic sewage. The cheese factory discharges approximately 0.016 MGD.

- Research Center: There is sizeable input (0.12 MGD) from the Kaiser Research Center, but available test data indicate it to be a waste of normal strength.
- Winery: The Villa Armondo Winery contributes the majority of its discharge (0.01 MGD) during the crushing season (fall and early winter). Data on this discharge have not been evaluated as of this date.
- Fair: The Alameda County Fair contributes a major portion of the industrial flow (0.25 MGD) during its month-long operation each summer. Available data indicate the possibility of high strength wastes.

2. Treatment Plant Process

The City of Pleasanton Sunol Sewage Treatment Plant (STP) utilizes physical and biological processes in the treatment of its sewage flow. This STP is unique in that it combines two biological waste treatment systems, fixed film and fluidized culture. The fixed film is conveniently termed "trickling filter" and the fluidized culture is termed "activated sludge". Additionally, the STP has aerated ponds which serve as polishing and equalization for the land application phase. Provisions have been made for odor control, such as lime addition, partial chlorination, and off-gas ozonation.

The biological treatment system, called contact biofiltration (CBF), appears to be a modification of a system described by Owen and Slechta in *Water and Sewage Works*, November 1975. The Sunol STP utilizes a variation of the combined trickling filter and activated sludge systems by using the system of contact stabilization. Figures IV. A-2 through IV. A-5 present the general plant layout flow scheme for liquid wastes and solids handling facilities.

The flow scheme proceeds as follows. After the raw influent passes through the chemical addition, pre-aeration, and primary sedimentation basins, the wastewater is combined with biological solids returned from the sludge aeration unit and biofiltration unit (trickling filter) recycled in the bio-filter sump to form a mixed liquor (similar to contact stabilization). This mixture (mixed liquor) is pumped from the bio-filter sump to the trickling filter, where it is distributed over a horizontal redwood slat filter media, reportedly creating an attached microorganism growth. The trickling filter underflow (effluent) proceeds to a second bio-filter sump where provisions exist for recycle of some portion of the flow back to the first bio-filter sump. The liquid that is not recycled proceeds to the secondary sedimentation basin where the biological solids are separated from treated wastewater. The biological solids are removed from the secondary sedimentation basins and some portions are wasted to the sludge digesters. The remaining sludge (biological solids) proceeds to the bio-unit sludge aeration tank where air is blown through the mixture. This stabilized sludge then proceeds to the first bio-unit sump, where it is mixed with fresh incoming clarified wastewater to undergo treatment in the trickling filter.

The liquid effluent from the secondary sedimentation basin is discharged to two aerated ponds in series. Water is pumped from the second pond to the spray irrigation fields.

3. Sunol STP Spray Effluent Quality

In general, the effluent quality as measured by Total Organic Carbon (TOC) and BOD values appeared to show significant seasonal variability. The average BOD and TOC concentrations during August-September 1975 were 35 and 48 mg/l, respectively, while during May-June 1976 the BOD average was 19 mg/l and the TOC was 33 mg/l. From October to December, 1976, the BOD values indicated effluent quality similar to the 1975 data, with monthly averages from 35 to 50 mg/l. After the first of the year, however, the effluent quality again improved, giving average BOD's from 19 to 24 mg/l.

The effluent quality as measured by organic parameters [TOC, COD (Chemical Oxygen Demand), BOD] and suspended solids appeared to improve during the Pre-Fair sampling period. The values showed slight declines during the first three weeks, then a marked decline from the third to the fourth week, and remained at this lowered level until the end of the sampling period.

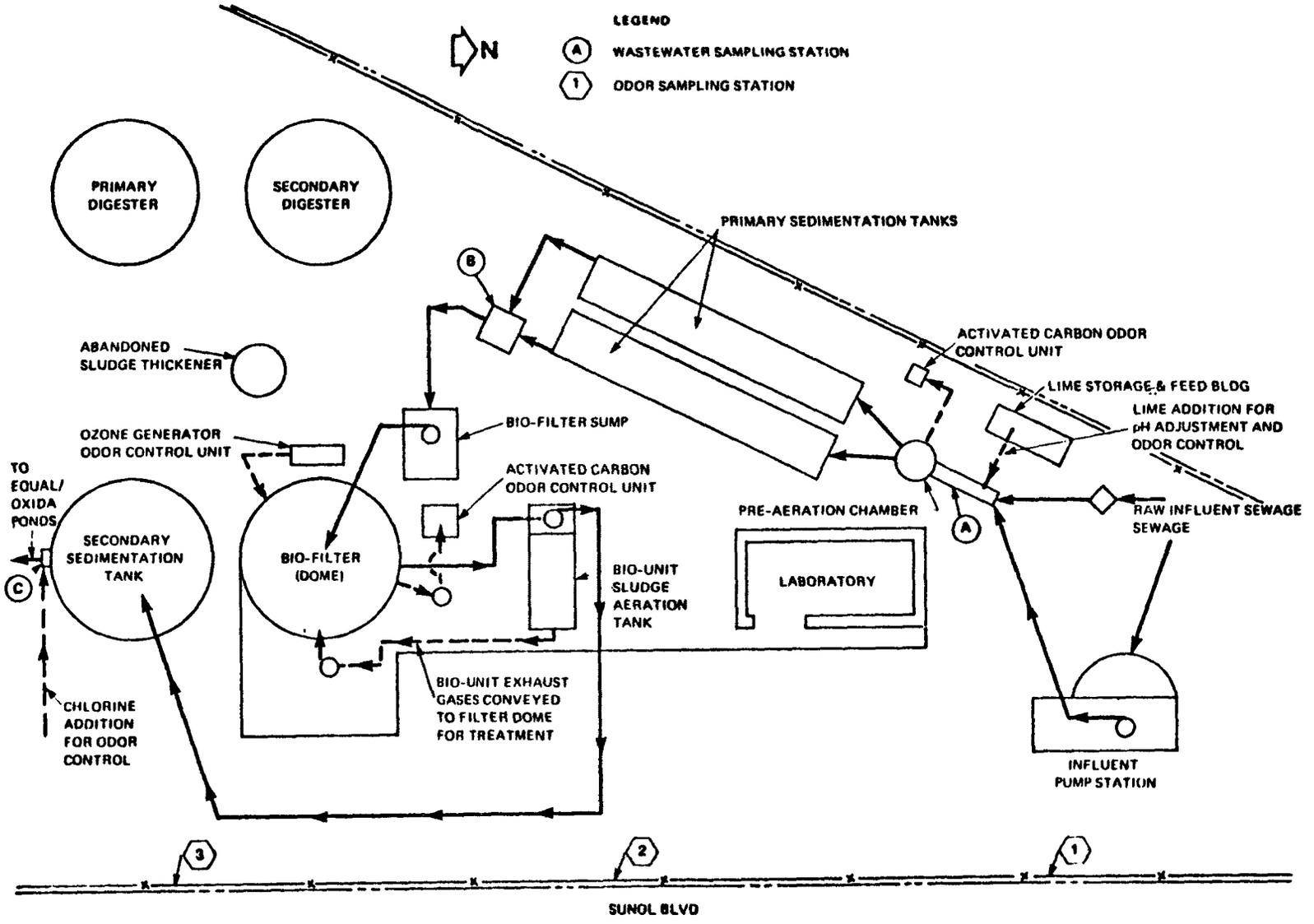


Figure IV.A-2.
PLANT LAYOUT AT PLEASANTON, CA

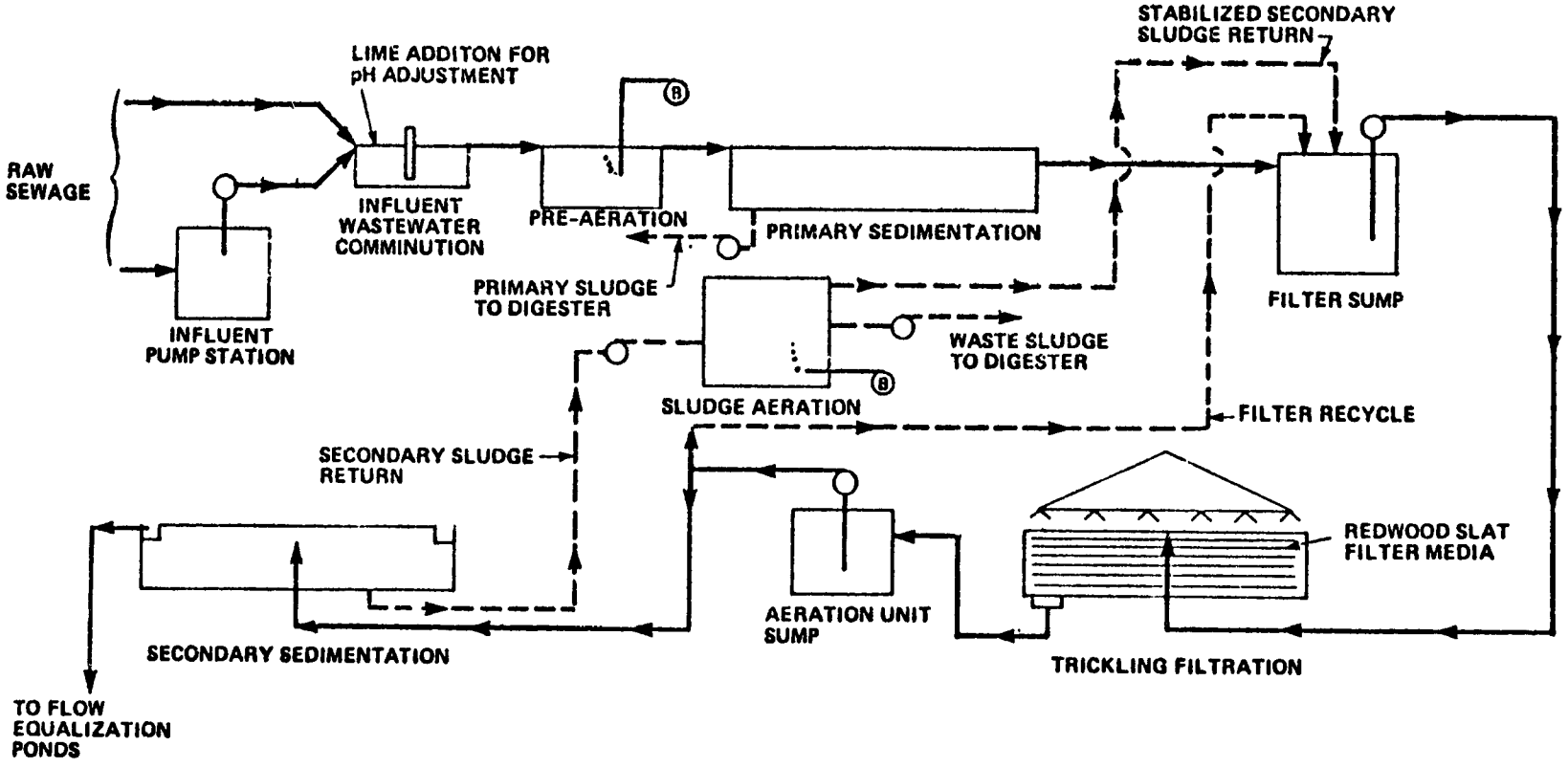


Figure IV.A-3.
CONTACT BIO-FILTER PROCESS (CBF)

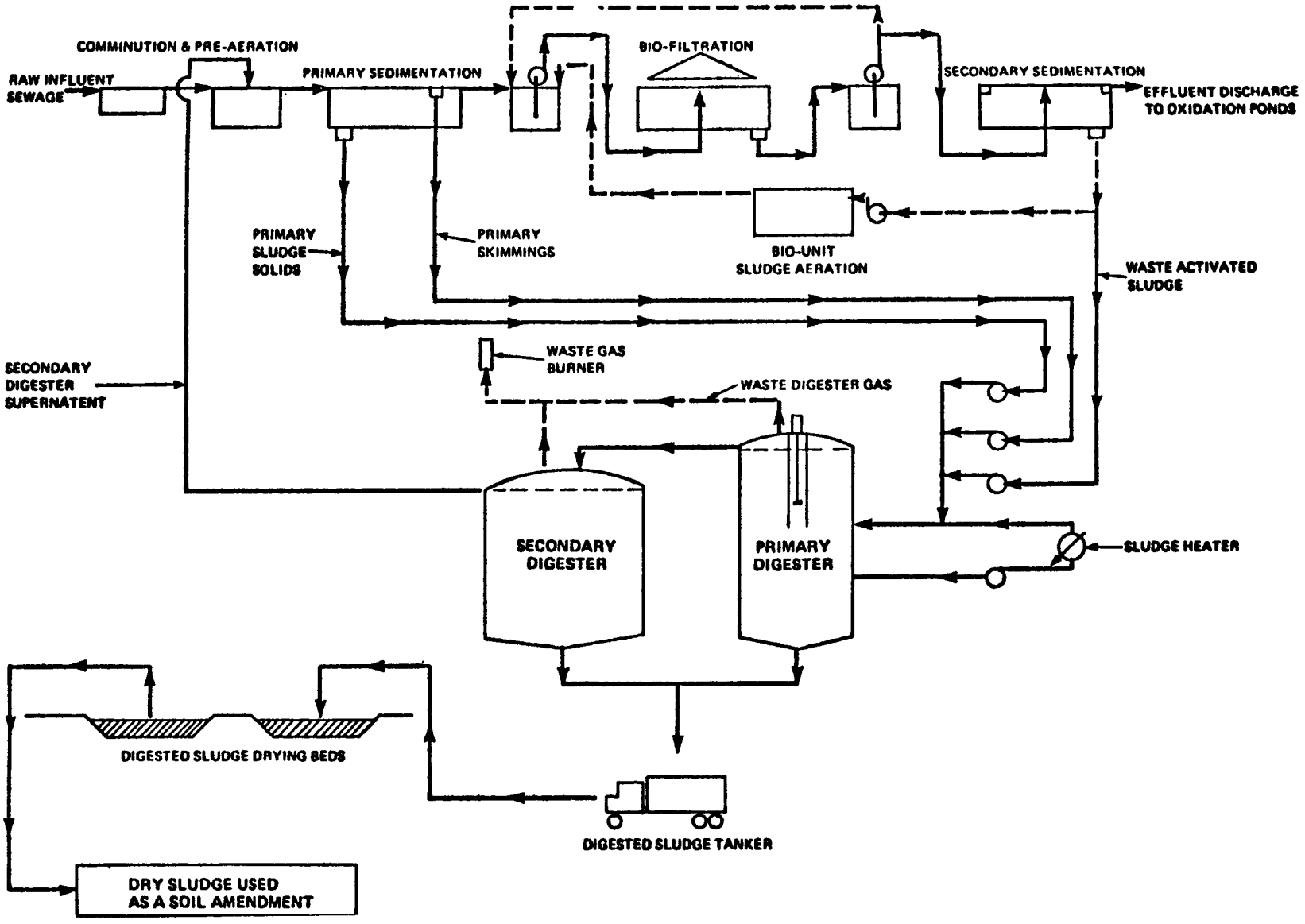


Figure IV.A-4.
SOLIDS HANDLING FACILITIES

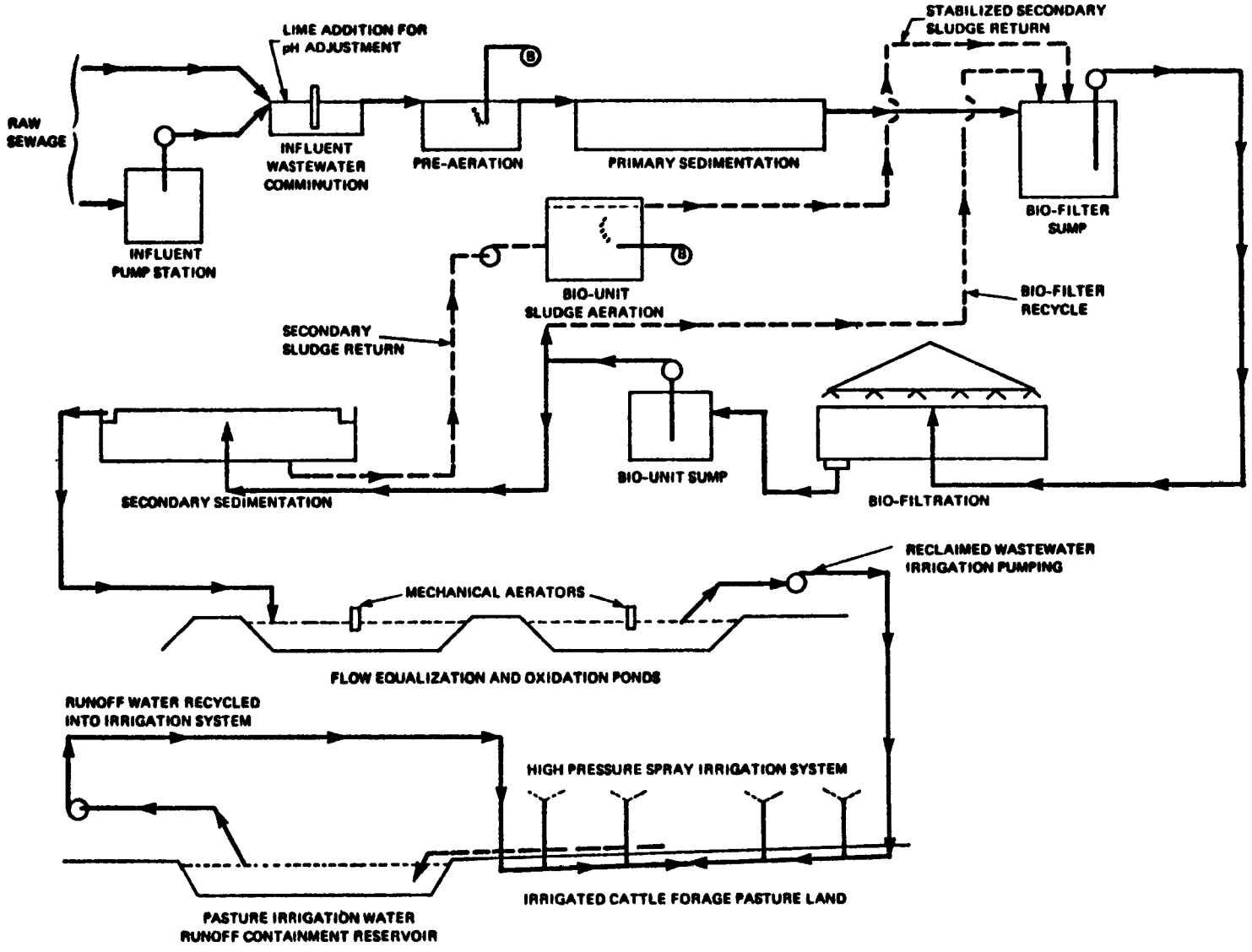


Figure IV.A-5.
PLANT AND DISPOSAL FACILITY FLOW SCHEMATIC

The weekly average values for BOD (during Pre-Fair) met the standard for secondary treatment as defined by EPA (30mg/l BOD), but exceeded the standard in the October through December, 1976 period. After this time, the levels decreased to be consistent with EPA guidelines. During the period of the fourth week until the seventh week of the Pre-Fair, the weekly average values for total suspended solids (TSS) also met the standard for secondary treatment of 30 mg/l TSS.

During the Pre-Fair sampling period, one sampling date, May 27th, coincided with sampling of the aerated pond effluent by plant operators. A comparison is made below:

	SwRI Data	Sunol STP Plant Data
Total Solids (mg/l)	618	631
Total Suspended Solids (mg/l)	12	15
BOD (mg/l)	11(May 28)	10

These values for BOD and TSS represent reasonably good quality effluent.

The Sunol STP plant operators sampled the effluent from the secondary sedimentation tanks. This sampling point does not represent overall plant effluent but represents influent to an additional treatment step -aerated ponds. The values for May, June, and July are presented in Appendix A, along with October through April analyses. A summary of these results is presented in the following table.

TABLE IV. A-1. SUMMARY OF SUNOL SECONDARY EFFLUENT PARAMETER ANALYSIS MONTHLY AVERAGE

Month/Year	BOD_5 (mg/l)	TSS (mg/l)	COD (mg/l)
Pre-Fair			
May, 1976	27	15	93
June	29	40	--
July	26	42	--
Post-Fair			
October	34	24	--
November	>34*	24	--
December	48	36	--
January, 1977	42	--	100
February	27	15	--
March	21	18	--
April	22	15	--

*one value reported as 54 mg/l.

These values suggest that some removal of organic material (suspended or dissolved) is taking place in the aerated ponds.

A listing of operational modifications by date is presented in Appendix B. These operational changes were primarily made as controls of the suspended solids in the mixed liquor. The addition of digester solids was made to increase the mixed liquor suspended solids (MLSS) concentration in order to maintain approximately 3,000 mg/l MLSS applied to the bio-filter. The wastage of the return activated solids (RAS) to the primary sedimentation tanks was made for the same purpose.

B. Spray Irrigation Operations

The spray equipment is designed so that a volume of water equal to the amount of incoming raw sewage, less evaporation losses, is sprayed onto cattle pastures daily. This is attained by keeping the level in the final effluent pond No. 2 (see Figure IV. B-1) receiving the secondary effluent as constant as possible by pumping more or less water to the irrigation fields. This is accomplished by adjusting the daily irrigation time from 12 to 18 hours. Two pumps, 100- and 75-HP, respectively, with a third standby pump, are used to move the water through 10-in. mains to the fields.

Table IV. B-1 lists the daily incoming raw sewage volumes versus the volume sprayed for the months of May and June, 1976. The volume of wastewater sprayed daily is estimated from the water levels in ponds 1 and 2.

TABLE IV. B-1 PLANT FLOW DATA
(thousands of gallons)

	MONTH			
	MAY		JUNE	
DAY	INFLOW	OUTFLOW	INFLOW	OUTFLOW
1	1,310	1,873	1,371	1,486
2	1,293	1,981	1,386	1,466
3	1,301	1,878	1,366	1,300
4	1,398	1,410	1,400	1,183
5	1,310	1,398	1,183	1,368
6	1,298	1,676	1,384	1,476
7	1,296	1,657	1,406	1,406
8	1,377	1,756	1,476	1,580
9	1,276	1,741	1,480	1,317
10	1,261	2,137	1,417	1,582
11	1,357	1,911	1,482	1,400
12	1,331	1,028	1,046	1,390
13	1,328	1,410	1,400	1,356
14	1,310	1,357	1,390	1,476
15	1,357	1,291	1,376	1,432
16	1,291	1,791	1,332	1,108
17	1,291	1,600	1,408	1,426
18	1,400	1,016	1,326	1,392
19	1,316	1,103	1,342	1,332
20	1,403	1,206	1,332	1,331
21	1,306	1,465	1,331	1,620
22	1,365	1,568	1,520	1,252
23	1,368	1,494	1,452	1,433
24	1,394	1,335	1,333	1,282
25	1,535	1,491	1,382	1,475
26	1,391	1,232	1,475	1,172
27	1,332	1,457	1,372	1,611
28	1,357	1,884	1,361	1,594
29	1,384	1,256	1,494	2,001
30	1,256	1,076	1,501	1,008
31	1,176	1,076		
TOTAL:	41,368	46,554	41,734	42,273
AVG.:	1,334	1,502	1,391	1,409

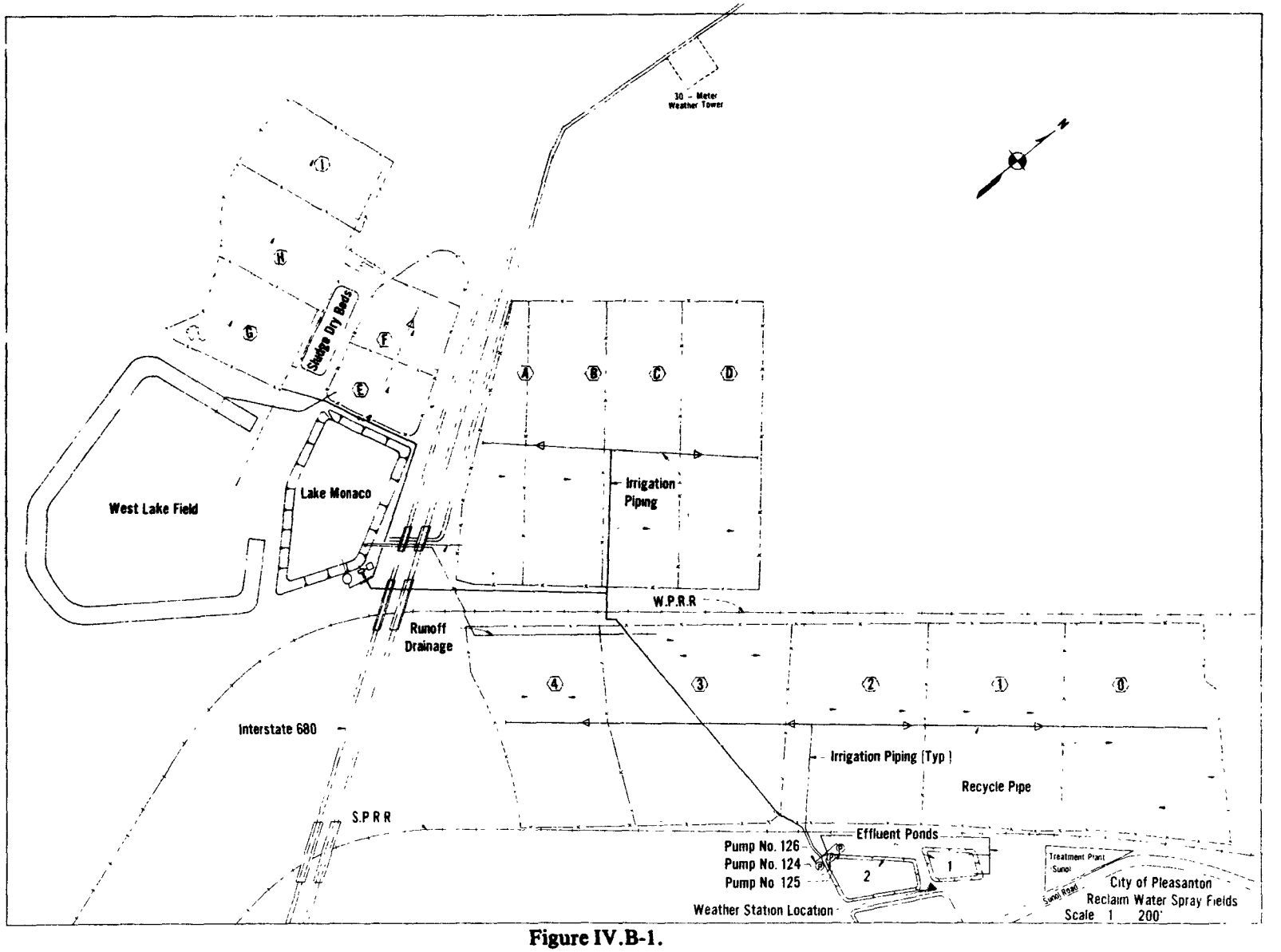


Figure IV.B-1.
SPRAY PATTERN FOR FIELDS DURING PRE-FAIR STUDY

Two major parcels and one smaller parcel of land are subdivided into smaller fields for the purposes of cattle management. The two larger areas are a 62-acre field labeled A,B,C, and D and a 100-acre field labeled 0,1,2, 3, and 4. The smaller fields across Interstate 680 are labeled E,F,G,H, and I.

The water is applied to these fields through Rainbird® No. 30 sprinkler heads which have been drilled to have 7/32- and 11/64-in. orifices. The spray heads , on 2-ft. high risers of 1-in. iron pipe, are connected to 3-in. aluminum irrigation pipes. The sprayers are located every 30 ft. along the irrigation pipe. Each spray wets an area approximately 18 meters in diameter and reaches a maximum height of approximately 5 meters.

The transit time of the water in the pipes ranges from 4 minutes, 40 seconds to the center sprays in field 3 to 24 minutes, 40 seconds to the furthermost sprays in field D. These transit times were measured by observing the appearance of dye at the sprays after its introduction at the pumps. Transit time to the fields across the Interstate was not measured, since no aerosol sampling was planned for that area.

The rate of application of water from individual sprayers depended primarily on their location along a line of sprayers. Measured rates ranged from a high of 86 l/min at individual sprayers next to the main water line (usually in the center of the line of sprayers) to a low of 30 l/min for the sprayers at the ends of sprayer line. These rates varied depending on the field and number of sprayers used.

As an example, Table IV. B-2 presents the flow measurements made in field B at every other spray starting from the northwest side of the field. These flows were determined by measuring the time required to fill a 20-l container directly from the individual spray heads. The entire flow from both nozzles on each sprayer was directed into a measuring container by two short lengths of tubing slip-sealed over the nozzles. The time required to fill the 20-l container was measured using a standard stopwatch.

TABLE IV. B-2. SPRAY VOLUME—FIELD B
(Every other Spray, Second Day Setting)

Spray Head No.	Liter/Minute Flow
1	35.3
3	35.8
5	36.7
7	40.5
9	39.9
11	42.9
13	43.6
15	46.2
17	50.0
19	53.6
21	51.1
23	69.8
25	74.1
Main irrigation supply line	
27	78.9
29	63.5
31	32.3
33	44.1
35	53.1
37	46.0
39	45.3
41	42.1
43	39.9
45	39.2
47	31.0
49	38.7
51	42.0

Effluent is simultaneously sprayed onto one field in each of three parcels of land (i.e., three separate fields) every day of the year. The dashed lines in Figure IV. B-1 represent the first position the spray lines would be in for each field if irrigation was scheduled for that particular field. Note that some fields have more than one spray line. The number of days each field is irrigated and the number of sprayers used is indicated in Table IV.B-3. It should also be noted that some fence lines were changed between Pre- and Post-Fair sampling periods.

TABLE IV.B-3. CHARACTERISTICS OF PRE-FAIR SPRAY FIELDS

Field No. or Letter	No. of Days Irrigated	No. of Spray Heads
0	7	70-90
1	6	66-69
2	6	68
3	4	59-69
4	5	70
A	7	35-51
B	7	51-53
C	7	52
D	7	52
E	6	13-17
F	7	17
G	8	18
H	8	18-19
I	7	19

The number of days irrigated and the number of sprayers used depends on the size, shape, and contour of the field being irrigated. The normal sequence of irrigation begins with fields 4, A, and E, with the spray lines being moved 60 ft. daily in the direction of the arrows as shown in Figure IV. B-1. Once any field (4, A, or E) has been irrigated, the spray lines are moved to the first position of the next field to be irrigated (i.e., 3, B, or F). This sequence is continued daily, always keeping the cattle in the dry field just ahead of the irrigation.

Runoff from the spray fields drains through steel pipe to a holding pond (Lake Monaco) located southwest of Interstate 680 (Figure IV. B-1). This pond, as well as West Lake Field, is used for additional holding capacity. This additional capacity is needed during rainy periods and as a reservoir for holding of effluent if problems occur with the spray operations. The plant may also irrigate from Lake Monaco when necessary.

C. On-Site Facilities

On-site facilities consisted of a large storage area and a mobile office trailer with laboratory space. The storage building was used as an assembly area for the maintenance and storage of all portable field equipment. The laboratory space in the mobile office trailer was utilized for sample preparation and on-site analysis. Various equipment in the laboratory essential to the operation of the Pre-Fair and Post-Fair studies are in Appendix D.

D. General Meteorological Conditions

1. Pre-Fair

May and June, 1976, were, from a meteorological standpoint, abnormally warm and dry in the general area of the sampling site and, indeed, in most of California. The period was marked by a persistent surface high pressure area centered in the Pacific Ocean well off the West Coast of the United States. This high pressure extended over California.

Aloft at 500 mb (about 18,000 ft), a low pressure area persisted over or in the vicinity of the Gulf of Alaska. Occasionally, this low pressure area would elongate generally north-south in the Pacific Coast area.

These synoptic features resulted in weak maritime cold fronts moving southeastward from the vicinity of the Gulf of Alaska and the area immediately to the south of the Gulf down over the Pacific Coast at intervals ranging from every one to three days. The weaker cool outbreaks tended to move primarily across the northern tier of states, with their western extremities moving through or close to the sampling site with an east-west orientation. Such fronts tended to dissipate in the general area of the sampling site. The stronger cool outbreaks tended to continue moving southeastward across the Rockies to the central United States. The accompanying western extension of the frontal zones moved through the sampling site with a northeast-southwest orientation. However, as the cool push continued into Utah, Arizona, and New Mexico, the trailing or western extremity of the front tended to pass through the sampling site and then stagnate with a north-south orientation paralleling the coastal hill ranges. This situation resulted in a temporary trough of low pressure lying near the sampling site, or moving eastward across the site and then regressing to the west.

Precipitation with the frontal passages was mainly confined to the states north of California. Very seldom during May and June did precipitation reach California and, when it did, it was generally confined to extreme coastal northern California.

The windrose pattern (percentage of time wind blows from a certain direction at several ranges of velocity) measured for the period of May and June, 1976 is presented in Figure IV. D-1. This pattern is significantly different than that measured for August and September, 1975. Calm winds were prevalent 20% of the time during the later period versus 70% of the time during August and September, 1975.

2. Post-Fair

The period of December 1976 through January 1977 was marked by a persistent surface high pressure area centered over the Great Basin to the east of Pleasanton. The 500-mb flow was predominantly from the northwest influenced by a quasi-permanent ridge just off the west coast. The result was a generally weak surface wind flow at the site from the east quadrant.

The period of February through April, 1976, for the most part, came under the influence of a surface high pressure area centered over the Pacific Ocean well off the west coast of the United States. This pressure system extended its influence over California and brought surface winds to the Pleasanton area from the northwest and north quadrants. The flow at 500 mb was also generally from the northwest, with ridging persisting over the Pacific Ocean. There were brief interludes of frontal passages during this period which were preceded by surface winds from the southwest in the Pleasanton area.

A set of synoptic weather maps is presented in **Appendix C**, in order to better depict the various synoptic regimes which occurred during each of the sampling trials conducted during the period of December 1976 through April 1977. The surface winds observed during this period were characterized by three distinct synoptic regimes as follows:

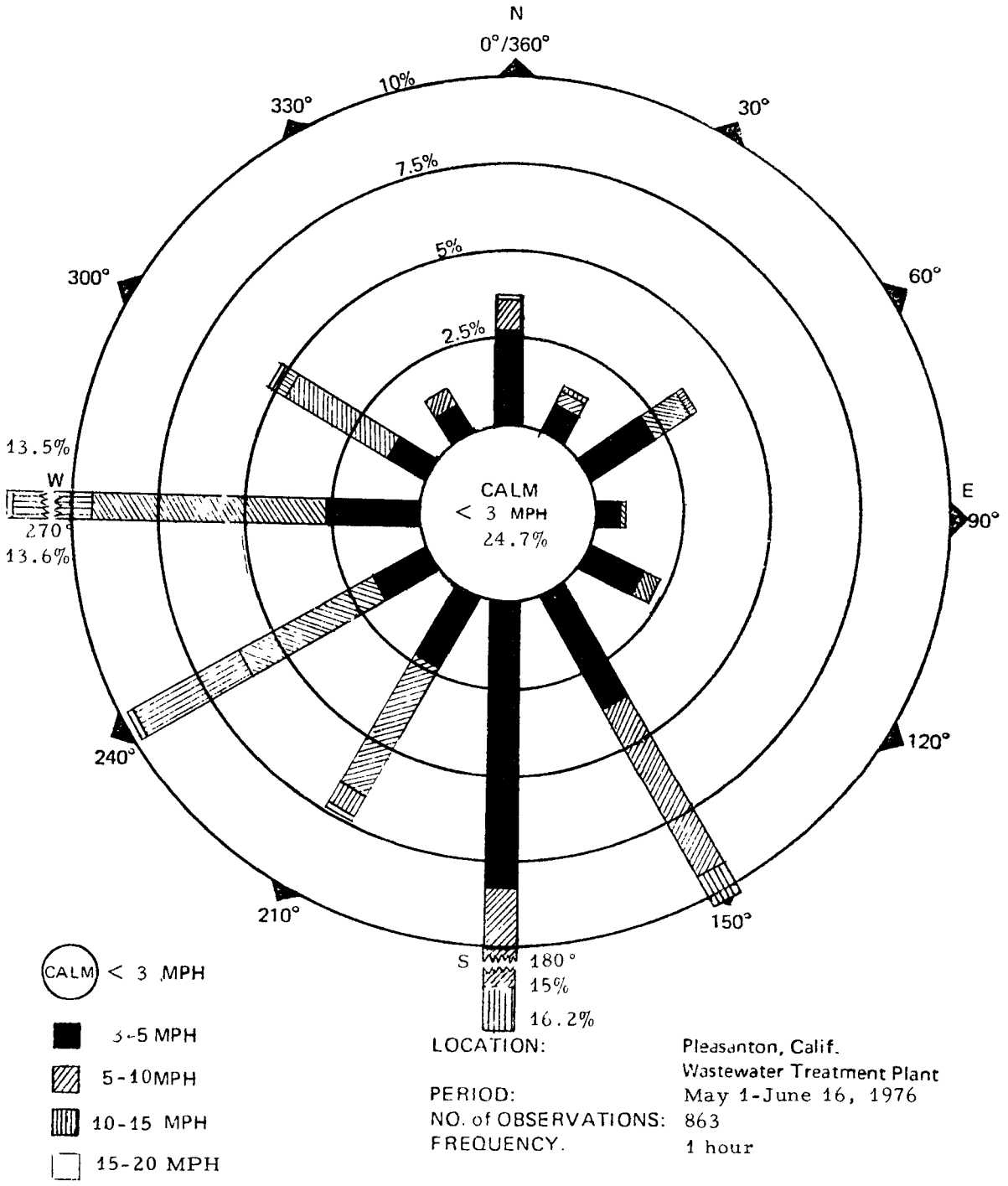
Synoptic Regime No. 1

A high pressure cell is centered over the Great Basin to the east or northeast of Pleasanton. The mean surface wind direction observed during the trial periods was from the east quadrant.

Synoptic Regime No. 2

A cold front approaches the Pleasanton area from the west or northwest. The mean surface wind direction observed was from the south quadrant.

Figure IV.D-1.
PRECENT DISTRIBUTION OF WIND DIRECTIONS AND WIND SPEEDS



Synoptic Regime No. 3

A post-frontal high pressure system lies to the west or northwest of Pleasanton. The mean surface wind direction observed during the trial periods was from the northwest and north quadrants.

During the period of testing, the surface winds over the site were generally light. Under this situation, the wind direction was quite variable because of the influence of local topography. Pleasanton is situated in a valley bounded on the near west by a low range of hills. Another range of hills lies to the southeast and is oriented northeast-southwest. The south opening of the valley leads to San Jose, and the terrain to the north slopes gradually upward. Thus, when the surface winds are light, there is an ill-defined flow generally from a northwesterly direction or its reciprocal broken by a sporadic flow from the northeast or southwest. A well-organized flow from the southeast occurs when a front is approaching and becomes northwesterly after the frontal passage.

The orientation of the spray line (northwest-southeast) is such that when the flow is well organized it parallels the spray line. Conversely, under light wind conditions the surface wind direction is variable, and the flow perpendicular to the spray line occurs only intermittently.

V. Methods and Materials

Note to Reader: Complete documentation of the methods and materials is necessary to establish the credibility of this study's results and to serve as a guide to future investigators. Inclusion of this lengthy section in the body of the report, however, hampered readability and was not necessary to permit reader comprehension of the study's findings. Thus, the complete Materials and Methods section has been included as Appendix D. The following synopsis of that section is provided to indicate what procedures, measurements and evaluations were performed and their reason for inclusion in this research effort. Appendix D follows the same outline format as this section to aid the reader requiring greater detail.

A. Sample Collection and Handling Methods

1. Meteorological Measurements and Instrumentations

On-site meteorological measurements were required to: 1) permit appropriate placement of the aerosol sampling equipment and (2) provide necessary input to the microbiological dispersion model. Measurements made during the study period included: wind velocity and direction, temperature, relative humidity, precipitation, and solar radiation. In addition, estimates of the atmospheric stability category during each aerosol run were made using meteorological data and observations to provide additional input to the microbiologial dispersion model. A site map giving meteorological measurement locations is presented in Figure V. A-1.

2. Wastewater Sampling Methods

A wastewater sampling and analysis program was conducted to document the quality of effluent during the study period and explore the possible relationships between effluent quality and aerosol concentrations.

a. Daily Composite Samples

During Pre-Fair studies only, daily composite effluent samples were collected from the wastewater being pumped from pond 2 to the spray fields. These samples underwent analysis for pH, chlorine (free and total), total organic carbon, solids (suspended and total), nitrogen series (nitrate, nitrite, ammonia, and organic nitrogen), phosphorus, BOD, COD, hardness and microbiological analyses.

b. Grab Samples

Three types of wastewater grab samples were collected during the study period.

1. Pond-chlorine. A single 500 mL grab sample was collected daily near the pond 2 pumping station for analyses of free and total chlorine (Pre-Fair only).

2. Pond-pathogen screen. A number of 20-liter large-volume grab (LVG) samples were collected from pond 2, in the vicinity of the spray field pumps, for detailed microbiological characterization. This permitted identification of prevalent pathogens in the effluent as candidates for later more routine analyses.

3. Spray line. To permit direct comparisons between effluent quality and aerosol concentration levels, wastewater samples were collected at the spray line during each aerosol sampling run.

3. Aerosol Sampling Methods

a. High-volume Samplers For Microorganism Aerosols

A total of 16-18 high-volume aerosol samplers were available during the study and usually

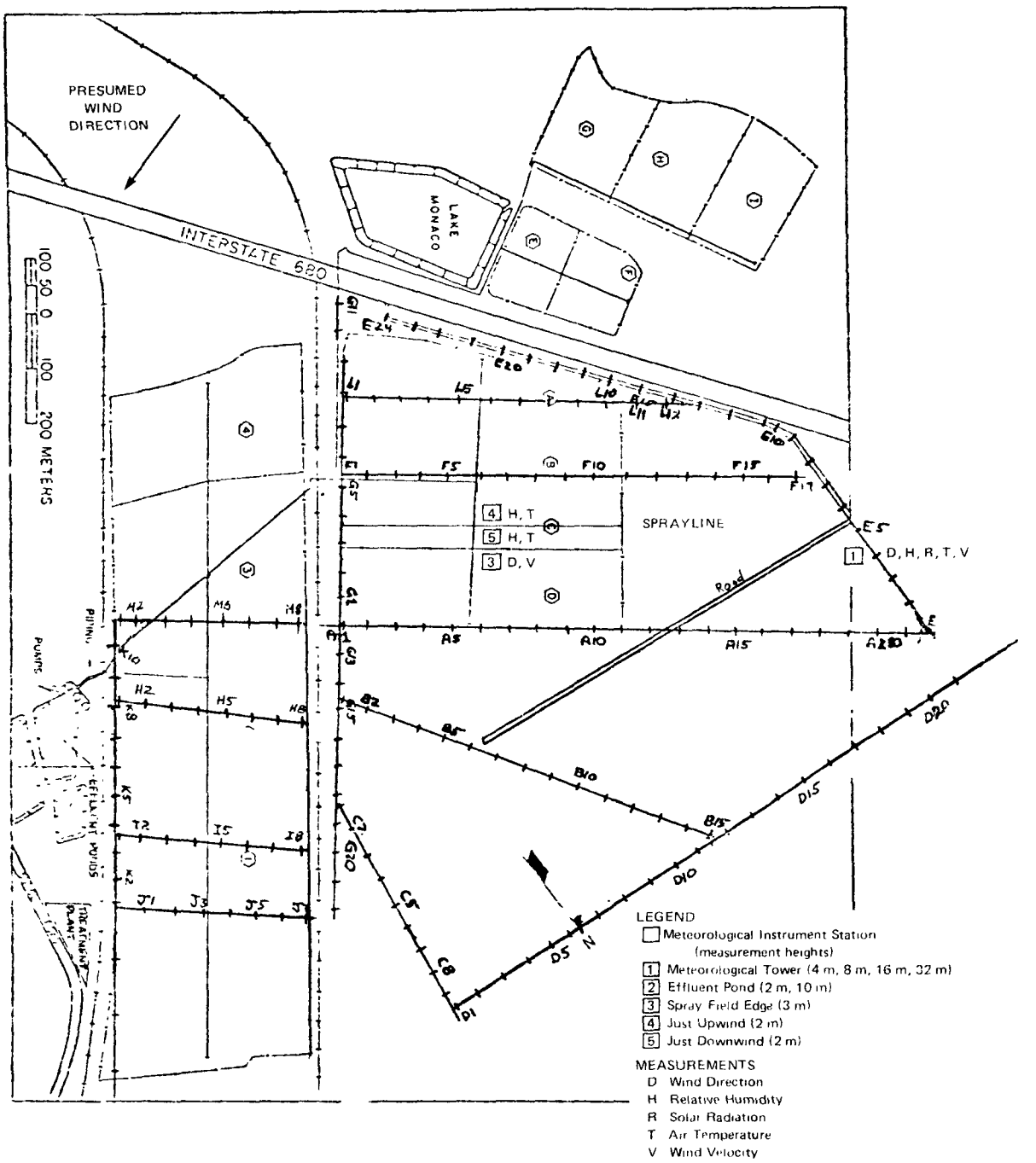


Figure V.A-1.
SITE MAP WITH ENVIRONMENTAL MEASUREMENT LOCATIONS

about eight were used simultaneously in most microbiological sampling efforts. Several were LEAP samplers, Model 3440, manufactured by Environmental Research Corporation, St. Paul, Minnesota. Most samplers were the Litton Model M Large-Volume Air Sampler, manufactured by Applied Science Division, Litton Systems, Inc., Minneapolis, Minnesota. Both samplers are designed to collect airborne particles by electrostatic attraction to a rotating disk on which they are concentrated into a thin, moving film of liquid. The nominal sampling rate of both instruments is 1000 liters per minute. Nominal sampling time per aerosol run was 30 minutes.

Appendix D provides extensive detail on description of samplers, selection of the liquid collection medium, sampler calibration, sampler disinfection procedures and sample handling.

b. All Glass Impinger (AGI) Samplers For Dye Aerosols

A 20-percent solution of Rhodamine WT dye was injected into the spray line and resultant aerosols sampled using 50-mL graduated all-glass impingers manufactured by Scientific Glass and Instruments, Houston, Texas. The purpose of these dye aerosol runs, conducted during a variety of meteorological conditions, was to determine the percent of the sprayed wastewater that leaves the site as aerosol (aerosolization efficiency).

c. Rotorod Samplers For Fluorescent Particle (FP) Tracer

During Post-Fair microbiological aerosol sampling runs, a fluorescent particle (FP) aerosol was generated near the spray line and sampled downwind, as a quasi-quantitative tracer, to document wind (and, consequently, aerosol plume) direction. A total of 40 rotorod samplers (Barber Colman type BYQM 2020) were available for sampling FP aerosols, and about 20 were used during each run.

B. Analytical Methods

1. Chemical Analysis of Wastewater Samples

Chemical analyses of wastewater samples fell under two categories, "routine" and "selective", based on the frequency these analyses were conducted during both Pre-Fair study periods. Tables V. B-1 and V. B-2 identify the parameters measured and reference the method used during both study periods.

In order to justify the shipment of TOC water samples to SwRI laboratories in San Antonio for subsequent analysis (maximum 14 days holding time) a holding time study was performed.

Fluorescent particles (FP), collected on rotorod samples during Post-Fair aerosol sampling, were analyzed microscopically. The rotorod FP samples were analyzed by comparison to six standard samples provided by Dugway Proving Grounds.

2. Microbiological Analyses

a. Wastewater

Most microbiological analyses of wastewater samples were conducted on a routine basis. Table V. B-3 lists the microorganisms that were routinely assayed from daily composite of spray line grab samples during the Pre-Fair and Post-Fair study periods. The enumeration of these microorganisms in wastewater samples provided a basis for evaluating their survival when aerosolized.

Most large-volume grab wastewater samples, collected from pond 2 primarily during Pre-Fair, underwent analysis for the 20 microbial types listed in Table V. B-4. This pathogen screen was intended to identify prevalent microorganisms in the effluent as improved candidates for routine analysis during later study periods. One large-volume sample was frozen prior to shipping and analyzed for respiratory viruses (adenoviruses, mumps virus, REO viruses, herpesviruses, cytomegalovirus, and measles virus).

b. Aerosols

Aerosol samples were submitted for analysis of the same microorganisms identified for wast-

Table V.B-1
ANALYTICAL METHODS FOR PRE-FAIR WASTEWATER CHEMICAL ANALYSES

Analysis	Analytical Laboratory*	Method Ref+	Method Page	Detection Limit mg/L	Remarks
Routine Parameters					
pH	SwRI-PL	1	276	NA	Glass electrode and Fischer Accumet pH meter.
Free chlorine	SwRI-PL	3		0.05	FACTS method. Optimal density determined by Hach DR-EL/2.
Total chlorine	SwRI-PL	1	129	0.1	DPD ferrous colormetric method. Optical density determined by Hach DR-EL/2.
Total Suspended Solids (TSS)	PEL	2	268	10	Gooch crucible with glass fiber filtration and dryness at 103 to 105°C.
Total Solids	PEL	2	270	10	Evaporation at 103 to 105°C.
Total Organic Carbon (TOC)	SwRI-SA	2	237	1	Analysis performed with a modified Beckman Carbon Analyzer after acidification and N_2 stripping.
Selective Parameters					
Chemical Oxygen Demand (COD-total)	SwRI-SA	1	495	2	Dichromate reflux followed by ferrous ammonium sulfate titration.
Biochemical Oxygen Demand (BOD)	PEL	1	489	1	5-day incubation at 20°C. Weston and Stack D.O. probe.
Total Phosphorus	SwRI-SA	1	523	0.1	Persulfate digestion, stannous chloride color development.
Nitrate Nitrogen (NO_3—N)	SwRI-SA	1	461	0.1	Brucine method.
Nitrate Nitrogen (NO_2—N)	PEL	2	215	0.01	Diazo dyemethod.
Ammonia Nitrogen (HH_3—N)	SwRI-SA	1	222,244	0.1	Distillation followed by Nesslerization.
Organic Nitrogen (Organic N)	SwRI-SA	1	244	0.1	Determined on residue from ammonia distillation. Digestion, distillation and Nesslerization.
Total Hardness ($CaCO_3$)	SwRI-PL	1	179	5	EDTA Titration.

* Analytical laboratories were:

SwRI-SA - Southwest Research Institute, San Antonio laboratories
SwRI-PL - Southwest Research Institute, Pleasanton, California facility
PEL - Pacific Environmental Laboratory, San Francisco, California

+ References and footnotes for analytical tests were:

(1) AWWA, APHA, WPCF, *Standard Methods for the Examination of Water and Wastewater*, Thirteenth edition, American Public Health Association, Washington, D.C. 1971.

(2) *Methods for chemical Analysis of Water and Wastes*, U. S. Environmental Protection Agency, Washington, D.C. 1974.

(3) Cooper, W.J., Sorber, C.A. and Meier, E.P., "A Rapid Specific Free Available Chlorine Test with Syringaldazine (FACTS), *Journal of American Water Works Association*, 67 (1), 34-39, January 1975.

Table V. B-2
ANALYTICAL METHODS FOR POST-FAIR WASTEWATER CHEMICAL ANALYSES

Analysis	Analytical Laboratory*	Method Ref+	Method Page	Detection Limit mg/L	Remarks
Routine Parameters					
pH	SwRI-PL	1	460	NA	Glass electrode and Fischer Accumet pH meter.
Total Organic Carbon (TOC)	UTSA	2	237	2	Samples acidified upon collection. N_2 stripped before analysis. Beckman Model 915A with Model 865 Infrared Analyzer.
	SwRI-HOU				Modified Beckman Model315 with Model 865 Infrared Analyzer.
Total Suspended Solids (TSS)	UTSA	1	94	10	Glass fiber filtration and dryness at 103 to 105°C.
Conductivity	SwRI-PL	1	71	5 mho/cm	Hach DR-EL/2 conductivity probe.
Selective Parameters					
Chemical Oxygen Demand (COD)	PEL	1	550	2	Dichromate reflux followed by ferrous ammonium sulfate titration.
Nitrate Nitrogen (NO_3—N)	PEL	1	427	0.1	Brucine method
Ammonia Nitrogen (NH_3—N)	PEL	1	417	0.1	Distillation followed by titration with standard acid.
Organic Nitrogen	PEL	1	437	0.1	Determined on residue from ammonia distillation. Digestion, distillation and titration with standard acid.
Nitrate Nitrogen (NO_2—N)	PEL	2	215	0.01	Diazo dye method.
Total Phosphorus (Total) P	PEL	1	473	0.1	Persulfate digestion, stannous chloride color development.
Total Solids	PEL	1	91	10	Evaporation at 103 to 105°C.
Biochemical Oxygen Demand (BOD_5)	PEL	1	543	1	5-day incubation at 20°C. Weston & Stack D.O. Probe.

*** Analytical laboratories were:**

SwRI-SA - Southwest Research Institute, San Antonio laboratories
SwRI-PL - Southwest Research Institute, Pleasanton, California facility
PEL - Pacific Environmental Laboratory, San Francisco, California

+ References and footnotes for analytical tests were:

(1) AWWA, APHA, WPCF, *Standard Methods for the Examination of Water and Wastewater*, Thirteenth edition, American Public Health Association, Washington, D.C. 1971.

(2) *Methods for chemical Analysis of Water and Wastes*. U. S. Environmental Protection Agency, Washington, D.C. 1974.

Table V.B-3
MICROORGANISMS ROUTINELY ASSAYED IN WASTEWATER

Microorganism	Pre-Fair	Post-Fair
Standard bacterial plate count	D,R	R
Total coliform	D,R	R
Fecal coliform	D,R	
Coliphage	D,R	R
Fecal streptococci	D	R
Pseudomonas	D	
Klebsiella	D	
Clostridium perfringens	D	
Mycobacteria		R
Enteroviruses-3 day count	D	
Enteroviruses-5 day count	D	

D—daily composite sample
R—spray line grab sample with each aerosol run

Table V.B-4
MICROBIALTYPES SOUGHT IN PATHOGEN SCREEN

Quantitative:
- *Klebsiella*
- *Pseudomonas*
- *Clostridium perfringens*
- Fecal Streptococci

Semi-quantitative:
- *Staphylococcus aureus*
- Mycobacteria
- *Leptospira*
- *Shigella*
- *Salmonella* (including Arizona)
- *Enterobacter*
- *Serratia*
- *Edwardsiella*
- *Escherichia*
- *Citrobacter*
- *Proteus*
- *Providencia*
- *Yersinia*
- *Neisseria* (pathogenic)
- *Aeromonas* and other oxidase-positive fermenters

ewater samples (10 assays on Pre-Fair samples, 5 assays on Post-Fair samples). Comparison of the wastewater and aerosol concentration data permitted evaluation of the survival properties of each microbial type. This included survival during the aerosolization process and, by comparion of concentration data at various downwind locations, long-term survival while in the aerosol state.

Several of the specific assay methods were different from those used for wastewater samples. This was largely due to the smaller volume of aerosol collection fluid available for assay.

C. Quality Assurance

A major goal of this research was to determine if wastewater measurements could be used as a basis for predicting the microbiological quality of aerosols from spray irrigation sites. This necessitated a definition of the accuracy and precision of all methods used. To accomplish this a quality assurance program was established which had two primary objectives:

(1) To determine the accuracy and precision of laboratory analyses, and

(2) To determine if there were systematic differences in high-volume aerosol sampler collection efficiency.

Quality assurance tasks for wastewater analyses included the use of spiked reference samples, the comparison of replicate samples sent to more than one laboratory and the comparison of replicate samples sent to a single laboratory. Quality assurance aerosol runs were conducted by placing all samplers at the same distance from the spray line on approximately one meter centers. Analyses of the sampler collection fluid were performed by several laboratories in a manner that permitted relative comparison of between-samplers and within-sampler precision.

D. Aerosol Sampling Protocols

1. Microbiological Aerosol Runs

Eight (when available) high-volume aerosol samplers were utilized during each aerosol sampling run. One was located well upwind to provide a measure of aerosol background levels. The remaining seven samplers were placed downwind to measure elevated aerosol levels due to operation of the spray source.

During Pre-Fair studies emphasis was placed on characterization of source strength and identification of pathogen survival characteristics upon aerosolization. The sampling configuration designed for the 20 Pre-Fair aerosol sampling runs is shown in Figure V. D-1. Following the eighth aerosol run, however, the five-meter station was eliminated and moved to 30 meters. This was due to the high frequency at which this station was hit with spray during wind gusts. Late in the Pre-Fair study period, it was determined that more duplicate samples at a given distance were required to better estimate source of variability. Thus, one of the following configurations was used during the last five aerosol runs:

Configuration A		Configuration B	
No. of Samplers	Distance	No. of Samplers	Distance
1	upwind	1	upwind
2	10 meters	2	20 meters
1	20 meters	1	30 meters
1	30 meters	1	40 meters*
1	40 meters*	2	50 meters*
2	50 meters*		

* double samplers at these distances were alternated on different runs.

Emphasis during Post-Fair studies was on modeling of downwind aerosol concentrations. Thus,

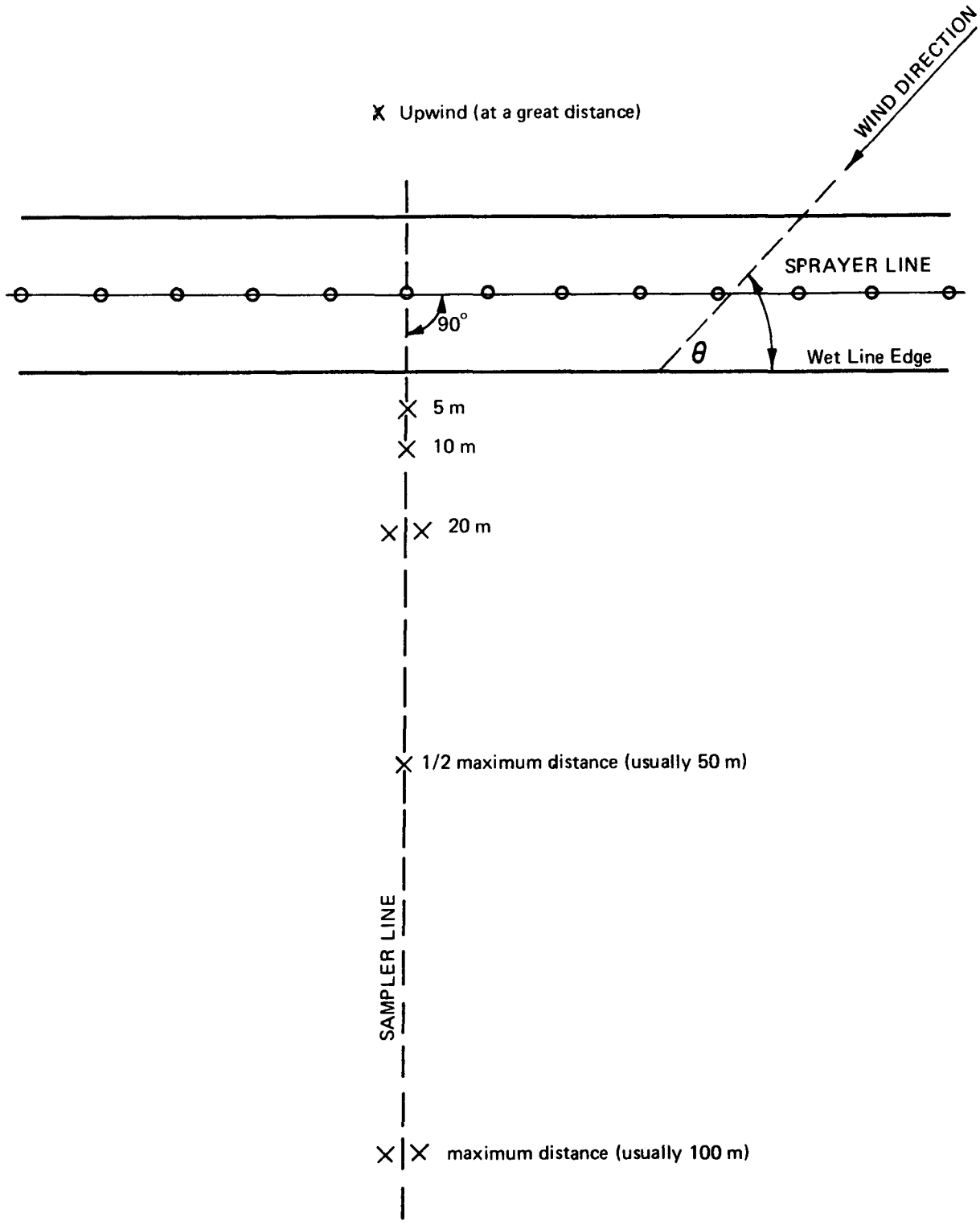


Figure V.D-1.
AEROSOL SAMPLING CONFIGURATION FOR PRE-FAIR

the configuration used for the 29 Post-Fair aerosol sampling runs (Figure V. D-2) required the placement of samplers at greater distances (up to 600 meters) from the spray source.

During both Pre- and Post-Fair studies, detailed protocols were prepared to provide guidance to field personnel. These included instructions by which the real-time on-site meteorological measurements should be used to determine: specific sampler locations; criteria for initiating or aborting aerosol runs, and whether collected samples should be shipped for analysis (good run) or discarded (bad run).

2. Dye Aerosol Runs

Dye aerosol runs were conducted during both Pre- and Post-Fair to determine the percent of sprayed wastewater that left the spray field as aerosol. Thus, a close-in configuration, as shown in Figure V. D-3 for the Post-Fair runs, was used for the eight AGI samplers used during Pre- and Post-Fair study periods respectively. Again detailed instructions were prepared to guide field personnel on the placement of samplers relative to wind direction and on the meteorological conditions necessary to initiate/accept as valid each run.

3. Quality Assurance Runs

Eight quality assurance runs were conducted (5 during Pre-Fair and 3 during Post-Fair studies). In each, all functioning samplers were placed side-by-side (separated by one meter in Pre-Fair and three meters in Post-Fair) at the same distance from the spray field. A randomized arrangement of samplers on both sides of the center line permitted evaluation of bacterial analyses precision (both within- and between-samplers) and a determination of systematic differences in the collection efficiency of the various high volume samplers.

4. Special Enteric Virus Aerosol Runs

During Pre-Fair studies, assays of the high-volume aerosol sampler collection fluid for enteric virus were consistently negative. This was due to their relatively low concentration in the wastewater and the inadequate sensitivity of the sampling procedure employed. Even at low concentrations, enteric virus aerosols could represent a significant hazard to human health. Therefore, a special sampling protocol was designed for Post-Fair studies to significantly improve the procedure's sensitivity and, thus, provide quantitative measurements of enteric virus aerosol concentrations. These special virus aerosol runs were designed to characterize source strength by placing all available aerosol samplers (12 or 13 samplers) side-by-side at a 50-meter distance from the spray source. The samplers were then operated for many consecutive aerosol runs (four for the first virus run and six for the second virus run). The 100 mL of collection fluid from each sampler, during each run, was placed in a common container. The total volume was then transported to the laboratory where it was concentrated and assayed to provide a single estimate of the enteric virus aerosol concentration. This represented an approximately 50-fold increase in the sampling procedure's sensitivity. Since all samples had to be pooled to obtain a single concentration estimate, the procedure could not provide an estimate of the variability associated with this measurement. Two such sampling runs were conducted during Post-Fair studies.

E. Data Flow, Processing and Analysis Methods

1. Sample Identification and Labeling

To develop valid findings from this large-scale field sampling and analysis effort, it is essential that the identity and integrity of the data be preserved. Accordingly, an integrated data system involving a unique sample code, computer-generated sample labels, field data reporting forms, and analytical data reporting forms was implemented. This system insured that each sample was uniquely identified (run number, sample location, medium, analysis, sampling period) and that this identity was transmitted with the analytical values from sample collection through processing, shipping , preparation, analysis, reporting, and statistical analysis. This uniform accounting and tracking procedure also made it impossible for laboratory personnel to iden-

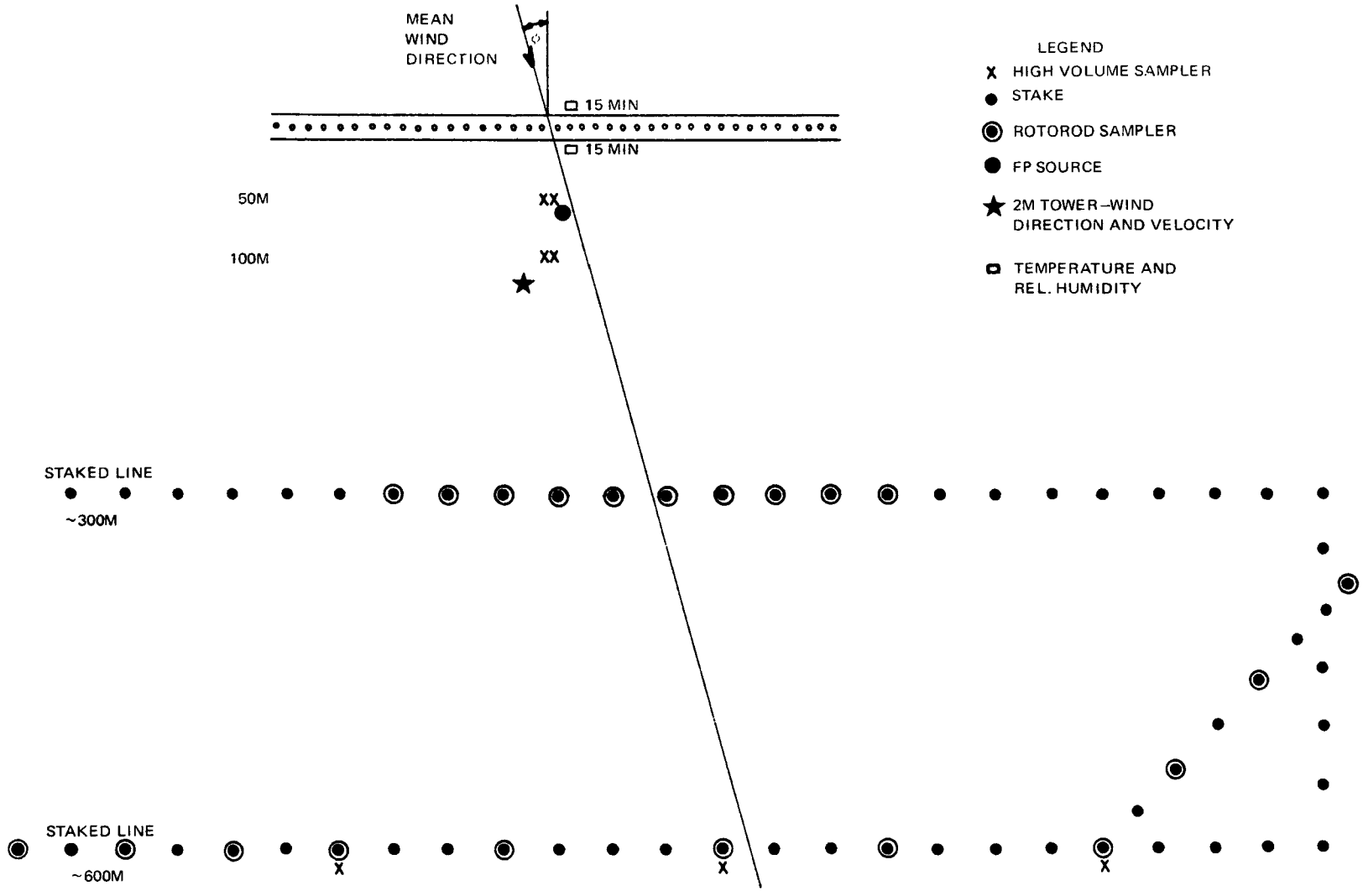


Figure V.D-2.
PRIMARY (MICROBIOLOGICAL) AEROSOL SAMPLING CONFIGURATION FOR POST-FAIR

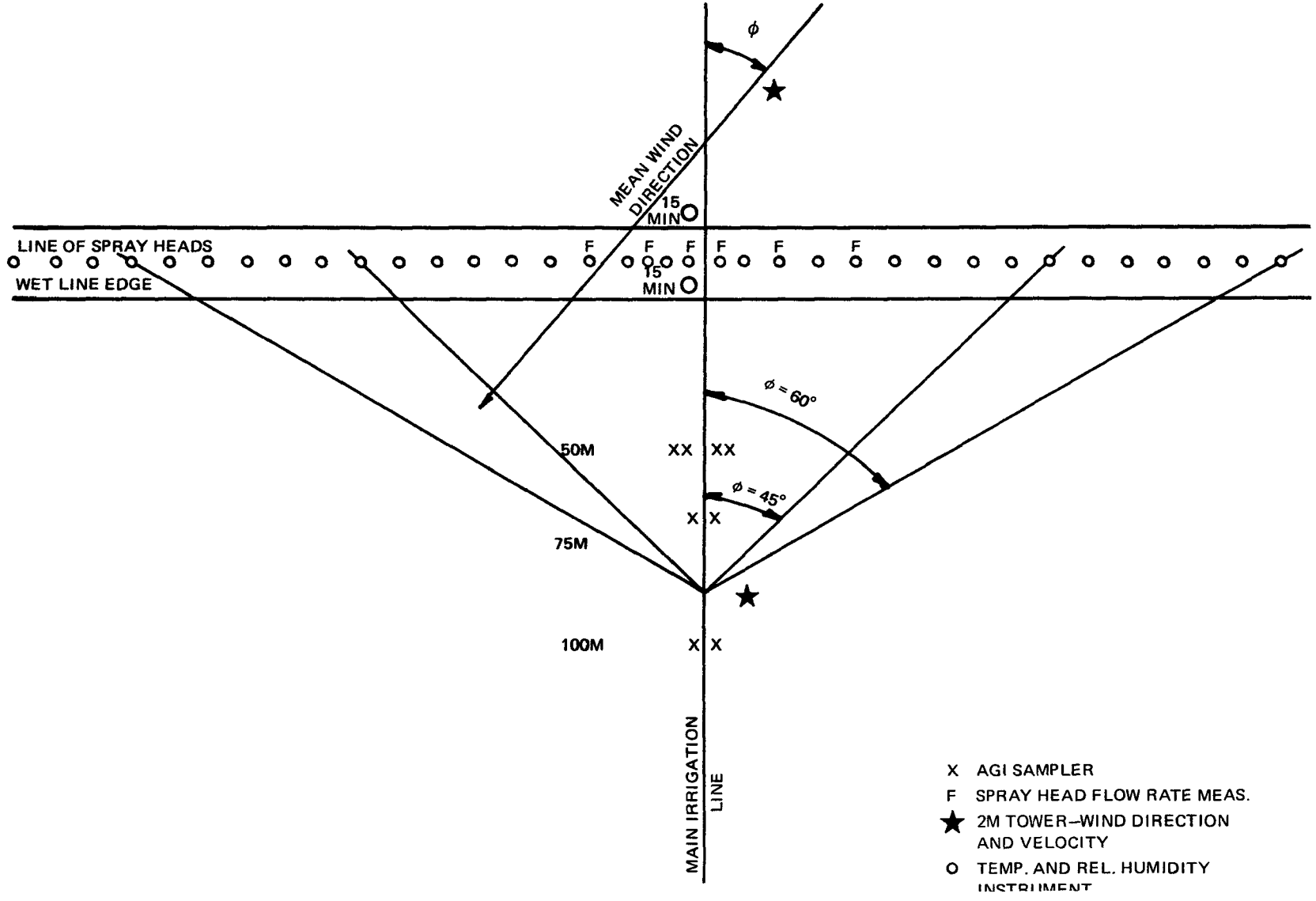


Figure V.D-3.
DYE AEROSOL SAMPLING CONFIGURATION FOR POST-FAIR

tify samples and, thus, introduce bias into their analyses. This was extremely important for the quality assurance samples which were also submitted using this code system.

2. Data Forms and Reporting System

In conjunction with the sample labeling system, a series of forms was prepared and a reporting procedure established to insure close coordination and control of the field and laboratory aspects of the study. Field and laboratory personnel submitted completed forms on a routine basis to the project statistician, where they were reviewed for data reporting deficiencies and for sampling and analysis problems. This provided a timely feedback mechanism to correct errors and to identify requirements to modify the study protocol.

3. Aerosol Data Processing

Ideally, the measured microbiological aerosol concentrations are presumed to accurately describe the microorganism levels emanating from the source under study (e.g., the line of sprayers). However, in a large-scale field program such as that conducted at Pleasanton, some measurements of microbiological aerosol concentrations are likely to reflect extraneous factors such as sampler/sample contamination or nonstudy sources that dominate the study source effect. In addition, the high variability of quantum microbiological measurements at levels close to the minimum detection limits of the methods used requires careful data smoothing techniques to avoid logical inconsistencies. The extraneous factors and variability-induced logical inconsistencies can have biasing and mathematically intractable consequences in data analyses such as the microbiological dispersion model development. Procedures developed and used to alleviate these data analysis problem areas include:

(a) a procedure for standard data processing and event notation,

(b) a procedure for use/rejection of data based on evidence of sampler/sample contamination, and

(c) data smoothing procedures used for application of the microbiological dispersion model.

4. Computational Techniques

The automated computational techniques which were used to analyze the extensive data generated during this project were chosen as being the most appropriate from numerous options that were available. SwRI currently maintains a special lease arrangement with the McDonnell Douglas (McAuto) computer facility in Huntington Beach, California for use of their CDC Cyber 70/74 equipment. This system is accessed through a CDC remote batch terminal at the Institute's Computer Laboratory. In addition, a Hewlett Packard 9810A programmable calculator with a limited package of statistical routines was utilized when the desired analysis was less involved and the quantity of data was sufficiently small to permit direct keyboard entry. The major automated computational procedures used in this project are listed in Table V. E-1.

5. Statistical Approach

The specific objectives given in Section III. D. have been addressed by applying suitable statistical methods to the study data to make appropriate inferences (i.e. findings). Statistical analyses relevant to many of the objectives were performed upon obtaining the Pre-Fair data. In some cases, the Pre-Fair analysis provided adequate information and its methods and results are presented in Section VI. In other cases, a repeated analysis encompassing both the Pre-Fair and the Post-Fair data was performed and is reported in Section VI. The Pre-Fair data analyses suggested the propriety of developing the microbiological dispersion model. Development and evaluation of this model was the major emphasis of the Post-Fair data analysis.

Table V.E-1
AUTOMATED COMPUTING PROCEDURES

Program	Source	Type	Computer System	Usage
LABELS	SwRI	New	Cyber 74	Generate sample labels.
DSTAT1	SwRI	Existing	HP9810A	Mean, standard deviation, and coefficient of variation.
DSTAT2	HP	Package	HP9810A	Calculate mean, standard deviation, skewness, kurtosis; grouped and ungrouped data.
CORREL	SwRI	New	HP9810A	Correlation coefficients
TPROB	SwRI	Existing	HP9810A	Percentiles of t-distribution.
ANOVA	HP	Package	HP9810A	Analysis of variance, one-way and two-way without interaction.
PRESTO	SwRI	Existing	Cyber 74	Stepwise multiple linear regression; correlation coefficients.
CANCORR	SPSS	Package	Cyber 74	Canonical correlation; correlation coefficients,
CONDESCRIPTIVE	SPSS	Package	Cyber 74	Calculate mean, standard deviation, skewness, kurtosis.
BMD08V	BMD	Package	Cyber 74	General analysis of variance models.
BIODCAY	SwRI	New	Cyber 74	Calculate estimates of microbiological dispersion model parameters I and λ.
MODEVAL	SwRI	New	DG Eclipse 300	Evaluates the microbiological dispersion model by comparing its prediction P against the observed value C-B.

VI. RESULTS

A. Wastewater Characteristics

1. Chemical Data and Patterns

The results of water quality chemical analyses indicated that the Pleasanton wastewater was generally typical of an undisinfected, secondarily treated wastewater. Mean values for certain parameters, determined during Pre-Fair studies, were as follows: BOD - 18.7 mg/L, COD - 99.5 mg/L, TOC 33.0 mg/L, ph 8.4, hardness 235.2 mg/L, TSS - 33.0 mg/L, total phosphorus - 5.6 mg/L and nitrite, nitrate, ammonia and organic nitrogen - 0.15 mg/L, 0.06 mg/L, 23.9 mg/L and 5.6 mg/L, respectively. Limited chemical analyses continued during Post-Fair studies and revealed no major differences in wastewater quality.

A strong relationship was observed among TOC, COD, and BOD. The significance level of the correlation between TOC and BOD was 0.006, and for the other pairs it was less than 0.001.

In general, no major wastewater quality differences were observed on a weekly or daily basis or during the conduct of any individual aerosol runs. Thus, the chemical quality of the wastewater was not considered to have a significant influence over the variability of the microbiological aerosol levels measured.

These results do not imply that water quality has no effect on microorganism survival. Chemical parameters may, in fact, have adverse (toxic) or beneficial (protection from desiccation) effects on the survival of aerosolized microorganisms. Thus, water quality may have had an overall impact on the actual levels of microbiological aerosols measured that could not be evaluated during this study. Since the water quality was relatively consistent throughout the study period, it was not considered a reliable indicator/predictor of microorganism (especially pathogen) levels. Thus, the chemical quality of the wastewater was not considered a relevant factor for inclusion in the microbiological dispersion model. A complete description of the wastewater chemical data and patterns is presented in Appendix E.

2. Microbiological Data and Patterns

a. Daily Composite Microbiological Data

Samples from the daily composite effluent sampler during Pre-Fair were sent to Pacific Environmental Laboratory (PEL) for microbiological analyses of standard indicator microorganisms, including total and fecal coliform and standard bacterial plate count, or the total aerobic bacteria plate count with standard methods medium. The concentration values obtained are shown in Table VI. A-1. Since the total coliform and fecal coliform analyses were performed using the membrane filter method, these values are reported as MFC/100 mL (membrane filter count per 100 milliliter of effluent).

The total and fecal coliform values shown are the average of a minimum of three repetitions, and often five or six. Standard bacterial plate count was generally performed in either duplicate or triplicate and the average value is shown for these as well. There was only one sample which gave unusual values, sample 78 from June 12, where there was apparently some contamination of the sample. The laboratory was unable to complete the analysis for fecal coliform on sample 74 due to an equipment malfunction.

As can be seen from the table, the concentrations were fairly consistent during given intervals within the sampling period. In addition, all three measurements exhibited similar indications of changes in the effluent microbiological constituents.

Portions of the daily composite effluent samples were shipped to the UTSA-CART laboratory for analyses for coliphage, selected pathogenic bacteria, and enteric viruses. The concentration values for these daily effluent samples are presented in Table VI. A-2. Following the table is a list of footnotes. The

Table VI.A-1.

DAILY COMPOSITE EFFLUENT CONCENTRATIONS OF MICROBIOLOGICAL INDICATOR PARAMETERS

Sample No.	Date	Total Coliform (MFC/100 ml X 10^3)	Fecal Coliform (MFC/100 ml X 10^3)	Standard Plate Count (No./100 ml X 10^6)
1-2	5-2	1700	117	130
2	3	2300	275	250
3	4	2500	210	100
4	5	2200	180	110
5*	7	1350	147	76.7
32*	8	750	93.3	77
33*	9	517	102.7	107
34*	10	832	126	91.7
35*	11	1200	143	157
36	11	913	68	180
37	12	255	36.7	57
98	13	817	82.7	237
71	14	958	123	120
1-3	15	1220	90	247
61	16	477	59.5	110
70	17	410	12.5	34.3
41	18	420	33.3	75.7
76	19	617	63	133
67	20	713	177	115
38	21	830	82.8	75
75	22	1200	152	393
64	23	1060	140	218
39	24	800	123	85
62	25	992	83.3	57
99	26	533	33.7	101
74	27	763	–†	13.5
63	28	1450	134	133
40	29	1430	220	90
97	30	565	53	62.7
60	31	817	76.7	96.7
73	6-1	703	57	133
42	2	293	24.3	102
69	3	267	24.3	35
66	4	833	59	107
95	5	530	35	63.5
68	6	257	13	157
77	7	417	59	11.3
72	8	160	22.3	36
65	9	760	85.7	19.3
96	10	798	80.4	15.7
43	11	290	31.3	11.7
78‡	12	2470	263	>300
50	13	490	70.3	14
51	14	743	93.2	12.7
52	15	620	79	21.3
53	16	340	82.3	14.7
56	17	530	63.3	6.13
58	22	353	64	8.05

*Composite sampler inoperative, grab sample taken morning after spraying.
†Equipment malfunction; laboratory unable to perform analysis.
‡Probable contamination.

Note: 1700 (MFC/100 ml X 10^3) = 1700 X 10^3 MFC/100 ml = 1.7 X MFC/100 ml

Table VI.A-2.
DAILY COMPOSITE EFFLUENT CONCENTRATIONS OF COLIPHAGE AND SELECTED PATHOGENIC BACTERIA AND VIRUSES

Sample Date	Coliphage Count, (PFU/l) X 10^3	Bacteria				Viruses		High Sample Temperature Upon Lab Receipt
		Klebsiella, (CFU/100 ml) X 10^3	Pseudomonas, (CFU/100 ml) X 10^3	Streptococci, (CFU/100 ml) X 10^3	Clostridium perfringens, (MPN/100 ml) X 10^3	3-Day Plaques (PFU/l)	5-Day Plaques (PFU/l)	
5-2-76	68		CS	CS	>24	9.9	TNTC	
5-3-76	370		100	>24†	11	4.6	TNTC	
5-4-76	CS		25	CS	11	340*	TNTC	
5-5-76	120		15	CS	>24	29	TNTC	
5-6-76	160		90	CS	4.6	589*	TNTC	
5-7-76	180		140	9.3†	1.1	18	36	10°C
5-8-76	110		88	CS	.46	25	TNTC	
5-9-76	87		110	11†	>2.4	26	28	
5-10-76	97		50	11†	.24	<2.4	4.7	
5-11-76	100		25	CS	.24	4.7	9.8	
5-12-76	140		13	2.3†	.093	12	16	7°C
5-13-76	540	25	80	>24†	11	33	31	9°C
5-14-76	350	39	75	4.6†	9.3	71	90	9°C
5-15-76	290	58	25	23†	9.3	<1.8	15	
5-16-76	230	41	15	10	4.6	20	43	
5-17-76	97	28	30	6.5	4.6	21	43	
5-18-76	360	36	60	5.1	4.6	19	26	
5-19-76	330	28	50	7.9	4.6	15	24	
5-20-76	340	52	25	13	7.5	39	39	
5-21-76	190	55	210	20	>24	18	18	
5-22-76	260	72	120	18	23	9.3	9.3	
5-23-76	120	66	250	10.4	4.3	1.3	3.3	
5-24-76	78	52	35	8.0	24	CS	CS	
5-25-76	130	55	120	2.8	46	CS	CS	
5-26-76	120	28	90	3.7	9.3	5.3	7.2	

*Possible contamination.
†MPN.

Table VI.A-2 (continued)

Sample Date	Coliphage Count, (PFU/l) × 10^3	Bacteria				Virus		High Sample Temperature Upon Lab Receipt
		Klebsiella, (CFU/100 ml) × 10^3	Pseudomonas, (CFU/100 ml) × 10^3	Streptococci, (CFU/100 ml) × 10^3	Clostridium perfringens, (MPN/100 ml) × 10^3	3-Day Plaques (PFU/l)	5-Day Plaques (PFU/l)	
5-27-76	180	94	30	6.8	4.3	15	15	
5-28-76	240	44	1700	9.6	4.3	4.7	CS	
5-29-76	410	39	2900	14.6	4.3	4.7	6.8	
5-30-76	290	25	450	2.5	2.3	18	18	
5-31-76	230	36	1900	10.3	7.5	<1.6	8.8	
6-1-76	160	41	100	5.7	9.3	4	66	
6-2-76	270	17	300	1.7	9.3	17	23	
6-3-76	280	30	200	2.3	4.3	45	50	
6-4-76	350	8.2	350	6.6	2.3	60	86	
6-5-76	280	17	260	4.3	2.3	56	87	
6-6-76	200	33	430	4.2	2.3	27	CS	
6-7-76	330	36	92	3.4	4.3	46	CS	
6-8-76	440	19	45	1.1	15	CS	CS	
6-9-76	570	50	130	5.7	9.3	5.9	CS	
6-10-76	490	50	120	12	9.3	<1 9	<4.0	
6-11-76	460	110	200	24	4.3	10	16	
6-12-76	360	55	150	3.0	4.3	22	22	
6-13-76	420	28	95	6.4	9.3	4.6	4.6	
6-14-76	350	58	30	17.1	9.3	7.9	7.9	
6-15-76	220	41	25	9.2	9.3	6.1	CS	
6-16-76	230	33	30	5.5	4.3	12	14	
6-17-76	220	94	20	4.0	9.3	12	14	

PFU —Plaque forming units. MPN —Most probable number. CS —Contaminated sample.

CFU —Colony forming units. TNTC —Too numerous to count. M —Missing

pathogenic bacteria selected for assay were *Klebsiella*, *Pseudomonas*, fecal streptococci, and *Clostridium perfringens*. The *Klebsiella* column of Table VI. A-2 consists of *Klebsiella pneumoniae* and *Klebsiella ozaenae*. The *Pseudomonas* reported in Table VI. A-2 are fluorescent.

The streptococci column gives the fecal streptococci assayed according to *Standard Methods for the Examination of Water and Wastewater*. The *Klebsiella, Pseudomonas*, and later streptococci assays are reported as CFU/100 ml (colony forming units for 100 milliliters of effluent). The early streptococci values and the *Clostridium perfringens* values were obtained through analyses requiring most probable number tables; these values are reported as MPN/100 ml (most probable number per 100 milliliters of effluent). Viruses in the effluent samples were plated on HeLa cell monolayers. Approximately 70% of each concentrated sample was observed for virus plaques after three days; the remainder of each sample was observed after five days. The 3- and 5-day virus counts in Table VI. A-2 are given in PFU/l (plaque forming units per liter of effluent). See Appendix D for a complete description of the microbiological analysis procedures performed on the effluent samples.

The first daily composite sample sent for coliphage analysis was concentrated. However, its titer was high enough (91% efficiency) to establish that concentration was unnecessary for the coliphage analyses of the remaining daily composite samples. Concentration was found to be necessary, and was conducted, on all the effluent samples assayed for viruses. The recovery efficiency of the daily poliovirus reference samples ranged from 15 to 100%. The quartiles of the recovery efficiency distribution were 39% (first quartile), 50% (median), and 65% (third quartile). The corrected virus plaque count shown in Table VI. A-2 was obtained by dividing the observed raw plaque count by the recovery efficiency for that day's reference sample. Since quantitative effluent concentrations were obtained on nearly every sample, the data are suitable for statistical analyses.

As anticipated with such a large-scale systematic sampling and analysis protocol, there were also a few problems. Some early bacteria and virus samples and several of the later virus samples were contaminated. Two or three of the early virus samples may have been contaminated with the reference poliovirus. Four of the early sample shipments were received at temperatures considerably above the desired 4°C. The elevated temperatures may have raised the bacterial pathogen levels and lowered the coliphage and virus levels of these samples in comparison with the samples shipped at about 4°C. Streptococci levels were determined by the MPN method for the first two weeks because the proper assay medium was unavailable.

Several special data symbols have been used in Table VI. A-2. When the sample assay was negative, the result has been reported as <(less than) the detection limit. The virus and coliphage plaque-forming units grow as they consume the host cells. When the individual virus plaques grew together, it was impossible to determine how many plaque-forming units there were; such results were reported as TNTC (too numerous to count).

It is important to note that a wide range of effluent concentration values was observed. The coliphage, bacteria, and virus concentrations in Table VI. A-2 and the coliform and standard bacterial plate count concentrations in Table VI. A-1 all exhibit at least a one order of magnitude range of values. Many approach a two orders of magnitude range. In fact, *Pseudomonas* and *Clostridium perfringens* vary over more than two orders of magnitude.

b. Distributional Characteristics

Summary statistics were calculated for all wastewater microorganism concentration data. The mean, standard deviation, skewness, and kurtosis statistics, given in Table VI. A-3, characterize the effluent sample distribution of each. The upper half of the table applies to the untransformed data. The arithmetic standard deviations are large relative to the arithmetic means for each parameter, which implies the orders of magnitude variation readily observed in Tables VI. A-1 and VI. A-2. The skewness and kurtosis

Table VI.A-3.
DISTRIBUTIONAL CHARACTERISTICS OF THE WEIGHTED DAILY AND LARGE—VOLUME EFFLUENT SAMPLE CONCENTRATIONS OF THE INDICATOR AND PATHOGENIC MICROBIOLOGICAL PARAMETERS

	No. of Samples with Numeric Analysis Results	Effluent Sample Statistics				P, Significance Levels of One-Sided Tests of Suitability of Normal Distribution Model	
		Mean	Standard Deviation	Skewness	Kurtosis	Skewness (H_0: $\sqrt{\beta_1} = 0$)	Kurtosis (H_0: $\beta_2 = 3$)
Untransformed Concentration		(Arithmetic)					
Total Coliform, (MFC/100 ml) X 10^3	54	927.9	650.9	1.5	5.0	<.01	<.01
Fecal Coliform, (MFC/100 ml) X 10^3	53	102.2	71.8	1.3	4.7	<.01	.01
Std Plate Count, (No./100 ml) X 10^6	53	106.3	95.9	2.1	9.6	<<.01	<<.01
Coliphage (PFU/l) X 10^3	53	258.1	130.8	0.5	2.4	.05	OK
Klebsiella, (CFU/100 ml) X 10^3	42	44.9	23.0	0.8	3.5	.01	OK
Pseudomonas, (CFU/100 ml) X 10^3	52	264.1	525.9	3.6	16.0	<<.01	<<.01
Streptococci, (CFU/100 ml) X 10^3	46	8.84	7.55	2.2	10.3	<<.01	<<.01
Clostridium perf , (MPN/100 ml) X 10^3	53	9.06	10.14	2.8	10.7	<<.01	<<.01
3–Day Virus Count (PFU/l)	48	20.6	28.9	5.5	42.1	<<.01	<<.01
5-Day Virus Count, (PFU/l)	39	27.9	35.4	4.3	27.3	<<.01	<<.01
Natural Log Transformed Concentrations		(Geometric)					
ln (Total Coliform)	54	748.5	1.24	0.02	2.68	OK	OK
ln (Fecal Coliform)	53	79.5	1.33	−0.41	2.84	OK	OK
ln(Std. Plate Count)	53	69.9	1.68	−0.57	2.63	.04	OK
ln (Coliphage)	53	223.9	1.17	−0.37	2.18	OK	.05
ln (Klebsiella)	42	38.8	1.19	−0.88	4.23	.01	.04
ln (Pseudomonas)	52	104.8	2.18	0.61	3.16	.03	OK
ln (Streptococci)	46	6.73	1.39	−0.40	3.49	OK	OK
ln (Clostridium perf)	53	5.39	2.02	−1.15	4.98	<<.01	<.01
ln (3-Day Virus Count)	48	12.11	1.81	−0.41	3.41	OK	OK
ln(5-Day Virus Count)	39	17.29	1.61	0.06	2.89	OK	OK

statistics test whether the distribution is normal. A skewed distribution has one tail that extends out farther than the other tail. Kurtosis measures whether the distribution has a very sharp peak or a very broad, flat top. The true normal distribution has a skewness parameter of 0 and a kurtosis statistic of 3. For each set of microbiological concentration data, tests of the null hypotheses that the data were normally distributed were conducted. The significance levels of the results are presented in the two right-hand columns of Table VI. A-3. All of the untransformed data exhibited positive skewness. Only the untransformed data for coliphage and possibly for *Klebsiella* had acceptable normal distribution kurtosis. This confirmed the requirement to transform the effluent microbiological data to permit valid correlation analysis.

The summary statistics under the natural log transformation are shown in the lower half of Table VI. A-3. To permit comparison with the untransformed data statistics, the geometric mean and the geometric standard deviation are given in Table VI. A-3, rather than the ln x mean and standard deviation. The geometric mean has the same units as the arithmetic mean. The geometric mean values are lower than the arithmetic mean because of the scale adjustment introduced by the logarithmic transformation.

The results of the skewness and kurtosis tests of the normal distribution null hypotheses are also given in Table VI. A-3 for the natural log transformed data. For most microorganism groups, both the skewness test and the kurtosis test indicate that the logarithmic transformed data have an acceptable normal distribution (i.e., the untransformed data have a lognormal distribution). For each of the others the natural

log data are more nearly normally distributed than are the untransformed data. Neither the natural log transformation, the square root transformation, nor the untransformed data for *Clostridium perfringens* followed a normal distribtuion. This may be due to the limited number of *Clostridium perfringens* values permitted in the MPN index probability tables rather than an actual characteristic of the concentration levels. Except for the effluent *Clostridium perfringens* data, each of the effluent microorganisms were generally considered to follow a lognormal distribution.

c. Wastewater Analysis Variability

A precision study for the three indicator microorganism group analyses was conducted during Pre-Fair in the same manner as for the chemical constituents. The replicate determinations from the daily effluent runs were used to obtain estimates of the standard deviations associated with analytical repeatability. The data used were transformed by the natural logarithms of the observed counts to achieve normality and homogeneity of variance. The standard deviations were then pooled and the results exponentiated to obtain the estimates of variance due to repeat analysis.

Ten samples were sent for analysis, five from each of two 500-ml grab samples (which were split from a single 1 liter grab) to determine the shipping and analysis variance component. The results for these samples and the standard deviations of the transformed data obtained are shown in Table VI. A-4. The standard deviations for the 2 pond grab samples are pooled into a single estimate as above and exponentiated to give an estimate of the percentage variation among the samples. The day-to-day variance estimate is taken from the summary statistics portion of Table VI. A-3. The three variance components estimated for each microorganism group are presented in Table VI. A-5.

The total coliform data show no tendency to be affected by the shipping process, giving approximately equal values for both the repeatability and between-sample components. The fecal coliform analysis has a higher standard deviation for shipping and analysis than repeatability, but an F-test performed in

Table VI.A-4.
MICROBIOLOGICAL INDICATOR QUALITY ASSURANCE PRECISION STUDY— ANALYTICAL RESULTS

		Parameter		
Grab	Sample	Total Coliform (MFC/100ml x 10^3)	Fecal Coliform (MFC/100ml x 10^3)	Standard Plate Count (no/100 ml x 10^6)
1	1	570	64.3	108
	2	543	77.8	57
	3	483	44.3	23.8
	4	577	55.0	230
	5	477	63.3	150
2	1	575	72.5	195
	2	650	46.7	160
	3	543	42.0	49.7
	4	603	56.7	108
	5	633	47.5	137
Coefficients of variation		8%	24%	109%

Table VI.A-5.
MICROBIOLOGICAL QUALITY ASSURANCE PRECISION STUDY—PRECISION ESTIMATES
(Percent Coefficient of Variation)

	Parameter		
	Total Coliform	Fecal Coliform	Standard Bacterial Plate Count
Variance Component			
Repeatability	11	11	12
Shipping and Analysis	08	24	107
Day to Day	24	33	118

the transformed scale does not indicate a significant difference between the two components. The standard bacterial plate counts are more variable. A variance component estimated from these data is 1.09, or 9-percent variation from sample to sample induced by the shipping and handling, plus between-sample variability.

The precision portion of the quality assurance study on the analysis for coliphage and pathogens was conducted using replicate portions from two effluent grab samples. The coliphage was analyzed in five replicates from each of the two samples and the pathogenic bacteria and viruses were analyzed in three replicates from each sample. The data are used to estimate analytical variability, within replicates, and to determine if there is a significant between-sample variance associated with these data. All analyses were performed in the logarithmic scale on the basis of the distributional nature of the data.

The coliphage data are presented in Table VI. A-6, along with the variance components calculated for the two components. The pooled within replicate standard deviation is 0.13 in the transformed data. Exponentiating gives 1.14, or 14% variability from sample to sample. The standard deviation between samples is 0.061, which is smaller than the replication error estimate and hence insignificant. The implication is that the observed variability is, in fact, replication error.

The data from the selected pathogenic bacteria analyses are presented in Table VI. A-7. As in the coliphage data above, standard deviations are calculated for the results from each sample, then pooled to obtain an overall estimate.

For *Klebsiella*, the pooled replication standard deviation is 0.26 in the transformed data, which when exponentiated gives 1.30 in the original scale. This implies that there is 30-percent variability from one analytical result to another from replicates of the same effluent sample. The difference between the means of the two groups gives an estimated standard deviation of 0.36, and again the between-sample term is insignificant.

The *Pseudomonas* data give a pooled standard deviation of 0.27 under the transformed scale, which gives 1.31 or 31-percent variability in the original scale. This is to be compared with the variability between the means of the two groups. This is estimated as 0.54, which is not significantly different. As a result, the six values may be considered replicates in the usual sense.

The streptococci results from within each 500-mL grab sample yield a pooled replication standard deviation of 0.13, and when exponentiated gives 1.14, or an estimated 14-percent variability between the samples within a given pond grab sample. The mean values give a standard deviation under the transformation of 0.06, and once again is less than the replication error and insignificant.

Table VI.A-6.
PRECISION QUALITY ASSURANCE STUDY FOR COLIPHAGE

Grab	Sample	Corrected Count (pfu/l × 10^5)	Standard Deviation (ln X)
1	6	4.5	0.06
	7	4.6	
	8	5.3	
	9	4.8	
	10	4.7	
2	11	4.3	0.17
	12	3.6	
	13	4.4	
	14	5.7	
	15	4.9	

The *Clostridium perfringens* results have a standard deviation of 0.21 in the transformed scale for replication error in analyzing the same effluent sample. This corresponds in the original scale to a value of 1.23, or 23-percent variability. The difference in the mean values is represented by a standard deviation of 0.39, which again represents an insignificant difference.

In summary, for all the bacterial analyses, no variation between samples was detectable, and the only discernable variability was due to analytical repeatability.

Table VI.A-7.
PRECISION QUALITY ASSURANCE STUDY FOR BACTERIA

		Parameter			
Grab	Sample	Klebsiella (cfu/100ml × 10^4)	Pseudomonas (cfu/100ml × 10^2)	Fecal Streptococci (cfu/100ml × 10^3)	C. Perfringens (MPN × 10^3)
1	6	5.2	1100	9.2	4.3
	7	3.8	1600	8.3	3.9
	8	4.4	800	11	3.9
Std. Dev. (ln x)		0.16	0.35	0.14	0.05
2	9	3.6	850	11	4.3
	10	3.3	850	9.0	2.3
	11	3.0	1100	9.7	2.3
Std. Dev. (ln x)		0.09	0.15	0.10	0.36

The results from the two virus analyses, 3-day count and 5-day count, are summarized in Table VI. A-8. Sample 11 was contaminated and not used in the analyses. Sample 10, though high, was not suggested to be contaminated and retained. The repeatability standard deviation for the natural logarithm of the data is 0.60, which calculates to a value of 1.82 in the original scale. This suggests that over 80-percent variability can be expected in replicate samples. The means represent a variability of only 0.16, and are much closer than the sample values. Again the between-sample term is insignificant and all error can be assumed to be replication error. This high between-sample variability is emphasized because it significantly impacts the statistical reliability of mathematical modeling efforts for virus aerosol concentrations presented later in this report.

For the 5-day count, the repeatability of the method is determined to be 0.55 in the transformed scale and 1.73 in the original scale. The between-sample component is estimated as 0.14, and no significant difference exists between these two values. Thus, for all the analyses, no differences could be found between the two samples. A summary of the estimated replication error for coliphage and all the pathogenic analyses is presented in Table VI. A-9.

Table VI.A-8.
PRECISION QUALITY ASSURANCE STUDY FOR VIRUS
(Corrected Count, pfu/l)

	Parameter		
Grab	Sample	3-Day Count	5-Day Count
1	6	24	35
	7	11	15
	8	18	25
Std.Dev. (ln x)		0.39	0.43
2	9	9.0	16
	10	31	45
	11*	852	926
Std.Dev. (ln x)		0.87	0.73

*contaminated sample

Table VI.A-9.
ESTIMATED REPLICATION ERROR FOR PATHOGENIC ANALYSES

Parameter	Coefficient of Variation, %
Coliphage	14
Klebsiella	30
Pseudomonas	31
Streptococci	14
Clostridium perfringens	23
3-Day Virus Count	82
5-Day Virus Count	73

d. Equivalence of Composite and Pond Grab Samples

The daily composite effluent samples, taken during Pre-Fair, represented an average of the effluent conditions in the pond near the pump over the time of spraying. At the time an aerosol run was made, however, an additional composite effluent sample was taken from a spray head on the sprinkler line. These represent the effluent quality, with respect to the microbiological indicators, over the 30-minute period of aerosol sampling. These two determinations of the microbiological constituents of the effluent were compared to indicate whether there was significant variability in these levels during a given day. This analysis was also conducted to determine the requirement for continued effluent sampling during aerosol runs or whether further studies could rely on microorganism concentration data from daily effluent composite samples.

The analysis was conducted in two parts. First, a correlation coefficient was calculated between the daily composite sample and the aerosol run composite sample and the significance of the correlation determined. Second, a comparative t-test was conducted to determine if the two types of samples could be estimating the same true mean. For these analyses, the results were transformed to normality using the natural logarithm in order to satisfy the assumptions of the statistical tests.

The comparative total coliform data are shown in Table VI. A-10 for the 25 aerosol runs conducted. The summary statistics from the two analyses are presented at the bottom of the table. The correlation coefficient for these data is estimated to be $r = 0.73$, and the t-test for significance gives a value of 5.13 with 23 degrees of freedom. These are significant at a level of less than 0.001, so that the daily and run samples can be said to be correlated. The t-test for equality of means is conducted by taking the difference between the two values for a given run and testing for a mean difference of zero. The t-statistic calculated for this test is 0.54 with 24 degrees of freedom and is clearly not significant. This implies that both samples are estimating the same mean level. Thus, the total coliform did not show a pattern of daily variability in these data.

The paired values and results of these statistical analyses for the fecal coliform are shown in Table VI. A-11. There are three missing values among these results when the laboratory was unable to perform the analyses due to equipment malfunction. The correlation coefficient for the remaining 22 pairs is estimated as $r = 0.40$, with a t-statistic of 1.97 with 20 degrees of freedom. This has a significance level of approximately 0.07, and, thus, is not significant at the 5-percent level. The t-test for equality gives a value of 0.50 with 21 degrees of freedom, which indicates that the two analyses are equivalent. The correlation analysis indicates that an increase in one is not necessarily accompanied by an increase in the other, but overall they can be said to be estimating the same level of fecal coliform in the effluent.

The standard bacterial plate count data are presented in Table VI. A-12. One value is missing since the laboratory did not perform the analysis. The estimated correlation coefficient is $r = 0.66$, and the t-statistic has a value of 4.17 with 22 degrees of freedom. This has a significance level of less than 0.001, and they may thus be considered to be correlated.

The t-test for difference of means for the standard bacterial plate count gives a test statistic of 2.96 with 23 degrees of freedom. This value is significant at the 0.01-percent level. By inspection, the aerosol effluent run samples can be seen to be estimating a higher mean level than the daily composite samples. It was noted that the pipes carrying the water to the spray fields were not free of material, and as a result, the water may be increasing in microbiological constituents, other than coliform, after it leaves the ponds.

The final indicator is the coliphage count data, presented in Table VI. A-13, for the twenty ordinary aerosol runs and the two quality assurance runs. On the first run, neither the daily nor the run sample produced a valid result, and on run 34, no analytical results were obtained for the run sample. The estimated correlation coefficient for the remaining 20 pairs of counts is 0.50, and the corresponding t-statistic is calculated as 2.42 with 18 degrees of freedom. This is a significant value at the 0.05 level, and the values may

be said to be correlated. The test for difference between means gives a test statistic of 0.68 with 19 degrees of freedom, and is clearly non-significant. The conclusion, therefore, is that the two samples are providing estimates of the same true mean levels.

In general, then, the results of the aerosol run effluent samples in the spray field were

Table VI.A-10.
COMPARATIVE TOTAL COLIFORM DATA—DAILY vs. RUN SAMPLE

Run Number	Date	Daily Sample (MFC/100 ml x 10^3)	Spray Sample (MFC/100 ml x 10^3)
1	5-4	2500	1480
2	4-5	2200	2070
3	5-5	2200	2140
4	5-13	817	690
5	5-13	817	720
6	5-17	410	930
7	5-17	410	700
8	5-21	830	950
9	5-24	800	1040
10	5-24	800	1280
11	5-25	992	1100
12	5-27	763	470
13	5-27	763	690
14	5-27	763	750
15	6-3	267	588
16-19	6-7	417	343
20-23	6-8	160	127
24-25	6-9	760	1100
26	6-10	798	758
27-30	6-13	490	267
31	6-14	743	970
32	6-15	620	265
33	6-15	620	170
34	6-16	340	550
35	6-17	530	350

Test	Correlation (r = 0.73)	Equality
t-statistic	5.13	0.54
degrees of freedom	23	24
significance level	<0.001	not significant

strongly related to those of daily composite samples from the effluent pond. The estimated correlation coefficients do not indicate a high degree of associativity, even though they are significant for the most part. The amount of the explained variation, estimated by r^2, has a maximum value of 54 percent for the parameters studied, which is not particularly high. The more important result is that for all the parameters except standard bacterial plate count, the two results are estimating the same mean value. From these data it was concluded that daily composite effluent samples were not necessary during Post-Fair studies.

Table VI.A-11.
COMPARATIVE FECAL COLIFORM DATA—DAILY vs. RUN SAMPLE

Run No.	Date	(MFC/100 ml x 10^3) Daily Sample	Run Sample
1	5-4	210	150
2	5	180	186
3	5	180	174
4	13	82.7	97
5	13	82.7	180
6	17	12.5	75
7	17	12.5	110
8	21	82.8	80
9	24	123	81
10	24	123	125
11	25	83.3	124
12	27	-- *	-- *
13	27	-- *	-- *
14	27	-- *	-- *
15	6-3	24.3	57
16-19	7	59	59.5
20-23	8	22.3	14
24-25	9	85.7	177
26	10	80.4	75.3
27-30	13	70.3	27.7
31	14	93.2	137
32	15	79	24
33	15	79	34
34	16	82.3	81
35	17	63.3	45

Test	Correlation (r=0.40)	Equality
t-statistic	1.97	0.50
degrees of freedom	20	21
significance level	0.07	not significant

* missing data

e. Relationship of Pathogen Levels to Indicator Organism Levels

An important Pre-Fair objective regarding the effluent sample analyses was to investigate the relationships of the pathogenic organism (*Pseudomonas*, streptococci, *Clostridium perfringens*, 3-day viruses, and 5-day viruses) effluent concentrations, to the indicator organism (total coliform, fecal coliform, standard bacterial plate count, and coliphage) effluent concentrations. A common microbiological data analysis technique is to assume the existence of proportional relationships. If a pathogenic level was found to be directly related to an indicator level in the Pleasanton effluent, then the indicator measurement and the identified relationship could substitute for assay of the pathogen in later phases of this study.

An effluent sample data base was constructed to investigate the potential pathogen-indicator

Table VI.A-12.
COMPARATIVE STANDARD BACTERIAL PLATE COUNT DATA
DAILY vs. RUN SAMPLE

Run Number	Date	Daily Sample (No./100 ml x 10^6)	Run Sample (No./100 ml x 10^6)
1	5-4	100	110
2	5	110	120
3	5	110	--*
4	13	237	101
5	13	237	167
6	17	34.3	76
7	17	34.3	46
8	21	75	70
9	24	85	104
10	24	85	145
11	25	57	35
12	27	13.5	34
13	27	13.5	26
14	27	13.5	73
15	6- 3	35	45
16-19	7	11.3	14
20-23	8	36	36.7
24-25	9	19.3	44
26	10	15.7	34.7
27-30	13	14	12
31	14	12.7	16
32	15	21.3	20
33	15	21.3	95
34	16	14.7	54
35	17	6.13	76

Test	Correlation (r = 0.66)	Equality
t-statistic	4.17	2.96
degrees of freedom	22	23
significance level	<0.01	<0.01

*missing data

relationships. This data base consisted of the 47 daily effluent samples (the composites and the grab sample substitutes when the composite sampler was inoperative) presented in Tables VI. A-1 and VI. A-2 and the seven large-volume effluent grab samples taken for microbial characterization during Pre-Fair.

Because the data for the parameters given in Table VI. A-2 differ in validity and informational content, a procedure for adjusting certain analysis results and weighting each result was developed and applied to obtain the effluent data base. The analysis values that were missing or could not be quantified (the

Table VI.A-13.
COMPARATIVE COLIPHAGE DATA—DAILY vs. RUN SAMPLE

Run No.	Date	(pfu/l x 10^3) Daily Sample	Run Sample
1	5-4	- - *	-- *
2	5	120	110
3	5	120	95
4	13	540	110
5	13	540	170
6	17	97	220
7	17	97	230
8	21	190	61
9	24	78	95
10	24	78	130
11	25	130	140
12	27	180	110
13	27	180	170
14	27	180	210
15	6-3	280	310
24	9	570	580
26	10	490	480
31	14	350	380
32	15	220	320
33	15	220	240
34	16	230	-- *
35	17	220	170

Test	Correlation (r = 0.50)	Equality
t-statistic	2.42	0.68
degrees of freedom	18	19
significance level	<0.05	not significant

* missing data

CS and TNTC designations in Tables VI. A-1 and VI. A-2) were assigned zero weight, i.e., excluded from the analysis. The extremely high 3-day virus values on the May 4 and May 6 daily samples were at first considered to be possible contamination with the concentration efficiency reference poliovirus. With the perspective of all the Pre-Fair effluent virus results, poliovirus contamination of these samples appears very likely. Since these extremely high values would have a major effect on any virus relationship present in the effluent data, these two virus values were also excluded from the analysis.

In the weighting procedure, the standard quantitative analysis values were given full weight. The inferior quantitative analysis values were assigned half the full weight. These inferior values consisted of results outside stated detection limits, results obtained by an analysis method inferior to the standard method (MPN for streptococci), results probably affected by an elevated temperature during shipment, and results reported as possible sample contamination. To quantify results outside the stated detection limits, analysis results below the lower detection limit were reset at half the lower detection limit, and analysis results above the upper detection limit were adjusted to be twice the upper detection limit. Unusually high 3- and 5-day virus values were reported for the first large-volume pond grab sample. Because these virus values were not so large as to be definite outliers, the likelihood of poliovirus contamination was less and these values were each given half weight.

A correlation analysis of the natural log transformed microbiological effluent concentration data was performed to seek relationships between pathogen-indicator parameter pairs. Of the 54 effluent samples, there were no missing values for total coliform and only one missing value each for fecal coliform, standard bacterial plate count, and coliphage. To facilitate the correlation, regression, and canonical correlation calculations, the geometric means of the data on the other 53 samples were substituted for the missing indicator value, and the substitute value was assigned half weight. A correlation coefficient was calculated for each pathogen-indicator pair over all those effluent samples for which there was a pathogen concentration value. The correlation coefficients obtained are presented in Table VI. A-14. The upper set of correlation coefficients in Table VI. A-14 are for the unweighted logarithmically transformed effluent data base. The lower set of correlation coefficients is calculated from the weighted logarithmically transformed effluent data base. The observation weight assigned each pathogen-indicator sample pair was the product of the pathogen sample weight and the indicator sample weight, standardized so that 1.00 was the average observation weight. The weighted correlation coefficients are considered more valid than the unweighted correlation coefficients, and have been used to make the relationship inferences.

Inspection of the correlation coefficients in Table VI. A-14 shows very little correlation between the pathogen and indicator effluent concentrations. There are negative correlations (between virus and coliform) as well as positive correlations (between some pathogenic bacteria and coliform). In contrast to the low correlations shown in Table VI. A-14, the correlations among some of weighted log transformed effluent indicator organisms were much higher: 0.879 for total coliform and fecal coliform, and 0.451 for total coliform and standard bacterial plate count.

The significance of the correlation coefficient between n pairs can be determined by testing the null hypothesis of no correlation between the parameters against the two-sided alternative using a t-distributed statistic with n-2 degrees of freedom. This test is only valid when at least one of the pair of variates is normally distributed. In Table VI. A-14, the significance levels of the correlation coefficients are presented for those pairs of weighted natural log transformed parameters for which the correlation coefficient was significant at the 0.05-percent level. Over the concentration ranges observed at Pleasanton, the only significant effluent pathogen-indicator correlations were: 0.362 between streptococci and total coliform ($P = .01$); 0.354 between streptococci and fecal coliform ($P = .05$); and -0.355 between the 5-day virus plaques and fecal coliform. Because the distributional analysis showed at least one parameter in every correlated pair to be lognormally distributed, the preceding correlation significance test is considered valid.

Although there were some significant effluent pathogen-indicator correlations, the indicated pairwise relationships were considered too weak to justify substitution of these relationships for future pathogen assays. However, for each of the pathogenic organisms that did have at least one significantly correlated indicator organism, a stepwise multiple linear regression was conducted on all the natural log transformed indicator organism data. The purpose was to see how well the best linear combination of the indicator organism concentration data might predict pathogenic concentrations. Weighted stepwise regression analyses were performed using as dependent variables the natural log transformations of the streptococci, *Clostridium perfringens*, and 5-day viruses effluent concentrations. In each regression, the potential regressor variables were the natural log transformations of the total coliform, fecal coliform, standard bacterial plate count, and coliphage effluent concentrations. In the weighted regression analysis, a weight must be assigned to each observation (i.e., the set of indicator and pathogen results for a sample). The observation weighting procedure used was to multiply the dependent parameter sample weight by a linear combination of the indicator parameter sample weights and divide by the standardizing average observation weight. It is desirable to give more weight to those samples for which the indicator organisms, more likely to be included in the regression equation, had quantitative values. Accordingly, the significantly correlated indicator organism weights were doubled in computing the indicator organism weight linear combination.

Table VI.A-14.

UNWEIGHTED AND WEIGHTED SAMPLE CORRELATIONS OF THE NATURAL LOG TRANSFORMED EFFLUENT CONCENTRATIONS OF THE INDICATOR AND PATHOGENIC MICROBIOLOGICAL PARAMETERS

	Pathogenic Bacteria			Pathogenic Viruses	
	Pseudo-monas	Strepto-cocci	Clostridium perfringens	3-Day Count	5-Day Count
No. of Daily and Large Volume Effluent Samples with Numeric Analysis Results	52	46	53	48	39
Correlation Coefficients of Unweighted Analyses:					
Indicator Parameters					
Total Coliform	.169	.276	.332	−.240	−.228
Fecal Coliform	.091	.264	.256	−.218	−.313
Std Plate Count	.161	.081	−.028	−.054	.166
Coliphage	.192	−.009	.121	.146	.147
Correlation Coefficients of Weighted Analyses:					
Indicator Parameters					
Total Coliform	.148	.362	.269	−.238	−.266
Fecal Coliform	.077	.354	.225	−.219	−.355
Std Plate Count	.163	.109	−.063	−.053	.126
Coliphage	.189	−.044	.143	.124	.188
Two-Sided Significance Level of the Significant Weighted Analyses Correlation Coefficients:					
Indicator Parameters					
Total Coliform	–	P=.01	P=.05	–	–
Fecal Coliform	–	P=.02	–	–	P=.03
Std Plate Count	–	–	–	–	–
Coliphage	–	–	–	–	–

To simplify presentation of the statistical analyses of the microbiological effluent and aerosol sample analyses, a consistent notation has been used. This notation is shown in Table VI. A-15.

The best equations generated through the weighted stepwise regression analysis are presented in Table VI. A-16. The best streptococci regression equation involves only total coliforms. It is:

$$\ln x_{ST_e} = 0.498 \ln X_{TC_e} + 2.136$$

This equation explains only 0.136 = 13.6% of the observed variation in the streptococci effluent concentration. Thus, while it is significantly better (P = .012) than no relationship, this streptococci-total coliform relationship is not very strong. The best *Clostridium perfringens* regression equation contains the total coliform and standard bacterial plate count indicators:

$$\ln X_{CL_e} = 0.658 \ln X_{TC_e} - 0.263 \ln X_{PC_e} + 4.457$$

This regression equation has less predictive ability than the streptococci regression. It only accounts for 11.5% of the variation in the effluent sample concentrations of *Clostridium perfringens*. The negative coefficient of the standard bacterial plate count variable could suggest a negative correlation between *Clostridium perfringens* and the non-coliform bacteria. However, with a t-statistic of -1.58, this coefficient is not significant at the 0.1 level, and such an interpretation is unwarranted. The best regression equation for the 5-day virus plaque effluent concentration is:

$$\ln X_{V5_e} = -0.622 \ln X_{FC_e} + 0.611 \ln X_{CP_e} + 0.302 \ln X_{PC_e} - 3.222$$

Table VI.A-15.
MICROBIOLOGICAL CONSTITUENT NOTATION

Microbiological Constituent	Mnemonic Notation
Indicators:	
Total Coliform	TC
Fecal Coliform	FC
Standard Plate Count	PC
Coliphage	CP
Pathogens:	
Pseudomonas	PS
Streptococci	ST
Clostridium perfringens	CL
Three-day virus plaques	V3
Five-day Virus plaques	V5
Aerosol Sample	a
Effluent Sample	e
Concentration	X

Table VI.A-16.
SUMMARY OF BEST MULTIPLE REGRESSION EQUATIONS FOR PREDICTING PATHOGEN EFFLUENT CONCENTRATION FROM THE INDICATOR EFFLUENT CONCENTRATIONS

Regressed Effluent Pathogen Concentration Variable	Best Predictive Regression Equation				
	Coefficient of Multiple Determination (R^2)	Significance of Regression F-Ratio	Term in Regression Equation		
			Indicator Regression Variable	Regression Coefficient b_i	Regression Coefficient t Statistic
$\ln X_{ST_e}$	.136	P=.012	$\ln X_{TC_e}$	0.498	2.63
			1*	2.136	
$\ln X_{CL_e}$	.115	P=.047	$\ln X_{TC_e}$	.0658	2.50
			$\ln X_{PC_e}$	-0.263	-1.58
			1*	4.457	
$\ln X_{V5_e}$	.275	P=.010	$\ln X_{FC_e}$	-0.622	-3.12
			$\ln X_{CP_e}$	0.611	2.17
			$\ln X_{PC_e}$	0.302	2.12
			1*	-3.222	

*1 represents the constant term in the regression equation

The equation explains 27.5% of the observed 5-day virus variation. It is significantly better (P= .01) than no regression relationship. The 5-day virus level seems to depend somewhat on the non-fecal coliform bacteria level and the coliphage level. However, the regression coefficient t-statistics are still too small to lend much credence to such an interpretation.

The stepwise multiple linear regression equations given above are an inadequate basis for relating the pathogen and indicator effluent concentrations over the microorganism concentration ranges observed in the Pleasanton effluent. Considerably more than half of the log transformed pathogen concentration variability (i.e., pathogen percentage variation) cannot be accounted for by the variability of the indicator organism concentrations.

A third method, canonical correlation, was also employed to seek those factors that the set of pathogen measurements and the set of indicator measurements have in common over all the effluent samples. Canonical correlation might identify indicator relationships for some combination of pathogens that could not be detected by the correlation and regression analyses, which only relate one pathogen at a time. As in the correlation and regression analyses, natural log transformations of each microorganism's effluent concentration were employed to construct the analysis variables. Observation weighting was accomplished by multiplying the indicator organism weight sum by the pathogenic organism weight sum and dividing by the average observation weight. Only the 36 effluent samples, for which all nine pathogen and indicator organisms had values, were included in the initial canonical correlation analysis. The results of this analysis are summarized in Table VI. A-17. One significant pair of canonical variables was identified. The canonical correlation is 0.638. This pair of canonical variables share 0.407 = 40.7% of their variation. Wilk's lambda statistic is transformed into a chi-square statistic to determine that P = 0.041 is the statistical significance of this canonical correlation. The coefficients of the canonical variables are shown in the lower part of Table VI. A-17. The important components of the pathogen canonical variate are 5-day virus (positive coefficient) and streptococci (negative coefficient). The important indicator canonical variate components are standard bacterial plate count (positive coefficient) and total and fecal coliform (negative coefficients).

Variants of the preceding case were also analyzed by canonical correlation. The pathogenic bacteria set was related to the indicator set, but there were no significant canonical correlations. The viruses set was related to the indicator set. The one significant canonical variable pair is very similar to the significant canonical variable pair displayed in Table VI. A-17. The variable coefficients have the same signs and nearly the same magnitudes. Pairwise deletion of missing data was used to construct the basic correlation coefficient matrix from all the observations for which both parameters in each pair had numerical values. The only nearly significant (P = 0.07) canonical variable pair emerging from this analysis which used all the available data was nearly identical to the significant canonical variable pair given in Table VI. A-17.

By comparing Table VI. A-17 with Table VI. A-16, it can be seen that the one significant canonical variate pair is basically a linear combination of the regression equations for 5-day viruses and streptococci. Substituting the regression equations into the expression $0.968\ \mathrm{Ln}X_{V5_e} - 0.422\ \mathrm{Ln}X_{ST_e}$ yields a linear combination of the natural log indicator parameter that agree in sign and compare well in magnitude with the indicator set coefficients given in Table VI. A-17. Thus, canonical correlation analysis has not identified any new pathogen-indicator relationships. In fact, it strongly suggests that, beyond the meager regression relationships given in Table VI. A-16, there are no more substantive relationships among the pathogen and indicator microorganism groups in the Pre-Fair effluent samples obtained at Pleasanton.

In summary, over the ranges of the effluent microbiological group concentrations obtained during the Pre-Fair sampling at Pleasanton (from one order of magnitude for coliphage and total coliform to well over two orders of magnitude for *Pseudomonas* and *Clostridium perfringens*), there are only the most tenuous of relationships between some pathogenic parameters and some indicator parameters. These

relationships are certainly an insufficient basis for discontinuing the pathogenic analyses of the effluent samples in later phases of the study.

f. Microbial Characterization

A thorough characterization of the treated sewage effluent at the Pleasanton site was conducted to identify the types of pathogenic bacteria and viruses. Few pathogenic organisms were isolated, despite the detection of relatively high levels of indicator bacteria in effluent and aerosol samples. The analytical methods employed in the present study were designed to provide definitive information on the types and approximate quantities of the bacterial population (including non-pathogens, opportunistic pathogens, and overt pathogens) present in the sewage effluent.

The levels of the routinely-assessed microorganism groups are given in Table VI. A-18 for

Table VI.A-17.
CANONICAL CORRELATION OF THE PATHOGEN EFFLUENT CONCENTRATION SET WITH THE INDICATOR EFFLUENT CONCENTRATION SET

Pair	Eigenvalue	Canonical Correlation	Wilk's Lambda	Significance of Canonical Correlation
1	0.407	0.638	0.354	P = 0.041
2	0.298	0.538	0.597	P = 0.191
3	0.121	0.348	0.840	P = 0.491

Coefficients of Significant Canonical Variables ($P < 0.05$)

	Pair 1
Indicator Set	
Ln X_{TC_e}	-0.623
LN X_{FC_e}	-0.475
Ln X_{PC_e}	0.679
Ln X_{CP_e}	0.162
Pathogen Set	
Ln X_{PS_e}	-0.045
Ln X_{ST_e}	-0.422
Ln X_{CL_e}	-0.113
Ln X_{V3_e}	-0.313
Ln X_{V5_e}	0.968

Table VI.A-18.
EFFLUENT CONCENTRATIONS OF USUAL MICROBIOLOGICAL CONSTITUENTS IN LARGE-VOLUME SAMPLES TAKEN FOR MICROBIAL CHARACTERIZATION

Sample Date	Total Coliform (MFC/100 ml) × 10^1	Fecal Coliform (MFC/100 ml) × 10^2	Standard Plate Count (No./100 ml) × 10^6	Coliphage (PFU/l) × 10^3	Klebsiella (CFU/100 ml) × 10^2	Pseudomonas (CFU/100 ml) × 10^1	Streptococci (CFU/100 ml) × 10^3	Clostridium perfringens (MPN/100 ml) × 10^1	3-Day Plaques (PFU/l)	5-Day Plaques (PFU/l)
4-27-76	670	56	M	210	CS	CS	CS	CS	244*	262*
5-5-76	3170	350	100	500	61	300	CS	11	40	28
5-13-76	240	27	40	260	66	120	9.3†	0.43	15	27
5-21-76	1300	115	61	490	88	300	18	11	7.2	13
5-24-76	2000	230	200	80	22	1000	23	11	1.8	2.5
6-1-76	1100	168	61	510	50	310	26	4.3	18	CS
6-22-76	473	85	11	190	6.9	120	5.1	7.5		

*Possible contamination.
†Most probable number.
M–Missing value.
CS–Contaminated sample.

the seven large-volume effluent samples taken for microbial characterization during Pre-Fair. These levels are representative of those obtained for the daily composite effluent samples.

A summary of all other bacterial types identified in these large-volume effluent samples is presented in Table VI. A-19. Data from the first large-volume sample and portions of the data from the second and third large-volume samples could not be obtained. These samples were received before the various differential and selective plating and diagnostic media required for the respective analyses were available. Large-volume samples obtained, on 5-21, 5-24, 6-1, 6-22, and 11-29, 1976 were characterized for every bacterial type or group listed, according to the procedures described in Appendix D, Methods and Materials. In addition, three of the daily composite effluent samples from Pre-Fair (i.e. sample dates 6-15, 6-16, and 6-17, 1976) were subjected to the same rigorous analyses. This provides additional data for comparison of the bacterial populations in the two types of effluent samples. It should be noted that the data from the microbial characterization of the aggregated large-volume aerosol sample (discussed as run M1-36 in Section VI.B.6) are also presented for comparison.

The data show that the components of the bacterial populations of the large volume (grab) and daily composite samples examined were qualitatively similar. Inspection of the quantitative data obtained for the selected microbial parameters (*Klebsiella, Pseudomonas*, fecal streptococci, and *Clostridium perfringens*) (Table VI.A-18) in the two types of effluent samples also suggests similarity. The approximate numbers of other bacterial types isolated from both types of samples were not appreciably different, with the exception of the isolation of *Leptospira, Salmonella,* and *Shigella* only from the large-volume samples. However, this observation may be a reflection of the small number of samples examined.

The small number of *Staphylococcus aureus* isolated from the effluent samples was surprising. Four of the eight large-volume and all of the daily composite samples plated to Mannitol Salt Agar were positive for this organism. Only the lowest dilution plates (0.1 mL) yielded numerous colonies, most of which were *Micrococcaceae*. The maximal number of the latter which proved to be *Staphylococcus aureus* was 40 cfu/ml in the composite for 6-16. In contrast, large numbers of colonies grew on the selective medium for *Neisseria* at 10^{-2} dilutions of the effluent samples. However, none of the representative colony types picked proved to be *Neisseria gonorrheae* or *Neisseria meningitidis*. Enrichment for *Leptospira* was positive in four of five large-volume samples but not the three daily composite samples examined. The negative results in the latter may be the result of lack of sensitivity of the test system employed. Enrichment tubes showing turbidity were examined by dark field microscopy. Failure to observe an organism with typical *Leptospira* morphology and motility in one of at least 15 randomly-selected fields constituted a negative test. However, only a very small fraction of an enrichment is examined when this is carried out microscopically, a fact complicated by the wet mounts required for dark field. Thus, positive enrichment tubes with light growth of *Leptospira* may not be detected. Of the positive samples, enrichment tubes from the large-volume sample of 5-24 yielded the greatest number of "typical" *Leptospira* (40/15 microscopic fields).

The genus *Mycobacterium* includes species that range from saprophytes widely distributed in soil and water to facultative and obligate intracellular parasites. Positive isolation of mycobacteria from effluent samples was expected. However, the large number of these organisms (approximately 10 CFU/mL) in every large-volume and daily composite sample examined was surprising. Isolation and identification of these organisms was facilitated by the treatment procedure which resulted in plates that were relatively free of other organisms. None of the isolates of the various colony types that were carried through the identification scheme (Figure III.B-2 in Appendix D) proved to be *Mycobacterium tuberculosis*. However, representatives of the following groups or species were identified: *Mycobacterium ulcerans, Mycobacterium gordanae,* Runyon Group I, Runyon Group II, Runyon Group III, *Mycobacterium marinum,* Runyon Group IV. A single confirmed *Mycobacterium ulcerans* was the only *Mycobacterium* isolated that is a pathogen of the same cat-

Table VI.A-19.
SUMMARY OF BACTERIAL IDENTIFIED—LARGE-VOLUME SAMPLES

Waste-water Sample Date	Clostridium perfringens	Fecal Streptococci	Staphylococcus aureus	Leptospira	Mycobacteria	Neisseria (patho-genic species)	Gram Neg. Non-Fermenters: Fluorescent Pseudomonads	Gram Neg. Non-Fermenters: Other Oxidase Pos. Glucose Oxidizers	Gram Neg. Non-Fermenters: Alcaligenes and Other Oxid. Pos. Glucose Inactive	Gram Neg. Non-Fermenters: Acinetobacter and Other Oxid. Neg.	Enterobacteriaceae: Klebsiella	Enterobacter	Serratia	Edwardsiella	Escherichia	Shigella	Salmonella	Arizona	Citrobacter	Proteus	Providencia	Yersinia	Aeromonas and Other Oxid. Pos. Fermenters
5-5-76	X	*	*	*	*	*	X	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
5-13-76	X	X	X	X	*		X	X	X	X	*	*	*	*	*	*	*	*	*	*	*	*	X
5-21-76	X	X		X	X		X	X	X	X	X	X			X	X					X		X
5-24-76	X	X		X	X		X	X			X	X	X		X	X			X	X			X
6-1-76	X	X		X	X		X		X		X	X	X		X				X	X			X
6-22-76	X	X	X		X		X	X	X		X	X			X	X	X		X	X			X
Aerosol (run #36)	X	X	X		X		X			X	X	X			X					X			X
6-16-76	X	X			*		X	X	X		X	X	X		X				X	X			X
6-17-76	X	X	X		X		X	X	X		X	X			X				X	X			X
6-18-76	X	X	X		X		X	X	X		X	X	X		X					X			X

NOTES: X - Positive isolations; blank indicates none detected; * - assay not run.

egory as *Mycobacterium tuberculosis*[23]. Others range from usually pathogenic (*Mycobacterium marinum*) to usually nonpathogenic.

The distribution of the various groups of Gram-negative nonfermenting organisms, the genera of *Enterobacteriaceae*, and the oxidase positive fermenting organisms was determined by characterization of colonies picked from moderately to highly selective enteric plating media (Tables VI. A-19 and VI. A-20). A different pattern, with a better representation of the coliforms, would probably have been obtained by extensive isolation and characterization of colonies from the nonselective media EMB, ENDO, and MacConkeys. However, the possibility of enhancing the frequency of isolation of the major enteric pathogens was desired. As illustrated in Table VI. A-20, 79 percent of the isolates from the direct platings on selective media were oxidase positive. The majority of these organisms (49 percent) were fermenters which would have been incorrectly identified as various genera of *Enterobacteriaceae* if the oxidase test had not been employed. In contrast, 82 percent of isolates picked from the selective media following enrichment in GN, selenite, or tetrathionate broths were *Enterobacteriaceae*.

The distribution of the various species of *Enterobacteriaceae* observed in the large-volume and daily composite effluent samples is summarized in Table VI. A-21. The data for the aggregated aerosol sample are shown for comparison. Isolates of both *Salmonella* and *Shigella* were detected only in large-volume sample obtained on 6-22-76. When the data are taken as a whole, most of the species were represented in, at least, one of the effluent samples. Table VI. A-22 is an analysis of the percentage of each species of *Enterobacteriaceae* isolated from the total of direct platings and enrichments of the large-volume samples. It should be noted that the enrichment procedure for *Yersinia enterocolitica* (an organism increasingly implicated in enterocolitis and mesenteric lymphadenitis) failed to yield a single isolate of this organism. The few colonies which appeared on the plating media were predominantly oxidase-positive Gram-negative fermenters. How-

Table VI.A-20.
GROUPS OF ORGANISMS FROM DIRECT PLATINGS AND ENRICHMENTS FOR ENTERICS
Large-Volume Samples

Group	% Isolates Direct Plating on XLD, HEK, BS, SS	% Isolates From Enrichments
All Enterobacteriaceae	19	82
Fluorescent Pseudomonads	14	6
Non-Fermenting, Oxidase Positive Glucose Oxidizers*	9	2
Alcaligenes and Other Oxidase Positive Glucose Inactive	9	1
Acinetobacter and Other Oxidase Negative Non-Fermenters	2	1
Aeromonas and Other Oxidase Positive Fermenters	47	8

* Other than fluorescent pseudomonads.

Table VI.A-21.
SPECIES OF ENTEROBACTERIACEAE IDENTIFIED—LARGE VOLUME AEROSOL SAMPLES

Wastewater Sample Date	Klebsiella pneumoniae	Klebsiella ozaenae	Klebsiella rhinoschleromatis	Enterobacter cloacae	Enterobacter aerogenes	Enterobacter hafniae	Acrogenic Ent. agglomerans	Anaerogenic Ent. agglomerans	Serratia marcescens	Serratia liquefaciens	Serratia rubidaea	Edwardsiella tarda	Escherichia coli	Escherichia coli A-D	Shigella (all species)	Salmonella (all species)	Arizona (Sal. arizonae)	Citrobacter freundii	Citrobacter diversus	Proteus vulgaris	Proteus mirabilis	Proteus morganii	Proteus rettgeri	Providencia alcalifaciens	Providencia stuartii	Yersinia enterocolitica	Yersinia pseudotuberculosis
5-5-76	x			x			x						x		x									x			
5-13-76	x						x	x			x		x		x			x		x	x	x	x				
5-21-76	x			x	x		x			x			x					x	x	x	x	x					
6-1-76	x	x		x	x		x						x		x	x		x			x	x					
Aerosol (run #36)	X			x			x	x					x									x					
6-16-76	x			x					x	x			x					x			x						
6-17-76	x	x		x			x	x					x					x			x						
6-18-76	X					x	x	x		x			x								x						

NOTES: X - Positive isolations; blank indicates none detected.

ever, a *Yersinia pseudotuberculosis* isolate and a *Shigella* isolate were identified from separate aerosol samples during a routine pick of colonies.

At the beginning of Post-Fair, a large volume sample was taken and analyzed by semi-quantitative procedures. The results of this microbiological screen are presented in Table VI. A-23.

g. Respiratory Virus

Five roller tubes showing viral cytopathology were subcultured successfully, indicating confirmation as viruses. The viruses from four of these five tubes were typed as ECHO 6. The virus in the other tube remains unidentified, since typing was unsuccessful.

Thirty-five (35) roller tubes survived uncontaminated through primary subculture without showing viral cytopathology. All subcultures were challenged with ECHO 11 for rubella. All showed typical ECHO 11 cytopathology and were reported as negative for rubella. Fluids from all subcultures were inoculated into four 10-day embryonated chicken eggs, incubated 72 hours and the allantoic fluids harvested and tested for hemagglutinins against crbc; all were negative. The control influenza strain gave 1:16 HA.

Table VI.A-22.
ANALYSIS OF ENTEROBACTERIACEAE
(Percent of Each Species Isolated From Total of Direct Platings and Enrichments—
Large Volume Samples of 5-21-76, 5-24-76, 6-1-76, 6-22-76)
Excluding *E. coli

	Percent		Percent
K. pneumoniae	9.2	Arizona	0
K. ozaenae	1.5		
K. rhinoscheromatis	0	C. freundii	16.0
		C. diversus	1.5
E. cloacae	11.4		
E. aerogenes	1.5	P. vulgaris	3.8
E. hafniae	0.8	P. mirabilis	16.8
E. agglomerans (aerogenic)	14.5	P. morganii	3.1
E. agglomerans (anaerogenic)	3.1	P. rettgeri	2.3
S. marcescens	0.8	P. alcalifaciens	0.8
S. liquefaciens	2.3	P. stuartii	0
S. rubidaea	0.8		
		Y. enterocolitica	0
E. tarda	0	Y. pseudotuberculosis	0
Shigella (all species)	3.1	Unidentified	6.1
Salmonella (all species)	0.8		

*E. coli was the predominant representative of the Enterobacteriaceae. Values are the percentage from direct platings on XLD, Hektoen, SS, and BS agars and from enrichments in GN, tetrathionate, and selenite broths.

Table VI.A-23.
SEMI-QUANTITATIVE MICROBIOLOGICAL SCREEN OF POST-FAIR SAMPLE (11-29-76)

Bacteria:	cfu/100ml
Arizona	<3.0x10^3 (ND)
Citrobacter Freundii	1.0x10^5
Edwardsiella	<3.0x10^3 (ND)
Enterobacter aerogenes	1.0x10^4
Enterobacter cloacae	3.0x10^6
Escherichia H_2S^+	1.0x10^5
Fecal Coliform	2.2x10^4
Fecal Streptococci	1.7x10^3
Klebsiella pneumoniae	7.0x10^4
Klebsiella ozaenae	4.0x10^4
Mycobacteria	1.4x10^4
Proteus	<3.0x10^3 (ND)
Providencia	<3.0x10^3 (ND)
Pseudomonas	2.0x10^4
Salmonella	<3.0x10^3 (ND)
Serratia	<3.0x10^3 (ND)
Shigella	<3.0x10^3 (ND)
Staphylococcus	4.0x10^3
Total coliform	1.2x10^6
Total Plate Count	1.5x10^7
Yersinia	* (ND)
Of the total number of colonies which were randomly picked and biochemically tested for Enterobacteriaceaa:	
Oxidae positive	46.5%
Klebsiella ozaenae	6.9%
Klebsiella pneumonia	13.9%
Enterobacter cloacae	13.9%
Enterobacter aerogenes	1.1%
Escherichia H_2S^+	2.3%
Citrobacter freundii	5.8%
No growth	9.3%
Viruses:	
Coliphage	4.8x10^5 pfu/l
Enteroviruses 3day	161 pfu/l
5day	242 pfu/l
Concentration Efficiency	100%
Total Organic Carbon:	
Filtered	33 mg/l
Unfiltered	39 mg/l

Note: ND - none detected
* - nonquantitative procedure

B. Aerosol Run Data Characteristics

1. Meteorological and Sampling Conditions

a. Meteorological Conditions

Thirty-six microbiological aerosol runs were attempted during the Pre-Fair sampling period. Five of the aerosol runs were quality assurance runs. Eleven aerosol runs had to be aborted because wind shifts violated one or more of the aerosol run criteria specified in the aerosol sampling protocol (see Appendix D). The 20 remaining aerosol sampling runs all met the protocol criteria. In addition to the microbiological aerosol runs, seven dye aerosol runs were successfully completed during the Pre-Fair sampling period. The environmental conditions during both the microbiological and dye aerosol runs appear in Table VI.B-1.

Table VI.B-1.
AEROSOL RUN METEOROLOGICAL AND SOURCE DATA SUMMARY
a. Temperature and Relative Humidity

Aerosol Runs			Air Temperature, °C				Relative Humidity, %					Wastewater Temperature, °C
Run No.	Run Date	Time of Run	2m at Effluent Pond	2m at Met. Tower	Just Upwind of Spray Line	Just Downwind of (Within) Spray Line	2m at Effluent Pond	2m at Met. Tower	Livermore	Just Upwind of Spray Line	Just Downwind of (Within) Spray Line	
Dye Aerosol Runs												
Pre-Fair												
D1-1	5-4-76	1355-1405	19.4	(19.4)	(19.9)	(18.3)	71	(39)	39	(54)	(59)	(21.7)
D1-3	5-28-76	1555-1625	18.3	(18.3)	(19.0)	(17.5)	(70)	(22)	22	(37)	(44)	(23.8)
D1-4	5-31-76	2030-2100	14.4	(14.4)	(15.8)	(14.7)	71	(40)	40	(55)	(60)	(22.7)
D1-5	5-31-76	2126-2156	13.3	(13.3)	(14.9)	(13.9)	72	(76)	76	(91)	(92)	(22.4)
D1-6	6-1-76	1805-1835	21.1	(21.1)	(21.3)	(19.5)	65	(24)	24	(39)	(46)	(23.5)
D1-9	6-14-76	1740-1810	33.9	(33.9)	(31.8)	(28.9)	45	(5)	5	(20)	(29)	28 0
D1-10	6-16-76	1640-1710	31.7	(31.7)	(30.0)	(27.3)	60	(19)	19	(34)	(41)	28.0
Post-Fair												
D2-1	12-2-76	1435-1505	16.7	16.1	18.3	15.0	(74)	31	(31)	51	59	16.0
D2-2	12-5-76	1253-1323	19.4	20.0	20.0	14.4	(70)	20	(20)	26	55	16.0
D2-4	12-6-76	1345-1415	15.6	16.1	22.8	17.2	(74)	31	54	53	65	15.5
D2-5	12-6-76	1520-1550	13.9	14.4	21.1	16.1	(77)	36	54	56	66	15.5
D2-6	12-6-76	1523-1553	18.9	18.3	20.6	14.4	63	23	31	23	38	14.0
D2-7	12-16-76	1303-1330	12.8	14.4	18.3	16.1	80	48	46	45	53	15.0
D2-8	12-16-76	1347-1417	13.3	14.4	18.3	16.1	79	48	47	45	53	15.0
D2-9	1-13-76	1312-1342	5.6	7.2	8.9	(8.3)	96	78	68	72	75	12.0
D2-10	1-13-76	1400-1430	4.4	7.2	8.3	7.8	96	78	73	83	83	12.0
D2-11	1-13-76	1835-1905	4.4	6.7	5.6	5.0	96	80	74	100	100	(11 5)
Microbiological Aerosol Runs												
Pre-Fair												
M1-1	5-4-77	1547-1617	21.1	(21.1)	(21.3)	(19.5)	72	(44)	44	(59)	(64)	(21.7)
M1-2	5-5-77	1538-1608	18.9	(18.9)	(19.5)	(17.9)	72	(38)	38	(53)	(58)	(21.7)
M1-3	5-5-77	1700-1730	18.3	(18.3)	(19.0)	(17.5)	71	(40)	40	(55)	(60)	(21.3)
M1-4	5-13-76	1625-1655	35.0	(35.0)	(32.7)	(29.7)	52	(12)	12	(27)	(32)	(22.3)
M1-5	5-13-76	1807-1837	32.2	(32.2)	(30.4)	(27.6)	52	(9)	9	(24)	(29)	(21.8)
M1-6	5-17-76	1923-1953	13.9	(13.9)	(15.4)	(14.3)	65	(85)	85	(100)	(100)	(21.7)
M1-7	5-17-76	2044-2114	10.0	(10.0)	(12.2)	(11.5)	75	(60)	60	(75)	(80)	(21.3)
M1-8	5-21-76	1538-1608	23.3	(23.0)	(23.1)	(21.1)	65	(32)	32	(47)	(52)	(23.2)
M1-9	5-24-76	1557-1627	17.2	(17.2)	(18.1)	(16.7)	76	(45)	45	(60)	(65)	(23.3)

NOTE: Values in parentheses have been substituted according to criteria described in the text.

Table VI.B-1. (cont'd)

Aerosol Runs			Air Temperature, °C				Relative Humidity, %					Wastewater Temperature, °C
Run No.	Run Date	Time of Run	2m at Effluent Pond	2m at Met. Tower	Just Upwind of Spray Line	Just Downwind of (Within) Spray Line	2m at Effluent Pond	2m at Met. Tower	Livermore	Just Upwind of Spray Line	Just Downwind of (Within) Spray Line	
Microbiological Aerosol Runs (cont'd)												
Pre-Fair (cont'd)												
M1-10	5-24-76	2032-2102	12.2	(12.2)	(14.0)	(13.1)	83	(86)	86	(100)	(100)	(22.0)
M1-11	5-25-76	2214-2244	15.6	(15.6)	(16.8)	(15.5)	71	(37)	37	(52)	(57)	(21.6)
M1-12	5-27-76	1717-1747	16.7	(16.7)	(17.7)	(16.3)	76	(46)	46	(61)	(66)	24.0
M1-13	5-27-76	2040-2110	10.6	(10.6)	(12.7)	(11.9)	80	(75)	75	(90)	(95)	24.0
M1-14	5-27-76	2150-2220	10.0	(10.0)	(12.2)	(11.5)	84	(80)	80	(95)	(100)	24.0
M1-15	6-3-76	2117-2147	10.0	(10.0)	(12.2)	(11.5)	76	(64)	64	(79)	(84)	(22.6)
M1-31	6-14-76	2010-2040	28.3	(28.3)	(27.2)	(24.8)	47	(5)	5	(20)	(25)	24.0
M1-32	6-15-76	1420-1450	37.2	(37.2)	(34.5)	(31.3)	50	(6)	6	(21)	(26)	26.0
M1-33	6-15-76	1635-1705	33.9	(33.9)	(31.8)	(28.9)	50	(20)	20	(35)	(40)	28.0
M1-34	6-16-76	2140-2210	21.1	(21.1)	(21.3)	(19.5)	65	(45)	45	(60)	(65)	(23.7)
M1-35	6-17-76	2242-2312	15.6	(15.6	(16.8)	(15.5)	77	(65)	65	(80)	(85)	26.0
Post-Fair												
M2-1	1-21-77	1600-1630	10.6	13.3	12.8	12.2	90	78	69	74	82	15.3
M2-2	1-25-77	1415-1445	10.0	12.1	13.3	12.2	87	75	60	82	82	15.9
M2-3	1-25-77	2012-2042	5.6	8.9	8.9	8.9	98	84	83	88	91	13.6
M2-4	1-30-77	1421-1451	(8.7)	9.7	10.0	10.0	91	69	57	77	79	13.3
M2-5	1-30-77	1603-1633	(8.5)	9.5	10.0	10.0	92	71	46	82	82	13.3
M2-6	2-9-77	1353-1423	13.3	15.0	14.4	14.4	87	72	(72)	71	78	15.5
M2-10	2-23-77	1835-1905	8.4	9.4	8.1	8.9	80	50	65	96	96	14.0
M2-11	2-24-77	1403-1433	11.7	13.3	13.9	13.9	74	35	45	48	53	16.2
M2-12	2-24-77	1513-1543	11.5	13.5	13.9	13.9	72	31	33	45	45	16.2
M2-13	2-28-77	2003-2033	5.6	7.8	(8.6)	(8.3)	87	55	69	(70)	(75)	13.8
M2-14	3-14-77	1625-1655	10.6	11.4	11.9	11.9	76	42	48	51	51	17.5
M2-15	3-16-77	1555-1625	9.4	10.0	12.2	11.4	88	52	62	59	63	17.3
M2-16	3-17-77	1616-1646	10.6	11.6	12.2	11.4	85	48	64	64	66	17.3
M2-17	3-18-77	1620-1650	10.6	18.9	13.9	13.6	78	27	33	54	56	17.6
M2-22	3-25-77	1344-1414	15.0	16.7	16.4	16.1	78	33	41	43	52	17.6
M2-23	3-26-77	1315-1345	19.4	20.6	20.0	18.9	69	21	24	35	40	17.9
M2-24	3-26-77	1634-1704	20.0	19.5	18.9	18.3	70	29	24	44	50	18.0
M2-25	3-27-77	1330-1400	17.2	17.8	17.5	17.5	79	36	53	63	65	17.8
M2-26	3-27-77	1518-1548	16.7	16.1	16.1	16.4	78	39	51	62	60	18.0
M2-29	4-11-77	1515-1545	22.2	21.7	21.9	21.1	75	29	38	31	38	19.8

Table VI.B-1. (cont'd)

Aerosol Runs			Air Temperature, °C				Relative Humidity, %					Wastewater Temperature, °C
Run No.	Run Date	Time of Run	2m at Effluent Pond	2m at Met. Tower	Just Upwind of Spray Line	Just Downwind of (Within) Spray Line	2m at Effluent Pond	2m at Met. Tower	Livermore	Just Upwind of Spray Line	Just Downwind of (Within) Spray Line	
Microbiological Aerosol Runs (cont'd)												
Post-Fair (cont'd)												
M2-30	4-12-77	1615-1645	22.8	23.3	21.9	21.4	69	21	28	17	19	19.8
M2-31	4-13-77	2106-2135	8.9	10.0	8.1	8.3	91	52	82	63	80	19.5
M2-32	4-14-77	2010-2040	(13.3)	13.3	10 8	11.4	(77)	37	55	65	66	18.0
M2-33	4-19-77	1640-1710	21.7	22.8	21.7	20.8	68	16	16	29	33	19.9
M2-34	4-19-77	2050-2120	15.6	13.3	11.4	11.7	68	25	21	43	44	19.4
M2-35	4-22-77	1410-1440	(26.7)	26.7	24.4	23.9	(65)	9	20	23	28	19.6
M2-36	4-23-77	1630-1700	(24.4)	24.4	23.3	22.5	(68)	15	22	26	29	19.9
M2-37	4-24-77	1335-1405	21.7	22.5	20.3	19.4	73	30	36	39	45	19.7
M2-38	4-24-77	1505-1535	18.9	19.5	18.9	17.5	74	23	32	36	45	19.6
Quality Assurance Aerosol Runs												
Pre-Fair												
M1-16-19	6-7-76	2104-2134	10.0	(10.0)	(12.2)	(11.5)	81	(74)	74	(89)	(94)	(23.0)
M1-20-23	6-8-76	1750-1820	18.3	(18.3)	(19.0)	(17.5)	64	(40)	40	(55)	(60)	17.0
M1-24-25	6-9-76	2259-2329	11.1	(11.1)	(13.1)	(12.3)	82	(70)	70	(85)	(90)	21.0
M1-26	6-10-76	2212-2242	11.1	(11.1)	(13.1)	(12.3)	84	(64)	64	(79)	(84)	22.0
M1-27-30	6-13-76	1707-1737	27.8	(27.8)	(26 8)	(24.4)	57	(9)	9	(24)	(29)	27.0
Post-Fair												
M2-8-9	2-16-77	1805-1835	17.2	16.7	(18.1)	(16.7)	78	58	51	73	(76)	17.8
M2-27-28	4-5-77	1635-1709	23.3	22.8	23.3	22.8	72	25	32	18	21	20.3
Virus Aerosol Runs												
Post-Fair												
V2-I.1	2-25-77	1753-1823	(11.1)	11.1			77	47		(62)		15.5
V2-I.3	2-26-77	1505-1535	(16.7)	16.7			70	36		(51)		16.8
V2-I.4	2-26-77	1545-1615	(16.7)	16.7			70	37		(52)		16.5
V2-I.5	2-26-77	1624-1654	(15.6)	15.6			72	36		(51)		16.3
V2-I.6	2-26-77	1703-1733	(14.4)	14.4			73	45		(60)		15.3
V2-II.1	4-9-77	1450-1520	(16.1)	16.1	16.1	15.8	78	35	41	55	59	19.5

Table VI.B-1. (cont'd)

Aerosol Runs			Air Temperature, °C				Relative Humidity, %					Wastewater Temperature, °C
Run No.	Run Date	Time of Run	2m at Effluent Pond	2m at Met Tower	Just Upwind of Spray Line	Just Downwind of (Within) Spray Line	2m at Effluent Pond	2m at Met. Tower	Livermore	Just Upwind of Spray Line	Just Downwind of (Within) Spray Line	
Virus Aerosol Runs (cont'd)												
Post-Fair (cont'd)												
V2-II.2	4-9-77	1530-1600	(16.1)	16.1	15.8	15 5	78	35	41	54	59	19.3
V2-II.3	4-9-77	1610-1640	(15.0)	15.0	15.0	14.4	78	38	41	56	63	19.2
V2-II.4	4-9-77	1655-1725	(14.4)	14.4	14.2	13.6	79	38	41	60	61	19.2
V2-II.5	4-9-77	1735-1805	(13.3)	13.3	13.9	13.3	80	40	41	59	61	19.2
V2-II.6	4-9-77	1815-1845	(12.1)	12.1	11.7	11.7	81	44	41	66	66	19.0

Table VI.B-1.
AEROSOL RUN METEOROLOGICAL AND SOURCE DATA SUMMARY
b. Wind Direction, Velocity, Stability and Solar Radiation

Aerosol Runs			Mean Wind Direction, deg		Wind Velocity, m/sec			Radiation				Wind Stability		
Run No.	Run Date	Time of Run	Relative to True North	Relative to Perpendicular to Spray Line	10m at Effluent Pond	4m at Met. Tower	2m in Spray Field	Cloud Cover in Eighths	Cloud Height, m	Net Radiation Index	Solar Radiation W/m²	Azimuth St. Dev. σ_A' (rad.)	Elevation St. Dev. σ_E' (rad.)	Pasquill Stability Class
Dye Aerosol Runs														
Pre-Fair														
D1-1	5-4-76	1355-1405	260	−40	8.9	(8.2)	(8 2)	4	High	4	(930)	0.24	0.052	3
D1-3	5-28-76	1555-1625	250	−30	6.0	(5.1)	(5 1)	<1		3	(800)	0.18	0.065	3
D1-4	5-31-76	2030-2100	250	−30	2.3	(1.5)	(1.5)	<1		−2	(17)	0.48	0.051	7
D1-5	5-31-76	2126-2156	220	0	1.8	(1.2)	(1.2)	<1		−2	(17)	0.38	0 051	7
D1-6	6-1-76	1805-1835	255	−35	2.1	(1.5)	(1.5)	5		1	(340)	0.45	0.065	3
D1-9	6-14-76	1740-1810	20	+20	2.3	(1.8)	(1.8)	<1		2	(520)	0.42	0.093	2
D1-10	6-16-76	1640-1710	261	−41	6.7	(5.1)	(5.1)	<1		3	(710)	0.18	0.065	3
Post-Fair														
D2-1	12-2-76	1435-1505	101	−61	1.1	2.0	1.1	0	Haze	2	290	0.23	0.093	3
D2-2	12-5-76	1253-1323	41	−1	4.1	4.1	3.6	1	High	2	430	0.24	0.075	3
D2-4	12-6-76	1345-1415	97	−37	4.3	2.8	1.8	0	Haze	2	410	0.30	0.093	3
D2-5	12-6-76	1520-1550	68	−28	4,0	3.1	2.2	0	Haze	1	105	0.21	0.052	4
D2-6	12-6-76	1523-1553	74	−34	3.8	3.3	3.1	0	Haze	1	170	0 10	0.052	4
D2-7	12-16-76	1303-1333	57	−17	2.9	2.8	1.8	0	Haze	2	(510)	0.27	0.093	3
D2-8	12-16-76	1347-1417	74	−34	3.6	2.5	1.6	0	Haze	2	(450)	0.28	0.093	3
D2-9	1-13-77	1312-1342	59	−19	3.7	2.8	1.8	8	Low	1	180	0.26	0.052	4
D2-10	1-13-77	1400-1430	66	−26	4.5	3.6	3.1	8	Low	1	126	0.16	0.052	4
D2-11	1-13-77	1835-1905	66	−26	2.9	2.1	1.1	8	150	−1	35	0 25	0 051	5
Microbiological Aerosol Runs														
Pre-Fair														
M1-1	5-4-77	1547-1617	290	−70	5.1	(4.3)	4.3	2		3	(730)	0.49	0.065	3
M1-2	5-5-77	1538-1608	260	−40	4.7	(3.7)	3.7	3		3	(750)	0.21	0.093	2
M1-3	5-5-77	1700-1730	275	−55	3.0	(2.3)	2.3	4		2	(570)	0.31	0.075	3
M1-4	5-13-76	1625-1655	265	−45	5.9	(5.0)	5.0	0		3	(700)	0.21	0.065	3
M1-5	5-13-76	1807-1837	240	−20	2.5	(1.8)	1.8	0		2	(370)	0.59	0.093	2
M1-6	5-17-76	1923-1953	175	+45	5.7	(4.5)	4.5	0		1	(112)	0.21	0.047	4
M1-7	5-17-76	2044-2114	145	+75	5.4	(4.2)	4.2	0		−2	(17)	0.28	0.038	5
M1-8	5-21-76	1538-1608	275	−55	3.2	(2.5)	2.5	0	Haze	3	(840)	0.63	0.093	2
M1-9	5-24-76	1557-1627	251	−31	5.4	(4.6)	4.6	1	Low	3	(750)	0.35	0.065	3
M1-10	5-24-76	2032-2102	215	+5	5.5	(4.3)	4.3	1	Low	−2	(17)	0.38	0.038	5

Table VI.B-1. (cont'd)

Aerosol Runs			Mean Wind Direction, deg		Wind Velocity, m/sec			Radiation				Wind Stability		
Run No.	Run Date	Time of Run	Relative to True North	Relative to Perpendicular to Spray Line	10m at Effluent Pond	4m at Met Tower	2m in Spray Field	Cloud Cover in Eighths	Cloud Height, m	Net Radiation Index	Solar Radiation, W/m²	Azimuth St. Dev σ_A' (rad.)	Elevation St Dev σ_E' (rad.)	Pasquill Stability Class
Microbiological Aerosol Runs (cont'd)														
Pre-Fair (cont'd)														
M1-11	5-25-76	2214-2244	210	+10	1 6	(1.1)	1.1	0		−2	(17)	0.21	0.051	7
M1-12	5-27-76	1717-1747	180	+40	4.8	(3.7)	3.7	1		2	(560)	0.52	0 075	3
M1-13	5-27-76	2040-2110	200	+20	8.7	(6.8)	6.8	1		−2	(17)	0.18	0 038	4
M1-14	5-27-76	2150-2220	210	+10	4.5	(3 3)	3.3	<1		−2	(17)	0.45	0.045	6
M1-15	6-3-76	2117-2147	188	+32	3.9	(1 1)	1.1	<1		−2	(17)	0.31	0.045	7
M1-31	6-14-76	2010-2040	150	+70	1.8	(0.5)	0.5	<1		1	(17)	1.01	0 065	3
M1-32	6-15-76	1420-1450	265	−45	4.5	(3 8)	3.8	3		4	(900)	0.28	0 110	2
M1-33	6-15-76	1635-1705	275	−55	6.9	(5.6)	5.6	3		3	(670)	0.31	0.065	3
M1-34	6-16-76	2140-2210	230	−10	3.0	(0.9)	0.9	<1		−2	(17)	0.28	0 045	7
M1-35	6-17-76	2242-2312	35	+5	3.1	(1.1)	1.1	<1		−2	(17)	0.31	0.045	7
Post-Fair														
M2-1	1-21-77	1600-1630	72	−32	1.3	1.7	1.3	7	Low	1	180	0 18	0.065	3
M2-2	1-25-77	1415-1445	87	−47	5.1	2 5	3.1	7	Low	0	430	0.35	0.056	4
M2-3	1-25-77	2012-2042	68	−28	3.0	1.9	2.0	0		−1	21	0.14	0 051	5
M2-4	1-30-77	1421-1451	68	−28	(2.3)	2 0	2.0	1	Haze	2	370	0.22	0.093	3
M2-5	1-30-77	1603-1633	68	−28	(2.3)	2 0	2.0	3	Haze	1	70	0.22	0.065	4
M2-6	2-9-77	1853-1423	159	+61	1.9	2 0	1.3	6		1	260	0.14	0.065	4
M2-10	2-23-77	1835-1905	239	−19	1 6	2.7	1.8	8	High	−1	0	0.26	0.051	5
M2-11	2-24-77	1403-1433	78	+38	1 8	4.2	3 1	7	7000	2	660	0.33	0 051	3
M2-12	2-24-77	1513-1543	75	+35	4.6	4.1	3 4	6	7000	1	540	0 28	0 075	4
M2-13	2-28-77	2003-2033	285	−65	1.7	3.6	2.2	0		−2	35	0.26	0.052	5
M2-14	3-14-77	1625-1655	229	−9	2.8	3.2	1.6	7	1900	1	112	0 30	0 045	4
M2-15	3-16-77	1555-1625	289	−69	3.4	4.7	3.6	4	1500	1	560	0 30	0 052	4
M2-16	3-17-77	1616-1646	65	+25	4.1	4.5	4.9	0	1800	2	370	0.44	0.075	3
M2-17	3-18-77	1620-1650	266	-46	4.5	6.0	4.5	<1	1200	2	300	0.18	0 065	4
M2-22	3-25-77	1344-1414	99	+59	6.0	5.6	4.9	< 1	1500	4	840	0.27	0.082	3
M2-23	3-26-77	1315-1345	86	+46	3.6	3.7	2 7	0	Haze	4	870	0.38	0.101	2
M2-24	3-26-77	1634-1704	274	−54	2.4	4 4	3.4	<1	Haze	2	430	0.17	0.075	3
M2-25	3-27-77	1330-1400	270	−50	6.3	6.2	5.4	0	9100	4	890	0 25	0 082	3
M2-26	3-27-77	1518-1548	254	−34	3.3	4.7	1.8	0		2	700	0.59	0.075	3
M2-29	4-11-77	1515-1545	268	−48	4.0	3.5	3.1	<1		3	680	0 30	0 093	3

Table VI.B-1. (cont'd)

Aerosol Runs			Mean Wind Direction, deg		Wind Velocity, m/sec			Radiation				Wind Stability		
Run No.	Run Date	Time of Run	Relative to True North	Relative to Perpendicular to Spray Line	10m at Effluent Pond	4m at Met. Tower	2m in Spray Field	Cloud Cover in Eighths	Cloud Height, m	Net Radiation Index	Solar Radiation, W/m²	Azimuth St. Dev. σ_A' (rad.)	Elevation St. Dev. σ_E' (rad.)	Pasquill Stability Class
Microbiological Aerosol Runs (cont'd)														
Post-Fair (cont'd)														
M2-30	4-12-77	1615-1645	268	−48	7.8	6.9	5.6	2		2	490	0 16	0.065	4
M2-31	4-13-77	2106-2135	170	+50	4.0	1.5	0.9	<1		−2	28	0.23	0.051	7
M2-32	4-14-77	2010-2040	168	+52	(3.0)	2.2	(1.7)	<1		−2	35	0.23	0.051	6
M2-33	4-19-77	1640-1710	264	−44	5.4	7.2	2.9	<1		2	430	0.17	0.047	4
M2-34	4-19-77	2050-2120	175	+45	4.0	1.9	1.1	<1		−2	<70	0.54	0.051	6
M2-35	4-22-77	1410-1440	215	+5	(5.2)	4.8	4.2	4	6100	2	850	0.29	0 075	3
M2-36	4-23-77	1630-1700	251	−31	(2.7)	4.1	2.2	3	6100	2	450	0.19	0 075	3
M2-37	4-24-77	1335-1405	251	−31	7.2	6.9	6.0	8	4600	2	490	0.16	0.065	4
M2-38	4-24-77	1505-1535	222	−2	5.5	3.4	3.6	8	4600	2	290	0.73	0.075	3
Quality Assurance Aerosol Runs														
Pre-Fair														
M1-16-19	6-7-76	2104-2134	250	−30	2.9	1.6	1.6	7		−1	14	0.38	0.051	6
M1-20-23	6-8-76	1750-1820	255	−35	5.5	2.0	2.0	5		1	390	0.42	0 047	4
M1-24-25	6-9-76	2259-2329	260	−40	1.3	1.1	1.1	<7		−1	14	0 63	0 051	6
M1-26	6-10-76	2212-2242	190	+30	2.8	2.2	2.2	<7		−1	14	0.52	0.051	5
M1-27-30	6-13-76	1707-1737	260	−40	4 9	5.1	5.1	7		1	460	0.31	0.047	4
Post-Fair														
M2-8-9	2-16-77	1805-1835	178	+42	(1.4)	1.7	0.9	5	High	−1	21	0.23	0.051	6
M2-18-21	3-22-77	1821-1851	155	+65	4.4	2.3	2.7	1	5500	−2	21	0.28	0.051	6
M2-27-28	4-5-77	1639-1709	263	−43	(2 1)	3.1	1.8	0		2	480	0.25	0.075	3
Virus Aerosol Runs														
Post-Fair														
V2-I.1	2-25-77	1753-1823	253	−33	(3.2)	5.0	2 6	8	High	−1	35	0.14	0.038	4
V2-I.3	2-26-77	1505-1535	281	-61	(3.1)	2.7	2.5	1	High	1	570	0.33	0.065	4
V2-I.4	2-26-77	1545-1615	274	−54	(3.7)	3 3	3.0	2	High	1	350	0.26	0.052	4
V2-I.5	2-26-77	1624-1654	270	−50	(2.8)	2.5	2.3	3	High	1	290	0.19	0.065	4
V2-I.6	2-26-77	1703-1733	260	−40	(3.3)	3.0	2 7	3	High	1	84	0.19	0.052	4
V2-II.1	4-9-77	1450-1520	266	-46	(6.9)	6.2	5 7	3	1900	3	710	0.23	0.065	4

Table VI.B-1. (cont'd)

Aerosol Runs			Mean Wind Direction, deg		Wind Velocity, m/sec			Radiation				Wind Stability		
Run No.	Run Date	Time of Run	Relative to True North	Relative to Perpendicular to Spray Line	10m at Effluent Pond	4m at Met. Tower	2m in Spray Field	Cloud Cover in Eighths	Cloud Height, m	Net Radiation Index	Solar Radiation, W/m²	Azimuth St. Dev. σ_A' (rad.)	Elevation St. Dev σ_E' (rad.)	Pasquill Stability Class
Virus Aerosol Runs (cont'd)														
Post-Far (cont'd)														
V2-II.2	4-9-77	1530-1600	273	−53	(7.3)	6.6	6.1	3	1900	2	670	0.18	0.065	4
V2-II.3	4-9-77	1610-1640	273	−53	(8 0)	7.2	6 6	3	1800	2	520	0.19	0 047	4
V2-II.4	4-9-77	1655-1725	169	+51	(6.9)	6 1	5.6	2	1800	2	360	0 16	0.065	4
V2-II.5	4-9-77	1735-1805	268	−48	(6.1)	5.4	4.9	<1	1700	1	200	0.16	0.047	4
V2-II.6	4-9-77	1815-1845	268	−48	(5 7)	5 0	4.6	<1		1	63	0.16	0 047	4

Fifty-two microbiological aerosol runs were attempted during the Post-Fair sampling period. Three were quality assurance aerosol runs and two were special virus aerosol runs, which involved a total of eleven 30-minute sampling periods. During these quality assurance and virus runs, all samplers were located side-by-side at a distance of 50 meters from the wet-line edge of the spray line. Eighteen microbiological aerosol runs had to be aborted when meteorological conditions violated the aerosol run criteria. Twenty-nine of the Post-Fair aerosol runs met the sampling protocol criteria. Ten dye aerosol runs were also successfully completed during the Post-Fair sampling period. The environmental conditions which existed at the time of the Post-Fair dye and microbiological aerosol runs also appear in Table VI.B-1.

The environmental conditions included in Table VI.B-1 represent measured, calculated, and estimated values. In addition to the time and date, eight descriptive environmental measurements are included. Five of these parameters were measured at several locations in and around the spray field, as indicated by the table headings and as located on the site map, Figure V.A-1. Because the statistical analysis required complete data sets, substitution rules were developed to replace missing values with the best available estimate. The substituted values have been placed in parentheses.

The mean wind direction was obtained from the 10-meter level of the effluent pond station in Pre-Fair and from the 4-meter level of the meteorological tower in Post-Fair. The direction in degrees is given with respect to true north, as well as relative to a line perpendicular to the spray line used for sampling. Wind velocity in meters per second is presented in Table VI.B-1 for three locations: (1) at the 10-meter level near the effluent pond station, (2) at the 4-meter level at the meteorological tower, and (3) at the height of 3-meter in the spray field. Both the wind direction and the velocity shown in Table VI.B-1 represent average values as determined from strip charts that were recorded over the period of each microbiological aerosol run. Those values of wind velocity measured in the field are considered the most accurate reflection of wind conditions affecting the aerosolization of the sprayed wastewater. The wind velocity measured at the 10-meter effluent pond tower is the least representative. Whenever a wind velocity was not available, it was calculated from the most representative value which was available by the relationship:

$$u_2 = u_1 \left(\frac{h_2}{h_1} \right)^p$$

where u_2 and u_1 are the respective wind velocities at heights h_2 and h_1, and p is the wind profile exponent as determined by H. E. Cramer Co. for each of the Pre- and Post-Fair runs. No adjustment for measuring location differences was attempted other than the height adjustment.

Four parameters relating to solar radiation are included in Table VI.B-1. Solar radiation was measured by a short-wave instrument on each Post-Fair run. The following regression equation, which has a coefficient of multiple determination, $r^2 = 0.877$, was developed from the Post-Fair run data and was used to predict solar radiation for the Pre-Fair runs.

$$R = 16.7 + 1095 \sin (SA) - 711 \left(\frac{\sin (SA) \times cc}{h} \right)$$

where
- R = solar radiation, W/m^2
- SA = solar altitude, deg.
- cc = fractional cloud cover
- h = cloud height factor, when cc is not equal to zero, where
  - h = 1 for low clouds
  - h = 2 for middle clouds
  - h = 3 for high clouds
  - h = 10 for haze

Solar altitudes were calculated for each run at the Pleasanton location from the date of the run and the time of day halfway through the run. Cloud cover factors were estimated for each run by the field sampling crew.

The standard deviations of the azimuth wind direction (σ_A) and of the elevation wind angle (^oE) were determined from the wind direction range, wind speed, and net radiation index, as described by H. E. Cramer Co. ([24] [25]). The H. E. Cramer meteorologists also estimated the Pasquill stability class for each run from these data. The FP rotorod data was used to confirm the field measurements of wind direction mean and standard deviation (σ_A) for the Post-Fair runs.

The air temperature for each Pre- and Post-Fair run is given for four locations in Table VI.B-1. Although differences exist between the sets of air temperature values, those values measured at one location tend to correlate well with values measured at another location. This fact was useful in developing regression equations which allowed the calculation of missing temperature measurements. Missing values of air temperatures upwind (TU) and downwind (TD) of the spray line were calculated from air temperatures measured at the effluent pond (TP) using the following regression equations:

$$TU = 0.82\,TP + 4.0 \qquad (r^2 = 0.523)$$
$$TD = 0.73\,TP + 4.2 \qquad (r^2 = 0.885)$$

Effluent pond air temperatures were directly substituted for missing values of air temperature at the meteorological tower (TM).

Table VI.B-1 also contains relative humidity values for four locations in the vicinity of the spray fields. In addition, relative humidity values were obtained from the Lawrence Laboratory at Livermore which coincide with the day and time of each run. As was the case of air temperature, relative humidity measured at different locations within a general local area tend to differ in a predictable pattern and therefore can be correlated. In order to predict missing values of relative humidity at the effluent pond (RHP) and downwind of the spray line (RHD), regression equations were developed which related those parameters with values of relative humidity which were measured at the meteorological tower (RHM) and upwind of the spray line (RHU), respectively. The following are the regression equations which were used to predict missing values:

$$RHP = 0.427\,RHM + 61 \qquad (r^2 = 0.865)$$
$$RHD = 0.887\,RHU + 11 \qquad (r^2 = 0.925)$$

Values of relative humidity measured at the meteorological tower (RHM) and those values reported by the Lawrence Laboratory at Livermore (RHL) were generally in agreement. Therefore, when one value was missing, the corresponding value at the other location was substituted. It was also determined that relative humidity measured just upwind of the spray line averaged 15 percentage points higher than values reported for the Livermore location; therefore, missing values for the upwind location were approximated by adding 15 percentage points to the corresponding Livermore value: RHU = RHL + 15.

Another measurement included in Table VI.B-1 is wastewater temperature, which was recorded at the spray line. Temperatures were observed to vary seasonally and by time of day. In order to calculate missing values, a two-step procedure was used: (1) measured values of wastewater temperature were normalized to the hottest part of the day (1-4 P.M.) and (2) a graph was drawn of the normalized temperature as a function of the date on which it was measured. It was possible to construct a graph that spanned the annual range since Pre-Fair runs were conducted in late spring and Post-Fair runs were conducted in the winter and early spring. Missing values of wastewater temperature were then read from the graph for the corresponding date of the run, and the value was corrected for the actual time of day during which the run was conducted.

b. Spray Line and Sampler Configurations

The location and general configuration of the Pre- and Post-Fair microbiological, dye, and quality assurance sampling runs and the Post-Fair virus trial runs are given in Tables VI.B-2 through VI.B-5. The spray fields and staked locations of the distant samplers are shown in Figure V.A-1. The line of spray heads that was rotated daily from one setting to the next through the lettered spray field (A, B, C and D) in a 28-day cycle was used for most of the aerosol sampling. Another line of spray heads was rotated daily through the numbered fields (0, 1, 2, 3 and 4). The side of the sprayer line on which the samplers were located during a run was designated as wet (SW of the sprayer line; still wet from the preceding days' irrigation) or dry (NE of the sprayer line).

For the first four weeks of the seven-week Pre-Fair sampling period, only seven high-volume aerosol samplers were available for use in the microbiological aerosol sampling. One or more of these samplers were often inoperative because of equipment failure or microbiological contamination. The sampling protocol called for eight high-volume samplers on each run; therefore, when it was necessary, the second sampler of the side-by-side sampler pairs was excluded. This is because the five quality assurance runs would provide better measurement variation estimates than could be obtained from the paired sampler data.

The sampler configurations for the Pre-Fair aerosol runs are shown in Table VI.B-3. The standard Pre-Fair distance configuration of the sampler line was used on Runs 1 and 3. A paired sampler configuration was employed on Runs 4 and 5, to assess the basic dye level variability at a given downwind distance due both to inherent variability in the sampled aerosol and to sampler variation. A modification of the standard configuration was used on Runs 6 and 10 when it became apparent that sampling within 10 meters of the wet-line edge yielded low dye concentrations. Configuration A was utilized for Run 9. While sprayer lines were also operating in field 2 on Runs 3 to 6, this had no effect on the dye experiment; the dye was only injected into the sprayer line directly upwind from the samplers. As shown in Table VI.B-3, the standard Post-Fair dye sampler configuration was used for all of the Post-Fair dye aerosol runs.

For the Pre- and Post-Fair quality assurance aerosol runs and for the Post-Fair virus trial runs, the samplers in each run were set side-by-side about 3 meters apart (1 meter apart in Pre-Fair) at a certain distance from the wet-line edge. In the Pre-Fair quality assurance runs, the sampler lines were set at different distances from the wet-line edge with the distances recorded in Table VI.B-4. For the Post-Fair quality assurance aerosol runs and the virus trial runs, the sampler lines were set at 50 meters from the wet-line edge as shown in Tables VI.B-4 and VI.B-5.

Concentration patterns and sampler locations for the Pre-Fair runs appear in Appendix B of the H. E. Cramer Report #TR-76-303-03 ([26]). Concentration patterns and sampler configurations for the Post-Fair runs appear in Appendices F and G of the H. E. Cramer Report #TR-77-309-01 ([27]).

For the Pre-Fair dye and microbiological aerosol runs, source strength profiles are included in Appendix C of the H. E. Cramer Report #TR-76-303-03 ([28]). The individual spray head flow rates are tabulated in Appendices B and C of the H. E. Cramer Report #TR-77-309-01 ([29]) for the Post-Fair dye, microbiological and virus runs.

2. Sampled Concentration Data

a. Dye Runs

The wastewater and aerosol sample dye concentrations obtained on the Pre- and Post-Fair dye runs are presented in Tables VI.B-6 and VI.B-7, respectively. These tables also contain the perpendicular distance of each AGI sampler from the wet-line edge. For the source dye concentration, wastewater samples were taken before and after a run, and the two values obtained were averaged to calculate the source dye concentration mean.

Table VI.B-2.
SAMPLING CONDITIONS FOR MICROBIOLOGICAL AEROSOL RUNS*

Pre-Fair

Aerosol Run No.	Sprayer Line Source Sampled		Location of Sampler Line		Sampler Line Configuration	Other Fields Operating
	Field	Setting	Side of Sprayer Line	Position (facing sprayer line)		Field-Setting
M1-1	B	1	Dry	Left	Standard	2-5
M1-2	B	2	Dry	Left	Standard	2-6
M1-3	B	2	Dry	Left	Standard	2-6
M1-4	C	3	Dry	Left	Standard	E-2
M1-5	C	3	Dry	Left	Standard	E-2
M1-6	C	7	Dry	Right	Standard	0-6 F-1
M1-7	C	7	Dry	Right	Standard	0-6 F-1
M1-8	D	4	Dry	Left	Standard	F-1
M1-9	3	1	Dry	Center	Standard	G-1
M1-10	3	1	Dry	Center	Mod Std	D-7, G-1
M1-11	3	2	Dry	Right	Mod Std	A-1 G-2
M1-12	3	4	Dry	Right	Mod Std	A-3, G-4
M1-13	3	4	Dry	Center	Standard	A-3
M1-14	3	4	Dry	Center	Standard	A-3, G-4
M1-15	B	3	Dry	Right	Mod Std	1-1, H-3
M1-31	C	7	Dry	Center	A	D-6, I-6
M1-32	D	1	Dry	Left	A	D-7, I-7
M1-33	D	1	Dry	Left	B	0-7, I-7
M1-34	D	2	Dry	Right	B	I-7
M1-35	D	3	Wet	Center	A	4-2, E-2

*See pages 26-28.

Post-Fair

Run No.	Sprayer Line Source Sampled		Location of Sampler Line		Distant Sampler Lines	Near Rotored Lines	Other Fields Operating	Other Spray Fields Contributing to Aerosol Sampled Downwind
	Field	Setting	Side of Sprayer Line	Position (facing sprayer line)				
M2-1	C	4	Wet	Left	E	L,E	1,I	
M2-2	D	1	Wet	Left	E	F	4,I	
M2-3	D	1	Wet	Center	E	F	4,I	
M2-4	D	6	Wet	Center	F	F	4,E,F	
M2-5	D	6	Wet	Left	E	F	4 E,F	
M2-6	B	2	Dry	Right	D	A	2,G	
M2-10	D	2	Dry	Left	C,I,K	H,B	4,I	4
M2-11	D	3	Wet	Right	G,E	F,G	4	
M2-12	D	3	Wet	Right	G,E	F,G		
M2-13	D	7	Dry	Left	G,I,K	H,B	3 E,F	
M2-14	B	7	Dry	Center	B	A	1,H	
M2-15	C	2	Dry	Left	K,H	M,A	1,H,WLF*	
M2-16	C	3	Wet	Center	E	L	1,H,WLF	
M2-17	C	4	Dry	Left	K,H	M	1,I,WLF	I
M2-22	D	4	Wet	Right	G,E	F,L	4,E F,WLF	E,F
M2-23	D	5	Wet	Right	G,E	F,L	4,E,F,WLF	
M2-24	D	5	Dry	Left	K,I	H,B	4,E F,WLF	
M2-25	D	6	Dry	Left	I,G,C	B,H	4,E,F,WLF	
M2-26	D	6	Dry	Left	I,G,C	B,H	4,E,F,WLF	E,F
M2-29	B	7	Dry	Left	H,B,K	M,A	1,H	
M2-30	C	1	Dry	Left	K,H,G	M,A	1,H	
M2-31	C	2	Dry	Right	D	A	1 H	
M2-32	C	3	Dry	Right	D	A	1,H	
M2-33	D	1	Dry	Left	K,I,G,C	H,G	4,I	I
M2-34	D	1	Dry	Right	D	B	4,I	
M2-35	D	4	Dry	Center	C,D	B	4,E,F	E,I
M2-36	D	5	Dry	Left	K,I,G	H,G	4,E,F	E F
M2-37	D	6	Dry	Left	I,C	H,G	4,E,F	
M2-38	D	6	Dry	Left	I,C	H,G	4,E,F	4

*West Lake Field

Aerosol dye concentrations versus downwind distance are plotted in Figure VI.B-1 for each of the seven Pre-Fair dye runs. The mean downwind distance from the wet-line edge used as the abscissa in these plots was obtained by dividing the perpendicular distance in Table VI.B-6 by the sine of the mean wind angle θ.

Some interesting observations can be made by scanning Figure VI.B-1. The relationship of aerosol dye concentration to downwind distance appears to differ substantially over the seven runs. On each of dye Runs D1-6 and D1-9, there is an obvious decrease of dye concentration with increasing distance from the wetline edge. It might be noted that these were the two dye runs located in the center sampler position. The other dye Runs (D1-1, D1-3, D1-4, D1-5 and D1-10) do not exhibit a consistent trend in the dye concentration with increasing downwind sampler distance.

Table VI.B-3.
SAMPLING CONDITIONS FOR DYE AEROSOL RUNS

Pre-Fair

Dye Run No.	Sprayer Line Source Sampled		Location of Sampler Line		Sampler Line Configuration
	Field	Setting	Side of Sprayer Line	Position (facing sprayer line)	
D1-1	B	1	Dry	Left	Standard
D1-3	2	1	Dry	Left	Standard
D1-4	2	4	Dry	Left	Paired
D1-5	2	4	Dry	Left	Paired
D1-6	2	5	Dry	Center	Mod Std
D1-9	C	7	Wet	Center	A
D1-10	D	2	Dry	Left	Mod Std

Post-Fair

Run No.	Sprayer Line Source Sampled		Location of Sampler Line		Sampler Line Configuration
	Field	Setting	Side of Sprayer Line	Position (facing sprayer line)	
D2-1	D	3	Wet	Center	Dye (50, 75, 100)
D2-2	D	6	Wet	Center	Dye (50, 75, 100)
D2-4	D	7	Wet	Center	Dye (50, 75, 100)
D2-5	D	7	Wet	Center	Dye (50, 75, 100)
D2-6	B	2	Wet	Center	Dye (50, 75, 100)
D2-7	B	3	Wet	Center	Dye (50, 75, 100)
D2-8	B	3	Wet	Center	Dye (50, 75, 100)
D2-9	B	3	Wet	Center	Dye (50, 75, 100)
D2-10	B	3	Wet	Center	Dye (50, 75, 100)
D2-11	B	3	Wet	Center	Dye (50, 75, 100)

The plots for Runs 1, 3, 4 and 9 disclose one or more apparently erratic sampler points within 50 meters downwind of the wet-line edge for which the sampled dye concentration differs appreciably from the distance pattern suggested by the other sampler points. It appears that dye samplers should not be located closer than 50 meters from the dye aerosol source.

b. Microbiological Runs

All of the adjusted and footnoted microbiological concentration data obtained on the 20 Pre-Fair aerosol runs and the 29 Post-Fair aerosol runs are presented in the tables of Appendix F. Each Appendix F table contains all of the analysis results (from every high-volume sample on all the runs for either Pre-Fair or Post-Fair) for a single type of analysis. The tables are formatted according to the downwind sampler distances from the wet-line edge that were used in the aerosol run sampler configuration. A list of footnotes, special data symbols, and microbiological units precedes the tables in Appendix F. When sample contamination affecting the microorganism concentration result was inferred, the analysis result has been underlined. The quality of the footnoted data must be evaluated taking the footnote(s) into account. Special data symbols accompany or replace the usual data value in the indicated circumstances. The units identify the type of assay procedure used to obtain the quantitative results.

A 30-minute composite wastewater sample was taken from a spray head in the sprayer line during each aerosol run. This sample was analyzed for four microorganism groups in Pre-Fair and for all five of the Post-Fair microorganisms. These results are shown in Appendix F, in the third column of the Pre-Fair

Table VI.B-4.
SAMPLING CONDITIONS FOR QUALITY ASSURANCE AEROSOL RUNS

Run No.	Sprayer Line Source Sampled		Location of Sampler Line		Distance from Wet Line Edge, m
	Field	Setting	Side of Sprayer Line	Position (facing sprayer line)	
PRE-FAIR					
M1-16-19	C	1	Dry	Center	25
M1-20-23	C	2	Dry	Center	30
M1-24-25	C	3	Dry	Center	25
M1-26	C	4	Dry	Center	20
M1-27-30	C	7	Dry	Left	40
POST-FAIR					
M2-8-9	C	2	Dry	Center	50
M2-18-21	D	1	Dry	Right	50
M2-27-28	B	1	Dry	Center	50

Table VI.B-5.
SAMPLING CONDITIONS FOR VIRUS AEROSOL RUNS
POST-FAIR

Run No.	Sprayer Line Source Sampled		Location of Sampler Line		Distance from Wet Line Edge, m
	Field	Setting	Side of Sprayer Line	Position (facing sprayer line)	
V2-I.3-6	D	5	Dry	Left	50
V2-II.1-6	B	5	Dry	Left	50

Table VI.B-6.
PRE-FAIR DYE AEROSOL RUN CONCENTRATION DATA

Dye Run No.	Wastewater Data	Aerosol Sampler Data	
	Source Dye Concentration (μg/l)	Perpendicular Distance from Wet Line Edge (m)	Aerosol Dye Concentration ($\mu g/m^3$)
D1-1	Mean: 91,700	5	1.77
	Before: 93,000	10	4.06
	After: 90,333	15	3.18
		20	15.89
		50	3.18
		100	1.94
D1-3	Mean: 85,000	5	1.29
	Before: 86,000	10	1.82
	After: 84,000	15	2.61
		20	1.71
		20	1.94
		50	1.65
		100	1.77
		100	1.71
D1-4	Mean: 96,500	10	2.88
		10	2.35
	Before: 97,000		
	After: 96,000	20	2.47
		20	2.00
		30	1.35
		30	0.88
		40	2.77
		40	2.82
D1-5	Mean: 72,000	15	2.94
		15	3.06
	Before: 76,000		
	After: 68,000	25	3.35
		25	3.06
		35	3.71
		35	3.77
		45	3.24

Table VI.B-6. (cont'd)

Dye Run No.	Wastewater Data: Source Dye Concentration (µg/l)	Aerosol Sampler Data: Perpendicular Distance from Wet Line Edge (m)	Aerosol Sampler Data: Aerosol Dye Concentration (µg/m^3)
D1-6	Mean: 96,000	10	9.89
	Before: 100,000	15	9.77
	After: 92,000		
		20	7.47
		20	9.12
		30	8.06
		50	4.35
		100	2.77
		100	5.28
D1-9	Mean: 17,100	10	2.29
		10	1.82
	Before: 18,000		
	After: 16,200	20	1.00
		30	1.71
		40	1.12
		40	1.06
		50	0.82
		50	1.00
D1-10	Mean: 18,800	10	0.13
	Before: 20,600	20	0.12
	After 17,000	20	0.13
		30	0.18
		40	0.18
		50	0.23
		100	0.18
		100	0.18

Table VI.B-7.
POST-FAIR DYE AEROSOL RUN CONCENTRATION DATA

Dye Run No.	Wastewater Data: Source Dye Concentration (µg/l)	Aerosol Sampler Data: Perpendicular Distance from Wet Line Edge (m)	Aerosol Sampler Data: Aerosol Dye Concentration ($\mu g/m^3$)
D2-1	Mean: 21,000	50	0.53
		50	0.59
	Before: 20,500		
	After: 21,500	50	0.53
		50	0.47
		75	0.29
		75	0.26
		100	0.14
		100	0.12
D2-2	Mean: 21,300	50	0.67
		50	0.69
	Before: 21,900		
	After: 20,600	50	0.62
		50	0.58
		75	0.41
		75	0.47
		100	0.34
		100	0.39
D2-4	Mean: 27,400	50	0.56
		50	0.54
	Before: 27,200		
	After: 27,600	50	0.46
		50	0.48
		75	0.29
		75	0.29
		100	0.19
		100	0.20
D2-5	Mean: 26,100	50	0.62
		50	0.52
	Before: 26,800		
	After: 25,400	50	0.54
		50	0.42
		75	0.29
		75	0.25
		100	0.18
		100	0.16

Table VI.B-7. (cont'd)

Dye Run No.	Wastewater Data: Source Dye Concentration (µg/l)	Aerosol Sampler Data: Perpendicular Distance from Wet Line Edge (m)	Aerosol Sampler Data: Aerosol Dye Concentration (µg/m^3)
D2-6	Mean: 22,100	50	0.49
		50	0.65
	Before: 21,200		
	After: 23,000	50	0.38
		50	0.45
		75	0.16
		75	0.12
		100	0.07
		100	0.15
D2-7	Mean: 25,600	50	0.62
		50	0.35
	Before: 18,600		
	After: 32,500	50	0.29
		50	0.48
		75	0.26
		75	0.28
		100	0.19
		100	0.16
D2-8	Mean: 31,000	50	0.65
		50	0.34
	Before: 29,500		
	After: 32,500	50	0.51
		50	0.53
		75	0.39
		75	0.24
		100	0.26
		100	0.13
D2-9	Mean: 25,500	50	0.33
		50	0.32
	Before: 23,900		
	After: 27,000	50	0.36
		50	0.47
		75	0.17
		75	0.19
		100	0.12
		100	0.12

Table VI.B-7. (cont'd)

Dye Run No.	Wastewater Data: Source Dye Concentration ($\mu g/l$)	Aerosol Sampler Data: Perpendicular Distance from Wet Line Edge (m)	Aerosol Sampler Data: Aerosol Dye Concentration ($\mu g/m^3$)
D2-10	Mean: 28,700	50	0.12
		50	0.14
	Before: 28,800		
	After: 28,500	50	0.22
		50	0.15
		75	0.18
		75	0.13
		100	0.09
		100	0.08
D2-11	Mean: 27,400	50	0.19
		50	0.17
	Before: 27,500		
	After: 27,300	50	0.18
		50	0.25
		75	0.14
		75	0.15
		100	0.20
		100	0.17

Figure VI.B-1.
PLOTS OF AEROSOL DYE CONCENTRATION WITH DOWNWIND DISTANCE

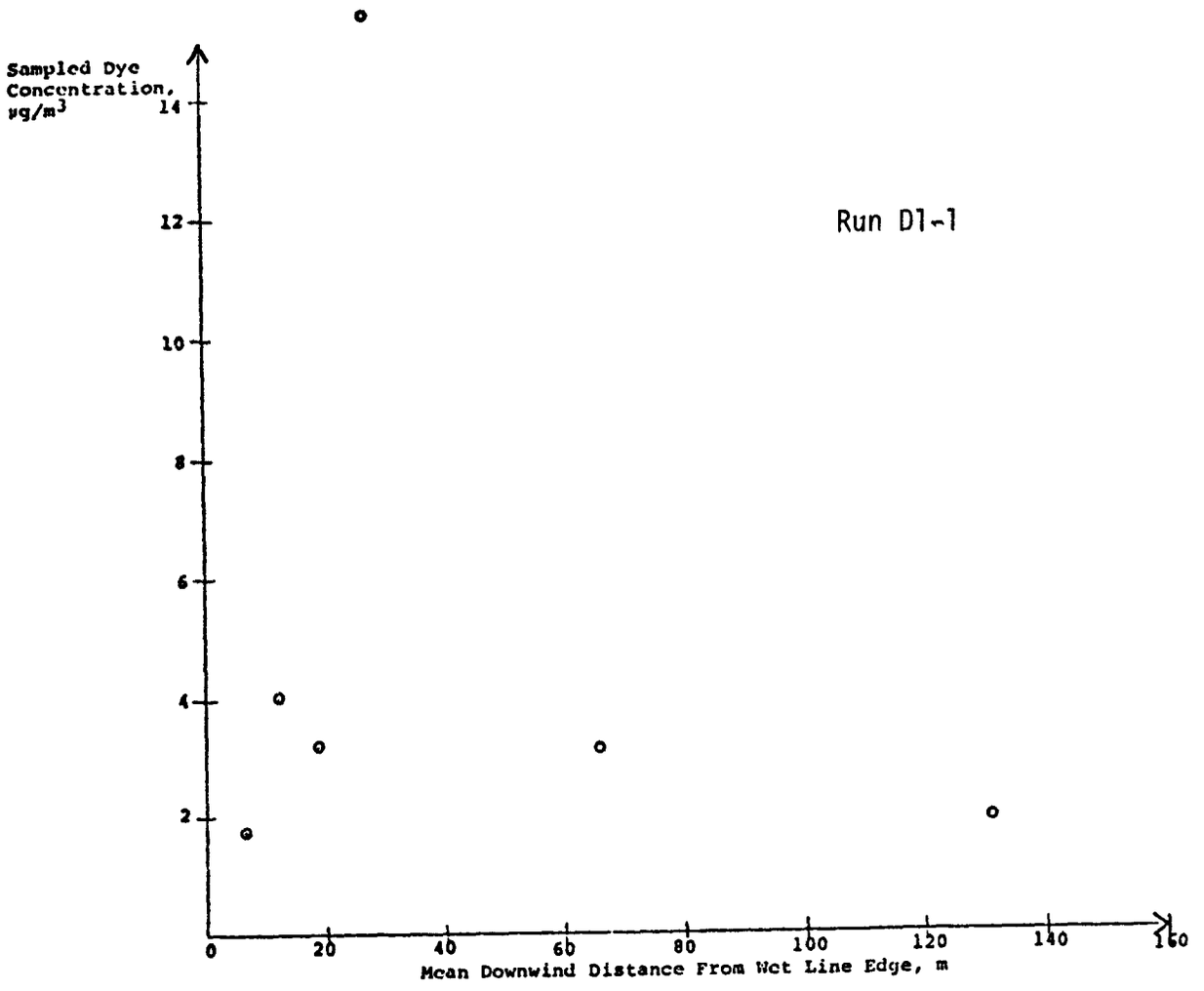
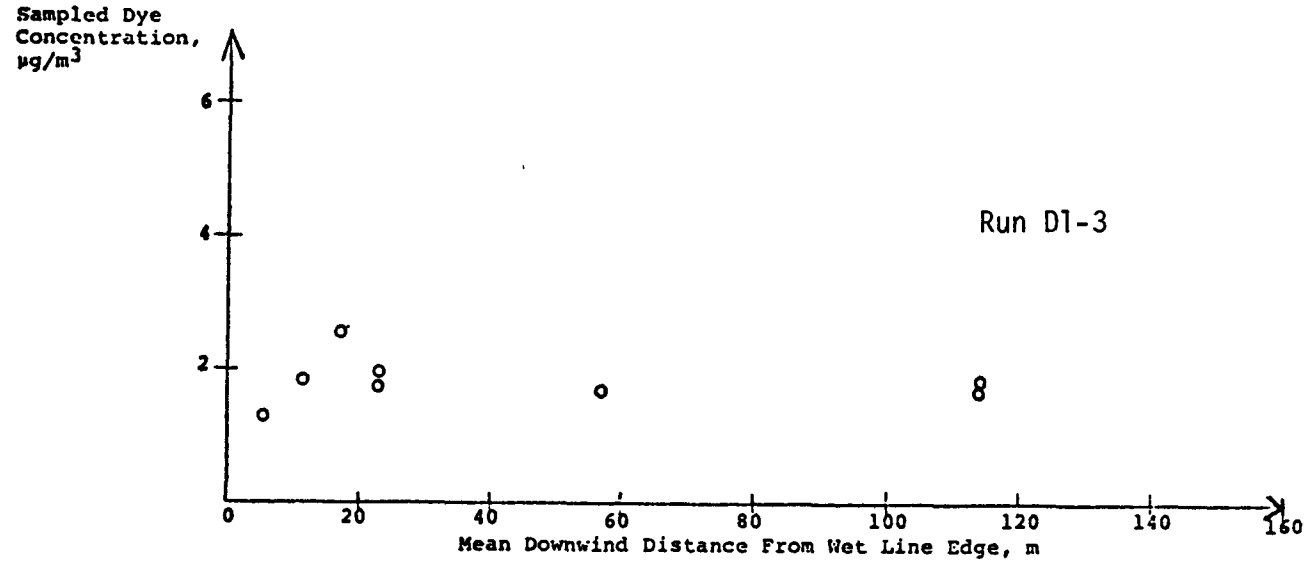


Figure VI.B-1. (continued)



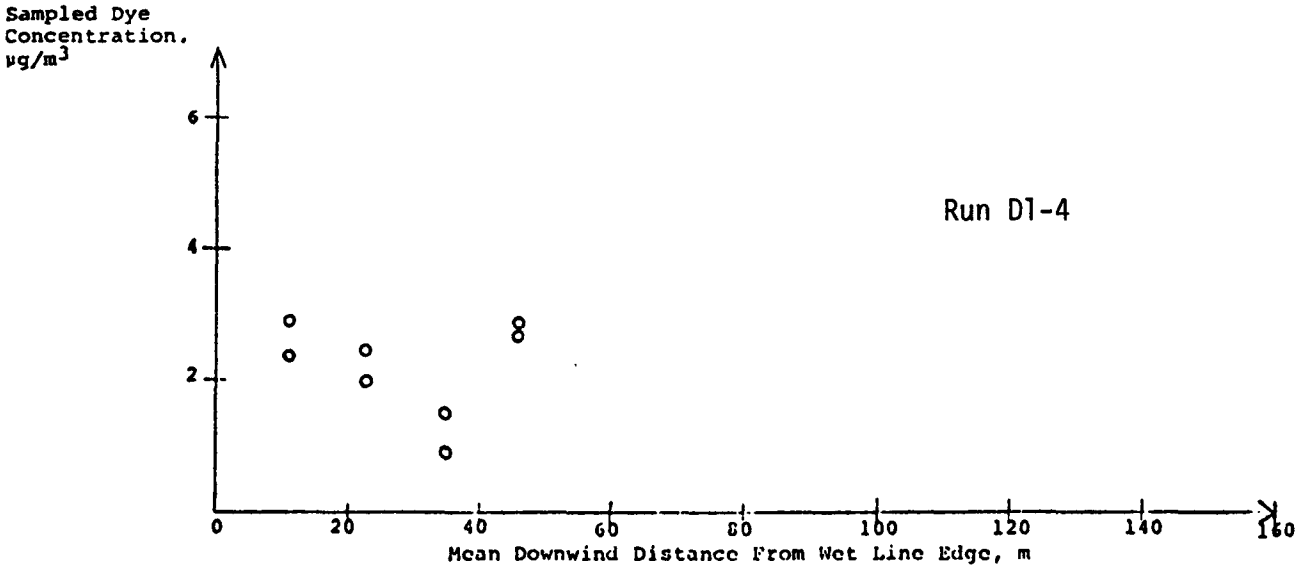
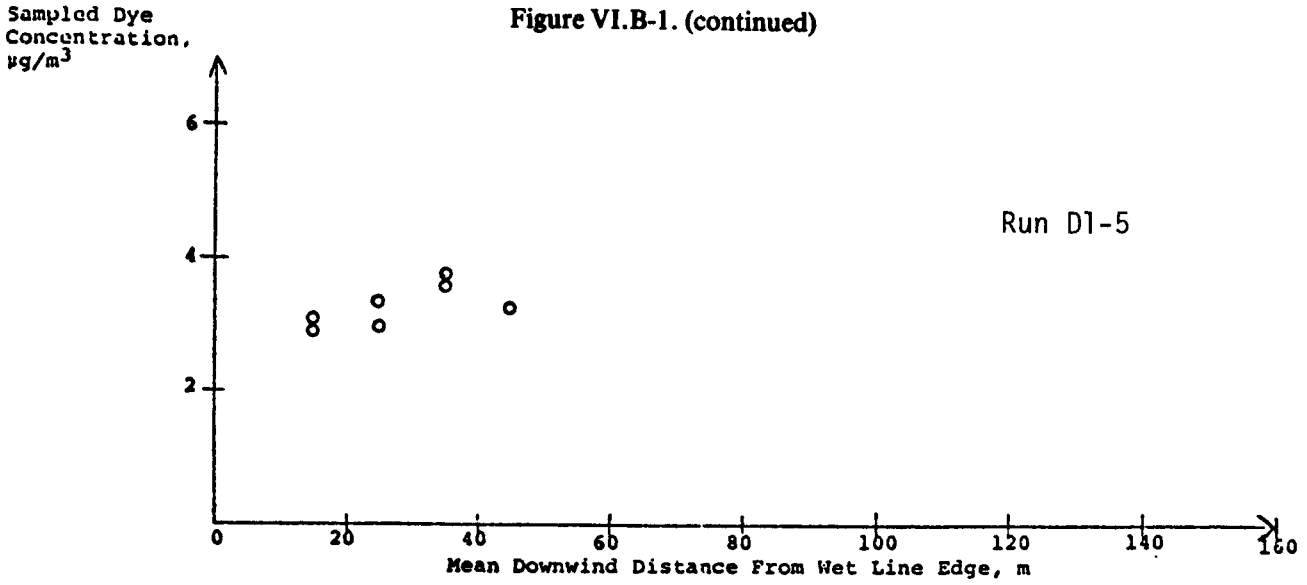


Figure VI.B-1. (continued)



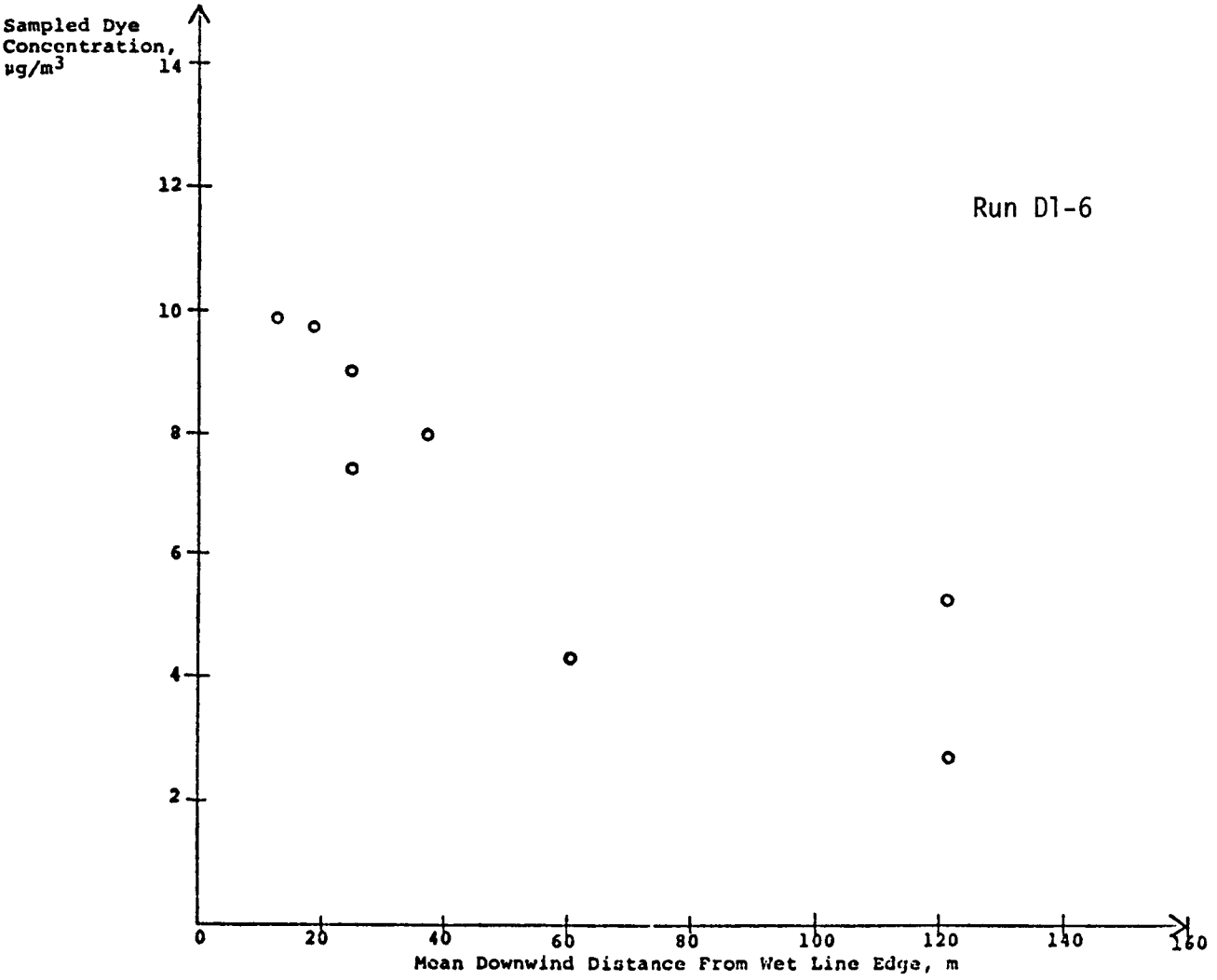
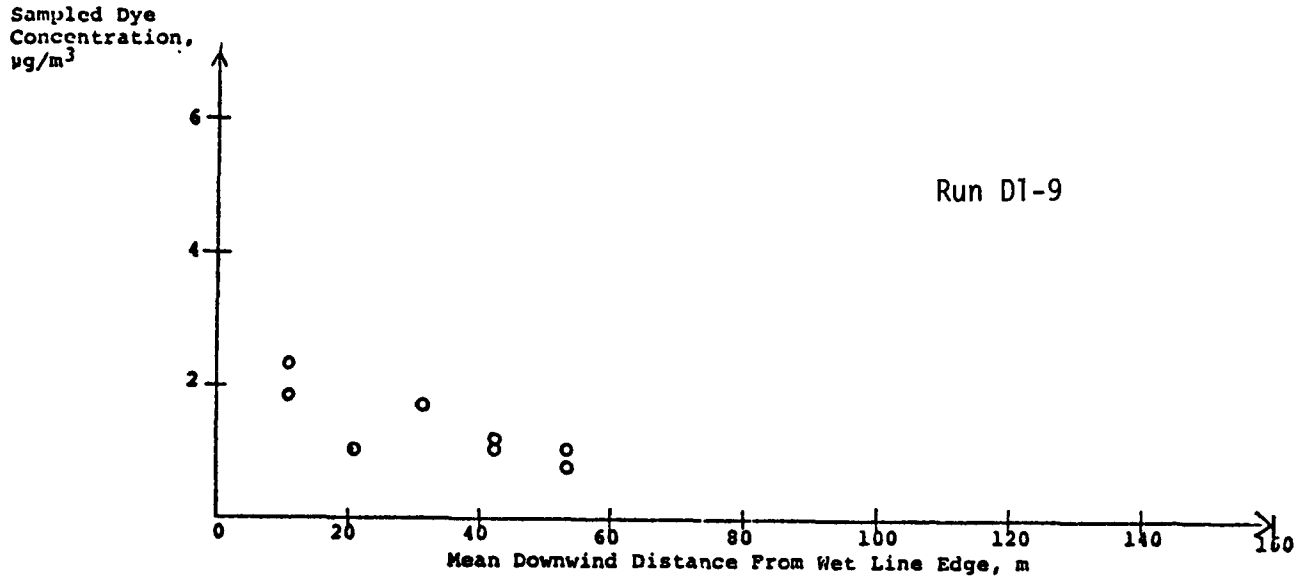
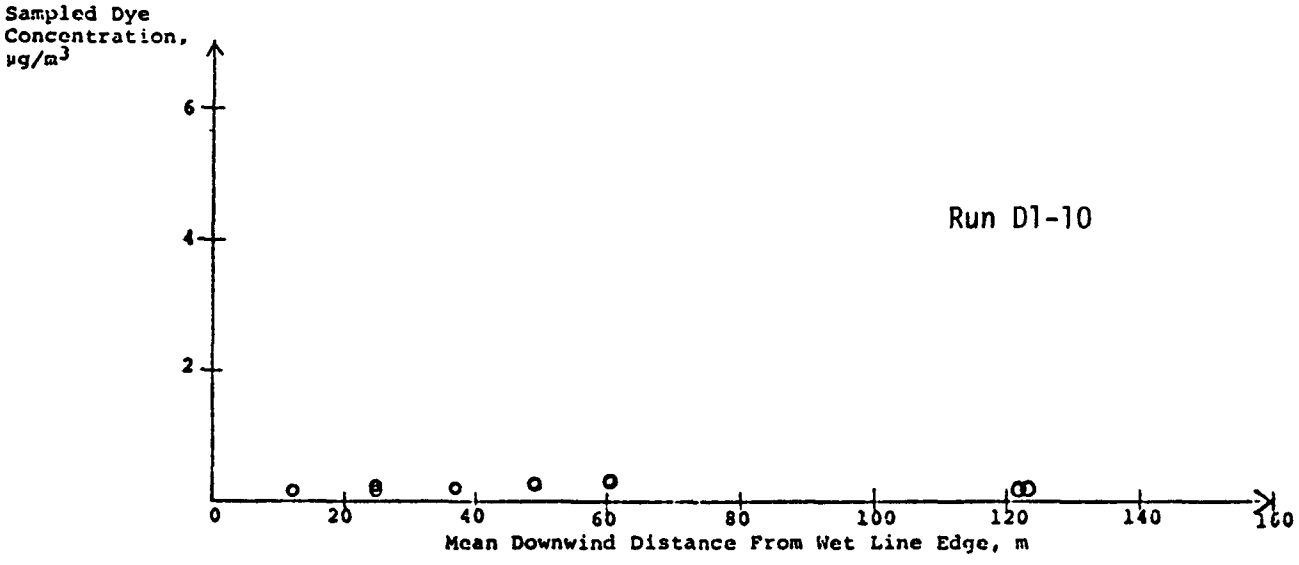


Figure VI.B-1. (continued)





tables for total coliform, fecal coliform, standard bacterial plate count, and coliphage, and on all of the Post-Fair tables.

The upwind sampler was placed at an appropriate upwind sampler location based on wind direction before the run. For Pre-Fair, there were three preselected locations in elevated wooded areas; the distance of the upwind sampler from the configured downwind samplers ranged from 1400 meters to greater than 3200 meters, with a median upwind distance of 1800 meters. During Post-Fair, there were five upwind sampler stations whose elevation, terrain and land usage were similar to the downwind sampler positions. The upwind sampler was also located closer to the configured downwind samplers and occasionally was located to one side of the samplers depending on the wind direction.

Within 100 meters of the wet-line edge, the aerosol concentrations of standard bacterial plate count, total coliform, fecal coliform, coliphage, fecal streptococci, *Pseudomonas,* and *Clostridium perfringens* were generally quantifiable above the minimum detection limit. Because the Post-Fair wastewater concentrations of mycobacteria were lower than anticipated, mycobacteria were seldom found in the aerosol samples on Runs M2-1 to M2-26 above the detection limit. However, in Runs M2-29 to M2-37, when the detection limit was lowered by a factor of 30, mycobacteria were found in almost every sample. Hence, the aerosol concentrations of these eight microorganism groups warrant statistical analysis.

Fourteen biochemical confirmation tests for *Klebsiella* were conducted on every mucoid colony from the aerosol samples on the 20 Pre-Fair microbiological aerosol runs. Through this exhaustive effort, *Klebsiella* was found in only four of the aerosol samples: *Klebsiella ozaenae* on Run M1-8 at 5 meters or Run M1-10 at 50 meters and on Run M1-14 at 50 meters; and *Klebsiella pneumoniae* on Run 12 at 20 meters. After confirmation testing for viruses, no positive three-day virus plaque counts were obtained from the Pre-Fair aerosol run samples. Only two of these aerosol samples had positive five-day virus plaques: the 10 meter sampler on Run M1-9 and the upwind sampler on Run M1-13. The discovery of *Klebsiella* and viruses in the aerosol is certainly important. However, the *Klebsiella*, three-day virus, and five-day virus aerosol concentrations are not suitable microorganism groups for the statistical analyses.

An examination of the Pre-Fair and Post-Fair aerosol concentration values with distance across a microbiological aerosol run in the quantitative aerosol data tables reveals a definite downwind distance pattern: a reduction in concentration with increasing downwind sampler distance. The microbiological concentration reduction with distance is more pronounced than the dye concentration reduction with distance exhibited in Figure VI.B-1. There clearly are microbiological decay factors in addition to the aerosol formation and dispersion factors. Also, it appears that microorganism die-off accumulates with distance from the source or aerosol age. The microbiological aerosol levels on the nighttime and evening runs were generally higher than on the afternoon runs. This suggests confirmation of the Phase I finding that solar radiation is a very significant microbiological decay factor. This topic will be given a more rigorous statistical treatment in Section VI.C.5.

As discussed in Appendix D, smoothing of the microbiological aerosol run data was necessary to make it amenable for developing the microbiological dispersion model. The smoothing procedures described in Appendix D (designating background and downwind samplers, computing the background concentration B, computing the downwind concentration C_d at sampler distance d, and excluding unusable runs) were applied to the Appendix F data for the eight microorganism groups that warrant statistical analysis. The resulting smoothed data for the microbiological aerosol runs are presented in Tables VI.B-8 through VI.B-15, with each microorganism group appearing on a separate table.

c. Quality Assurance Runs

The purpose of the aerosol quality assurance runs in Pre-Fair and Post-Fair was to determine whether there were systematic differences or biases among the high-volume aerosol samplers used in the aero-

Table VI.B-8.
SMOOTHED STANDARD BACTERIAL PLATE COUNTS BY SAMPLER DISTANCE FROM MICROBIOLOGICAL AEROSOL RUNS

Aerosol Run Number	Run Date	Wastewater Concentration (No./100ml)	Aerosol Concentrations (No./m^3 of air)								Distant Sampler Distance
			Background B	Downwind Concentrations C_d at Sampler Distance d							
				5m	10m	20m	30m	40m	50m	100m	Distant	
M1-1	5-4-76	110 x 10^6	140	9800	1400	830						
M1-2	5-5-76	120 x 10^6	2000	6700	6000	8600			5500	6500		
M1-3	5-5-76	110 x 10^6	680		4900	5800			1500	1300		
M1-5	5-13-76	167 x 10^6	1200	2600	2100	2000						
M1-6	5-17-76	76 x 10^6	380	1700	1600	3600						
M1-7	5-17-76	46 x 10^6	620	2000	2000	1700						
M1-8	5-21-76	70 x 10^6	100		1000	3200			340 360			
M1-9	5-24-76	104 x 10^6	1450	6300	3000				1800			
M1-10	5-24-76	145 x 10^6	1600		4500		2200		2000			
M1-11	5-25-76	35 x 10^6	840		3000		2000		2200	1400		
M1-12	5-27-76	34 x 10^6	150		850	2100			750	580		
M1-13	5-27-76	26 x 10^6	64			2000			790	1400	980	(200m)
M1-15	6-3-76	45 x 10^6	2100			2580						
M1-31	6-14-76	16 x 10^5	220		3500	3000	3800		2000 2700			
M1-32	6-15-76	20 x 10^5	570		1500	1200	3800	6300				
M1-33	6-15-76	95 x 10^5	680			5600 5000	5300	3300				
M1-34	6-16-76	54 x 10^6	1600			1650						
M1-35	6-17-76	76 x 10^6	300		5200 1000	2900	1600	1900	1300 1300			
M2-1	1-21-77	15 x 10^6	310						830	830	430	(220m)
M2-3	1-25-77	113 x 10^6	1560						1820	4500 3800		
M2-4	1-30-77	27 x 10^6	650						1280			
M2-6	2-9-77	7.0x 10^6	85						480 290	92 500		
M2-12	2-24-77	2.1x 10^6	90						95	500		
M2-15	3-16-77	73 x 10^6	180						1400 330	380		
M2-16	3-17-77	105 x 10^6	38						1100 1300	150 83	130	(290m)
M2-17	3-18-77	76 x 10^6	130						950 720	2400	360	(340m)
M2-22	3-25-77	2.2x 10^6	47						60 72	56		
M2-23	3-26-77	20 x 10^6	39						66	97 110		
M2-24	3-26-77	41 x 10^6	48						190 190	60		
M2-25	3-27-77	26 x 10^6	53						310 270	98 150		
M2-29	4-11-77	44 x 10^6	370						1300 1900	1700 3200		
M2-31	4-13-77	67 x 10^6	365						1000	500 530		
M2-32	4-14-77	75 x 10^6	380						520	520		
M2-34	4-19-77	15 x 10^6	930						4800 2300	1900		
M2-35	4-22-77	6.9x 10^6	145						170	500 430		
M2-38	4-24-77	15 x 10^6	210						2100	870 1200	300	(435m)

Table VI.B-9.
SMOOTHED TOTAL COLIFORM CONCENTRATIONS BY SAMPLER DISTANCE FROM MICROBIOLOGICAL AEROSOL RUNS

Aerosol Run Number	Run Date	Wastewater Concentration (MFC/100ml)	Aerosol Concentrations (MFC/m³ of air)								Distant Sampler Distance
			Background	Downwind Concentrations C_d at Sampler Distance d							
			B	5m	10m	20m	30m	40m	50m	100m	Distant
M1-2	5-5-76	2070 x 10^3	0.15	9.2	10.5	17.2 20.0			8.3	5.1	
M1-3	5-5-76	2140 x 10^3	0.15	12.7	8.1	10.9 12.6			3.0	0.5	
M1-4	5-13-76	690 x 10^3	0.07	1.7	2.1	0.2					
M1-5	5-13-76	720 x 10^3	0.07	5.8	1.4	0.2					
M1-6	5-17-76	930 x 10^3	0.1	6.5	4.6	7.7				0.6	
M1-7	5-17-76	700 x 10^3	0.1	4.3	1.2	5.2				0.5	
M1-8	5-21-76	950 x 10^3	0.1	10.8	15.5	1.1			0.45		
M1-9	5-24-76	1040 x 10^3	0.15	18.0	38				5.2	4.4	
M1-10	5-24-76	1280 x 10^3	0.5		57	93	46		32	4.9	
M1-11	5-25-76	1100 x 10^3	0.1		43	46	53		8.3	0.15	
M1-12	5-27-76	470 x 10^3	0.1		2.0	6.5			1.4	0.15	
M1-13	5-27-76	690 x 10^3	0.15			11.7			6.2	4.3	2.6 (200m)
M1-14	5-27-76	750 x 10^3	0.1			47			14.5	4.1	2.2 (200m)
M1-15	6-3-76	588 x 10^3	0.1		3.2	2.2			2.1	0.9 2.0	
M1-31	6-14-76	970 x 10^3	0.05		13.2	4.7	2.6		0.4 1.5		
M1-32	6-15-76	265 x 10^3	0.03		0.53 0.67		0.45				
M1-33	6-15-76	170 x 10^3	0.08			0.75		1.3			
M1-34	6-16-76	550 x 10^3	0.08			1.7 2.3 3.8	0.8				
M1-35	6-17-76	350 x 10^3	0.1		19.3 30.5	12.0	6.6	5.1	2.0 2.6		
M2-1	1-21-77	60 x 10^3	0.025						6.7 7.5	0.4	
M2-2	1-25-77	280 x 10^3	0.02						2.0 1.5	0.4 1.6	
M2-3	1-25-77	450 x 10^3	0.025						37 31	15.2 10.5	0.3 (360m)
M2-4	1-30-77	260 x 10^3	0.01						6.5 5.8	2.0 1.3	
M2-5	1-30-77	340 x 10^3	0.02						4.8 5.5	2.6 3.1	
M2-6	2-9-77	220 x 10^3	0.05						2.1 1.9	0.2 0.2	
M2-10	2-23-77	410 x 10^3	0.1						1.3 5.5	4.7 5.3	
M2-11	2-24-77	340 x 10^3	0.02						3.1 2.2	0.9 1.3	
M2-12	2-24-77	360 x 10^3	0.02						0.3 2.9	1.3 0.2	
M2-13	2-28-77	390 x 10^3	0.05						1.7 2.7	2.8 3.3	
M2-14	3-14-77	420 x 10^3	0.01						13.0 10.8	1.1 1.3	
M2-15	3-16-77	690 x 10^3	0.01						5.3 4.5	1.6 1.1	
M2-16	3-17-77	1100 x 10^3	0.02						9.7 12.2	4.3 1.8	0.2 (290m)
M2-17	3-18-77	1200 x 10^3	0.1						5.7 7.7	5.7 2.4	0.2 (340m)
M2-22	3-25-77	860 x 10^3	0.02						2.0 2.0	0.4 0.2	
M2-24	3-26-77	740 x 10^3	0.05						1.8 0.6	0.1	
M2-25	3-27-77	730 x 10^3	0.01						2.2 3.8	0.35	
M2-26	3-27-77	670 x 10^3	0.017						0.3 0.4	0.025	
M2-29	4-11-77	1600 x 10^3	0.01						0.1		
M2-30	4-12-77	640 x 10^3	0.01						1.2 0.7	2.0 0.6	
M2-31	4-13-77	450 x 10^3	0.05						7.8 7.5	4.8	0.2 0.2 0.2 (435/505/580m)
M2-32	4-14-77	1200 x 10^3	0.01						2.0 2.3	1.5 1.5	
M2-33	4-19-77	390 x 10^3	0.02						14.0 13.5	0.2	
M2-34	4-19-77	540 x 10^3	0.45						6.7		
M2-35	4-22-77	360 x 10^3	0.025						2.0	2.0 1.7	0.1 (655m)
M2-36	4-23-77	410 x 10^3	0.02						1.3	0.1	
M2-37	4-24-77	450 x 10^3	0.01						1.7 2.5	0.25	
M2-38	4-24-77	410 x 10^3	0.01						1.1 0.7	0.025	

Table. VI.B-10.
SMOOTHED FECAL COLIFORM CONCENTRATIONS BY SAMPLER DISTANCE FROM MICROBIOLOGICAL AEROSOL RUNS

Aerosol Run Number	Run Date	Wastewater Concentration (MFC/100ml)	Aerosol Concentrations (MFC/m^3 of air)							
			Background	Downwind Concentrations C_d at Sampler Distance d						
			B	5m	10m	20m	30m	40m	50m	100m
M1-2	5-5-76	186 x 10^3	0.02	2.0	2.1	4.4 2.1			1.2	0.2
M1-3	5-5-76	174 x 10^3	0.05	0.9	1.7	3.0 1.0			0.075	
M1-5	5-13-76	180 x 10^3	0.02	1.1	0.7	0.1				
M1-6	5-17-76	75 x 10^3	0.075	1.1	0.3	1.4				
M1-7	5-17-76	110 x 10^3	0.075	0.6	0.75					0.25
M1-8	5-21-76	80 x 10^3	0.02	0.8						
M1-9	5-24-76	81 x 10^3	0.075	3.1	2.3	2.0			1.2	0.2
M1-10	5-24-76	125 x 10^3	0.3		9.5	12.2	3.8		5.6	0.5
M1-11	5-25-76	124 x 10^3	0.06		6.6	6.2	1.3		0.5	
M1-15	6-3-76	57 x 10^3	0.02		1.8	1.9	0.2		0.5	0.1
M1-31	6-14-76	137 x 10^3	0.05		2.9	0.6	0.6		0.2 0.2	
M1-32	6-15-76	24 x 10^3	0.02		0.15	0.33				
M1-34	6-16-76	81 x 10^3	0.075			0.4 1.0 0.6	0.25		0.7	
M1-35	6-17-76	45 x 10^3	0.05		4.9 4.4	9.9	1.2	0.6	0.4 0.5	

Table VI.B-11.
SMOOTHED COLIPHAGE CONCENTRATIONS BY SAMPLER DISTANCE FROM MICROBIOLOGICAL AEROSOL RUNS

Aerosol Run Number	Run Date	Wastewater Concentration (PFU/l)	Aerosol Concentrations (PFU/m³ of air)									Distant Sampler Distance
			Background B	Downwind Concentrations C_d at Sampler Distance d								
				5m	10m	20m	30m	40m	50m	100m	Distant	
M1-3	5-5-76	95 x 10^3	0.0		0.15	0.2						
M1-5	5-13-76	170 x 10^3	0.0		0.05					0.1		
M1-6	5-17-76	220 x 10^3	0.0	1.0	0.6	0.8				0.05		
M1-7	5-17-76	230 x 10^3	0.0	0.1		0.3			0.025	0.1		
M1-8	5-21-76	61 x 10^3	0.0	0.7	0.4	0.1 0.3			0.05			
M1-9	5-24-76	95 x 10^3	0.0	1.1	0.1	1.2			0.8	0.3		
M1-10	5-24-76	130 x 10^3	0.0		2.0	3.7 1.1	1.4		2.6	0.2		
M1-11	5-25-76	140 x 10^3	0.0		2.3	0.8 1.4	1.1		0.6	0.4		
M1-12	5-27-76	110 x 10^3	0.0		0.9	0.7 0.3			0.2	0.1		
M1-13	5-27-76	170 x 10^3	0.0			1.1			1.0	0.7	0.2	(200m)
M1-14	5-27-76	210 x 10^3	0.0			3.3			0.8	0.6	0.2	(200m)
M1-15	6-3-76	310 x 10^3	0.0		0.1	0.7	0.3		0.3	0.2 0.1		
M1-31	6-14-76	330 x 10^3	0.0		0.7	0.6	0.7		0.013			
M1-32	6-15-76	320 x 10^3	0.0		0.025 0.2	0.1		0.013				
M1-33	6-15-76	240 x 10^3	0.0			0.1 0.4	0.2	0.025				
M1-35	6-17-76	170 x 10^3	0.0		1.8	0.8	1.1	0.5	0.2 0.6			
M2-1	1-21-77	180 x 10^3	0.0						0.4 0.3	0.5 0.3		
M2-2	1-25-77	94 x 10^3	0.075						0.7 0.4	0.2		
M2-3	1-25-77	160 x 10^3	0.1						0.8 0.4	0.2		
M2-4	1-30-77	120 x 10^3	0.025						0.8 0.7	0.15		
M2-5	1-30-77	170 x 10^3	0.0						0.4 0.1	0.3		
M2-10	2-23-77	300 x 10^3	0.0						0.1 1.1	0.9 1.2		
M2-11	2-24-77	390 x 10^3	0.0						0.4 0.7	0.3 0.1		
M2-12	2-24-77	230 x 10^3	0.0						0.3 1.1	0.4 0.1	0.3	(220m)
M2-13	2-28-77	310 x 10^3	0.0						1.1 1.3	1.2 0.9		
M2-14	3-14-77	190 x 10^3	0.025						2.8 2.1	0.8 0.5		
M2-15	3-16-77	120 x 10^3	0.075						0.3 0.4	0.1 0.3		
M2-16	3-17-77	140 x 10^3	0.0						0.9 0.7	0.55		
M2-17	3-18-77	190 x 10^3	0.0						0.5 0.5	0.1 0.4	0.1	(340m)
M2-22	3-25-77	170 x 10^3	0.0						1.1 0.4	0.05		
M2-23	3-26-77	380 x 10^3	0.025						0.15	0.05		
M2-24	3-26-77	700 x 10^3	0.0						1.6 2.1	0.2 0.7		
M2-25	3-27-77	1200 x 10^3	0.0						2.5 2.2	0.7 0.3		
M2-26	3-27-77	930 x 10^3	0.033						0.15			
M2-29	4-11-77	400 x 10^3	0.0						0.7 0.4	0.05		
M2-30	4-12-77	180 x 10^3	0.0						0.1 0.3	0.15		
M2-31	4-13-77	240 x 10^3	0.0						0.8 0.8	0.8 0.1		
M2-32	4-14-77	240 x 10^3	0.0						0.8 0.4	0.013		
M2-33	4-19-77	450 x 10^3	0.0						0.3 0.025	0.4		
M2-34	4-19-77	200 x 10^3	0.0						0.1 0.2	0.3 0.2		
M2-35	4-22-77	1200 x 10^3	0.0						2.6 2.8	1.1 1.1		
M2-36	4-23-77	780 x 10^3	0.0						0.4 0.1	0.3 0.1		
M2-37	4-24-77	480 x 10^3	0.0						0.4 0.4	0.1 0.4		
M2-38	4-24-77	430 x 10^3	0.0						0.4 0.4	0.3 0.7		

Table VI.B-12.
SMOOTHED FECAL STREPTOCOCCI CONCENTRATIONS BY SAMPLER DISTANCE FROM MICROBIOLOGICAL AEROSOL RUNS

Aerosol Run Number	Run Date	Wastewater Concentration (CFU/100ml)	Aerosol Concentrations (CFU/m^3 of air)								Distant Sampler Distance
			Background B	Downwind Concentrations C_d at Sampler Distance d							
				5m	10m	20m	30m	40m	50m	100m	Distant
M1-6	5-17-76	6.5×10^3	0.4	3	0.8	3					
M1-8	5-21-76	20×10^3	0.15						0.25		
M1-9	5-24-76	8.0×10^3	0.15	6.0	1.6	0.7				0.25	
M1-10	5-24-76	8.0×10^3	0.15		4.2	4.0 2.0	2.7		4.7	3.1	
M1-11	5-25-76	2.8×10^3	0.6		3.1	5.7 2.7	2.7		1.5		
M1-12	5-27-76	6.8×10^3	0.1		15	30 25			13	10	
M1-13	5-27-76	6.8×10^3	0.3			1.3 1.7			8.5	0.6	
M1-14	5-27-76	6.8×10^3	0.1			14			90	26	6.8 (200m)
M1-15	6-3-76	2.3×10^3	0.3		2.1	3.0	2.8		1.8	2.3 1.8	
M1-31	6-14-76	17.1×10^3	1.5		3.3	1.9	3.3		3.2 2.1		
M1-33	6-15-76	9.2×10^3	0.1			0.5 0.3	0.7	0.55			
M1-34	6-16-76	5.5×10^3	0.6			0.8 1.8 4.3	5.1	1.2			
M1-35	6-17-76	4.0×10^3	0.3		4.7 4.4	2.7	2.4	3.3	6.5 2.4		
M2-1	1-21-77	9.7×10^3	0.4						1.0 1.0	1.8 0.45	
M2-2	1-25-77	4.8×10^3	0.3						1.4 0.4	0.9 0.7	0.4 (300m)
M2-3	1-25-77	1.3×10^3	2.0						3.3 7.7	8.3 7.3	3.7 (360m)
M2-4	1-30-77	6.0×10^3	0.2						1.0 0.7	0.25	
M2-5	1-30-77	12.0×10^3	0.3						1.3 1.3	1.0 1.1	
M2-6	2-9-77	3.3×10^3	0.1						0.4 0.4	0.2 0.6	
M2-12	2-24-77	1.2×10^3	0.01						0.025	1.1 0.4	
M2-13	2-28-77	5.7×10^3	0.1						0.4 2.1	0.8 0.6	
M2-14	3-14-77	2.0×10^3	0.03						0.3	0.4 0.7	0.1 (490m)
M2-15	3-16-77	7.0×10^3	0.01						0.1 0.2	0.6 0.1	0.1 (280m)
M2-16	3-17-77	12.0×10^3	0.01						0.8 0.8	0.2 0.4	0.1 (290m)
M2-17	3-18-77	7.2×10^3	0.01						0.6 0.3	0.8 0.4	
M2-22	3-25-77	4.3×10^3	0.03						0.2		
M2-24	3-26-77	10.0×10^3	0.03						0.2 0.4	0.3 0.6	0.1 (420m)
M2-25	3-27-77	11.0×10^3	0.025						0.7 0.9	0.1	
M2-29	4-11-77	4.3×10^3	0.025							0.175	
M2-31	4-13-77	6.2×10^3	0.77						0.85	3.7 1.8	
M2-32	4-14-77	3.0×10^3	0.16						0.35		
M2-33	4-19-77	2.2×10^3	0.01						0.05	0.1	
M2-34	4-19-77	1.9×10^3	0.3						0.4		
M2-35	4-22-77	0.63×10^3	0.01						0.2	0.1 0.3	
M2-36	4-23-77	3.0×10^3	0.17						0.3 0.3		
M2-37	4-24-77	1.6×10^3	0.01						0.6 0.6	0.2 0.025	0.2 (425m)
M2-38	4-24-77	0.93×10^3	0.1						4.9 5.1	1.6 7.0	0.2 (435m)

Table VI.B-13.
SMOOTHED *PSEUDOMONAS* CONCENTRATIONS BY SAMPLER DISTANCE FROM MICROBIOLOGICAL AEROSOL RUNS

Aerosol Run Number	Run Date	Wastewater Concentration (CFU/100ml)	Aerosol Concentrations (CFU/m^3 of air)							
			Background	Downwind Concentrations C_d at Sampler Distance d						
			B	5m	10m	20m	30m	40m	50m	100m
M1-4	5-13-76	80 x 10^3	4	190	13	33				51
M1-5	5-13-76	80 x 10^3	2	140	1300	330				
M1-6	5-17-76	30 x 10^3	2	430	750	4			6	77
M1-7	5-17-76	30 x 10^3	4			380				77
M1-8	5-21-76	210 x 10^3	15	240	290	235				
M1-9	5-24-76	35 x 10^3	4	140	26				37	
M1-10	5-24-76	35 x 10^3	4		570		150		220	210
M1-11	5-25-76	120 x 10^3	2			83	68		100	30
M1-13	5-27-76	30 x 10^3	4			165			230	15
M1-15	6-3-76	200 x 10^3	2				17		8	9
M1-31	6-14-76	30 x 10^3	2			9	8		9 9	
M1-32	6-15-76	25 x 10^3	1	4						
M1-33	6-15-76	25 x 10^3	1			4.5				
M1-34	6-16-76	30 x 10^3	15			91 76	180	100		
M1-35	6-17-76	20 x 10^3	3.5		250	210	100	260	89 61	

Table VI.B-14.
SMOOTHED *CLOSTRIDIUM PERFRINGENS* CONCENTRATIONS BY SAMPLER DISTANCE FROM MICROBIOLOGICAL AEROSOL RUNS

Aerosol Run Number	Run Date	Wastewater Concentration (MPN/100ml)	Aerosol Concentrations (MPN/m³ of air)								Distant Sampler Distance
			Background	Downwind Concentrations C_d at Sampler Distance d							
			B	5m	10m	20m	30m	40m	50m	100m	Distant	
M1-5	5-13-76	11,000	0.06	5.1	6.0	3.0				5.9		
M1-6	5-17-76	4,600	0.06	2.4	1.0	1.3				2.3		
M1-7	5-17-76	4,600	0.06	1.2								
M1-8	5-21-76	48,000	0.06	0.8		3.3 2.0			1.2 0.8			
M1-9	5-24-76	24,000	0.06	1.9	1.8	1.3			0.23			
M1-10	5-24-76	24,000	0.06		2.3	3.0 1.3	1.9		1.05			
M1-11	5-25-76	4,600	0.06		1.0	0.95			0.5	4.4		
M1-12	5-27-76	4,300	0.06		2.3	3.3 5.0			1.0	0.23		
M1-13	5-27-76	4,300	0.06			1.3 2.3			1.0	0.23	1.8	(200m)
M1-14	5-27-76	4,300	0.06			1.3			2.3	0.45		
M1-15	6-3-76	4,300	0.06		0.9	1.3	1.1		0.33			
M1-31	6-14-76	9,300	0.9		2.7	1.9			1.2 1.2			

Table VI.B-15.
SMOOTHED MYOBACTERIA CONCENTRATIONS BY SAMPLER DISTANCE FROM MICROBIOLOGICAL AEROSOL RUNS

Aerosol Run Number	Run Date	Wastewater Concentration (CFU/100ml)	Aerosol Concentrations (CFU/m³ of air)							
			Background	Downwind Concentrations C_d at Sampler Distance d						
			B	5m	10m	20m	30m	40m	50m	100m
M2-15	3-16-77	11.0×10^3	0.2						11 11	
M2-29	4-11-77	1.0×10^3	0.5						1.15	0.65
M2-30	4-12-77	10.0×10^3	0.1						0.15	0.3 0.7
M2-31	4-13-77	5.0×10^3	0.4						0.5	1.7 1.0
M2-32	4-14-77	7.0×10^3	0.2						0.3 2.3	2.0 1.0
M2-33	4-19-77	7.5×10^3	0.1						2.3 1.0	
M2-34	4-19-77	7.6×10^3	0.15						0.4	1.7 1.0
M2-35	4-22-77	1.0×10^3	0.4						4.3 2.7	1.0 0.7
M2-36	4-23-77	6.0×10^3	0.05						1.7 0.7	1.0 1.0
M2-37	4-24-77	7.3×10^3	0.35						1.0 1.3	1.3
M2-38	4-24-77	5.0×10^3	0.2						11 11	

sol sampling runs and to quantify the sources of variation associated with sampling the microorganism group concentrations.

Tables VI.B-16 through VI.B-23 show the concentration data gathered for Pre-Fair and Post-Fair. The three-day and five-day virus counts were below the detection limit for all samples and the *Clostridium perfringens* levels were also undetected; tables for these groups are not included. Analysis for fecal coliform, *Klebsiella*, and *Pseudomonas* was performed only in Pre-Fair, and the results are shown in Tables VI.B-18, VI.B-21 and VI.B-25. Table VI.B-22 shows the results from the analysis of mycobacteria which was performed only in Post-Fair. Analyses for standard bacterial plate count, total coliform, coliphage, and fecal streptococci were performed in Pre-Fair and Post-Fair periods. The results from these analyses are in Tables VI.B-16, VI.B-17, VI.B-19 and VI.B-20. Also, on the Post-Fair Runs M2-8-9 and M2-27-28, the samples were received by the laboratory at a temperature higher than the specified 4°C.

The 100 mL samples from each sampler on the Pre-Fair and Post-Fair quality assurance runs were divided into portions (usually 25 mL) in the field and then given run numbers. Some of these portions were then divided into smaller parts, called aliquots by the lab. The run numbers and portion numbers are shown in the columns labeled as such. After analysis the data were converted to the measured unit per m^3 of air by the method described in Appendix D using the sampling time and the flow rate. The footnotes used in the quality assurance tables are given in Figure VI.B-2.

d. Virus Runs

Two special virus runs were conducted during the Post-Fair sampling period. Table VI.B-24 shows all of the concentration data gathered from these two runs. Besides being analyzed for enteroviruses, the wastewater and aerosol samples were also checked for standard bacterial plate count, total coliform, fecal streptococci, coliphage, and mycobacteria.

The enterovirus concentrations were about three orders of magnitude lower than coliphage, so a large volume of air had to be sampled. This required the use of high-volume aerosol samplers and special sample concentrating procedures. The special enterovirus runs established the ability to detect enteroviruses in wastewater aerosols and provided a quantitative measure of their concentration coming from a known wastewater aerosol source.

The identifications of the confirmed enterovirus isolates obtained from the aerosol samples on the Post-Fair virus aerosol runs are presented in Table VI.B-25. During Pre-Fair, the single confirmed viral isolate from the 10-meter downwind sampler on Run M1-9 and both confirmed isolates from the upwind sampler on Run M1-12 were identified as poliovirus 1. Since poliovirus 1 was used in the assay laboratory to determine the efficiency of the sample concentration procedure, the Pre-Fair poliovirus 1 isolates may be the result of laboratory contamination and thus are not reported in Table VI.B-25. On virus aerosol Run V2-I, the single confirmed three-day viral isolate was identified as poliovirus 2; of the three confirmed five-day enterovirus isolates, two were identified as poliovirus 2. Of the seven confirmed five-day enterovirus isolates from virus Run V2-II, one was identified as poliovirus 1 and another was identified as coxsackievirus B-3. The process of identifying the remaining confirmed five-day enterovirus isolates from Runs V2-I and V2-II was terminated before completion because of laboratory reorganization. The variety of enteroviruses identified from the aerosol samples of the virus aerosol runs increases the likelihood that the sprayed wastewater was the enterovirus source.

Table VI.B-16.
STANDARD BACTERIAL PLATE COUNTS FROM QUALITY ASSURANCE AEROSOL RUNS

Aerosol Run No.	Run Date	Wastewater Sample (No./100 ml)	Sampler Distance		Aerosol Sample Portion No	Aerosol Concentrations from Samplers Aligned from Left to Right (No /m³ of air)											
			From Wet Line Edge	Between Samples													
PRE-FAIR																	
M1-16-19	6-7-76	14×10^6	25 m	1 m													
					16	16500[d]	8000	6000	5000	3600	4000	3600	3300	2700			
					17	7500[d]	2900	1700	2000	2500	2000	1900	1600	1900			
					18	6700[d]		1700		1900		2500					
M1-20-23	6-8-76	37×10^6	30 m	1 m													
					20	2800	1700	1400	2300	1800	2400	1600	2100				
					21	2700	1200	1500	2100	2000	2400	1700	2200				
					22		1100		1900		2300		2000				
					23		1300		1300		2000		1600				
M1-24-25	6-9-76	44×10^6	25 m	1 m													
					24	2600	1500	1200	2000	1300	>89000[k]	1600	1900				
M1-26	6-10-76	35×10^6	20 m	1 m													
					26	1300	1400	1500	>89000[k]	1400	27000	1500	1500				
M1-27-30	6-13-76	12×10^6	40 m	1 m													
					27	4300	930	2000	900	1600	2100	740					
					28	4000	980	2100	1900	1600	12200	740					
					29	6900		1700		3800		1200					
					30	11000		4100		1400		2400					
POST-FAIR																	
M2-18-21	3-22-77	88×10^6	50 m	3 m													
					18	>10000[p]	280	900	330	1800	5300	530	830	330	600	830	570
							270	870	470	2100	5000	570	1200	370	430	730	570
					19	>10000[p]	530	1100	370	430	10000	500	1200	430	670	1200	700
							670	1300	300	570	9300	500	1400	430	670	1100	570
								1100									
					20	M[t]	830	1600	670	320	3700	970	1200	570	1100	2900	830
							570	1700	630	320	3000	1000	1300	670	1000	2800	830
					21	M[t]	730	1900	670	430	2800	1500	1500	970	930	8000	1600
							800	1700	670	330		1500	1400	1100	1100	8300	1300

Table VI.B-17.
TOTAL COLIFORM CONCENTRATIONS FROM QUALITY ASSURANCE AEROSOL RUNS

Aerosol Run No.	Run Date	Wastewater Sample (MFC/100 ml)	Sampler Distance		Aerosol Sample Portion No.	Aerosol Concentrations from Samplers Aligned from Left to Right (MFC/m^3 of air)											
			From Wet Line Edge	Between Samples													
Pre-Fair																	
M1-16-19	6-7-76	343×10^3	25 m	1 m													
					16	13.2[d]	9.2	7.8	9.8	10.7	4.3	6.3	12.9	4.9			
					17	7.2[d]	15.0	9.1	9.6	5.5	6.7	10.8	5.1	9.3			
					18	8.0[d]		7.6		5.8		7.8					
M1-20-23	6-8-76	127×10^3	30 m	1 m													
					20	1.2	1.2	4.2	3.9	0.6	1.2	2.3	3.0				
					21	1.2	1.0	1.2	1.7	0.6	2.4	1.5	1.1				
					22		1 2		3.8		1.2		1.9				
					23		1.9		3.1		3.0		1.6				
M1-24-25	6-9-76	1100×10^3	25 m	1 m													
					24	19.5	7.5	14.7	9.6	12.1	22.0	16.1	13.4				
M1-26	6-10-76	758×10^3	20 m	1 m													
					26	6.1	9.4	8.9	11.2	8.6	13.0	8 4	13.8				
M1-27-30	6-13-76	267×10^3	40 m	1 m													
					27	<0.5	<0.4	0.9	<0.5	<0.6	<0.6	0.7					
					28	<0.5	1.0	<0.5	1 3	<0.6	0.8	<0.5					
					29	<0.5		<0.5		1.0		0.4					
					30	0.7		<0.5		<0.4		<0.5					
Post-Fair																	
M2-18-21	3-22-77	673×10^3	50 m	3 m													
					18	8.0[p]	13.3	8.3	7.0	6 7	19.3	10.0	12.7	9.0	8.3	5.3	7.7
						8.3[p]	14.7	8.7	5.3	4.7			8.0	7.3	7.3	5.3	4.0
					19	10.0[p]	8.3	12 7	10 0	9.7	10.0	9 0	8 7	8.7	14.7	11.3	8.0
							10.0	14.7	14.7	13.3	10.0		8.7	9.3	14.0		8.7
					20	M[t]	18.0	15.0	12.7	9.0	11 3	12.0	9 3	10.7	9.7	12.0	10.0
							21.0	14.7	15.3	12.0	10 7	10.0		11.3	12.7	13.3	10.7
					21	M[t]	28.0	23.0	21 0	7 7	22.0	29.0	22 0	22.0	22.0	25.0	15.7
								27.0		6 0	19.3	27.0			21.0		12.7

Table VI.B-18.
FECAL COLIFORM CONCENTRATIONS FROM QUALITY ASSURANCE AEROSOL RUNS

Aerosol Run No.	Run Date	Wastewater Sample (MFC/100 ml)	Sampler Distance		Aerosol Sample Portion No.	Aerosol Concentrations from Samplers Aligned from Left to Right (MFC/m^3 of air)								
			From Wet Line Edge	Between Samples										
Pre-Fair														
M1-16-19	6-7-76	60×10^3	25 m	1 m										
					16	1.8[d]	1 3	0.8	2 4	2.2	0.8	0.3	0 3	0.9
					17	2.4[d]	0.7	2.3	<0.3	0.8	1.8	1.2	0.8	5.6
					18	4.4[d]		2.7		1 9		2.1		
M1-20-23	6-8-76	14×10^3	30 m	1 m										
					20	0.5	<0 3	0 3	0 8	<0.3	0.3	0 3	0 3	
					21	0.4	0.6	0.3	0 2	0 5	0.2	0.6	0.2	
					22		0.5		0 3		<0.2		0.2	
					23		0.2		0.4		0.3		0.2	
M1-24-25	6-9-76	177×10^3	25 m	1 m										
					24	3.0	1.1	7.7	2.6	4.3	3 5	7.5	2.0	
M1-26	6-10-76	75×10^3	20 m	1 m										
					26	0.9	2.1	2.5	1.6	1 6	1.6	1 7	1 7	
M1-27-30	6-13-76	28×10^3	40 m	1 m										
					27	<0.3	<0.3	<0.3	<0.3	<0.3	<0 2	<0.3		
					28	<0.3	<0.3	<0.2	0 2	<0.3	<0 2	<0 2		
					29	<0.3		<0.3		0.3		<0.2		
					30	<0.3		<0.2		<0 2		<0.2		

Table VI.B-19.
COLIPHAGE CONCENTRATIONS FROM QUALITY ASSURANCE AEROSOL RUNS

Aerosol Run No.	Run Date	Wastewater Sample (PFU/ℓ)	Sampler Distance: From Wet Line Edge	Sampler Distance: Between Samples	Aerosol Sample Portion No	Aerosol Concentrations from Samplers Aligned from Left to Right (PFU/m³ of air)												High Sample Temperature Upon Lab Receipt
PRE-FAIR																		
M1-24-25	6-9-76		25 m	1 m	24	4.3	2.8	2 0	2 0	2.6	2.4[k]	2 3	4.1					
M1-26	6-10-76		20 m	1 m	26	1.4	1.8	0 8	1 2	1 4	2 0	2.0	1.3					
POST-FAIR																		
M2-8-9	2-16-77	170 x 10^3	50 m	3 m	8	0.3	0.5	0 3	0 2	5 9	1.5[l]	1 0	0 5	0 5				8°C
					9	0.1		0 3	0 7	2.1			0.3					8°C
M2-27-28	4-5-77	550 x 10^3	50 m	3 m	27	0 4	0.4	0 9	0 5	0 7	0 2	0 3	0.7	1 0	<0 2	0.4	0 7	9°C
					28		0.3		0.3		0 1		0.4		0 3		0.7	9°C

Table VI.B-20.
FECAL STREPTOCOCCI CONCENTRATIONS FROM QUALITY ASSURANCE AEROSOL RUNS

Aerosol Run No.	Run Date	Wastewater Sample (CFU/100 ml)	Sampler Distance: From Wet Line Edge	Sampler Distance: Between Samples	Aerosol Sample Portion No	Aerosol Concentrations from Samplers Aligned from Left to Right (CFU/m³ of air)												High Sample Temperature Upon Lab Receipt
PRE-FAIR																		
M1-24-25	6-9-76		25 m	1 m	24	1.8	3.2	0.6	1.1	1.2	2.7	1.5	0.6					
M1-26	6-10-76		20 m	1 m	26	0 6	0 3	0.3	0.3	0 3	0.8	1.1	<0 3					
POST-FAIR																		
M2-8-9	2-16-77	5.0 x 10^3	50 m	3 m	8	0.4	1 4	>330	0.7	0 6	0 6[i]	1.0	1.0	0 7	2.4			8°C
					9		0.4		0 4		1 1[i]		0 7		1 7			8°C
M2-27-28	4-5-77		50 m	3 m	27	0 1	<0.1	1 7	0.4	0 7	1 1	0 1	0.2	0.2	<0 1	<0.1	0.1	9°C
					28	0 2		0 6		3 3		0 3		0 1		<0.1		9°C

Table VI.B-21.
PSEUDOMONAS CONCENTRATIONS FROM QUALITY ASSURANCE AEROSOL RUNS

Aerosol Run No	Run Date	Sampler Distance: From Wet Line Edge	Sampler Distance: Between Samples	Aerosol Sample Portion No	Aerosol Concentrations from Samplers Aligned from Left to Right (CFU/m³ of air)							
PRE-FAIR												
M1-24-25	6-9-76	25m	1m	24	86	68	101	41	30	118	95	89
M1-26	6-10-76	20m	1m	26	190	110	120	30	210	120	160	240

Table VI.B-22.
MYCOBACTERIA CONCENTRATIONS FROM QUALITY ASSURANCE AEROSOL RUNS

Aerosol Run No.	Run Date	Wastewater Sample (CFU/100 ml)	Sampler Distance: From Wet Line Edge	Sampler Distance: Between Samples	Aerosol Sample Portion No	Aerosol Concentrations from Samplers Aligned from Left to Right (CFU/m³ of air)											High Sample Temperature Upon Lab Receipt
POST-FAIR																	
M2-8-9	2-16-77	1.3×10^3	50 m	3 m													
					8	<11	<11	<11	<11	<11	11	11	11	11	<11		8°C
					9a	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11		8°C
					9b	<11	<11	<11	22	<11	<11	<11	<11	<11	<11		8°C
M2-27-28	4-5-77	2.0×10^3	50 m	3 m													
					27	0.7	<0.3	1.0	<0.3	<0.3	0.3[i]	0.3	0.7	0.7	<0.3	<0.3	0.3	9°C
					28	0.3	0.7	2.0	0.3	1.7	2.3[i]	0.3	0.7	0.3	1.0	0.3	1.0	9°C
					28a	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	9°C
					28b	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	9°C

3. Nature of Aerosol Data

a. Distributional Characteristics

This section provides descriptive statistics which help characterize the distributions of the data for each of the microorganism groups. The descriptive statistics are given for values at specified distances because the data analysis is concerned with determining if the spray line is a source of the microorganisms and what effect distance has on the concentrations of the microorganisms.

All the aerosol concentration data in the Appendix F tables are used except underlined (indicating contamination) values, *Klebsiella*, three-day enterovirus, and five-day enterovirus from the Pre-Fair data and Runs 1-26 and 38 for mycobacteria from the Post-Fair data. The data from these groups are not included because very few values are above the detection limit. All data used have been lognormally transformed (lnC).

Table VI.B-23.
***KLEBSIELLA* CONCENTRATIONS FROM QUALITY ASSURANCE AEROSOL RUNS**

Aerosol Run No.	Run Date	Sampler From Wet Line Edge	Distance Between Samples	Aerosol Sample Portion No.	Aerosol Concentrations from Samplers Aligned from Left to Right (CFU/m³ of air)							
PRE-FAIR												
M1-24-25	6-9-76	25 m	1 m	24	2.5	0.3	2.6	4.5	2.0	M^k	0.5	1.4
M1-26	6-10-76	20 m	1 m	26	0.3	1.2	2.2	M^k	0.6	3.5	1.3	1.7

Table VI.B-24.
MICROBIOLOGICAL CONCENTRATIONS ON VIRUS AEROSOL RUNS

Virus Aerosol Run No.	Run Date (Time)	Microbiological Parameter	Concentration: Wastewater Sample	Concentration: Aerosol Sample (50 m)
V2-I	2-26-77 (1505-1733)	Enterovirus–3-day count	23 PFU/ℓ	0.0036 PFU/m³
		Enterovirus–5-day count	45 PFU/ℓ	0.011 PFU/m³
		Standard Plate Count	1.7×10^6/100 ml	10,000,000 /m³
		Total Coliform	190×10^3 MFC/100 ml	4.6 MFC/m³
		Fecal Streptococci	4.6×10^3 CFU/100 ml	<0.1 CFU/m³
		Coliphage	470×10^3 PFU/ℓ	0.4 PFU/m³
		Mycobacteria	1.3×10^3 CFU/100 ml	<11 CFU/m³
V2-II	4-9-77 (1450-1845)	Enterovirus–3-day count	250 PFU/ℓ	<0.0025 PFU/m³
		Enterovirus–5-day count	330 PFU/ℓ	0.017 PFU/m³
		Standard Plate Count	1.9×10^6/100 ml	430,000 /m³
		Total Coliform	64×10^3 MFC/100 ml	28 MFC/m³
		Fecal Streptococci	1.0×10^3 CFU/100 ml	0.2 CFU/m³
		Coliphage	370×10^3 PFU/ℓ	1.5 PFU/m³
		Mycobacteria	1.2×10^3 CFU/100 ml	<11 CFU/m³

(S_{lnC}), skewness, and kurtosis. Skewness indicates clustering of the data; if the statistic is negative, the data are clustered to be right of the mean; if positive, clustering is to the left of the mean; if 0 (zero), the data are clustered about the mean. Kurtosis indicates peakedness of the data; a positive value indicates a distribution that is more peaked or narrower than the normal distribution; a negative value indicates a flatter distribution; the normal distribution has zero kurtosis.

The results of the analyses are given in Table VI.B-26 for Pre-Fair and Post-Fair data. For skewness, most of the values are between -1 and 1. These are within the acceptable range for a normal distribution. Small sample sizes (<25) can have a value slightly greater than + 1 and be within the acceptable range. The acceptable range of a normal distribution for kurtosis is about -1.5 to 1.5 for small sample sizes (for sample sizes greater than 50, the range is -1 to 1); most values fall within that range. Large skewness and kurtosis values for several of the groups (such as total coliform at upwind) can be accounted for by the large number of values below the detection limit. Since the log-transformed data usually have a distribution that is approximately normal, the microorganism aerosol concentration data can be considered approximately lognormally distributed.

b. Relative Prevalence

The relative prevalence of the microorganism groups in the wastewater and aerosol samples for Pre-Fair and Post-Fair is indicated by the geometric means or lower detection limit in Table VI.B-27 and by the ratio of the group geometric mean to total coliform geometric mean in Table VI.B-28. The geometric

Figure VI. B-2

FOOTNOTES FOR UNUSUAL EVENTS

Footnotes

a MPN method used instead of normal assay method.
b Excessive sampler arcing observed; no data adjustment made.
c Data adjusted for extreme sampler arcing.
d Data adjusted for nonstandard volume of air sampled.
e Data adjusted for sampler power supply problems.
f Data adjusted for loss of BHI sampler fluid by means other than evaporation.
g Possible sample contamination; too close to spray line.
h Possible sample contamination; loss or foaming over of BHI.
i Possible sample contamination; equipment malfunction (pump/hose readjustment, open top, tubing problems).
j Possible sample contamination; external sources (vehicle traffic, train dust, wind shift, insects, smoke bomb, cattle too close, operator upwind).
k Possible sample contamination observed by analysis laboratory.
l Probable sample contamination; external source (nearby truck or dirt road).
m Probable sample contamination; equipment malfunction (glass view on plate broken, top opened, tubing broken, contaminated intake).
n Certain sample contamination; improper cleaning procedures (clorox residual left).
o Certain sample contamination; improper collection procedures used.
p Certain sample contamination; sample dropped.
q No sample collected because sampler developed problems during the run.
r Sample amount was insufficient for analysis.
s No analysis performed.
t No analysis result reported because laboratory observed sample contamination.
u Analysis results lost.
v Elevated sample temperature after shipment (8-9°).

means for the aerosol concentrations are listed by distance. Values preceded by a less than (<) sign are the detection limit for the parameter and indicate that the geometric mean of the parameter at that distance is below the detection limit. The ratio of microorganism geometric mean to total coliform geometric mean does not include the upwind values. Values preceded by a less than sign were calculated using the microorganism's detection limit.

For the Pre-Fair downwind data, *Pseudomonas* are 15 times as prevalent as total coliform in the aerosol. Fecal streptococci and *Clostridium perfringens* are between one-third and one-half as prevalent as total coliform in the aerosol. Fecal coliform and coliphage are less prevalent than any of the above three. The *Klebsiella* aerosol concentration detection limit is slightly below the total coliform geometric mean. For the Post-Fair data, mycobacteria is about one and one-half times as prevalent as total coliform in the aerosol. Coliphage and fecal streptococci are about one-third and one-half as prevalent, respectively, as total coliform.

c. *Systematic Sampler Differences*

The testing of the high volume microbiological samplers for collection efficiency at NBL provides a basis for evaluating whether there is need for sampler correction factors to adjust for any collection efficiency bias. The data from the nine runs, as shown in Appendix D, are adjusted for the actual flow

Table VI.B-25.
IDENTIFICATION OF CONFIRMED ENTEROVIRUS ISOLATES FROM AEROSOL SAMPLES

	Post-Fair	
Aerosol Run Number	V2-I	V2-II
Run Date	2-26-77	4-9-77
Sampler Distance	50m	50m
Three-Day Enteroviruses		
Confirmed Isolates	1	0
Poliovirus 1 Poliovirus 2 Poliovirus 3	1	
Five-Day Enteroviruses		
Confirmed Isolates	3	7
Poliovirus 1		1
Poliovirus 2	2	
Poliovirus 3		
Coxsackievirus B-3		1
Not Identified*	1	5

* Identification process terminated prior to completion.

Table VI.B-26.
DISTRIBUTIONAL CHARACTERISTICS OF THE NATURAL LOG TRANSFORMED MICROORGANISM GROUP CONCENTRATIONS
PRE-FAIR

Upwind	N	$\overline{\ln C}$	$S_{\ln c}$	Skewness	Kurtosis[1]
Std. Bacterial Plate Count	18	6.692	1.360	-0.280	-1.031
Total Coliform	19	-1.357	0.691	2.393	6.658 [2]
Fecal Coliform	16	-1.657	0.474	0.264	-0.777
Coliphage	18	-2.557	0.531	1.486	0.545
Pseudomonas	15	2.516	1.159	1.805	2.324
Fecal Streptococci	16	-0.720	0.935	1.244	1.392
Clostridium perfringens	17	-0.672	0.061	2.373	3.633 [2]
5-20 m Downwind					
Std. Bacterial Plate Count	43	7.861	0.683	0.200	-1.031
Total Coliform	53	1.744	1.335	-0.243	-0.134
Fecal Coliform	47	0.003	1.250	0.055	-0.829
Coliphage	53	-1.069	1.136	0.070	-0.940
Pseudomonas	38	4.270	2.102	0.168	-1.138
Fecal Streptococci	52	0.365	1.175	0.732	-0.226
Clostridium perfringens	49	0.376	0.940	-0.050	-1.112
30-50 m Downwind					
Std. Bacterial Plate Count	29	7.355	0.877	-0.982	1.439
Total Coliform	29	0.889	1.504	0.268	-0.481
Fecal Coliform	29	-1.004	1.074	0.803	0.038
Coliphage	31	-1.441	1.186	0.195	-1.323
Pseudomonas	25	3.525	1.555	0.165	-1.411
Fecal Streptococci	32	0.528	1.239	0.904	1.494
Clostridium perfringens	23	-0.086	0.619	1.167	1.662
100-200 m Downwind					
Std. Bacterial Plate Count	16	6.784	0.832	0.092	1.253
Total Coliform	17	0.179	1.205	-0.168	-1.636
Fecal Coliform	12	-1.437	0.503	0.095	-0.907
Coliphage	17	-1.729	0.732	0.056	-0.637
Pseudomonas	11	3.766	1.693	0.824	0.169
Fecal Streptococci	14	0.676	1.336	0.653	-0.731
Clostridium perfringens	16	0.065	0.891	0.471	-1.203

1 - 3 is subtracted

2 - The high skewness and kurtosis for these parameters can be attributed to the number of runs below the detection limit.

rates from the pin-wheel anemometer test, and an average value calculated for each sampler tested. These are shown in Table VI.B-29.

The sample variance among these sample means is $S^2 = 11{,}606.9$. For comparison, the nine values for Sampler 7 are used to calculate an S^2 of 5,657.0, and an F-test gives a ratio of 2.05 with 7 and 8 df.

Table VI.B-26. (cont'd)

POST-FAIR

Upwind	N	$\overline{\ln C}$	$S_{\ln c}$	Skewness	Kurtosis[1]	
Std. Bacterial Plate Count	24	5.711	1.417	-.220	-1.003	
Total Coliform	25	-2.104	.698	3.249	8.896	2
Coliphage	28	-2.857	.434	3.038	7.867	2
Fecal Streptococci	29	-1.871	1.363	1.058	.142	
Mycobacteria	9	-1.190	.964	.898	-.592	
50 m Downwind						
Std. Bacterial Plate Count	45	6.104	1.308	-.103	-1.153	
Total Coliform	56	.912	1.374	-.666	.295	
Coliphage	56	-.722	1.014	-.371	-.088	
Fecal Streptococci	55	-1.077	1.360	.083	-.643	
Mycobacteria	18	-.219	1.022	-.177	-1.106	
100 m Downwind						
Std. Bacterial Plate Count	48	5.803	1.239	.215	-.763	
Total Coliform	56	-.441	1.499	.059	-1.180	
Coliphage	55	-1.479	1.041	-.057	-1.205	
Fecal Streptococci	57	-1.243	1.417	.438	-.437	
Mycobacteria	18	-.201	.966	-.468	-.113	
Distant Downwind						
Std. Bacterial Plate Count	76	5.865	1.404	-.216	-.505	
Total Coliform	85	-2.072	.524	2.832	7.983	2
Coliphage	81	-2.778	.554	2.694	6.231	2
Fecal Streptococci	85	-1.863	1.213	.780	-.460	
Mycobacteria	27	-.710	.932	-.105	-1.414	

1 - 3 is subtracted

2 - The high skewness and kurtosis for these parameters can be attributed to the number of runs below the detection limit.

Table VI.B-27.
GEOMETRIC MEANS AND RATIOS OF WASTEWATER AND AEROSOL CONCENTRATIONS OF MICROORGANISM GROUPS

	Geometric Mean Wastewater Conc. (#/ml)	Geometric Mean Aerosol Concentrations (#/m^3 of Air)				Aerosol/Wastewater Ratio of Geometric Mean Concentration		
Pre Fair Runs		Upwind	5-20 m	30-50 m	100-200 m	5-20 m	30-50 m	100-200 m
Standard Plate Count	699,000	805	2590	1560	880	0.0037	0.0022	0.0013
Total Coliform	7,500	<0.5	5.7	2.4	1.2	0.0008	0.0003	0.0002
Fecal Coliform	800	<0.3	1.00	0.37	<0.3	0.0013	0.0004	<0.0004
Coliphage	220	<0.1	0.34	0.24	0.18	0.0012	0.0007	0.0006
Fecal Streptococci	67	<0.6	1.4	1.7	1.9	0.021	0.025	0.028
Pseudomonas	1,050	<15	72	34	43	0.068	0.032	0.041
Klebsiella	390	<5	<5	<5	<5	<0.01	<0.01	<0.01
Clostridium perfringens	54	<0.9	1.5	0.9	1.1	0.027	0.017	0.020
3-Day Enterovirus	0.012	<0.2	<0.2	<0.2	<0.2	<10	<10	<9
5-Day Enterovirus	0.017	<0.2	<0.2	<0.2	<0.2	<7	<7	<6

Post Fair Runs		Upwind	50 m	100 m	Distant	50 m	100 m	Distant
Standard Plate Count	158,000	300	450	330	350	0.0028	0.0021	0.0022
Total Coliform	4,900	<0.2	2.52	0.59	<0.2	0.0005	0.0001	<0.00004
Coliphage	290	<0.1	0.51	0.23	<0.1	0.0017	0.0008	<0.0003
Fecal Streptococci	34	0.15	0.34	0.29	0.15	0.0100	0.0085	0.0044
Mycobacteria	46	0.30	0.80	0.82	0.51	0.0176	0.0178	0.0110
3-Day Enterovirus	0.076		0.002			0.026		
5-Day Enterovirus	0.12		0.014			0.11		

Table VI.B-28.
RELATIVE PREVALENCE OF MICROORGANISM GROUPS

	Ratio of Group Geometric Mean to Total Coliform Geometric Mean	
Pre Fair Runs	Wastewater (#/ml)/(MFC/ml)	Downwind Aerosol (#/m^3)/(MFC/m^3)
Std. Bacterial Plate Count	93.4	530
Total Coliform	1.000	1.000
Fecal Coliform	1.106	0.174
Coliphage	0.030	0.061
Fecal Streptococci	0.009	0.47
Pseudomonas	0.140	15.2
Klebsiella	0.052	<0.78
Clostridium perfringens	0.007	0.359
3-Day Enterovirus	0.000002	<0.035
5-Day Enterovirus	0.000002	<0.035
Post Fair Runs		
Std. Bacterial Plate Count	32.6	788
Total Coliform	1.000	1.00
Coliphage	0.060	0.35
Fecal Streptococci	0.0070	0.49
Mycobacteria	0.0094	1.41
3-Day Enterovirus	0.000016	0.0008
5-Day Enterovirus	0.000024	0.006

Table VI.B-29.
MEAN NORMALIZED FLAVOBACTERIUM COUNTS, ADJUSTED FOR FLOW RATE (CFU/l)

Sampler							
1	5	6	7	8	9	10	11
288	544	503	297	270	423	475	432

This is not a significant value; thus, there is no indication of a need for a sampler correction factor.

The aerosol data for indicator microorganisms from the Pre-Fair quality assurance aerosol runs are used to further investigate the need for a correction factor for a sampler bias. An ANOVA is performed for each type of data on each run with samplers and portions as factors. Only the PEL analyses are used, because the additional factor of laboratories could interfere with the analysis. The F-ratios are summarized in Table VI.B-30.

For the total coliform and fecal coliform data, no significant F-ratios are obtained. The standard bacterial plate count data provide two significant F-ratios, however, and these are investigated further.

From Run M1-20-23 a means separation technique is used to determine which samplers are different. From this, it is determined that Sampler 1 is higher than all the others, which can be considered equivalent. On Run M1-27-30, a similar analysis shows that Samplers 8 and 10 are high, relative to the others. However, some of these high values were subsequently identified as affected by sampler contamination by the usual contamination inference procedure, so that the significant F-ratios may be due to contaminated samplers.

From the above, the conclusion drawn is that no systematic bias exists among the samplers. In general, variation among samplers is no greater than can be explained by sample-to-sample variation, and where a sampler term is significant, the usual contamination inference procedure often deletes the value prior to data analysis. Thus, there is no evidence of a need for a bias correction factor.

4. Aerosol Measurement Precision from Quality Assurance Program

Inspection of the dye and microbiological aerosol concentration data in Appendix F shows considerable variation, even between paired samplers. Special quality assurance aerosol runs (Tables VI.B-16 through VI.B-23) were conducted to more carefully determine the uncertainty in an aerosol concentration measurement for each microorganism group. After the quality assurance runs, several aliquots were sometimes analyzed from up to four separate portion bottles each containing the BHI sample collection fluid from one of many samplers placed side-by-side at the same distance from the wet-line edge. Therefore, it is of practical value to determine the total uncertainty in an aerosol concentration measurement (measurement variation), that fraction of measurement variation retained in the portions (portion variation), and that fraction of the portion variation retained in the aliquots (aliquot variation). Measurement variation results from differing levels of the microorganism sampled by the samplers, variation in sampler operator procedures, undetected sampler contamination, bottle variation, shipping variation, analytical technique of the laboratory

Table VI.B-30.
SUMMARY OF F-RATIOS

Aerosol Run No.	Analysis Parameter	F	df		Significance Level
			f_1	f_2	
16–19	Total Coliform	0.96	3	13	OK
	Fecal Coliform	0.79	3	13	OK
	Plate Count	1.78	8	13	OK
20–23	Total Coliform	2.34	7	16	OK
	Fecal Coliform	0.75	7	16	OK
	Plate Count	7.9	7	16	<0.005
27–30	Total Coliform	0.41	6	15	OK
	Fecal Coliform	1.20	6	15	OK
	Plate Count	3.10	6	15	0.05

doing the analysis, and random error. Because portions were taken from the same sample, but submitted to the laboratory on the quality assurance runs as being different, portion variation is due to bottle variation, shipping variation, analytical technique variation, and random error. Aliquots are taken from the same portion during sample preparation by the laboratory; therefore, aliquot variation is due to random error in the analytical measurement process.

The appropriate random effects model for the full nested design (aliquots k within portions j with samplers i) occurring on some quality assurance runs is

$$X_{ijk} = \mu + A_i + B_j + \varepsilon_{ijk} \qquad (1)$$

Sampler $i = 1, \ldots, a$ $\quad A_i \frown N(0, \sigma_A)$

Portion $j = 1, \ldots, b_i$ $\quad B_j \frown N(0, \sigma_B)$

Aliquot $k = 1, \ldots, m$ $\quad \varepsilon_{ijk} \frown N(0, \sigma)$

Then in terms of the preceding definitions, the measurement variation variance is $\mathrm{Var}(X_{ijk}) = \sigma_A^2 + \sigma_B^2 + \sigma^2$, while the portion variation variance is $\sigma_B^2 + \sigma^2$, and the aliquot variation variance is σ^2.

On most quality assurance runs, one aliquot of each portion from each sampler was analyzed and reported separately. The standard analysis of variance of this nested design would yield mean square estimates of the "between sampler" and "within sampler" sources of variation. The within sampler mean square does estimate the variance due to portion variation, $\sigma_B^2 + \sigma^2$. However, the between sampler mean square must be suitably adjusted and combined to yield an estimate of the measurement variation, $\sigma_A^2 + \sigma_B^2 + \sigma^2$.

The analysis of distributional characteristics in Section VI.B.3 suggests that the aerosol concentration data for each microorganism group may be considered to follow a lognormal distribution. When replicate measurements are made at each of several levels i to estimate the true values μ_i for a log-normally distributed variable, then the true coefficient of variation $\beta_i = \sigma_i/\mu_i$ for each grouped data set is the same value β ([30]). Thus, a coefficient of variation analysis ([31]) is used to estimate and report each type of variation.

In a coefficient of variation analysis, the coefficient of variation is estimated over k groups of size n_i $(x_1, x_2 \ldots, x_{n_i})$ as

$$\hat{\beta} = \sum_{i=1}^{k} w_i \hat{\beta}_i / k = \sum_{i=1}^{k} w_i (a_i s_i / \bar{x}_i)/k \qquad (2)$$

where

$\bar{x}_i$ = sample mean of group $i = \Sigma x/n$

s_i = sample standard deviation of group $i = [\Sigma (x - \bar{x}_i)^2 /(n - 1)]^{1/2}$

α_i = bias correction factor $= (2/n_i)^{1/2}\Gamma(n_i/2)/\Gamma[(n_i - 1)/2]$

$\hat{\beta}_i$ = sample coefficient of variation of group $i = \alpha_i s_i/\bar{x}_i$

w_i = group weight $\propto n_i/\alpha_{n_i}^2$; $\Sigma w_i = k$

The bias correction factor α_i adjusts for the bias in the sample standard deviation s_i as an estimator of the population standard deviation σ_i ([32]) Zeigler ([33]) tabulates these correction factors, which approach 1.0 as the sample size increases: $C_2 = 1.253$, $C_3 = 1.128$, $C_4 = 1.058$, $C_8 = 1.036$, and $C_{20} = 1.013$. The weights w_i are computed ([34]) to be proportional to the inverse of the variance of the group estimator $\hat{\beta}_i = \alpha_i s_i/\bar{x}_i$. In a coefficient of variation analysis, the estimates $\hat{\beta}_i$ are ordered by increasing group mean $\bar{x}_i$ and tabulated to check the assumption that β_i is independent of μ_i.

In the aerosol measurement precision analysis, most groups consisted only of a single pair of concentration measurements. To facilitate presentation of the analysis, similar pairs were combined via equation (2) and tabulated as a single group. In such cases, the arithmetic mean was taken as the group mean for a

quality assurance run group (when the true mean was presumably the same), and the geometric mean was used as the group mean for basically unrelated pairs (e.g., all Post-Fair pairs at 50 meters).

a. Dye Aerosol Concentrations

(1) Dye Analysis Accuracy

To ensure that the dye measurements made on both the wastewater and the aerosol dye samples were representative of the true concentration, a study was conducted using simulated samples at known concentrations. The samples were prepared using a basic stock solution of dye and the medium in which the actual samples would be analyzed, i.e., deionized water for the aerosol samples, and wastewater for the run composite samples. Samples were randomized to ensure that the analyst did not know at which level he was working.

For the aerosol simulation, a total of 18 sample portions were prepared, with 2 blanks and 4 portions at each of 4 concentrations: 1.8, 18, 72 and 360 μg/l. The values obtained are shown in Table VI.B-31. For each level, the mean and standard deviation are calculated and a 95-percent confidence interval constructed. The measurements are said to be accurate if the actual value falls within the confidence limits shown.

As can be seen, the measurements satisfy the accuracy criterion above at all levels. However, in all cases there is slight positive bias. On a relative basis, this ranges from 1.6 to 4.2 percent of the true value, and averages 2.5 percent. However, this is within the limits of the precision of the determination.

Similarly, 4 portions of wastewater were prepared at an actual level of 59,500 to represent the run composite samples taken. These were submitted to the same analysis as the samples above and the results are summarized in Table VI.B-32. The actual concentration was unknown to the analyst.

As in the previous study, the confidence interval contains the actual value of the sample, and thus can be said to be accurate. The bias is calculated as before and is similar to that found in the simulated aerosol samples. The possibility thus exists that there is a slight, 2 to 3-percent, high-side bias in the measurements, but with only 4 portions analyzed at a given level, there is insufficient information to ascertain this using ordinary parametric tests.

A chi-square test can be used to give further information, however. If the differences are calculated between each determined value and the actual value, it is noted that of the 20 non-blank determinations, 3 is equal to, 2 are less than, and the remaining 15 are greater than the actual value. For the deter-

Table VI.B-31.
DYE ACCURACY RESULT SUMMARY, SIMULATED AEROSOL SAMPLES

Sample No.	Actual Level, (μg/l) 0	1.8	18	72	360
1	0.0	1.5	18.5	74.0	360
2	0.0	2.0	18.5	72.5	361
3	–	2.0	18.5	74.0	380
4	–	2.0	18.0	72.0	370
Mean	0.0	1.88	18.38	73.13	367.75
Std Dev	0.0	0.25	0.25	1.03	9.32
95% CI	–	(1.48, 2.28)	(17.98, 18.78)	(71.49, 74.77)	(352.92, 382.58)
Bias, %	0.0	4.2	2.1	1.6	2.2

Table VI.B-32.
DYE ACCURACY RESULT SUMMARY, SIMULATED EFFLUENT SAMPLES (μg/l)

Sample No.	Actual Value, 59,500
A	60,000
B	60,800
C	58,000
D	60,000
Mean	59,700
Std Dev	1,194
95% CI	(57,800, 61,600)
Bias, %	3.4

minations to be accurate, approximately equal numbers should fall above and below the true value, or 8.5, discounting the equal values. A test of significance is used to determine the likelihood of obtaining a 2 and 15 split if the expected number of each were actually 8.5. The chi-square statistic has a value of 9.94 with 1 degree of freedom, and this gives a significance level of about 0.002. Thus there is evidence that the bias is real, though slight. The indicated 2-percent positive bias in both the wastewater and aerosol dye concentrations will have no net effect on aerosolization efficiency estimates because of cancellation (see equation (4) of Section VI.C).

(2) Dye Measurement Precision

A preliminary scan of the paired values in the dye run plots of Figure VI.B-1 suggests that the magnitude of the paired value variability is approximately proportional to the average of the paired dye levels. When the sample standard deviation is proportional to the sample mean, the coefficient of variation approach is an appropriate analysis methodology. Coefficients of variation were computed to estimate the measurement variation from the paired samplers on the dye runs. Separate estimates were calculated for the Pre-Fair pairs, the Post-Fair sets at 50 meters (after adjusting for diffusion concentration D), the Post-Fair pairs at 75 meters, and the Post-Fair pairs at 100 meters. The portion coefficient of variation was estimated from the aerosol simulation in the dye accuracy study (Table VI.B-31).

The dye measurement and portion coefficients of variation are presented in Table VI.B-33. The measurement coefficient of variation estimates appear to be nearly constant over the aerosol concentration range of 0.1 to 2.5 $\mu g/m^3$. A weighted dye measurement coefficient of variation of 0.17 = 17 percent was obtained using equation (2). The measurement coefficient of variation is considerably larger than the portion coefficient of variation (5 percent). Most of the dye measurement variation appears to be due to sampling factors (and perhaps to localized aerosol concentration anomalies), rather than to analytical factors.

b. Microbiological Aerosol Concentrations

Because no techniques of preparing microbiological samples of a known stable concentration were available, the accuracy of microbiological aerosol measurements could not be determined. The log-normally distributed nature of the aerosol concentration data for each microorganism group indicates that a coefficient of variation analysis is warranted to estimate the precision of the aerosol measurement process. The precision has been determined for each microorganism group in terms of measurement variation, portion variation, and aliquot variation, when appropriate, for each quality assurance aerosol run and for suitable groups of paired samples from the microbiological aerosol runs.

All of the applicable aerosol concentration data, except those values inferred to have been

affected by sample contamination, were used in calculating the precision variation. The single exception was that the calculation of measurement and portion variation for standard bacterial plate count and total coliform on quality assurance Run M2-18-21 was only based on portions 18 and 19. The data for this run (see Tables VI.B-16 and VI.B-17) show a general increasing trend with portion number for most samplers. The portions were refrigerated until analysis was begun. Total coliform analyses were prepared over a three-hour period in the morning and standard bacterial plate count analyses were prepared over a two-hour period in the afternoon. The portion bottles were removed from the refrigerator at the beginning of the analysis period and prepared sequentially starting with portion 18. The increased levels in standard bacterial plate count and total coliform for portions 20 and 21 from each sampler (often exceeding a factor of 2) is apparently due to the lengthened exposure to room temperature prior to analysis. It should be noted that the preparation duration on M2-18-21, in which 48 aerosol sample portions were analyzed, exceeded those of the other quality assurance runs and any microbiological aerosol run (typically, eight aerosol sample portions).

The microbiological aerosol concentration precision results are presented in Tables VI.B-34 (standard bacterial plate count), VI.B-35 (total coliform), VI-B-36 (fecal coliform), VI.B-37 (fecal streptococci), VI-B-38 (coliphage), and VI.B-39 (pathogens - *Pseudomonas*, mycobacteria, *Klebsiella*, and *Clostridium perfringens*). When the measured aerosol concentrations are sufficiently above the detection limit, the measurement variation of a microorganism group tends to have a constant coefficient of variation, regardless of the run group mean. This can be seen especially for total coliform above 3 MFC/m^3 in Table VI.B-35 and for fecal streptococci above 0.6 CFU/m^3 in Table VI.B-37. Nearer the detection limit, the precision coefficients of variation tend to be higher because the sampling resembles a Poisson process with few events per interval.

Weighted estimates of the precision coefficients of variation calculated using equation (2) are provided on the bottom line of the precision tables. These weighted estimates indicate the extent of variation of each type for the microorganism group over the aerosol concentration range examined. Thus, for example, the precision coefficients of variation for total coliform in aerosols over the range from 0.4 to 14 MFC/m^3 were 0.50 for measurement variation, 0.49 for portion variation, and 0.15 for aliquot variation. This means that the standard deviation of a total coliform aerosol measurement due to all sources of uncertainty in the measurement process is about 0.50 = 50 percent of the true aerosol concentration (which is best estimated by

Table VI.B-33.
DYE AEROSOL CONCENTRATION PRECISION

RUN GROUP	MEAN ($\mu g/m^3$)	COEFFICIENT OF VARIATION	
		MEASUREMENT VARIATION	PORTION VARIATION
Post-Fair at 100m	0.17	0.21	
Post-Fair at 75m	0.25	0.15	
Post-Fair at 50m	0.44	0.18	
Pre-Fair Pairs	2.5	0.15	
Dye Accuracy Study	---		0.05
WEIGHTED ESTIMATE		0.17	0.05

Table VI.B-34.
STANDARD BACTERIAL PLATE COUNT AEROSOL CONCENTRATION PRECISION

RUN GROUP	MEAN ($No./m^3$)	COEFFICIENT OF VARIATION		
		MEASUREMENT VARIATION	PORTION VARIATION	ALIQUOT VARIATION
Post-Fair at 100m	330	0.54		
Post-Fair at 50m	620	0.46		
QA:M2-18-21	740	0.60	0.36	0.11
QA:M1-26	1430	0.06		
Pre-Fair Pairs	1700	0.31		
QA:M1-24-25	1730	0.29		
QA:M1-20-23	1890	0.27	0.12	
QA:M1-27-30	2690	0.98	0.49	
QA:M1-16-19	3100	0.56	0.59	
WEIGHTED ESTIMATE		0.50	0.37	0.11

the concentration value obtained). In a portion, the standard deviation of the uncertainty in the analyzed total coliform value is about 0.49 = 49 percent of the value. In an aliquot, the standard deviation of the uncertainty in the analyzed value is about 0.15 = 15 percent of its value.

The weighted estimates for the microorganism groups are presented in Table VI.B-40; the groups are arranged by increasing measurement coefficient of variation. The microbiological aerosol measurement variation for each of the microorganism groups is much greater than the dye aerosol measurement variation. The microbiological portion variations are also much greater than the dye portion variation. Thus, there is much less precision in the microorganism aerosol concentrations than in the dye aerosol concentrations. Grouping of the microorganisms by measurement variation in Table VI.B-40 shows better precision for total coliform and standard bacterial plate count than for fecal coliform, *Pseudomonas*, and *Clostridium perfringens*. Aerosol measurement of mycobacteria, fecal streptococci, *Klebsiella*, and coliphage has the least precision. Microbiological aerosol measurement precision seems to improve as expected when the measurements consistently exceed the detection limit and when the more routine measurements are made.

By making the assumption that measurement variation and portion variation were calculated for the same distribution of aerosol concentrations, one can quantify the contributions of analytical sources of variation and of field sampling sources of variation to the total measurement variation. Under this assumption, the square of the measurement variation equals the sum of the squares of portion variation (representing shipping and analytical sources) and of sampling variation (representing field sampling sources). The sampling variation thus obtained is shown in Table VI.B-40. Comparing the relative magnitudes of sampling variation and portion variation shows that the dye measurement variation is mostly due to field sampling sources.

Table VI.B-35.
TOTAL COLIFORM AEROSOL CONCENTRATION PRECISION

RUN GROUP	MEAN (MFC/m^3)	COEFFICIENT OF VARIATION		
		MEASUREMENT VARIATION	PORTION VARIATION	ALIQUOT VARIATION
QA:M1-27-30	0.4	0.85	0.84	
Post-Fair at 100m	1.0	0.65		
QA:M1-20-23	1.9	0.55	0.37	
Pre-Fair Pairs	2.5	0.57		
Post-Fair at 50m	3.0	0.37		
QA:M1-16-19	8.5	0.37	0.38	
QA:M2-18-21	9.9	0.34	0.35	0.15
QA:M1-26	9.9	0.27		
QA:M1-24-25	14.4	0.35		
WEIGHTED ESTIMATE		0.50	0.49	0.15

Table VI.B-36.
FECAL COLIFORM AEROSOL CONCENTRATION PRECISION

RUN GROUP	MEAN (MFC/m^3)	COEFFICIENT OF VARIATION	
		MEASUREMENT VARIATION	PORTION VARIATION
QA:M1-20-23	0.3	0.47	0.49
Pre-Fair Pairs	0.8	0.46	
QA:M1-16-19	1.7	0.84	0.83
QA:M1-26	1.7	0.27	
QA:M1-24-25	4.0	0.64	
WEIGHTED ESTIMATE		0.58	0.65

Table VI.B-37.
FECAL STREPTOCOCCI AEROSOL CONCENTRATION PRECISION

RUN GROUP	MEAN (CFU/m^3)	COEFFICIENT OF VARIATION	
		MEASUREMENT VARIATION	PORTION VARIATION
QA:M1-26	0.5	0.74	
QA:M2-27-28	0.5	1.47	0.81
Post-Fair at 100m	0.6	0.78	
Post-Fair at 50m	0.6	0.55	
QA:M2-8-9	0.9	0.64	0.52
QA:M1-24-25	1.6	0.62	
Pre-Fair	2.0	0.58	
WEIGHTED ESTIMATE		0.77	0.67

Table VI.B-38.
COLIPHAGE AEROSOL CONCENTRATION PRECISION

RUN GROUP	MEAN (PFU/m^3)	COEFFICIENT OF VARIATION	
		MEASUREMENT VARIATION	PORTION VARIATION
Post-Fair at 100m	0.3	0.88	
Pre-Fair Pairs	0.3	0.87	
QA:M2-27-28	0.5	0.63	0.51
Post-Fair at 50m	0.5	0.56	
QA:M2-8-9	1.0	1.32	0.63
QA:M1-26	1.5	0.29	
QA:M1-24-25	2.8	0.33	
WEIGHTED ESTIMATE		0.73	0.56

In contrast, for each microorganism group, the portion variation is larger than the sampling variation. Shipping and analytical sources are apparently responsible for more of the microorganism aerosol measurement variation than are the field sampling sources. For total coliform and standard bacterial plate count, aliquot variation represents only a small part of the portion variation; for these microorganism groups, most of the "shipping and analytical variation" is attributable to factors such as bottles, analysts, and day-to-day procedural variations rather than to variations in repeated analyses. The differences in Table VI.B-40 between measurement variation, portion variation, and the more frequently reported aliquot variation emphasize the need for quality assurance runs and pairing of samplers if one is to accurately estimate aerosol measurement precision and assess its contributing factors.

Table VI.B-39.
PATHOGEN AEROSOL CONCENTRATION PRECISION

MICROORGANISM GROUP / RUN GROUP	MEAN (CFU/m^3)	COEFFICIENT OF VARIATION: MEASUREMENT VARIATION	COEFFICIENT OF VARIATION: PORTION VARIATION
PSEUDOMONAS			
Pre-Fair Pairs	22	0.82	
QA:M1-24-25	79	0.40	
QA:M1-26	150	0.47	
Pseudomonas Weighted Estimate		0.58	
MYCOBACTERIA			
QA:M2-27-28	0.6	0.86	0.92
Post-Fair at 100m	1.0	0.59	
Post-Fair at 50m	1.4	0.86	
Mycobacteria Weighted Estimate		0.81	0.92
KLEBSIELLA			
QA:M1-26	1.6	0.73	
QA:M1-24-25	2.0	0.76	
Klebsiella Weighted Estimate		0.74	
CLOSTRIDIUM PERFRINGENS			
Pre-Fair Pairs	1.5 MPN/m^3	0.60	

Table VI. B-40.
AEROSOL CONCENTRATION PRECISION SUMMARY

MICROORGANISM GROUP	Coefficient of Variation			
	Total (Measurement Variation)	Sampling (Sampling Variation)	Analytical (Portion Variation)	Replication (Aliquot Variation)
Dye	17%	16%	5%	
Total Coliform	50%	9%	49%	15%
Standard Bacterial Plate Count	50%	34%	37%	11%
Fecal Coliform	58%	Little	65%	
Pseudomonas	58%			
Clostridium perfringens	60%			
Coliphage	73%	46%	56%	
Klebsiella	74%			
Fecal Streptococci	77%	39%	67%	
Mycobacteria	81%			

5. Particle Size Distributions

The data available for particle size analysis come from Phase I of the program. A more extensive study of particle size using six-stage Andersen samplers was planned for Phase II, but had to be deleted because of funding limitations. In the Phase I sampling, two-stage Andersen samplers were used to obtain the samples, with the size ranges of >7.0 μm (Stage I) and 1.05-7.0 μm (Stage II), which roughly correspond to nonrespirable and respirable particles, respectively. While some information is obtained on the distribution, it is less definitive than would have been available using the six-stage sampler. However, the purpose of the aerosol runs made with these samplers in Phase I was not originally aimed to obtain particle size data. In some instances, the purpose was to determine the correct times for the aerosol sampling in subsequent phases of the program. On other runs, the Andersen sampler was paired with AGI and LEAP samplers to allow an evaluation of the different means of obtaining aerosol samples. This breakout of the data by size is intended only to give a general idea of the distribution and to provide comparative data with other studies.

Two collection media were used in the Phase I study: EMB agar for total coliform and Casitone agar for total count. The summary of the total coliform results is shown in Table VI.B-41, while for total count the results are shown in Table VI.B-42. For those counts that fell either above or below the detection limit, no percentage calculation was made. It should be noted that not all the runs made during Phase I are summarized here. If the number of usable results was small, or in some cases nonexistent, the run was not included in these summaries.

The particle size distributions for total coliform and total count are shown in Tables VI.B-43 and VI.B-44, respectively. For purposes of summarizing the results, the upwind samples and those beyond 200 meters for total count and beyond 100 meters for total coliform are considered to represent background levels. The results are summarized as percent respirable (1.05-7.0 μm).

For total count, the percent of the total number of particles which can be considered to be respirable range from a low of 14 percent to a high of 76 percent for the background. The median percent was 43.5 with a mean value of 43.6 percent, based on 20 values. The total count particle size distribution is broken

down into two ranges for further study; close downwind (5 to 25 meters) and far downwind (50 to 200 meters). The close downwind results gave a range from 65 percent respirable to 76 percent respirable, based on four results. The median was 70 percent with a mean of 70.2 percent. The far downwind results had ten data points, ranging from 20 percent to 74 percent respirable. The median for this data set was 43 percent, with a

Table VI.B-41.
AEROSOL PARTICLE SIZE DISTRIBUTION FROM TWO-STAGE ANDERSEN SAMPLERS— TOTAL COLIFORM (No./m³)

Run	Distance	Stage I (Non-Respirable)	Stage II (Respirable)
7	100	1.4×10^2	3.9×10^2
	100	1.1×10^1	2.0×10^2
8	u	7.7×10^1	$>2.4 \times 10^2$
	20	1.4×10^2	6.0×10^2
	20	1.3×10^2	6.4×10^2
	75	7.1×10^1	4.9×10^2
	75	2.8×10^1	5.6×10^1
	75	3.5×10^1	1.4×10^1
	390	$<3.5 \times 10^1$	7.1×10^2
	390	2.1×10^1	1.4×10^2
	390	4.7×10^1	9.4×10^1
9	u	1.7×10^2	1.7×10^2
	u	1.1×10^2	8.0×10^1
	825	3.5×10^1	1.4×10^1
	825	1.9×10^1	1.6×10^1
	825	3.5×10^1	1.9×10^1
10	500	$<2.0 \times 10^0$	$<2.0 \times 10^0$
	500	2.1×10^2	8.5×10^1
	500	5.9×10^1	--
	1500	1.5×10^2	3.5×10^2
	1500	--	4.9×10^1
	1600	$<3.5 \times 10^1$	$<3.5 \times 10^1$
	1600	1.4×10^1	4.2×10^1
	1600	2.0×10^0	2.4×10^1
11	1	$<7.1 \times 10^1$	$<7.1 \times 10^1$
	1	1.1×10^2	1.8×10^2
	1	2.8×10^1	6.4×10^1
	1	$<2.0 \times 10^0$	1.6×10^1
	1600	$<7.0 \times 10^0$	$<7.0 \times 10^0$
	1600	2.0×10^0	5.0×10^0

Note: u = upwind.

Table VI.B-42.
AEROSOL PARTICLE SIZE DISTRIBUTION FROM TWO-STAGE ANDERSEN SAMPLERS—TOTAL COUNT

Run	Distance, m	Stage I (Non-Respirable)	Stage II (Respirable)
1	u	1.6×10^1	5.0×10^0
	5	4.2×10^2	1.0×10^3
	25	9.9×10^2	2.3×10^3
	50	4.2×10^2	2.6×10^2
	100	4.7×10^1	1.2×10^1
	150	7.0×10^0	$<7.0 \times 10^0$
2	u	4.7×10^1	1.3×10^1
	10	6.4×10^0	1.2×10^2
	50	1.6×10^2	2.1×10^2
	100	1.4×10^1	1.5×10^1
	200	8.8×10^1	5.9×10^1
	500	5.5×10^1	8.0×10^1
	700	--	6.0×10^1
3	u	8.0×10^0	2.6×10^1
	20	8.0×10^1	2.6×10^2
	100	1.9×10^2	1.6×10^2
	200	1.1×10^2	1.1×10^2
	600	1.2×10^1	1.4×10^1
	1000	$>2.4 \times 10^2$	1.6×10^2
	1600	2.9×10^1	5.3×10^1
4	u	2.9×10^1	1.6×10^1
	40	2.2×10^2	$>4.7 \times 10^1$
	100	1.2×10^1	3.5×10^1
	450	2.7×10^1	3.5×10^1
	800	3.5×10^1	3.6×10^1
	1200	$>2.4 \times 10^2$	1.2×10^2
5	u	1.4×10^2	1.8×10^2
	310	$>2.4 \times 10^2$	$>2.4 \times 10^2$
8	u	$>2.4 \times 10^2$	$>2.4 \times 10^2$
	20	--	--
	20	$>1.4 \times 10^3$	$>1.4 \times 10^3$
	75	2.3×10^3	7.4×10^2
	75	8.5×10^2	4.0×10^2
	75	$>4.7 \times 10^2$	2.8×10^2
	390	4.2×10^2	1.8×10^2
	390	6.7×10^2	4.4×10^2
	390	$>4.7 \times 10^2$	$>4.7 \times 10^2$
9	u	$>1.4 \times 10^3$	8.8×10^2
	u	$>4.7 \times 10^2$	$>4.7 \times 10^2$
	825	5.7×10^2	5.7×10^2
	825	$>4.7 \times 10^2$	$>4.7 \times 10^2$
	825	$>2.4 \times 10^2$	$>2.4 \times 10^2$
10	500	4.2×10^2	$<3.5 \times 10^1$
	500	9.9×10^2	8.8×10^2
	500	2.9×10^2	4.7×10^2
	1500	7.8×10^2	1.3×10^2
	1500	$>4.7 \times 10^2$	--
	1600	2.8×10^2	1.4×10^2
	1600	3.5×10^2	1.1×10^2
	1600	1.3×10^2	9.4×10^1
11	1	1.4×10^2	$<7.1 \times 10^1$
	1	7.4×10^2	2.8×10^2
	1	3.6×10^2	1.8×10^2
	1	2.8×10^1	4.2×10^1
	1600	4.5×10^2	2.1×10^2
	1600	$>4.7 \times 10^2$	$>4.7 \times 10^2$

mean of 42.1 percent. As can be seen, the far downwind results resembled the size distribution of the background samples for percent respirable, while the close downwind samples collected a higher percentage of respirable (small) particles.

For total coliform, the background was represented by two upwind samples and ten samples taken more than 100 meters from the spray line. The distances of these samples ranged from 390 to 1600 meters. Percentage of the total particles in the respirable range for the background ranged from 29 percent to 92 percent, with a median of 48 percent and a mean of 53.8 percent. The samples taken close to the spray line were at distances of 20, 75 and 100 meters and no further division of these values was made. The percent respirable at these distances went from a low of 29 percent to a high of 87 percent, based upon seven observations. The

Table VI.B-43.

AEROSOL PARTICLE SIZE DISTRIBUTION FROM TWO-STAGE ANDERSON SAMPLERS —TOTAL COLIFORM (Percent)

Run	Distance, m	Stage I (Non-Respirable)	Stage II (Respirable)
7	100	26	74
	100	36	64
8	u	--	--
	20	19	81
	20	17	83
	75	13	87
	75	33	67
	75	71	29
	390	--	--
	390	60	40
	390	48	52
9	u	50	50
	u	58	42
	825	71	29
	825	54	46
	825	65	35
10	500	--	--
	500	71	29
	500	--	--
	1500	30	70
	1500	--	--
	1600	--	--
	1600	25	75
	1600	8	92
11	1	--	--
	1	38	62
	1	30	70
	1	--	--
	1600	--	--
	1600	29	71

Table VI.B-44.
AEROSOL PARTICLE SIZE DISTRIBUTION FROM TWO-STAGE ANDERSEN SAMPLERS—TOTAL COUNT
(Percent)

Run	Distance, m	Stage I (Non-Respirable)	Stage II (Respirable)
1	u	76	24
	5	30	70
	25	30	70
	50	74	26
	100	80	20
	150	--	--
2	u	78	22
	10	35	65
	50	43	57
	100	48	52
	200	60	40
	500	41	59
	700	--	--
3	u	24	76
	20	24	76
	100	54	46
	200	50	50
	600	46	54
	1000	--	--
	1600	35	65
4	u	64	36
	40	--	--
	100	26	74
	450	44	56
	800	49	51
	1200	--	--
5	u	44	56
	310	--	--
8	u	--	--
	20	--	--
	20	--	--
	75	76	24
	75	68	32
	75	--	--
	390	70	30
	390	60	40
	390	--	--
9	u	--	--
	u	--	--
	825	50	50
	825	--	--
	825	--	--
10	500	--	--
	500	53	47
	500	38	62
	1500	86	14
	1500	--	--
	1600	67	33
	1600	76	24
	1600	58	42
11	1	--	--
	1	72	28
	1	67	33
	1	40	60
	1600	68	32
	1600	--	--

median percent respirable was 74 percent, with a mean of 72.7 percent. In general, then, there was a higher percentage of respirable total coliforms close to the spray line.

The results can be compared to other studies conducted at Ft. Huachuca, Arizona, and at Chicago, Illinois. In each of these studies, the six-stage Andersen sampler was used, but the division according to respirable (Stage 1) and nonrespirable (Stages 2-6) particles can be made. In the Ft. Huachuca study, the respirable portion of the total viable particles accounted for 70-80 percent of the total particles for three of the four runs. On the fourth run, only 50-60 percent of the particles were in the respirable range. These are similar to the data obtained in this study.

At the Chicago site both standard bacterial plate count and total coliform data were obtained. For standard bacterial plate count, the upwind sample gave a respirable portion of 35 percent, slightly below the median for this study. The results showed median values of 67 percent for close downwind (10-20 meters) and 55 percent respirable for farther downwind (100 meters), similar to those for this study. No samples were obtained beyond 200 meters in that study.

For total coliform, only at one distance were nonrespirable particles isolated at 97 meters downwind. However, the actual counts obtained at all distances fell below the recommended minimum of 30 particles and these data provide little comparative utility.

6. Aerosol Microbial Characterization

A special aerosol run, M1-36, was made with eight samplers set side-by-side at 20 meters from the wet-line edge to collect a high-volume aerosol sample for pathogen screen. Run M1-36 was conducted on the dry side of Field 3-1 at night (2137-2207) on June 21, 1976, at a temperature of 12°C, a relative humidity of 82 percent, and a wind velocity of 1.6 m/s. The BHI sample collection fluid from all samplers was aggregated and analyzed both by the qualitative pathogen screen procedure used for the high-volume wastewater samples and by the usual quantitative procedure. The aerosol microbial characterization results are presented as Run 36 in Tables VI.A-19 and VI.A-21 for comparison with the wastewater microbial characterization. The aerosol concentrations obtained from this sample are given in Table VI.B-45. The mycobacteria aerosol concentration was surprisingly high on this run. The prevalence of mycobacteria in this aerosol sample and in the wastewater microbial characterization sample during Pre-Fair was the basis for its selection as a microorganism group to be routinely monitored in the Post-Fair sampling. In addition to the microorganism groups listed in Table VI.B-45, the pathogen screen identified *Proteus, Enterobacter,* "other oxidase-negative gram-negative nonfermenters," and "other oxidase positive fermenters." While the aerosol bacteria identified were representative of those found in the wastewater microbial characterizations, fewer bacterial types were found in the aerosol, as expected.

Table VI.B-45.
AEROSOL CONCENTRATIONS FOR THE AEROSOL MICROBIAL CHARACTERIZATION RUN

MICROORGANISM GROUP	AEROSOL CONCENTRATION
Std. Bacterial Plate Count	7800 /m^3
Total Coliform	43 MFC/m^3
Fecal Coliform	6.0 MFC/m^3
Fecal Streptococci	2.3 CFU/m^3
Pseudomonas	240 CFU/m^3
Mycobacteria	280 CFU/m^3
Klebsiella	4.1 CFU/m^3
Clostridium perfringens	4.3 MPN/m^3
Staphylococcus aureus	9 CFU/m^3

C. Aerosol Data Analyses

1. Microbiological Dispersion Model

This section presents a microbiological dispersion model that may be applicable for prediction of the viable aerosol concentration of any measurable microorganism group emanating from any sprayed wastewater aerosol source. This microbiological dispersion model incorporates the microbiological aerosol characteristics derived from the extensive aerosol sampling program of this study. Considering the imprecision and cost of measuring microorganism aerosol concentrations from spray irrigation by field sampling, using predictions of the microbiological dispersion model supplemented with minimal field sampling does appear to be a preferable alternative to extensive field sampling when the sprayed wastewater does not contain residual chlorine.

The microbiological dispersion model extends the standard air pollutant dispersion models[35], and predecessor microbiological models[36,37,38,39,40] by incorporating a site-specific parameter for aerosolization efficiency and microorganism-specific parameters for microbiological die-off.

a. Model Derivation

Consider the aerosol concentration, $C_g(r,d)$, of a microorganism group or dye, g, sampled under the environmental conditions of aerosol run r at a perpendicular distance d downwind from the wet-line edge of the spray line. This sampled aerosol concentration should equal the sum of the predicted microorganism concentration, $P_g(r,d)$, emanating from the spray line to that distance on the run, the existing background concentration, $B_g(r)$, of the microorganism under the run conditions, and a random error, ε_g, with a mean of zero to express the uncertainty of the relationship:

$$C = P + B + \varepsilon \qquad (1)$$

where:

- d = perpendicular distance from the spray wet-line edge, (m)
- g = measured microorganism group or dye
- r = environmental conditions during the aerosol run
- C = C_g (r,d) = sampled aerosol concentration of microorganism g at distance d during run r, (cfu/m^3, colony forming units per cubic meter)
- B = B_g (r) = sampled background aerosol concentration of microorganism g during run r (upwind of the spray line), (cfu/m^3)
- P = P_g (r,d) = predicted aerosol concentration of microorganism g emanating from the spray line to a distance d during run r obtained using the microbiological dispersion model, (cfu/m^3)
- ε = ε_g = random error with zero mean representing the measurement errors in C and B and the prediction error in P, (cfu/m^3).

The variance of ε is the sum of the variances of C, B, and P under the reasonable assumption of independence. The measured concentrations C and B have a relatively constant coefficient of variation, α_g, which was estimated in Section VI. B.4.b. as the measurement variation for each microorganism, g. Thus, the variance of ε is:

$$\mathrm{Var}(\varepsilon_g) = \alpha_g^2(C_g^2 + B_g^2) + \mathrm{Var}(P_g)$$

Since α_g ranges from 0.50 for total coliform and standard bacterial plate count to 0.81 for mycobacteria, and since aerosol model predictions may be expected to have coefficients of variation in the same range, the standard deviation of εg probably ranges between 70 and 110 percent of the aerosol concentration, C. Thus, equation (1) contains a substantial degree of uncertainty.

Figure VI.C-1 depicts the transport of aerosol downwind of a spray line to a position, x. Let:

- a = a(r,d) = aerosol age at distance d during run r, (s)
- u = mean wind velocity during run r, (m/s)
- w = wet-line edge distance during run r, (m)
- ϕ = angle of the wind direction with the perpendicular to the line of the spray heads.

Then the aerosol age, a, is computed as

$$a = \frac{w + d}{u \cos \phi} \tag{2}$$

At d = 50m, for example, $a_{50} = (w + 50m)/(u \cos \phi)$.

The following multiplication model for the predicted concentration, P, coming from the spray line is postulated:

$$P = D \times E \times I \times e^{\lambda a} \tag{3}$$

where the model parameters are:

- D = D_g (r,d) = physical diffusion model aerosol concentration of microorganism g emanating from the spray line to a distance d during run r, assuming all the sprayed wastewater (including its measured wastewater concentration of microorganism g) becomes aerosol, and assuming no microbiological die-off, (cfu/m^3)
- E = E(r) = aerosolization efficiency factor: the fraction of the sprayed wastewater that is aerosolized during run r, ($0 < E \leq 1$)

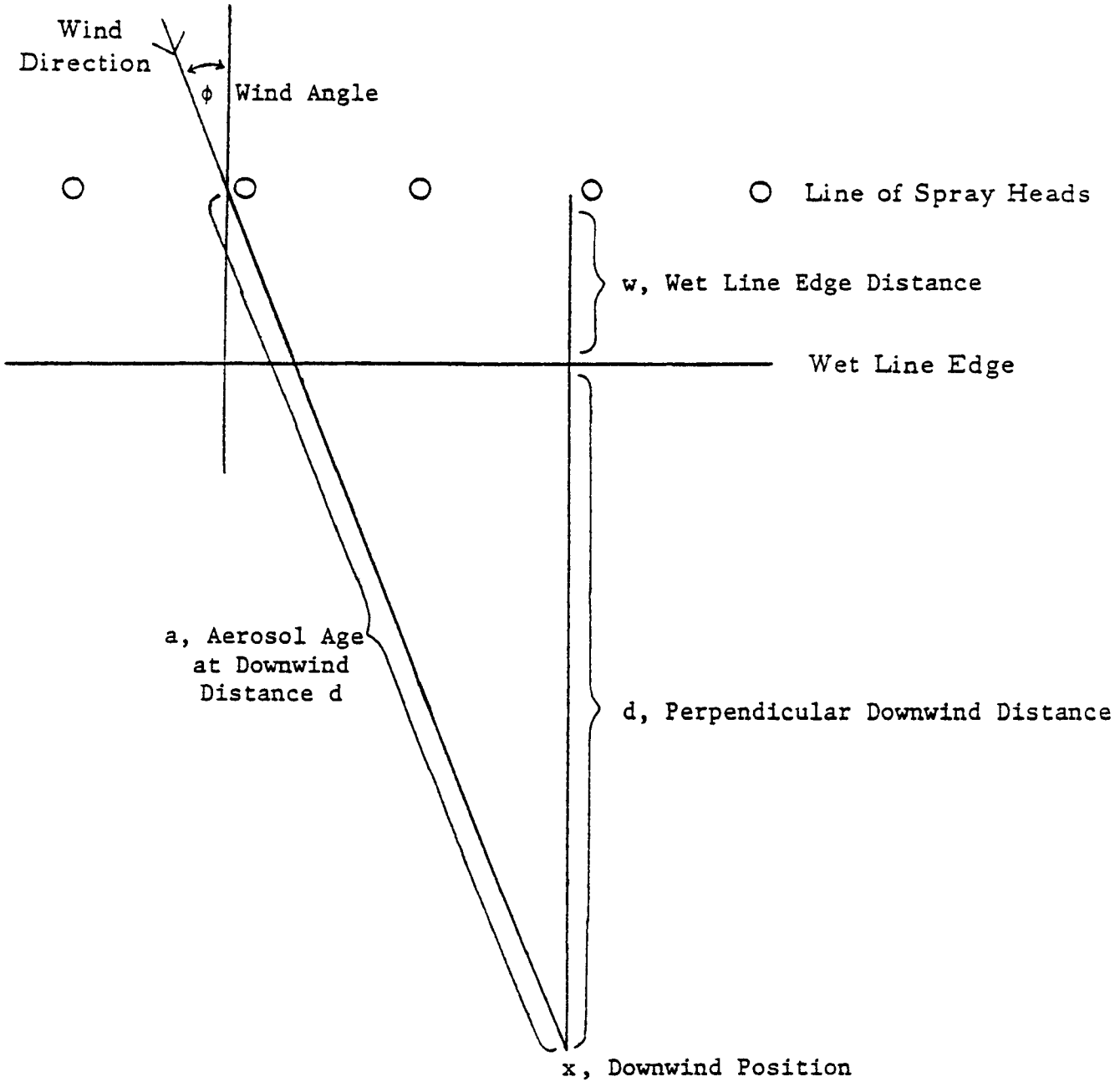


Figure VI.C-1.
SCHEMATIC OF AEROSOL TRANSPORT DOWNWIND OF A SPRAY LINE

I = $I_g(r)$ = microbiological impact factor: the proportion of the aerosolized microorganisms of group g that remain viable immediately after aerosolization during run r, ($I>0$)

λ = $\lambda_g(r)$ = microbiological age decay rate: rate at which the microorganisms of group g die off with aerosol age during run r, ($\lambda \leqslant 0$).

The exponential microbiological viability decay factor, $e^{\lambda a}$, expresses the reasonable assumption that the run, r, environmental conditions kill a constant proportion, λ, of the remaining number of the viable microorganisms of group g in the aerosol cloud with unit increase in the aerosol age, a.

Dispersion models generally[41, 42] contain a source strength term Q, in units of mass per unit time, to express the pollutant emission rate at the source. For spray irrigation, Q is calculated as the product of four factors: the wastewater concentration of the microorganism, the wastewater application rate, the aerosolization efficiency (E), and the impact factor (I) for the microorganism. In the microbiological dispersion model, the wastewater concentration and application rate factors are included in D, while E and I are specified separately. This permits separate estimation of the E and I factors.

b. Effect of Each Model Factor

The effect of each factor of the microbiological dispersion model, P, is depicted schematically in Figure VI. C-2. The physical diffusion model calculates a very high microbiological aerosol concentration, D, because it assumes that all of the sprayed wastewater is converted to aerosol and that all of the microorganisms measured in the wastewater remain viable while entering and being transported in the aerosolized state.

Actually, only a small fraction, E, of the sprayed wastewater becomes aerosolized. The aerosolization efficiency factor, E, depends upon the type of spray equipment employed, the spray head pressure, and such meteorological conditions, r, as the ambient wind velocity and air temperature. Typical values of E are in the range of 0.001 to 0.01 (i.e., 0.1% to 1% of the sprayed wastewater). Thus, when the amount of wastewater actually aerosolized is taken into account, a much lower microbiological aerosol concentration, $D \times E$, is obtained.

Further impact changes, I, in microbiological aerosol concentration occur during entry into the aerosolized state. Originally it was assumed that these impact changes were reductions, varying in magnitude for different microorganism groups, g, and environmental conditions, r, that would result from the shock of entry into the inhospitable aerosol environment and from possible collection inefficiency in the aerosol samplers. However, the estimates of I to be presented later consistently exceed 1.0 for the hardy microorganisms (i.e., those microorganisms that survive best in the aerosol environment). As will be discussed later, the impact factor, I, is actually an empirical catchall for various microorganism-specific initial effects. With the impact factor contribution, the resulting aerosol concentration at the spray line is $D \times E \times I$.

Finally, there is gradual exponential die-off of microorganisms after aerosolization, $e^{\lambda a}$, which is presumed to occur at a constant decay rate, λ, with aerosol age. This microbiological die-off accumulates as a result of continued exposure to hostile environmental factors, such as solar radiation and low relative humidity, throughout the travel of the aerosol cloud. Taking this exponential die-off factor into account, the aerosol concentration curve for the complete predicted concentration, $P = D \times E \times I \times e^{\lambda a}$, is obtained. Adding the background concentration, B, to the predicted concentration, P, yields the sampled aerosol concentration, $C = P + B$.

The microbiological dispersion model equations (1) and (3) were used to estimate values of the model parameters, D, E, I, and λ for each appropriate microorganism group from the aerosol sampling

$$P = D \times E \times I \times e^{\lambda a}$$

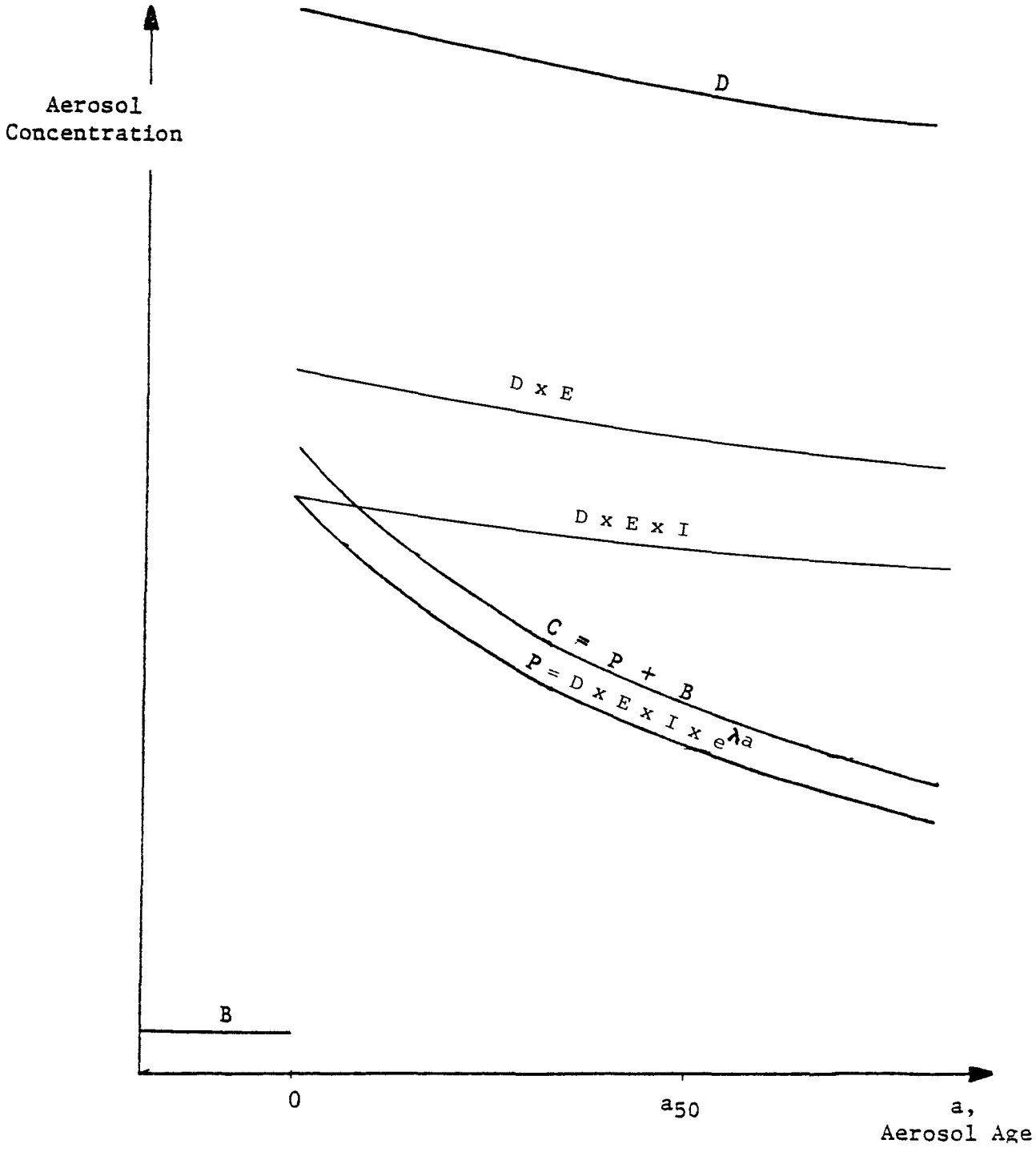


Figure VI.C-2.
SCHEMATIC OF EFFECTS OF MODEL FACTORS

program. The estimation procedures used and the model parameter estimates obtained are presented in the following three sections.

2. Diffusion Model Concentration D

The H. E. Cramer Company participated in the research effort and calculated the physical diffusion model terms, D, for each microorganism group at each sampler location during each run[43, 44] using their Volume-Source Diffusion Models Program. D is calculated using the total volume of sprayed wastewater and the measured wastewater concentration of each microorganism. Each sprayer along the sprayer line was modeled individually as a separate source. The diffused concentration contributions from each sprayer that reached a given location were then summed to compute D for that location.

a. Approach

Specific objectives of the H.E. Cramer Company work included:

- Calculations of the model concentrations, D, and the aerosolization efficiency, E, of the wastewater spray system for the Rhodamine WT dye tracer at each sampler during each dye aerosol run.
- Calculations of model concentrations, D, for each microorganism group sampled at each sampler during each microbiological, quality assurance, and special enterovirus aerosol run.
- Calculations of normalized concentration isopleth patterns downwind from the spray lines for each of the runs.

The concentrations, D, were calculated by means of a diffusion model under the assumptions that all of the material is aerosolized and that no material is lost by decay, gravitational deposition or other depletion processes (i.e., all aerosol particles are small enough to be treated as a gas having no settling velocity over the aerosol ages sampled). The model calculations were made by using the Volume-Source Diffusion Models Program developed for Dugway Proving Ground by the H.E. Cramer Company from the generalized models described by Cramer[45].

A mathematical description of the Volume-Source Diffusion Models Program is presented in Appendix A of the H.E. Cramer Company technical reports[46, 47]. In the model calculations, each spray head was treated as an individual source and the model was used to calculate the composite concentration pattern produced by the discharges from all spray heads in use during each aerosol run. Meteorological inputs used in the diffusion model calculations were presented in Table VI. B-1 of this report.

b. Source Inputs

The source geometry of the spray lines and the positions of the active spray lines relative to the samplers were based on field measurements and observations. The spray heads were spaced about 9.1 meters apart and were about 0.6 meters above the ground. The top of the wastewater spray cone from each head was observed to be about 5 meters above the ground and the radius of the spray circle produced by each head was about 9 meters.

Volume sources with the above dimensions and spacing were used to simulate the spray lines in the model. The height and radius of the spray cone were divided by 2.15 to obtain the initial source dimensions, $\sigma_{zR} = 2.0$ meters and $\sigma_{yR} = 4.2$ meters (the standard deviations of the vertical and the lateral aerosol distributions at the source). The source height H was assumed to be 0.6 meters above ground. The distance from the projected virtual point source over which rectilinear expansion was assumed was $X_{ry} = X_{rz} = 50$ meters. The separation distance between model volume sources was set equal to 9.1 meters, and the coordinates of the spray lines and downwind sampling locations were entered on the model calculation grid.

Estimates of the source strengths (emission rates) of the dye and various microorganism

groups contained in the spray were based on measurements of their wastewater concentrations during the run. To obtain emission rates for individual spray heads, these concentrations were multiplied by a flow rate. Flow-rate measurements were made for each spray head on the line directly upwind from the aerosol samplers at least once each time the spray line was moved to a new spray field. During some of the microbiological and virus aerosol runs, spray lines in other fields were operating and could have contributed to the measured concentrations at one or more of the samplers. Since flow-rate measurements were not made on these lines, the flow-rate data for those lines on other runs in the Post-Fair and Pre-Fair programs were used to estimate the flow rates for use in the model calculations.

The flow rate profiles used in the source strength calculations for the dye, microbiological and virus runs are given in Appendix C of the Cramer Pre-Fair report[48] and Appendices B, C, and D of the Cramer Post-Fair Reports[49].

c. Calculation Procedure and Model Concentrations

The Volume Source Diffusion Model was used with the meteorological, FP rotorod, and source inputs to produce concentration isopleth patterns for each trial. Concentrations were calculated for an array of grid points located at distances up to 1000 meters downwind from the main spray line. As noted above, grid points corresponding to the locations of the aerosol samplers were included in the array. Computer plots of the calculated concentration isopleths for the dye and microbiological trial runs are presented in Appendix B of the Cramer Pre-Fair report and in Appendices F and G of the Cramer Post-Fair report.

In this report, Figure VI. C-3 illustrates the dye concentration isopleths, D, obtained from the diffusion model for a typical dye aerosol run. The normalized isopleths calculated from the diffusion model are presented in Figures VI. C-4 and VI. C-5 for two of the microbiological aerosol runs. When these normalized isopleths are multiplied by the wastewater concentration of a microorganism group, the diffusion model microorganism aerosol concentration D is obtained.

Diffusion model concentrations, D, are presented and compared with the sampled concentrations C for each sampler on each run for each microorganism group in Appendix D of the Cramer Pre-Fair report and in Appendix E of the Cramer Post-Fair report. While the results varied considerably between runs, the diffusion model concentration D at 100 meters generally was from 50 to 80 percent of the model concentration D at 50 meters.

The model calculation procedure for the two special virus runs was modified slightly to conform to the enterovirus concentration, which was based on pooling the sample from all samplers over four and six successive 30-minute run sub-periods. The measured aerosol concentrations for the virus runs given in Table VI. B-24 thus represent an average over the sub-periods as well as over the 13 sampling locations. A similar averaging scheme was used to calculate the model concentrations. First, a set of normalized concentrations was calculated for each sub-period using model inputs derived from meteorological measurements made during the 30-minute sampling periods. The calculated concentrations for all samplers in all sub-trials were then averaged to obtain an average normalized concentration for the virus trial. Average aerosol concentrations for the enteroviruses and other microorganism groups were then obtained by multiplying the normalized concentration by their corresponding wastewater sample concentrations.

3. Aerosolization Efficiency Factor E

a. Dye Run Aerosolization Efficiency Estimates

An aerosolization efficiency estimate was computed for each dye aerosol run. Since the dye is not present in the background, and since it does not degrade significantly over the aerosol ages sampled, $B = 0$, $I = 1$, and $\lambda = 0$ for the dye runs. Thus, the aerosolization efficiency estimate at a dye run sampler location derived from the model equations (1) and (3) is:

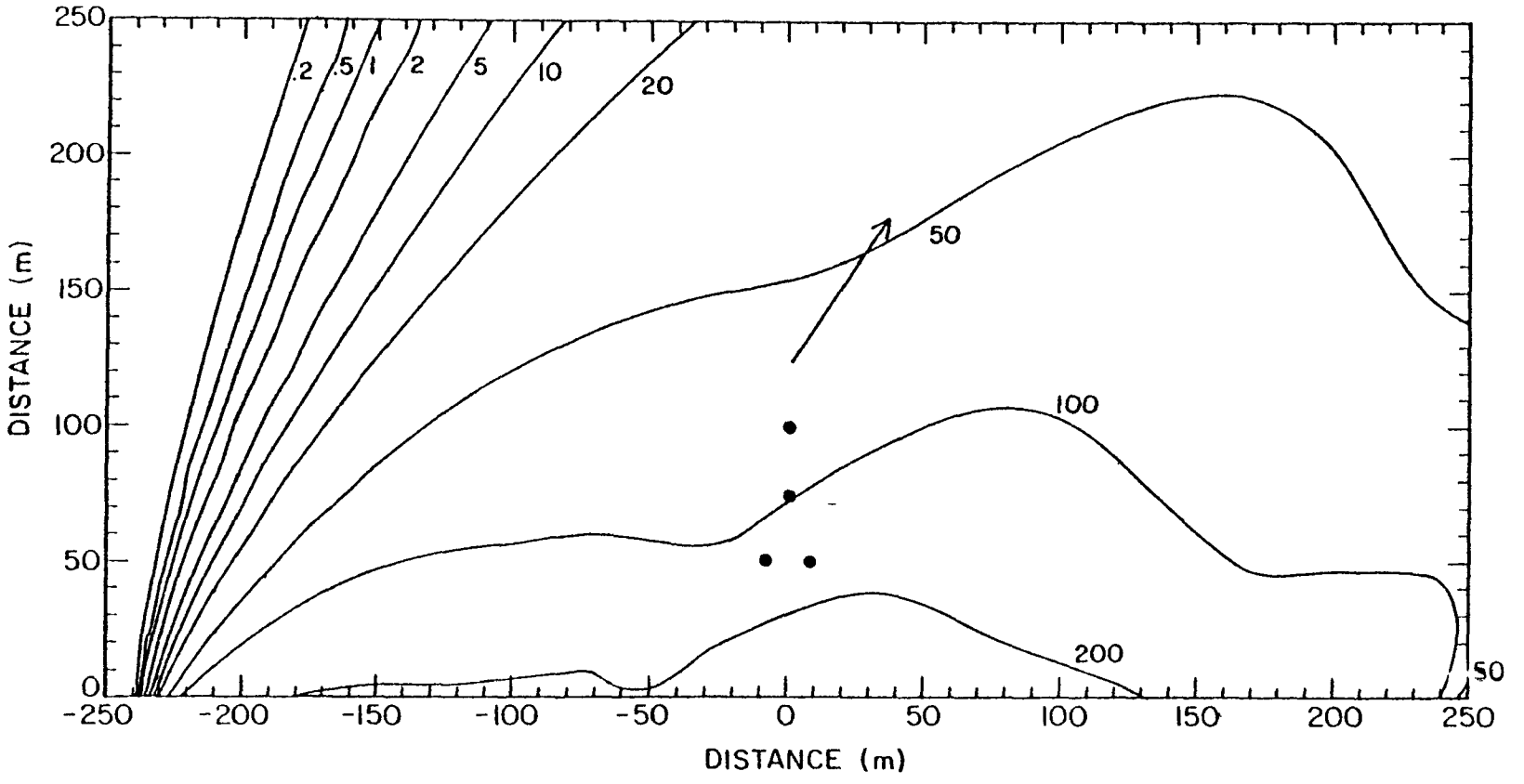


Figure VI.C-3.
CONCENTRATION ISOPLETHS OF DIFFUSION MODEL D FOR A TYPICAL DYE RUN

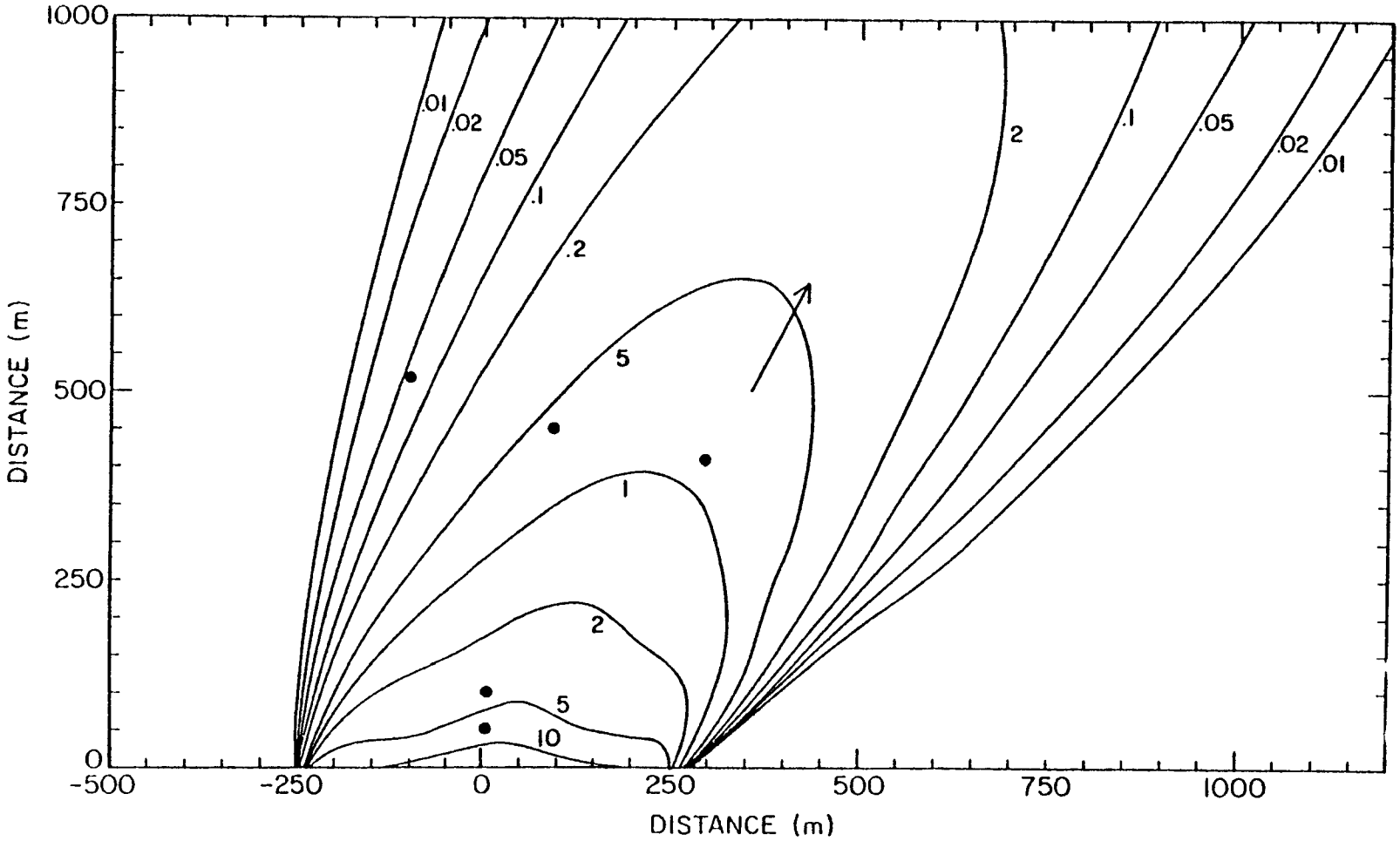


Figure VI.C-4.
NORMALIZED ISOPLETHS OF DIFFUSION MODEL D FOR A TYPICAL MICROBIOLOGICAL AEROSOL RUN

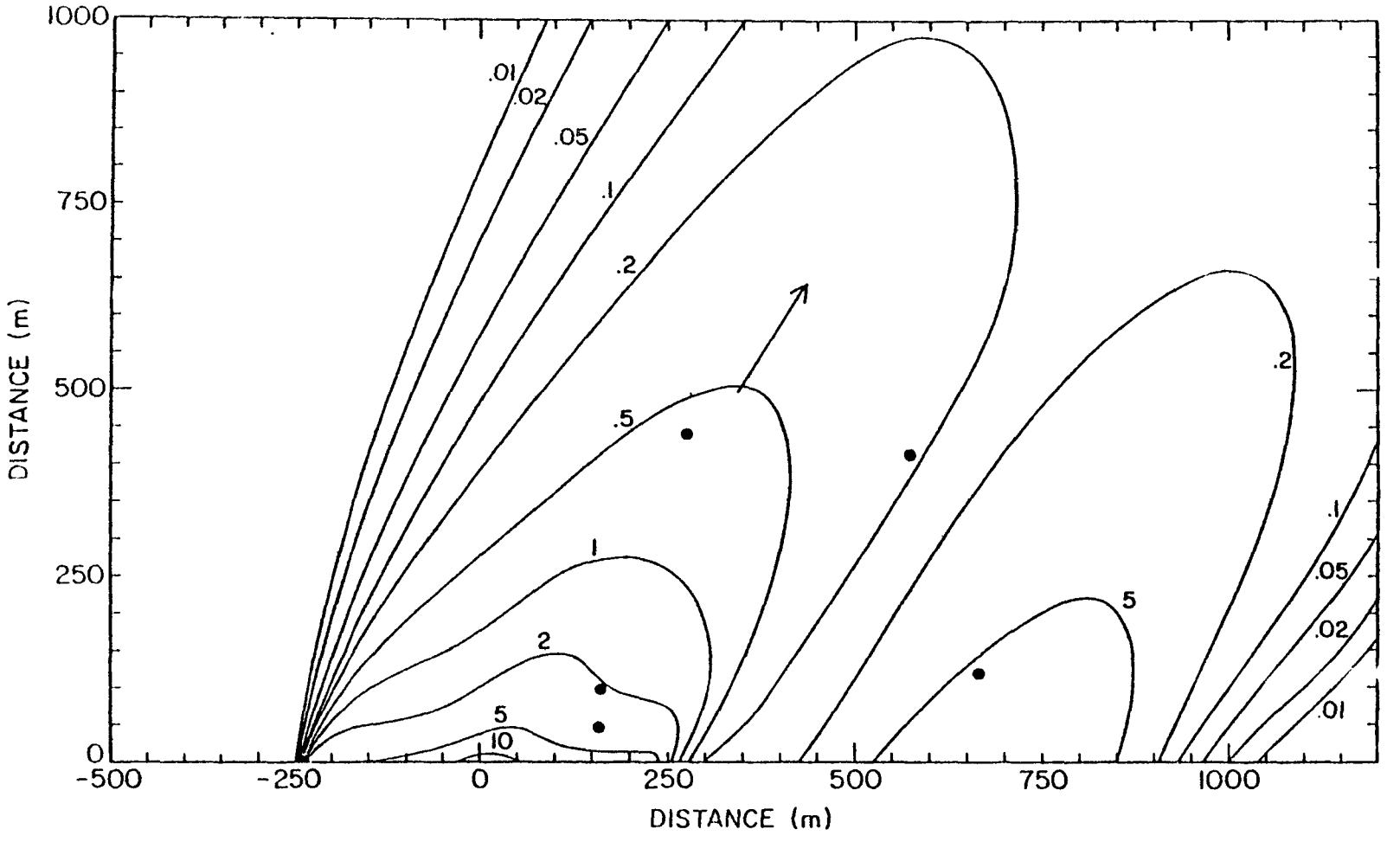


Figure VI.D-5.
NORMALIZED ISOPLETHS OF DIFFUSION MODEL D FOR A MICROBIOLOGICAL AEROSOL RUN HAVING TWO SPRAY LINE CONTRIBUTIONS

$$E = C/D \quad (4)$$

The H.E. Cramer Company computed aerosolization efficiency E values for each sampler on each dye run using equation (4). These sampler values are presented in Table D-1 of Appendix D of the Cramer Pre-Fair report and in Table E-1 of Appendix E of the Cramer Post-Fair report.

The geometric mean of the E values obtained at each sampler location beyond d = 5 meters from the wet-line edge was taken as the aerosolization efficiency estimate E for the dye run. These dye run estimates are presented in Table VI. C-1. Note that the aerosolization efficiency on the Pre-Fair dye runs conducted in late spring (May and June) were more variable and generally higher than the aerosolization efficiency for the Post-Fair dye runs during December and January.

The distribution of the 17 dye run estimates of aerosolization efficiency is summarized in Table VI. C-2. The median aerosolization efficiency value obtained at Pleasanton was 0.0033 (0.33%). There was over an order of magnitude of variation in E values from the tenth percentile (0.09% to the ninetieth percentile (1.8%). Thus, the fraction of wastewater that was aerosolized varied considerably from one run to another.

b. Microbiological Run Aerosolization Efficiency Predictions

To separate the effects of E and I during the microbiological aerosol runs conducted at Pleasanton, it was necessary to estimate E independent of the microbiological aerosol data. Thus, a regression equation was sought from the 17 dye runs to relate the E values occurring at Pleasanton to potentially relevant meteorological and operating conditions. The conditions considered were wind velocity, air temperature, relative humidity, solar radiation, wastewater temperature, spray head pressure, and seasonal bias.

Table VI.C-1.
ESTIMATES AND PREDICTIONS OF DYE RUN AEROSOLIZATION EFFICIENCY E

Dye Aerosol Runs		
Aerosol Run Number	Aerosolization Efficiency E	
	Dye Run Estimate	Regression Prediction
D1-1	.0272	.0172
D1-3	.0067	.0072
D1-4	.0019	.0022
D1-5	.0038	.0021
D1-6	.0067	.0040
D1-9	.0160	.0110
D1-10	.0059	.0168
D2-1	.0041	.0030
D2-2	.0062	.0047
D2-4	.0033	.0031
D2-5	.0023	.0023
D2-6	.0022	.0035
D2-7	.0021	.0027
D2-8	.0025	.0026
D2-9	.0014	.0013
D2-10	.0009	.0012
D2-11	.0008	.0011

Table VI.C-2.
DISTRIBUTION OF AEROSOLIZATION EFFICIENCY VALUES E

	Number of Values (Dye Runs)	Distribution of Values -- Percentiles (Median)				
		10%	25%	50%	75%	90%
E, Aerosolization Efficiency at Pleasanton	17	0.0009	0.0019	0.0033	0.0064	0.018

The potential regressor variables considered for inclusion in the aerosolization efficiency regression were:

UM	—	wind velocity at meteorological tower, 4 meter level
US	—	wind velocity in spray field, 3 meter level
P	—	spray head pressure, Pascals
PERIOD	—	1 = Pre-Fair, 2 = Post-Fair
TM	—	air temperature at meteorological tower, 2 meter level
TP	—	air temperature at effluent ponds, 2 meter height
RHM	—	relative humidity at meteorological tower, 2 meter level
RHU	—	relative humidity upwind, 2 meter height
RHL	—	relative humidity at Lawrence Laboratory in Livermore
WT	—	wastewater temperature at end of spray line, °C
R	—	solar radiation at meteorological tower, W/m^2
UMSQ	—	UM squared
USSQ	—	US squared
TMSQ	—	TM squared
TPSQ	—	TP squared
RSQ	—	R squared
RHMSQ	—	RHM squared
TMXRHM	—	product of TM and RHM
UMXP	—	product of UM and P
UMXTM	—	product of UM and TM
UMXR	—	product of UM and R

Most of these variables are environmental conditions whose values were given in Table VI.B-1. The average spray head pressure variable was calculated from Darcy's formula using the flow rate that was measured at the spray line for each run. Since the orifice diameters were the same for all spray heads, the expression for spray head pressure reduced to:

$$P = 21.028 \times (\text{Avg Spray Head Flow Rate})^2 \text{ Pascals}$$

where the flow rate is in liters per minute. PERIOD is a dummy variable to take into account any unmeasured systematic factors that differed between the late spring Pre-Fair dye runs and wintertime Post-Fair dye runs.

The dependent variable $\log_{10}E$ was utilized. Inspection of the dye run E values in Table VI.C-1 suggests the uncertainty in an E estimate may be proportional to the estimated value. Thus, regression on $\log_{10}E$ should yield a relatively constant error term.

Stepwise regression yielded numerous candidate regression equations involving a similar set of regressor variables, many of which were highly correlated with each other. Because its regressor variables were less correlated, the following regression equation was selected to predict the aerosolization efficiency E at Pleasanton over the wide range of meteorological conditions encountered:

$$\log_{10}E = 0.031\ t + 0.000096\ u{\bullet}r - 3.10 \qquad (5)$$

where t = air temperature, °C
u = wind velocity, m/s
r = solar radiation, W/m²

With a coefficient of multiple determination $R^2 = 0.801$, this regression explains 80 percent of the observed variation in $\log_{10}E$. The standard error SE = .194 implies that the true aerosolization efficiency E will be between 0.64 $\hat{E}$ and 1.56 $\hat{E}$ of the equation (5) estimate $\hat{E}$ for about two-thirds of the estimates.

Predicted values of aerosolization efficiency obtained using regression equation (5) are also provided in Table VI.C-1 for each dye run. The Pleasanton aerosolization efficiency predicted by regression was usually within a factor of two of the dye run estimates.

Using equation (5), predictions of the aerosolization efficiency were also made for each microbiological aerosol run. These predictions are presented in Table VI.C-3.

4. Impact Factor I and Aerosol Viability Decay Rate λ

a. Estimation Procedures for I and λ

(1) Standard Estimation Procedure

The values of I and λ for a microorganism group during a run were estimated jointly by simple linear regression using the aerosol concentrations obtained from each of the samplers. Substituting the equation (1) expression for P into equation (3), rearranging terms, and taking natural logarithms, one obtains

$$\ln\left(\frac{C_d - B}{D_d \cdot E}\right) = \ln I + \lambda a_d \tag{6}$$

This equation has the form of a simple linear regression model

$$y_d = b_o + b_1 a_d + \varepsilon_d \tag{7}$$

where values of the dependent variable, $y_d = \ln [(C_d - B)/(D_d \bullet E)]$, and of the independent variable, a_d= aerosol age, can be computed as an observation pair (y_d, a_d) for each downwind sampler at distance, d, that obtained a detectable microorganism concentration, C_d, above the background level, B. Since $b_0 = \ln I$ and $b_1 = \lambda$, the estimates of I and λ are readily calculated from the coefficient estimates for b_0 and b_1 resulting from the simple linear regression on the n observation pairs for the microorganism and run:

$$\begin{aligned} \hat{I} &= \exp(\hat{b}_o) \\ \hat{\lambda} &= \hat{b}_1 \end{aligned} \tag{8}$$

where $\hat{b}_1 = \dfrac{\Sigma a_d y_d - \Sigma y_d \Sigma a_d / n}{\Sigma a_d^2 - (\Sigma a_d)^2 / n}$ $\qquad$ $\hat{b}_o = \bar{y} - \hat{b}_1 \bar{a}$

The uncertainty in the equation (8) estimates of I and λ for a microorganism group on a run can be estimated from the deviation of the y_d values about the linear regression equation (7) estimates $\hat{y}_d$. Using the expressions for the standard errors of the regression coefficients[50] s.e($\hat{b}_0$) and s.e($\hat{b}_1$), and the usual variance functional transformation[51],

$$\mathrm{Var}(y) \approx \left(\frac{dy}{dx}\right)^2_{x = E(x)} \cdot \mathrm{Var}(x)$$

the standard errors of the estimators $\hat{I}$ and $\hat{\lambda}$ are

$$s.e.(\hat{I}) \approx \hat{I}\, s.e.(\hat{b}_o) = \hat{I}\sqrt{\frac{\Sigma a_d^{\,2}}{n\Sigma(a_d - \bar{a})^2}}\sqrt{\frac{\Sigma(y_d - \hat{y}_d)^2}{n-2}} \qquad (9)$$

$$s.e.(\hat{\lambda}) = s.e.(\hat{b}_1) = \frac{1}{\sqrt{\Sigma(a_d - \bar{a})^2}}\sqrt{\frac{\Sigma(y_d - \hat{y}_d)^2}{n-2}}$$

The standard estimation procedure was to jointly estimate I, λ, and their standard errors for each microorganism group on each run using equations (8) and (9). This procedure was applied if

Table VI.C-3.
REGRESSION PREDICTION OF AEROSOLIZATION EFFICIENCY E FOR THE MICROBIOLOGICAL AEROSOL RUNS

Microbiological Aerosol Runs			
Aerosol Run Number	Regression Prediction E	Aerosol Run Number	Regression Prediction E
M1-1	.0072	M2-1	.0018
M1-2	.0057	M2-2	.0021
M1-3	.0039	M2-3	.0012
M1-4	.021	M2-4	.0017
M1-5	.0091	M2-5	.0015
M1-6	.0024	M2-6	.0023
M1-7	.0016	M2-8-9	.0027
M1-8	.0067	M2-10	.0015
M1-9	.0058	M2-11	.0034
M1-10	.0019	M2-12	.0030
M1-11	.0024	M2-13	.0012
M1-12	.0041	M2-14	.0018
M1-13	.0017	M2-15	.0028
M1-14	.0016	M2-16	.0024
M1-15	.0016	M2-17	.0025
M1-16-19	.0016	M2-18-21	.0019
M1-20-23	.0035	M2-22	.0066
M1-24-25	.0018	M2-23	.0065
M1-26	.0018	M2-24	.0050
M1-27-30	.0096	M2-25	.0092
M1-31	.0060	M2-26	.0054
M1-32	.024	M2-27-28	.0058
M1-33	.021	M2-29	.0074
M1-34	.0036	M2-30	.0085
M1-35	.0024	M2-31	.0015
		M2-32	.0021
		M2-33	.0074
		M2-34	.0025
		M2-35	.0132
		M2-36	.0068
		M2-37	.0079
		M2-38	.0050
		V2-I	.0028
		V2-II	.0045

there were more than n = 2 valid observation pairs for the microorganism group at two or more distances on the aerosol run, if the viability decay rate estimate from equation (8) was negative ($\hat{\lambda} < 0$), and if each of the observation pairs were considered to have roughly equivalent weight.

(2) Special Estimation Procedures

For the hardier microorganism groups, joint estimation of I and λ by equations (8) often yielded a positive value for the viability decay rate λ. A positive λ implies growth rather than die-off of the microorganism with lengthening exposure to the hostile aerosol environment. Significant net growth in aerosols over durations up to several minutes is highly unlikely. Thus, positive λ values probably result from the substantial uncertainty error in the model equations (1) and (3) in those situations when the actual decay rate was too slight to be detected. This uncertainty error is attributable to measurement variation, localized microbial aerosol sources, and estimation errors in D and E. Accordingly, positive λ values obtained by equations (8) were considered indistinguishable from $\lambda = 0$ and denoted as $\lambda = X$

Substituting $\lambda = 0$ in equation (6) yields a distinct value $I_d = (C_d - B)/(D_d \bullet E)$ for each downwind sampler. The geometric mean of these downwind sampler values was taken as the impact factor I estimate when the λ estimate was considered indistinguishable from zero:

$$\hat{\lambda} = X$$
$$\hat{I} = \text{Geometric Mean } (I_d) = \exp(\bar{y}) \quad \text{over all d} \tag{10}$$

Assuming $\lambda = 0$ were known, the standard error of the equation (10) $\hat{I}$ is $\text{s.e.}(\hat{I}) \approx \hat{I}\, s/\sqrt{n}$ where $s^2 = \Sigma(y_d - \bar{y})^2/(n - 1)$. Because λ is actually indeterminate in such cases, the $\hat{I}$ standard error is probably larger than this, and may be better reflected by the equation (9) estimator. Thus, when joint estimation by equations (8) yield a positive λ estimate, equations (10) were used to estimate I and λ and the standard error of $\hat{I}$ was estimated as

$$\text{s.e.}(\hat{I}) = \text{Max}\,[\hat{I}s/\sqrt{n}, \hat{I}\,\text{s.e.}(b_o)] \tag{11}$$

When there were only n = 2 observation pairs per run, and when the observation pairs did not have roughly equivalent weight, variants of the standard joint estimation procedure [equations (8) and (9)] and the indeterminate decay rate estimation procedure [equations (10) and (11)] were used. For n = 2, equation (6) represents n = 2 equations in two unknowns (I and λ). $\hat{I}$ and $\hat{\lambda}$ were estimated as the exact solution of these simultaneous equations. When $\hat{\lambda}$ was positive, equations (10) and (11) with n = 2 were used for the estimation.

Because the sampling designs used spread the samplers farther apart at the greater distances, d, the observation at the most distant of the designated downwind samplers usually had the greatest effect on the estimated viability decay rate, λ. However, the concentration, C_d, at this sampler was occasionally below the detection limit and, thus, arbitrarily close to the background concentration, B. On such a regression, the most distant observation y value had considerably greater uncertainty than did the others and should hence receive less weight. In these cases, a weighted regression estimation procedure was used[52]. The weighted simple linear regression model is

$$\sqrt{w_d}\,y_d = b_0\sqrt{w_d} + b_1\sqrt{w_d}\,a_d + \sqrt{w_d}\,\epsilon$$

where the observation, d, weight, w_d, was based on the number of CFUs (colony forming units) obtained during analysis of the aerosol sample:

CFUs Found	Raw Weight
0	0.125
1	0.5
2	0.75
3	0.9
≥4	1.0

The raw weights were standardized so that $\Sigma w_d = n$. The estimation procedure can still be represented by equations (8) and (9) when $\hat{\lambda} < 0$ and by equations (10) and (11) when the equations (8) λ were positive, provided each summation in these equations is replaced by a weighted summation [e.g., $\Sigma a_d y_d$ becomes $\Sigma w_d a_d y_d$, Σy_d becomes $\Sigma w_d y_d$, and $\Sigma(y_d - \hat{y}_d)^2$ becomes $\Sigma w_d(y_d - \hat{y}_d)^2$].

b. Impact Factor I

The estimated impact factor $\hat{I}$ and its standard error s.e. $(\hat{I})$ were obtained for each microorganism group on each aerosol run by applying the appropriate estimation procedure to the data of Tables VI.B-8 through VI.B-15. The run estimates of $\hat{I}$ and s.e.$(\hat{I})$ are presented in Table VI.C-4.

Comparison of an individual impact factor estimate $\hat{I}$ against its corresponding standard error s.e.$(\hat{I})$ throughout Table VI.C-4 shows there is considerable uncertainty in the individual impact factor estimates. The standard error is generally of the same order of magnitude as the impact factor estimate. Considering the sizable measurement variation in the aerosol concentrations C (cf. Section VI.B.4.b), the large

Table VI.C-4.
RUN ESTIMATES OF MICROORGANISM GROUP IMPACT FACTOR I AND STANDARD ERROR SE(I)
a. Pre-Fair Runs

Aerosol Run Number	Standard Plate Count		Total Coliform		Fecal Coliform		Coliphage		Fecal Streptococci		Pseudomonas		Clostridium Perfringens	
	$\hat{I}$	SE($\hat{I}$)	$\hat{I}$	SE($\hat{I}$)	$\hat{I}$	SE($\hat{I}$)	$\hat{I}$	SE($\hat{I}$)	$\hat{I}$	SE($\hat{I}$)	$\hat{I}$	SE($\hat{I}$)	$\hat{I}$	SE($\hat{I}$)
M1-1	2.0	4.3												
M1-2	.357	.082	.046	.014	.126	.051								
M1-3	.25	.13	.048	.014	.062	.095	.0077	.0040						
M1-4			.028	.074							1.07	.78		
M1-5	.022	.010	.84	.29	.16	.16	.0006	.0012			14	14	1.22	.28
M1-6	.34	.13	.153	.056	.13	.10	1.18	.30	3.2	3.9	40	92	4.9	1.8
M1-7	.347	.054	.053	.038	.073	.015	.094	.052			210			
M1-8	.23	.28	.13	.13			.62	.33			5.1	1.2	.34	.19
M1-9	.089	.063	.040	.021	.088	.023	.134	.077	.64	.45	2.7	3.7	.237	.079
M1-10	.20	.15	.59	.20	1.19	.70	1.53	.87	2.65	.51	52	28	.62	.31
M1-11	.139	.033	1.08	.86	.54	.35	.277	.073	4.4	2.8	2.2	1.0	.064	.021
M1-12	.21	.13	.12	.12			.38	.13	21.2	5.3			7.3	4.1
M1-13	1.48	.46	.252	.042			1.42	.30	7	11	400	610	6.5	3.3
M1-14			.57	.29			1.34	.56	70	72			6.0	7.0
M1-15			.102	.023	.62	.49	.17	.10	25.8	6.9	1.14	.89	7.2	5.4
M1-31	.71	.14	.084	.066	.091	.047	.33	.53	.26	.12	1.09	.19	.736	.049
M1-32	.21	.13	.0037	.0010	.016	.018	.031	.045						
M1-33	.163	.077	.013	.012			.17	.47	.101	.028				
M1-34			1.1	1.8	.048	.035			1.9	4.1	26.3	9.5		
M1-35	.18	.12	1.06	.23	2.9	1.9	1.00	.61	6.1	1.9	94	62		

uncertainty in the impact factor estimates $\hat{I}$ is not surprising. The ratio of the estimate $\hat{I}$ to its standard error s.e.($\hat{I}$) has the t distribution with n-2 degrees of freedom, where n is the number of observation pairs regressed for the microorganism on the run. Table VI.C-5 summarizes these t statistics, $t = \hat{I}/s.e.(\hat{I})$, by microorganism group. For most microorganism groups, less than half of the impact factor estimates exceed twice their standard error. The exception is the total coliform $\hat{I}$ estimates, 62 percent of which exceed twice their standard error. Hence, the individual impact factor estimates generally have low reliability. However, the majority of $\hat{I}$ estimates (ranging from 63 percent for coliphage to 91 percent for *Clostridium perfringens*) do exceed their standard error. Thus, aggregating the individual run $\hat{I}$ estimates for a microorganism group as an I-value distribution for the microorganism group should provide a good representation of the central tendency of I for the microorganism group and a fair representation of its dispersion under different run conditions. The empirical distribution of these impact values for each microorganism is presented in Table VI.C-6.

The microorganism groups are arranged in Table VI.C-6 according to the magnitude of their median and quartile impact factor values. The microorganisms differ substantially with respect to their middle range of impact factor values. The "indicator" microorganism groups—total coliform, fecal coliform, standard bacterial plate count, and coliphage—all had a similar middle range of impact factor values. The

Table VI.C-4.
RUN ESTIMATES OF MICROORGANISM GROUP IMPACT FACTOR I AND STANDARD ERROR SE(I)
b. Post-Fair Runs

Aerosol Run Number	Standard Plate Count		Total Coliform		Coliphage		Fecal Streptococci		Mycobacteria		Enteroviruses	
											3-Day	3 & 5 Day
	$\hat{I}$	SE($\hat{I}$)	$\hat{I}$	SE($\hat{I}$)	$\hat{I}$	SE($\hat{I}$)	$\hat{I}$	SE($\hat{I}$)	$\hat{I}$	SE($\hat{I}$)	$\hat{I}$	$\hat{I}$
M2-1	.35	.14	22.2	3.3	.163	.044	.8	2.5				
M2-2			.08	.11	1.2	1.2	.84	.61				
M2-3	.11	.11	1.05	.12	.68	.81	34	14				
M2-4			.47	.19	2.0	.36	9.4	6.2				
M2-5			.225	.044	.17	.10	.97	.10				
M2-6	.8	3.1	.573	.046			.52	.49				
M2-10			.148	.064	.34	.22						
M2-11			.143	.067	.34	.38						
M2-12	.2	1.1	.032	.090	.18	.12	1.7	2.4				
M2-13			.162	.053	.91	.16	3.0	2.4				
M2-14			1.97	.44	2.8	1.4	2.3	1.1				
M2-15	.102	.085	.143	.053	.45	.90	.46	.23				
M2-16	.055	.059	.184	.061	.72	.19	.71	.21				
M2-17	.25	.16	.175	.052	.35	.16	1.31	.40				
M2-22	.069	.066	.104	.071	3.6	5.1						
M2-23	.19	.10			.016	.038						
M2-24	.288	.003	.20	.34	.74	.85	.283	.088				
M2-25	.18	.13	.24	.18	.62	.51	5.5	1.9				
M2-26			.30	.13								
M2-29	.217	.072			.70	.56			2.5	8.7		
M2-30			.0110	.0054	.061	.071			.14	.11		
M2-31	.209	.072	.130	.023	.45	.82	1.0	1.2	.83	.76		
M2-32	.025	.022	.0161	.0018	12	20			1.15	.88		
M2-33					.018	.017	.12	.11				
M2-34	1.4	2.1			.071	.025			.76	.53		
M2-35	.053	.055	.0160	.0037	.0635	.0039	.73	.68	31	24		
M2-36			.32		.014	.015			.95	.51		
M2-37			.281	.154	.08	.10	1.10	.77	.81	.19		
M2-38	.88	.16	1.31	.88	.101	.027	59	26				
V2-I											44	69
V2-II											<4.7	24

inter-quartile ranges were (0.06—0.6) for total coliform, (0.07—0.6) for fecal coliform, (0.1—0.4) for standard bacterial plate count, and (0.09—0.9) for coliphage. It is important to note that all of the pathogenic bacteria and virus groups had substantially higher middle ranges of I than did these indicator microorganisms. The bacterial pathogen impact factor middle ranges extended from (0.8—2) for mycobacteria, (0.2—7) for *Clostridium perfringens*, and (0.7—6) for fecal streptococci to (2—70) *Pseudomonas*. While only two impact factor estimates were obtained for each enterovirus group, the enteroviruses appear to have a middle range at least as high as any bacterial pathogen evaluated. The five-day enteroviruses (when polioviruses were not suppressed) appear to have a middle range that is more than 100 times as high as any indicator microorganism group.

Frequent impact factor values exceeding 1.0 were not expected for any microorganism group. However, over half of the I estimates for enteroviruses, *Pseudomonas*, fecal streptococci, and *Clostridium perfringens* exceeded 1.0. Possible explanations for the consistent occurrence of pathogen I values above 1.0 are presented and discussed in Section VII.C.

The impact factor estimates I for most microorganism groups exhibited about two orders of magnitude of variation in value between their tenth and ninetieth percentiles on the Pleasanton runs. The standard bacterial plate count impact values were somewhat more consistent than those of the more specific microorganism groups.

c. Viability Decay Rate λ

The individual viability decay rate estimates $\hat{\lambda}$ and their standard errors s.e.($\hat{\lambda}$) calculated for

Table VI.C-5.
RELIABILITY OF IMPACT FACTOR ESTIMATES I

Microorganism Group	No. of ($\hat{I}$, s.e.($\hat{I}$)) Estimates	Percentage of Estimates Exceeding Two Std. Errors t>2	Std. Error t>1
Std. Bacterial Plate Count	33	39%	76%
Total Coliform	42	62%	86%
Fecal Coliform	13	23%	77%
Coliphage	43	30%	63%
Fecal Streptococci	31	45%	77%
Pseudomonas	12	33%	67%
Clostridium perfringens	11	45%	91%
Mycobacteria	8	13%	88%

Note: $t = \hat{I}/s.e.(\hat{I})$

Table VI.C-6.
DISTRIBUTIONS OF AEROSOL IMPACT FACTOR, I

Impact Factor, I	Number of Estimates (Aerosol	Distribution of Values - Percentiles										
Microorganism Group	Runs)	5%	10%	25%		40%	50%	60%		75%	90%	95%
Fecal Coliform	13		.029	.068		.090	.13	.15		.58	2.0	
Total Coliform	44	.012	.016	.060		.13	.16	.23		.55	1.1	1.6
Std. Bacterial Plate Count	33	.021	.036	.11		.19	.21	.24		.35	1.2	1.7
Coliphage	43	.009	.017	.094		.18	.34	.52		.91	1.8	3.4
Mycobacteria	8		~.13	.77		.82	.89	1.0		2.1	34	
Clostridium perfringens	11		.085	.24		.71	1.2	5.1		6.5	7.3	
Fecal Streptococci	31	.11	.27	.71		.97	1.7	2.7		6.1	32	64
Pseudomonas	13		1.1	1.7	(33%)	4.1	14	32	(67%)	73	320	
3-Day Enteroviruses	2				<4.7		~10		44			
5-Day Enteroviruses	2				24		~40		68			

~x -- Interpolated or extrapolated value x.

each microorganism and run from the data of Tables VI.B-8 through VI. B-15 are presented in Table VI.C-7. The percentage of indeterminate viability decay rates ($\hat{\lambda}$ =X) in Table VI.C-7 is summarized in Table VI.C-8. For all microorganism groups except total and fecal coliform, indeterminate decay rates were prevalent. We interpret these indeterminate values $\hat{\lambda}$ =X as reflecting a very slight (negative) decay rate which could not be estimated because of the large uncertainties in the aerosol concentrations C and the background concentration B, relative to the limited range of sampled aerosol ages. Their prevalence suggests that many of the estimated negative decay rates may also be indistinguishable from no decay ($\lambda = 0$).

For the estimated negative decay rates, their t statistics, $t = \hat{\lambda}/s.e.(\hat{\lambda})$, are summarized by microorganism group in Table VI.C-9. Only for total coliform and fecal coliform do more than half of the negative viability decay rate estimates exceed twice their standard error. However, over 60 percent of the estimates exceed one standard error for all frequently estimated groups.

The distribution of the Table VI.C-7 viability decay rates for each microorganism group is presented in Table VI.C-10. The indeterminate viability decay rates are indicated by an X in Table VI.C-10. Question marks have been placed in parentheses after the smallest negative viability decay rates, since the prevalence of X's suggests these values may also be indistinguishable from $\lambda = 0$.

Based on their viability decay rates, the microorganism groups seem to fall into three categories. Total coliform and fecal coliform exhibit the most rapid decay and their decay rates could most frequently be estimated. Furthermore, based on their t statistics, the viability decay rate estimates for total and fecal coliform are also more reliable than for the other microorganism groups. Viability decay appeared to occur on about 50 percent of the runs for coliphage, *Clostridium perfringens* and standard bacterial plate county; their rates of decay were also slower than the coliform decay rates. Decay with aerosol age could seldom be detected for mycobacteria, *Pseudomonas*, and fecal streptococci.

For all microorganism groups, the upper portion of the λ distribution, which represents the

Table VI.C-7.

RUN ESTIMATES OF MICROORGANISM GROUP VIABILITY DECAY RATE λ AND STANDARD ERROR SE(λ), s^{-1}

a. Pre-Fair Runs

Aerosol Run Number	Standard Plate Count		Total Coliform		Fecal Coliform		Coliphage		Fecal Streptococci		Pseudomonas		Clostridium Perfringens	
	$\hat{\lambda}$	SE($\hat{\lambda}$)	$\hat{\lambda}$	SE($\hat{\lambda}$)	$\hat{\lambda}$	SE($\hat{\lambda}$)	$\hat{\lambda}$	SE($\hat{\lambda}$)	$\hat{\lambda}$	SE($\hat{\lambda}$)	$\hat{\lambda}$	SE($\hat{\lambda}$)	$\hat{\lambda}$	SE($\hat{\lambda}$)
M1-1	−.19	.13												
M1-2	X		X		−.032	.020								
M1-3	−.014	.010	−.0316	.0066	−.023	.070	X							
M1-4			−.33	.44							X			
M1-5	−.018	.030	−.383	.023	−.227	.079	X				X		X	
M1-6	X		−.062	.019	X		−.108	.088	X		−.02	.12	X	
M1-7	X		−.003	.013	X		X				−.003			
M1-8	−.042	.036	−.089	.039			−.051	.021			X		X	
M1-9	−.134	.070	−.025	.032	−.072	.017	X		−.081	.046	X		−.118	.047
M1-10	−.165	.070	−.094	.023	−.144	.039	−.065	.041	X		X		−.039	.052
M1-11	−.0050	.0038	−.077	.014	−.068	.017	−.0078	.0049	−.022	.017	−.005		X	
M1-12	X		−.093	.042			−.022	.016	X				−.048	.037
M1-13	X		X				−.008	.011	−.039	.15	−.17	.13	X	
M1-14			−.025	.013			−.016	.011	X				−.020	.049
M1-15			X		−.021	.012	X		X		X		−.018	.017
M1-31	X		−.0081	.0028	−.0073	.0019	−.0037	.0078	X		X		−.0036	.0002
M1-32	X		X		X		−.15	.13						
M1-33	−.037	.037	X				−.19	.25	X					
M1-34			−.095	.043	X				X		X			
M1-35	−.004	.017	−.0497	.0053	−.063	.015	−.028	.014	X		−.014	.015		

X Viability decay rate estimate was positive.

slower die-off rates, cannot be quantified based on the Pleasanton study. Table VI.C-10 suggests that $-0.01s^{-1}$ to $-0.02s^{-1}$ was the lowest viability decay rate which could be detected at Pleasanton. For the hardier and infrequently measured microorganisms, such as mycobacteria, *Pseudomonas,* and fecal streptococci, perhaps $-0.06s^{-1}$ was the lowest detectable viability decay rate.

Because λ is an exponential multiplier, $e^{2\lambda a} = (e^{\lambda a})^2$; doubling the decay rate λ squares the exponential decay factor. Thus, when considering sizable aerosol ages, even differences in λ of a factor of two have a substantial effect. Using an average wind speed of 4 m/s, the median viability decay of total coliform per 100 meters (25s) is $\exp(-.032s^{-1} \times 25s) = 0.45$, slightly more than a two-fold reduction per 100 meters. The quartile viability decay of total coliform per 100 meters is $\exp(-.094s^{-1} \times 25s) = 0.10$, a ten-fold reduction per 100 meters. The corresponding decay rates for coliphage (a median 30 percent reduction per 100 meters and a quartile 3-1/2 fold reduction per 100 meters) are substantially less. Consequently, Table VI.C-10 indicates that age decay is considerably more rapid and prevalent for the coliforms than for coliphage, *Clostridium perfringens*, and standard bacterial plate count. The pathogens fecal streptococci, *Pseudomonas*, and mycobacteria seldom exhibited detectable die-off with aerosol age.

Table VI.C-7.
RUN ESTIMATES OF MICROORGANISM GROUP VIABILITY DECAY RATE λ AND STANDARD ERROR SE(λ), s^{-1}
b. Post-Fair Runs

Aerosol Run Number	Standard Plate Count		Total Coliform		Coliphage		Fecal Streptococci		Mycobacteria	
	$\hat{\lambda}$	SE($\hat{\lambda}$)	$\hat{\lambda}$	SE($\hat{\lambda}$)	$\hat{\lambda}$	SE($\hat{\lambda}$)	$\hat{\lambda}$	SE($\hat{\lambda}$)	$\hat{\lambda}$	SE($\hat{\lambda}$)
M2-1	−.0044	.0039	−.0757	.0026	X		−.015	.048		
M2-2			−.014	.025	−.030	.020	X			
M2-3	X		−.0162	.0011	−.031	.024	X			
M2-4			−.0253	.0076	−.0397	.0038	−.067	.014		
M2-5			−.0082	.0038	X		X			
M2-6	−.022	.040	−.0393	.0008			X			
M2-10			X		X					
M2-11			−.033	.017	−.048	.041				
M2-12	X		−.02	.10	X		X			
M2-13			X		X		X			
M2-14			−.1137	.0079	−.055	.018	X			
M2-15	X		−.0197	.0068	−.019	.038	X			
M2-16	−.006	.027	−.0394	.0086	X		−.0062	.0078		
M2-17	X		−.0379	.0069	X		X			
M2-22	−.007	.033	−.083	.022	−.116	.050				
M2-23	X				−.008	.070				
M2-24	−.1069	.0004	−.133	.053	−.061	.033	X			
M2-25	−.090	.031	−.158	.036	−.119	.035	−.174	.017		
M2-26			−.250	.020						
M2-29	X				−.089	.023			−.009	.091
M2-30			X		X				X	
M2-31	−.0185	.0034	−.0015	.0005	−.011	.020	X		X	
M2-32	X		X		−.091	.037			X	
M2-33					X		X			
M2-34	−.010	.024			X				X	
M2-35	X		−.0077	.0032	−.0329	.0033	X		−.134	.042
M2-36			−.17		X				X	
M2-37			−.214	.039	−.042	.081	X		X	
M2-38	−.0032	.0027	−.237	.028	X		−.0127	.0068		

X Viability decay rate estimate was positive.

Table VI.C-8.
PERCENTAGE OF INDETERMINATE VIABILITY DECAY RATE ESTIMATES ($\hat{\lambda} = X$)

Microorganism Group	Number of $\hat{\lambda}$ Estimates	Percentage of Indeterminate Estimates ($\hat{\lambda} = X$)
Std. Bacterial Plate Count	33	45%
Total Coliform	44	20%
Fecal Coliform	13	31%
Coliphage	43	40%
Fecal Streptococci	31	74%
Pseudomonas	13	62%
Clostridium perfringens	11	45%
Mycobacteria	8	75%

Table VI.C-9.
RELIABILITY OF NEGATIVE VIABILITY DECAY RATE ESTIMATES $\hat{\lambda} < 0$

Microorganism Group	No. of Negative ($\hat{\lambda}$, s.e.($\hat{\lambda}$)) Estimates	Percentage of Negative Estimates Exceeding	
		Two Std. Errors $\lvert t \rvert > 2$	Std. Error $\lvert t \rvert > 1$
Std. Bacterial Plate Count	18	2%	61%
Total Coliform	34	9%	85%
Fecal Coliform	9	7%	89%
Coliphage	26	1%	73%
Fecal Streptococci	8	3%	75%
Clostridium perfringens	6	3%	67%

Note: $t = \hat{\lambda}/\text{s.e.}(\hat{\lambda})$.

Table VI.C-10.
DISTRIBUTIONS OF VIABILITY DECAY RATE λ, s^{-1}

Viability Decay Rate λ, s^{-1}	Number of Estimates (Aerosol Runs)	Distribution of Values - Percentiles									
Microorganism Group		5%	10%		25%	40%	50%	60%	75%	90%	95%
Total Coliform	44	-.31	-.23		-.094	-.050	-.032	-.020	-.004(?)	X	X
Fecal Coliform	13		-.19		-.070	-.045	-.023	-.016(?)	X	X	X
Coliphage	43	-.14	-.11		-.051	-.029	-.011	X	X	X	X
Clostridium perfringens	11		-.10		-.039	-.019	-.004(?)	X	X	X	
Std. Bacterial Plate Count	33	-.17	-.12		-.020	-.006	-.004(?)	X	X	X	X
				(20%)							
Mycobacteria	8		~-.15	-.027(?)	-.009(?)	X	X	X	X		
Pseudomonas	13		-.077	-.016(?)	-.008(?)	X	X	X	X	X	
Fecal Streptococci	31	-.12	-.060(?)	-.014(?)	-.006(?)	X	X	X	X	X	X

X -- Indeterminate viability decay rate.

~v -- Extrapolated value v.

v(?) -- Questionable value, perhaps indistinguishable from 0.

5. Prediction Using the Microbiological Dispersion Model

Equation (3) and the data from Tables VI.C-6 and VI.C-10 define the microbiological dispersion model. Considerations involved in using this model to predict the microbiological aerosol concentrations P downwind of any spray irrigation site are discussed in this section.

a. Usage Considerations

(1) Assumptions

In developing and using the microbiological dispersion model, several assumptions are made.

- The major physical and biological processes that affect microorganism aerosol levels emanating from a sprayed wastewater source are adequately represented by the multiplicative form of the microbiological dispersion model equation (3) out to distances of 500 meters to 1000 meters (aerosol ages of 100 seconds to 500 seconds) from the spray location.
- The die-off of a microorganism group that occurs during aerosolization and transport in the aerosol state is caused by factors such as meteorological conditions that have the same effect at any spray irrigation site. Thus, a given level of the controlling factors will produce the same reduction in viable aerosolized microorganisms (i.e., yield the same values of the parameters I and λ) at any spray site.
- The microbiological aerosol concentrations obtained in field studies vary somewhat depending on the sampling, shipping, and assay procedures employed. Since the distributions of the microbiological parameters I and λ were derived from the Pleasanton study, the concentrations P predicted by the model assume the use of the methods of the Pleasanton study (high volume electrostatic precipitator samplers, special and standard assay methods, etc.).

(2) Procedure

Several steps are involved in the process of predicting microorganism aerosol concentrations P in the wastewater aerosol downwind from a spray irrigation site under specified meteorological conditions using the microbiological dispersion model.

- *Microorganism Wastewater Concentrations.* Select the prevalent and relevant microorganism group(s) and determine typical microorganism concentrations in the wastewater.
- *Diffusion Model Aerosol Concentration D.* Apply an appropriate atmospheric dispersion model to project each microorganism wastewater concentration to its corresponding diffused aerosol concentration at the downwind location. Validated atmospheric dispersion models of varying sophistication[50,54,55,56,57] are available to calculate D. Model input generally includes the configuration of sprayers, spray trajectory, wastewater spray rate, the microorganism wastewater concentrations, pertinent meteorological conditions for the case considered, the local topography, and the distance to the downwind location. Usually the centerline (peak) concentration at the downwind distance is computed as D.

- *Aerosolization Efficiency E.* Select an aerosolization efficiency estimate for the case considered based on the type of spray equipment, spray head pressure, and pertinent meteorological factors. Once a median estimate of E is developed for a spray site, equation (5) may be used to help select an E value for the case based on the specified meteorological conditions.
- *Impact Factor I and Viability Decay Rate λ.* For each microorganism group, select the proper I and λ percentile values from their distributions given in Tables IV.C-6 and IV.C-10. Section V, Discussion, provides guidance in choosing the proper percentile based on the relevant meteorological conditions. Because of uncertainties in the estimation process, selecting values of I and λ outside their middle ranges (25th to 75th percentiles) is not recommended.
- *Predicted Microorganism Aerosol Concentration P.* The predicted microorganism concentration in the wastewater aerosol is then calculated from the estimates of D, E, I, and λ using equation (3).

b. Examples

(1) Pleasanton Residential Example

The microbiological dispersion model was used to obtain order-of-magnitude estimates of the microorganism aerosol concentrations to which the residents are typically exposed in the Pleasanton subdivision nearest the spray fields. The edge of this subdivision is located about 650 meters east and southeast from the edges of the nearest spray fields.

The model input conditions, parameter values, and predicted aerosol concentrations are presented in Table VI.C-11 for a typical summer nighttime case for total coliform, mycobacteria, and enteroviruses. Typical observed values were used for the wastewater concentrations and wastewater spray rate. The H. E. Cramer Company Volume-Source Diffusion Models Program was used to calculate the centerline concentration D for each microorganism group at the subdivision edge, taking into account the orientation of the sprayer line when the subdivision would be downwind. A median aerosolization efficiency E = 0.0033 was obtained from equation (5). As will be discussed later in the Discussion Section, the set of meteorological conditions for this summer nighttime case tends to be associated with values of both I and λ in the upper tails of their distributions. Thus, the 60th percentile values of I in Table VI.C-6 and of λ in Table VI.C-10 were selected as typical values for the three microorganism groups. The centerline aerosol concentrations P predicted at the subdivision edge are 0.01 mfc/m^3 for total coliform, 0.09 cfu/m^3 for mycobacteria, and 0.006 pfu/m^3 for enteroviruses.

As a comparison, the aerosol concentrations of these microorganism groups at the subdivision edge are also presented for a typical summer midday case in Table VI.C-12. Equation (5) yields an aerosolization efficiency E = 0.016 for these meteorological conditions. Since the summer midday conditions tend to give I and λ values in the lower tails of their distributions, 40th percentile values for the microorganisms were selected for this typical midday case. The predicted centerline aerosol concentrations were 0.001 mfc/m^3 for total coliform, 0.06 cfu/m^3 for mycobacteria, and 0.002 pfu/m^3 for enteroviruses.

(2) Deer Creek Campsite Example

The microbiological dispersion model can also be applied at other spray irrigation sites. For example, one can obtain order-of-magnitude estimates of the microorganism level extremes to which

Table VI.C-11.
PREDICTION OF TYPICAL NIGHTTIME MICROORGANISM AEROSOL LEVELS ENTERING PLEASANTON RESIDENTIAL AREA

Model Input Conditions	Summer Nighttime Case		
Season and Time	summer night		
Air Temperature	20° C		
Relative Humidity	70%		
Solar Radiation	0 W/m^2		
Wind Velocity	2 m/s		
Stability Class	E		
Mixing Height	30 m		
Residential Distance	650 m		
Residential Direction	E to SE		
Aerosol Age, a	325 s		
Wastewater Spray Rate	70 ℓ/s		
MICROORGANISM GROUP	Total Coliform	Mycobacteria	Enteroviruses
Wastewater Concentration	1×10^7 mfc/ℓ	80,000 cfu/ℓ	50 pfu/ℓ
Model Parameter Values			
D (Centerline)	12,000 mfc/m^3	100 cfu/m^3	0.06 pfu/m^3
E	0.0033	0.0033	0.0033
I	0.23	1.0	60
λ	-0.020 s^{-1}	-0.004* s^{-1}	-0.002* s^{-1}
$e^{\lambda a}$	0.0015	0.27	0.52
Predicted Aerosol Concentration			
P (Centerline)	0.01 mfc/m^3	0.09 cfu/m^3	0.006 pfu/m^3

*Interpolated value between the quantified range of the model parameter and zero.

Table VI.C-12.
PREDICTION OF TYPICAL MIDDAY MICROORGANISM AEROSOL LEVELS ENTERING PLEASANTON RESIDENTIAL AREA

Model Input Conditions	Summer Midday Case		
Season and Time	summer midday		
Air Temperature	30° C		
Relative Humidity	40%		
Solar Radiation	1000 W/m^2		
Wind Velocity	4 m/s		
Stability Class	B		
Mixing Height	High		
Residential Distance	650 m		
Residential Direction	E to SE		
Aerosol Age, a	162.5 s		
Wastewater Spray Rate	70 ℓ/s		
MICROORGANISM GROUP	Total Coliform	Mycobacteria	Enteroviruses
Wastewater Concentration	1 x 10^7 mfc/ℓ	80,000 cfu/ℓ	50 pfu/ℓ
Model Parameter Values			
D (Centerline)	1800 mfc/m^3	15 cfu/m^3	0.009 pfu/m^3
E	0.016	0.016	0.016
I	0.13	0.82	30
λ	-0.050 s^{-1}	-0.007*s^{-1}	-0.004*s^{-1}
$e^{\lambda a}$	0.0003	0.32	0.52
Predicted Aerosol Concentration			
P (Centerline)	0.001 mfc/m^3	0.06 cfu/m^3	0.002 pfu/m^3

*Interpolated value between the quantified range of the model parameter and zero.

campers are exposed at an 80-100 trailer campsite located from 700 to 900 meters northeast of the spray field at Deer Creek Lake, Ohio.

Spray irrigation is accomplished at Deer Creek Lake with 96 Rainbird® impact sprayers arranged in an 8 × 12 grid in a 3-acre square field (sides of 150 meters). The U.S. Army conducted a field sampling and assay program at the Deer Creek Lake site in the summer of 1976[58]. Wastewater samples were assayed routinely for indicator microorganisms and occasionally for pathogens. The microbiological aerosol runs were performed using twelve Andersen samplers and two Litton Model M high volume samplers, with samples only assayed for total aerobic bacteria. Four dye aerosol runs using fluorescein dye were also made.

Predictions of campsite microorganism aerosol level extremes emanating from the spray field made by using the microbiological dispersion model are presented in Table VI.C-13 for a daytime and a nighttime case. Typical values for the site were used for the wastewater concentrations of total coliform and fecal streptococci and for the wastewater spray rate. Calculations based on applying the Volume-Source Diffusion Models Program to the sampling data[59] were used to estimate the centerline concentration D at the campsite edge and typical aerosolization efficiencies E for the daytime and nighttime cases. Considering the meteorological conditions, the extreme values selected for I and λ were the 25th percentiles for the daytime case and the 75th percentiles for the nighttime case. The predicted extreme daytime and nighttime aerosol concentrations P at the campsite edge are, respectively, 2×10^{-9} and 0.05 cfu/m^3 for total coliform, and 0.0004 and 0.01 cfu/m^3 for fecal streptococci.

6. Preliminary Evaluation of Distance and Solar Radiation Factors

Prior to developing the microbiological dispersion model, several preliminary analyses of the aerosol concentration data were performed to investigate some of the fundamental assumptions of the model. These analysis results are presented in detail in Appendix G. The following synopsis indicates the purpose and findings of each analysis.

a. Analysis of Variance

The significance of distance and solar radiation as factors affecting microbiological aerosol levels was investigated by analysis of variance of the Post-Fair data. Except for the standard bacterial plate count, aerosol levels varied significantly with sampler distance. The aerosol levels of standard bacterial plate count, total coliform, and fecal streptococci were significantly reduced on runs made during high solar radiation.

b. Source and Distance Analysis

The pairs of aerosol measurements from two sampler locations were compared using paired comparison tests. Comparison of upwind versus close downwind concentrations demonstrated that the spray line was a significant aerosol source of each of the microorganism groups monitored. Comparison of close downwind versus distance downwind data only yielded significant aerosol concentration decreases with distance for total coliform, fecal coliform, coliphage, and fecal streptococci. Failure to find a significant decrease with distance for the other microorganism groups reflects the variability of their aerosol concentration measurements and/or a small number of runs.

7. Preliminary Assessment of Factors Affecting Microbiological Aerosol Levels

A preliminary assessment was conducted after the Pre-Fair sampling of the environmental factors affecting microbiological aerosol levels on the Pre-Fair aerosol runs. Stepwise multiple linear regression was used to select, from a large list of plausible candidates, these environmental variables in the D, E, I, and λ categories which best fit the microorganism aerosol data. A full description of the analysis and its results is given in Appendix G.

Table VI.C-13.
PREDICTION OF MICROORGANISM AEROSOL LEVEL EXTREMES ENTERING DEER CREEK LAKE CAMPSITE

Model Input Conditions	Daytime Case		Nighttime Case	
Season and Time	summer midday		summer night	
Air Temperature	30° C		20° C	
Relative Humidity	40%		70%	
Solar Radiation	1,000 W/m^2		0 W/m^2	
Wind Velocity	4 m/s		2 m/s	
Stability Class	B		E	
Mixing Height	high		30 m	
Residential Distance	700 m		700 m	
Residential Direction	NNE to ENE		NNE to ENE	
Aerosol Age, a	175 s		350 s	
Wastewater Spray Rate	30 ℓ/s		30 ℓ/s	
MICROORGANISM GROUP	Total Coliform	Fecal Streptococci	Total Coliform	Fecal Streptococci
Wastewater Concentration	180,000 cfu/ℓ	1,000 cfu/ℓ	180,000 cfu/ℓ	1,000 cfu/ℓ
Model Parameter Values				
D (Centerline)	40 cfu/m^3	0.2 cfu/m^3	100 cfu/m^3	0.6 cfu/m^3
E	0.009	0.009	0.004	0.004
I	0.06	0.71	0.55	6.1
λ	-0.094 s^{-1}	-0.006 s^{-1}	-0.004 s^{-1}	-0.001* s^{-1}
$e^{\lambda a}$	7×10^{-8}	0.35	0.25	0.70
Predicted Aerosol Concentration				
P (Centerline)	2×10^{-9} cfu/m^3	0.0004 cfu/m^3	0.05 cfu/m^3	0.01 cfu/m^3

* Interpolated value between the quantified range of the model parameter and zero.

Similar factors were identified as explaining much of the observed variation in the aerosol levels of total coliform, fecal coliform, and coliphage. The diffusion model D was found to be important for all three microorganisms. Temperature was indicated to have an important effect on wastewater aerosolization efficiency, while low pond relative humidity and middle upwind relative humidity both had lethal impact effects. Viability decay occurred primarily at middle relative humidities and at high temperature.

There is some evidence that the three pathogenic bacteria, *Pseudomonas,* fecal streptococci, and *Clostridium perfringens,* may also be affected by similar environmental variables. All appear to be initially reduced in viability by strong, dry winds. Desiccation also appears to play a role in their viability decay with aerosol age or distance.

This preliminary assessment of environmental factors was important, both because it justified the form of the microbiological dispersion model equation (3), and because it represents the most comprehensive analysis to date of the effects that atmospheric and operating conditions have on the microorganism-specific parameters I and λ, and thereby on the aerosol concentration.

D. Evaluation of the Microbiological Dispersion Model

The predictive value of the microbiological dispersion model is determined by how well its prediction, P, of the microorganism aerosol concentration from a spray source agrees with C-B, the measured concentration corrected for background. In this section, model predictions are compared to the aerosol concentration data from the Pleasanton runs, which were not used in the model development, and from field sampling programs conducted at Deer Creek Lake, Ohio[60] and Fort Huachuca, Arizona[61,62,63]. Information is presented on the accuracy and precision of the model predictions.

1. Evaluation Data

To evaluate the suitability of the model for a microorganism group, it is necessary to obtain many pairs (C-B,P) of the net measured concentration, C-B, and the model-predicted concentration, P, for the microorganism group from various distances, runs and sites. Consistent procedures were used for calculating values of C-B and P and for deciding whether to use these values in the model evaluation.

From the Pleasanton sampling program, the smoothed concentration values, C , not used in developing the microbiological dispersion model, were potentially usable in the model evaluation. These consisted of a few values from the microbiological aerosol runs in the tables of Appendix F and all the quality assurance run values (Tables VI.B-16 through VI.B-23). Nearly all the concentration values above background from the Deer Creek Lake and Fort Huachuca sampling programs were potentially usable in the model evaluation. The exceptions were several cases of presumed contamination in which very high outlier values relative to the surrounding values were obtained, and several cases in which chlorinated effluent was sampled. At all sites, a line of samplers, or at least a pair of samplers, was often located at the same distance from the spray source. Sometimes a pair of samplers consisted of one high-volume aerosol sampler and one Andersen sampler. At Ft. Huachuca, several downwind samplers were deployed along an arc at 10° to 40° intervals at the same distance from the single spray source. These situations were treated by averaging the concentrations from paired high-volume and Andersen samplers, by averaging the concentrations along a line of samplers, and by averaging the concentrations over an arc of samplers. Thus, no more than one net concentration value, C-B, was used at a given sampler distance on a run. The measured concentration values from all sites were used for model evaluation only if they exceeded both the run background ($C\text{-}B > 0.5B$) and the minimum detection limit ($C\text{-}B > DL$).

Predicted values corresponding to the net measured concentration value were computed from equation (3), $P = D \bullet E \bullet I \exp(\lambda \bullet a)$, using the procedures given in Section VI.C.5.a.2. Diffusion model aerosol concentrations at each sampler position were calculated by H.E. Cramer Company, using their Vol-

ume Source Diffusion Model.[64] Calculation of aerosolization efficiency from the dye runs at Deer Creek Lake and Fort Huachuca, using equation (4) E = C/D, with Cramer's modeled D values, showed that the aerosolization efficiencies of the Deer Creek Lake and Fort Huachuca spray systems were similar to Pleasanton. Consequently the aerosolization efficiency, E, during each microbiological run at Deer Creek Lake and Fort Huachuca was computed from equation (5).

The solar radiation measurement, r, used in equation (5) should be recorded by a short-wave instrument of the vertical Eppley-type for consistency with the Belfort short-wave instrument used at Pleasanton. The incoming radiometer readings, in millivolts, from the Deer Creek Lake and Fort Huachuca reports, were adjusted to equivalent short-wave values based on hourly cumulative vertical-Eppley readings provided by Mr. Marmon of the Atmospheric Sciences Laboratory, White Sands, New Mexico. A percentile of the I and λ distributions was selected for each run following the guidance given in Section VII, below. Solar radiation was used as the primary criterion in selecting the 25th, 40th, 50th, 60th or 75th percentile, with consideration occasionally given to additional factors, such as run selectivity at Pleasanton and effluent chlorination at Fort Huachuca. In a few cases, no predicted value could be computed to correspond to a C-B value, either because the run was not diffusion-modeled or because no wastewater concentration was measured.

The numbers of data pairs (C-B,P) for the model evaluation are presented in Table VI.D-1 by microorganism group and site. The first column gives the number of pairs for which a detectable measured value and model prediction were obtained; these are the potential evaluation data. The number of these pairs, for which the measured concentration is detectable (C-B > 0.5B and C > DL) is given in the second column; these pairs were used to evaluate the precision of the model predictions. The number of these pairs for which the predicted concentration would also be detectable (P > 0.5B and P > DL) is shown in the next column; these were used to analyze the accuracy of the model predictions. Table VI.D-1 shows that standard bacterial plate count, total coliform, and possibly coliphage are the only microorganism groups having enough data pairs to perform an adequate model evaluation. For the other microorganism groups, nearly all of the usable data pairs come from the Pleasanton sampling program.

It should be noted that the sampling and analytical methods used at Deer Creek Lake and Fort Huachuca differed from those employed at Pleasanton. The aerosol samplers for the Deer Creek Lake and Fort Huachuca runs were mainly six-stage Andersen samplers supplemented by one or two high-volume aerosol samplers, whereas only high-volume samplers were used at Pleasanton. Presumptive identification of total coliforms using Endo agar or Endo broth was used at Deer Creek Lake and Fort Huachuca; at Pleasanton, presumptive total coliforms on lactose broth were confirmed on brilliant green bile . At Fort Huachuca, coliphage measurements were obtained by seeding the wastewater with coliphage f2 and sampling this aerosol with Andersen and high-volume samplers. Since laboratory studies indicated that the recovery efficiency of airborne f2 from Andersen samplers was about 25 percent of that of liquid impaction samplers,[65] the field coliphage f2 concentrations obtained with the Andersen samplers were multiplied by four. At Pleasanton, the natural coliphage present in the wastewater were sampled and assayed. The preceding differences might affect the model evaluation, since the I and λ coefficients of the microbiological dispersion model are based on the Pleasanton sampling and analytical methods.

Each data pair used in the model evaluation (i.e. those with detectable measured net concentrations C-B for which totals are given in the second column of Table VI.D-1) is tabulated in Appendix H. The tables of Appendix H are arranged by microorganism group, site, and analytical method. Displayed in the Appendix H tables for each data pair are the aerosol run number, key meteorological parameters (temperature, relative humidity, solar radiation, and wind velocity), the selected percentile of the I and λ distributions, the measured aerosol concentration, C-B, the model-predicted aerosol concentration, P, and the orders of magnitude of discrepancy statistic, OMD.

In comparing the measured and predicted aerosol concentrations, proportional differences are more relevant than absolute differences. For example, the discrepancies between the microorganism aerosol concentration pair (10,000/m^3 vs. 1,000/m^3) and between the pair (10/m^3 vs. 1/m^3)might be considered

Table VI.D-1.
NUMBER OF DATA PAIRS FOR MODEL EVALUATION

Microorganism Group / Site	Number of Data Pairs: Run/Distance Combinations Measured and Modeled	Detectable* Measured Concentrations Number	Detectable* Measured and Predicted Concentrations Number	Detectable* Measured and Predicted Concentrations Effective No†
Std. Bacterial Plate Count				
Pleasanton	9	7	6	6
Deer Creek Lake	60	54	45	28.5
Ft. Huachuca	50	37	34	24.5
All Sites and Methods	119	98	85	59
Total Coliform				
Pleasanton	8	8	8	8
Presumptive on Endo Agar:				
Ft. Huachuca	30	24	17	13.5
Presumptive on Endo Broth:				
Deer Creek Lake	10	8	0	0
Ft. Huachuca	13	9	7	6.5
All Endo Broth	23	17	7	6.5
All Sites and Methods	61	49	32	28
Fecal Coliform				
Pleasanton	6	5	5	5
Ft. Huachuca	4	2	1	1
All Sites and Methods	10	7	6	6
Coliphage				
Pleasanton	5	5	5	5
Ft. Huachuca (seeded f2)	16	15	15	10.5
All Sites and Methods	21	20	20	15.5
Fecal Streptococci				
Pleasanton	10	8	8	8
Ft. Huachuca	8	2	0	0
All Sites and Methods	18	10	8	8
Pseudomonas				
Pleasanton	4	4	4	4
Clostridium perfringens				
Pleasanton	3	2	2	2
Mycobacteria				
Pleasanton	5	4	4	4

* Above background ($C-B \geq 0.5B$, $P > 0.5B$) and detection limit ($C \geq DL$, $P \geq DL$), and excluding presumably contaminated samples.

† Presumed equivalent number of independent observations.

equivalent because both are "factor of 10" or "one order of magnitude" discrepancies. Hence, the model evaluation is based on these two proportional difference statistics which are easily interpreted. The discrepancy factor statistic, F, is defined as the larger of (C-B)/P and P/(C-B):

$$F = \text{Max}\left(\frac{C-B}{P} \ , \ \frac{P}{C-B}\right) \tag{14}$$

The orders of magnitude of discrepancy statistic is defined as:

$$OMD = \log_{10}\left(\frac{C-B}{P}\right) \tag{15}$$

These statistics are mathematically related since $OMD = \log_{10}F$ and $F = 10^{|OMD|}$. Thus, three orders of magnitude discrepancy (OMD = ± 3) is equivalent to a discrepancy factor of 1000 : $F = 10^3 = 1000$.

A scan of the Appendix H tables shows that the greater majority of model predictions differ from the measured aerosol concentrations by less than an order of magnitude ($-1 < OMD < 1$). The accuracy and precision of the model predictions are more fully characterized in the following sections.

Applicability of the model to chlorinated effluent aerosols can be inferred from the model predictions for standard bacterial plate count on several runs at Fort Huachuca, in which the effluent was chlorinated prior to spraying. The results are presented in Table VI.D-2. Measurement of the aerosol concentrations of standard bacterial plate count on the chlorinated runs is quite uncertain, because the low net measured concentrations of nearly 0 to 30/m³ were less than the measured background concentrations of 19 to 170/m³ on each run, except Run 74-6. Also, the diffusion modeling of some of these runs could only provide approximate concentrations because the wind direction varied so widely. Yet the microbiological dispersion model regularly predicted an aerosol concentration several orders of magnitude lower than the measured value ($OMD \geq +1.5$). The marked underprediction with chlorinated effluent contrasted with the relatively

Table VI.D-2

COMPARISON OF MODEL PREDICTIVE ABILITY OF CHLORINATED AND UNCHLORINATED EFFLUENT

	Aerosol Run Number	Wastewater Chlorination (mg/l) Total	Free	Standard Bacterial Plate Count Effluent (CFP/ml)	Aerosol Conc (CFP/m) C-B	P	OMD $\log_{10}\left(\frac{C-B}{P}\right)$
Average Chlorination (Ft. Huachuca)							
	74-5	6.3	1.5	50	2.3	0.08	+1.5
	74-6	0.8	0.0	220	30	0.05	+2.8
	75-6	6.0	0.7	88	29	0.06	+2.7
	75-5	6.5	0.8	87	5	0.05	+2.0
	75-15	6.0	0.6	50	0	0.4	?
Low Chlorination (Deer Creek Lake)	45 pairs	0.1-0.4	--	29,000			+0.26
Unchlorinated (Ft. Huachuca)	34 pairs	--	--	310,000			+0.14
Unchlorinated (Pleasanton)	6pairs	<0.1	<0.05	610,000			+0.02

good model predictions for low chlorination, and especially for unchlorinated effluents, are summarized in Table VI.D-2. Thus, it appears that the microbiological dispersion model does not give valid predictions of microbiological aerosol concentrations from highly chlorinated effluents.

An hypothesis is offered to explain model underprediction for chlorinated effluent aerosols. The types of microorganisms that are most susceptible to die-off during aerosolization may be the same types that are generally killed by chlorination of wastewater. Hence, little impact die-off would occur ($I \approx 1$) during aerosolization of chlorinated effluent.

2. Accuracy of Model Predictions

The predictions of the model will intuitively be regarded as being accurate if the predicted microorganism concentrations fall randomly above and below the net measured concentrations with no detectable bias. It is assumed that each OMD calculated from equation (15) for a group of data pairs is sampled from the same normal distribution with unknown population mean μ_{OMD} and variance σ^2_{OMD}. If the mean μ_{OMD} is not significantly different from 0, the model predictions may be considered accurate in a statistical sense. The null hypothesis of model prediction accuracy ($\mu_{OMD} = 0$) can be tested against the two-sided alternative, $\mu_{OMD} \neq 0$, using the test statistic $t = \overline{OMD}/(S_{OMD}/\sqrt{n})$ which has the t distribution with n-1 degrees of freedom, provided the OMD's are independent observations from the same normal distribution. Here $\overline{OMD} = (\Sigma\ OMD)/n$ and $S^2_{OMD} = \Sigma\ (OMD - \overline{OMD})^2/(n - 1)$ are the sample mean and sample variance, respectively.

An analysis of model prediction accuracy using the t statistic was conducted for each microorganism group at each site and for the total OMD observations for each microorganism group over all three sites. To prevent an artificial selection bias, the accuracy analysis was based on those data pairs from the Appendix H tables, for which both the net measured concentrations and the predicted concentrations were detectable.

A scan of the Appendix H data reveals that the OMD values of a microorganism group for the different sampling distances on an aerosol run are related. The OMD values at the various sampler distances on a run all tend to have similar values, especially for samplers that were located close to each other. Consider, for example, the OMD values for standard bacterial plate count from the Deer Creek Lake study on Run 15 [—0.25 (30m), —0.39 (50m)] and on Run 16 [0.84 (30m), 0.88 (50m), 0.78 (200m)]. Since the OMD values at different distances on a run are similar, the OMD values obtained for a group are not independent observations, as is assumed in calculating a probability value for the t statistic by the usual procedure. Because the OMD values for a run are related, in effect there are less than n independent observations in a group of data pairs. The "effective number of independent observations" was calculated by assuming that runs with OMD values at three sampler distances provided two independent observations, that runs with OMD values at two distances provided one and one half independent observations, and that runs with a single OMD value contributed one independent observation. Both the actual number of OMD values and the presumed effective number of independent observations are presented in the last two columns of Table VI.D-1 for each group of detectable measured and predicted concentrations on which an accuracy analysis was conducted. The t statistic and its two-sided significance level (p) were computed for both the number of observations and the effective number of independent observations. Although similar values were obtained for both cases, the effective number is regarded as more valid, and inferences are based on this procedure.

Provided the number of independent observations is correctly determined, the p value is the probability of getting a more discrepant t statistic than the value obtained for a random sample of equal size from a population with zero mean ($\mu_{OMD} = 0$). Thus, p indicates the accuracy of the model predictions relative to the net measured aerosol concentration. A p value above 0.20 suggests little or no consistent bias, while a p value below 0.05 suggests there is a consistent bias in the model predictions.

The results of the accuracy analyses for standard bacterial plate count, total coliform, fecal col-

iform, coliphage, and the pathogenic microorganisms are presented respectively in Tables VI.D-3 through VI.D-7. The geometric mean of the P/(C-B) ratios is tabulated to indicate the average discrepancy between the predicted and measured values. The OMD provides another measure of this discrepancy. For groups in which the number of detectable data pairs differed from the effective number of independent observations, the t statistic and its two-sided p value are presented for both cases.

Table VI.D-3 shows that the accuracy of the model predictions for standard bacterial plate count differ considerably among the study sites. At Pleasanton, there is no evidence of bias in the model predictions. At Fort Huachuca, the model predictions tended to be 72 percent as large as the measured net concentrations, but there was sufficient variability that no consistent bias was detected ($p = 0.16$). At Deer Creek Lake, the model predictions of standard bacterial plate count were consistently ($p = 0.0016$) less than the measured values, averaging 55 percent as large. Referring to Table VI.D-2, the slight model prediction underestimation with low chlorination at Deer Creek Lake is consistent with the apparent two orders of magnitude model underestimation for heavily chlorinated Fort Huachuca effluent. Thus, chlorination may be responsible for the slight underprediction at Deer Creek Lake. The summarized results over all three sites are similar to those for Deer Creek Lake, which provided the majority of the data pairs. With $p = 0.0012$, the model predictions of standard bacterial plate count were found to be consistently smaller than the measured values, averaging 64 percent as large.

Table VI. D-3

ANALYSIS OF THE ACCURACY OF STANDARD BACTERIAL PLATE COUNT MODEL PREDICTIONS

	Sites			All Sites
	Pleasanton	Deer Creek Lake	Ft. Huachuca	and Methods
Number of Detectable* Measured and Predicted Concentration Pairs	6	45	34	85
$\frac{P}{C-B}$ Geometric Mean	0.95	0.55	0.72	0.64
OMD Mean, $\overline{OMD}$	0.02	0.26	0.14	0.20
OMD Standard Deviation	0.56	0.40	0.48	0.45
$t = \overline{OMD}/(S_{OMD}/\sqrt{n})$	0.02	4.40	1.72	4.08
p value (two-sided)	>0.90	0.00005	0.10	0.0001
Effective+ Number	6	28.5	24.5	59
$t = \overline{OMD}/(S_{OMD}/\sqrt{n'})$		3.50	1.46	3.40
p value (two-sided)		0.0016	0.16	0.0012

Orders of Magnitude of Discrepancy, $OMD = \log_{10}\left(\frac{C-B}{P}\right)$

*Above background ($C-B \geq 0.5B$, $P > 0.5B$) and detection limit ($C \geq DL$, $P \geq DL$), and excluding presumably contaminated samples.

+ Presumed equivalent number of independent observations.

The analysis given in Table VI.D-4 provides no evidence of a consistent bias in model predictions of total coliform aerosol concentration. With unchlorinated wastewater aerosols assayed for confirmed total coliforms at Pleasanton and for presumptive total coliforms at Fort Huachuca both on Endo agar and on Endo broth, no consistent proportional differences were found between the measured values and the model predictions. Thus, prediction of total coliform aerosol concentrations with the microbiological dispersion model for unchlorinated wastewater aerosols apparently yields accurate estimates of the measured values that could be obtained using a variety of aerosol sampler types and assay methods.

Accuracy analysis of fecal coliform model predictions in Table VI.D-5 shows that the model tends to underestimate measured fecal coliform aerosol concentrations by about a factor of $1/0.40 = 2.5$. The impact factor distribution, I, for fecal coliform is based on only 13 Pre-Fair aerosol runs at Pleasanton, which may be insufficient to characterize this distribution.

The accuracy analysis in Table VI.D-6 indicates that model predictions of total coliphage aerosol concentrations tended to overpredict the measured values at Pleasanton by a factor of 2.4, while possibly underpredicting seeded coliphage aerosol concentrations at Fort Huachuca at about half the measured value. However, considering both sites together, there is no evidence of consistent prediction bias.

Table VI. D-4
ANALYSIS OF THE ACCURACY OF TOTAL COLIFORM MODEL PREDICTIONS

	Assay Methods			
	Confirmed	Presumptive		
		Endo Agar	Endo Broth	All Sites
	Pleasanton	Ft. Huachuca	Ft. Huachuca	and Methods
Number of Detectable* Measured and Predicted Concentration Pairs	8	17	7	32
$\frac{P}{C-B}$ Geometric Mean	1.38	0.62	0.52	0.73
OMD Mean, $\overline{OMD}$	-0.14	0.21	0.28	0.14
OMD Standard Deviation	0.55	0.51	0.63	0.55
$t = \overline{OMD}/(S_{OMD}/\sqrt{n})$	-0.71	1.67	1.17	1.39
p value (two sided)	~ 0.50	0.13	~ 0.30	0.18
Effective+ Number	8	13.5	6.5	28
$t = \overline{OMD}/(S_{OMD}/\sqrt{n'})$		1.49	1.13	1.30
p value (two sided)		0.16	~ 0.30	0.21

Orders of Magnitude of Discrepancy, $OMD = \log_{10}\left(\frac{C-B}{P}\right)$

*Above background ($C-B \geq 0.5B$, $P > 0.5B$) and detection limit ($C \geq DL$, $P \geq DL$), and excluding presumably contaminated samples.

+Presumed equivalent number of independent observations.

Analyses of the accuracy of model predictions at Pleasanton of fecal streptococci, *Pseudomonas, Clostridium perfringens*, and mycobacteria are given in Table VI.D-7. The analyses suggest a tendency for the model to overpredict fecal streptococci and *Pseudomonas* aerosol concentrations. However, many of the data pairs available for model evaluation of the bacterial pathogens were from "left over" runs in which few detectable measured aerosol concentrations were obtained. It appears that this bias in selecting data pairs has produced biased (C-B,P) observations that may have confounded the accuracy analysis for each of these bacterial pathogens.

The accuracy of the model predictions over all methods and sites is summarized in Table VI.D-8 for each microorganism group. An assessment of the probable bias that results from using the microbiological dispersion model to predict microorganism aerosol concentrations is given in the last column of Table VI.D-8.

Model predictions tend to slightly underestimate the standard bacterial plate count and fecal coliform aerosol concentrations, giving predictions that average about half as large as the anticipated measured values. Given the imprecision of measured aerosol concentrations of standard bacterial plate count and fecal coliform (i.e. coefficients of variation of 50 and 58 percent respectively, were presented in Table VI.B-40), and the variety of sampling methods, analytical methods, and effluent aerosols evaluated, the slight underestimation bias in model predictions of standard bacterial plate count and fecal coliform aerosol concentrations is not considered to be of practical significance.

The accuracy analysis of the model predictions of the bacterial pathogens was based on few observations (C-B,P), some of which may not be valid for assessing accuracy. Thus, the accuracy of model predictions of bacterial pathogen aerosol concentrations is still regarded as unknown.

Table VI. D-5.
ANALYSIS OF THE ACCURACY OF FECAL COLIFORM MODEL PREDICTIONS

	Sites		
	Pleasanton	**Ft. Huachuca**	**All Sites and Methods**
Number of Detectable* Measured and Predicted Concentration Pairs	5	1	6
$\frac{P}{C-B}$ Geometric Mean	0.41	0.36	0.40
OMD Mean, $\overline{OMD}$	0.39	0.44	0.40
OMD Standard Deviation	0.31		0.28
$t = \overline{OMD}/(S_{OMD}/\sqrt{n})$	2.83		3.52
p value (two-sided)	0.048		0.017

Orders of Magnitude of Discrepancy, $OMD = \log_{10}\left(\frac{C-B}{P}\right)$

*Above background ($C-B \geq 0.5B$, $P > 0.5B$) and detection limit ($C \geq DL$, $P \geq DL$), and excluding presumably contaminated samples.

+ Presumed equivalent number of independent observations.

Although varying results were obtained at different sites using different methods, the accuracy analyses for all sites did not detect a consistent significant bias in the model predictions of the aerosol concentrations of total coliform and coliphage. While the overall accuracy analyses involved enough (C-B,P) observations to detect a substantial bias if it had existed, only slight site-specific or nonspecific biases were found. Thus, for most model applications, the predictions of total coliform and coliphage aerosol concentrations with the microbiological dispersion model appear to be sufficiently accurate estimates of the measured aerosol concentrations that would be obtained with various types of aerosol samplers and assay methods.

3. Precision of Model Predictions

The precision of the model predictions refers to how close the predicted aerosol concentrations tend to be to the net measured aerosol concentrations regardless of which value tends to be larger. Thus, the precision of a prediction relative to C-B, can be measured in terms of the discrepancy factor statistic F defined in equation (14) or of the absolute value of the orders of magnitude of discrepancy statistic |OMD| .

An analysis of model prediction precision was conducted for each microorganism group at each site and for all three sites. All the data pairs given in the Appendix H tables (i.e., all pairs for which the measured aerosol concentration was detectable) were considered valid measures of microbiological dispersion model precision and thus were utilized in the precision analysis.

Table VI. D-6
ANALYSIS OF THE ACCURACY OF COLIPHAGE MODEL PREDICTIONS

	Sites		
	Natural	Seeded f2	
	Pleasanton	Ft. Huachuca	All Sites and Methods
Number of Detectable* Measured and Predicted Concentration Pairs	5	15	20
$\frac{P}{C-B}$ Geometric Mean	2.4	0.49	0.73
OMD Mean, $\overline{OMD}$	-0.38	0.31	0.14
OMD Standard Deviation	0.10	0.48	0.52
$t = \overline{OMD}/(S_{OMD}/\sqrt{n})$	-8.5	2.51	1.19
p value (two-sided)	0.0011	0.0025	0.25
Effective⁺ Number	5	10.5	15.5
$t = \overline{OMD}/(S_{OMD}/\sqrt{n'})$		2.10	1.05
p value (two-sided)		0.06	~0.32

Orders of Magnitude of Discrepancy, $OMD = \log_{10}\left(\frac{C-B}{P}\right)$

*Above background ($C-B \geq 0.5B$, $P > 0.5B$) and detection limit ($C \geq DL$, $P \geq DL$), and excluding presumably contaminated samples.

⁺Presumed equivalent number of independent observations.

Table VI. D-7
ANALYSIS OF THE ACCURACY OF PATHOGENIC MICROORGANISM MODEL PREDICTIONS AT PLEASANTON

	Fecal Streptococci	*Pseudomonas*	*Clostridium Perfringens*	Mycobacteria
Number of Detectable* Measured and Predicted Concentration Pairs	8	4	2	4
$\frac{P}{C-B}$ Geometric Mean	4.2	5.9	7.5	0.38
OMD Mean, $\overline{OMD}$	-0.62	-0.77	-.088	0.43
OMD Standard Deviation	0.36	0.24	0.81	0.53
$t = \overline{OMD}/(S_{OMD}/\sqrt{n})$	-4.89	-6.53	-1.52	1.70
p-value (two-sided)	0.0018	0.007	~0.35	0.19

Orders of Magnitude of Discrepancy, $OMD = \log_{10}\left(\frac{C-B}{P}\right)$

*Above background ($C-B \geq 0.5B$, $P > 0.5B$) and detection limit ($C \geq DL$, $P \geq DL$), and excluding presumably contaminated samples.

+ Presumed equivalent number of independent observations.

Table VI. D-8
SUMMARY OF MODEL PREDICTION ACCURACY FOR ALL SITES AND METHODS

Microorganism Group	Detectable Measured and Predicted Pairs	Factor P/(C-B) Geometric Mean	Mean of Orders of Magnitude of Discrepancy	P Value (two-sided) for Effective No. of Obs.	Assessment of Prediction Bias
Standard Bacterial Plate Count	85	0.64	0.20	0.0012	slight underestimate
Total Coliform	32	0.73	0.14	0.21	unbiased
Fecal Coliform	6	0.40	0.40	0.017	slight underestimate
Coliphage	20	0.73	0.14	~ 0.32	unbiased
Fecal Streptococci	8	4.2	-0.62	0.0018	unknown
Pseudomonas	4	5.9	-0.77	0.007	unknown
Clostridium perfringens	2	7.5	-0.88	~ 0.35	unknown
Mycobacteria	4	0.38	0.43	0.19	unknown

The results of the precision analyses for standard bacterial plate count, total coliform, fecal coliform, coliphage, fecal streptococci, and the pathogenic microorganism assayed only from Pleasanton are presented respectively in Tables VI.D-9 through VI.D-14. The mean of |OMD| and the largest OMD value summarize the prediction precision for a data group in terms of the orders of magnitude of the discrepancy. The geometric means of the discrepancy factors F give the average size of the factor of discrepancy between C-B and P. The distribution of the discrepancy factors is described by presenting the percentages of these factors below 2 (i.e., C-B and P within a factor of 2 of each other), below 5, and below 10.

The precision analysis of standard bacterial plate count model predictions is presented in Table VI.D-9 separately for Pleasanton, Deer Creek Lake and Fort Huachuca, and together for all three sites. Satisfactory and very similar precision was found in the standard bacterial plate count model predictions at all three sites. The geometric mean of the discrepancy factors ranged only from 2.64 at Fort Huachuca to 2.89 at Pleasanton, with an average discrepancy of a factor of 2.70 between P and C-B over all 98 data pairs at the three sites. At each site, 43 percent of the (C-B,P) pair values differed from each other by less than a factor of 2. Ninety-five percent of the data pairs at the three sites had a discrepancy factor below 10 (i.e., less than a one order of magnitude discrepancy). Thus, the microbiological dispersion model predicts aerosol concentrations of standard bacterial plate count from unchlorinated and slightly chlorinated wastewater ($\leq$ 0.4 mg/l total chlorine) quite well. The model's predictive ability for standard bacterial plate count appears to be equivalent at Pleasanton, Deer Creek Lake, and Fort Huachuca.

The precision analysis given in Table VI.D-10 for total coliform model predictions shows considerable differences in predictive precision from one site to another. The precision of model predictions of confirmed total coliforms at Pleasanton compares favorably with standard bacterial plate count precision at

Table VI. D-9
ANALYSIS OF THE PRECISION OF STANDARD BACTERIAL PLATE COUNT MODEL PREDICTIONS

	Sites			
	Pleasanton	**Deer Creek Lake**	**Ft. Huachuca**	**All Sites and Methods**
(C-B,P) Pairs with Detectable* Measured Concentrations	7	54	37	98
Mean of |OMD|	0.46	0.43	0.42	0.43
Largest OMD	+0.92	+1.15	+1.23	+1.23
Percentage of Discrepancy Factors:				
Below 2	43%	43%	43%	43%
Below 5	57%	80%	76%	77%
Below 10	100%	93%	97%	95%
Geometric Mean of Discrepancy Factors F	2.89	2.72	2.64	2.70

*Above background (C-B $\geq$ 0.5B) and detection limit (C $\geq$ DL) and excluding presumably contaminated samples.

Table VI. D-10

ANALYSIS OF THE PRECISION OF TOTAL COLIFORM MODEL PREDICTIONS

	Assay Methods				All Methods at Unchlorinated Sites
	Confirmed	Presumptive			
		Endo Agar	Endo Broth		
	Pleasanton	Ft. Huachuca	Ft. Huachuca	Deer Creek Lake	
(C-B,P) Pairs with Detectable* Measured Concentrations	8	24	9	8	41
Mean of |OMD|	0.41	0.72	0.65	1.50	0.64
Largest OMD	-1.18	+2.37	+1.43	+2.09	+2.37
Percentage of Discrepancy Factors:					
Below 2	50%	21%	33%	0%	29%
Below 5	88%	67%	67%	0%	71%
Below 10	88%	71%	67%	13%	73%
Geometric Mean of Discrepancy Factors F	2.55	5.2	4.5	32	4.4
Total Chlorine in Wastewater (mg/l)	>0.1	"none"	"none"	0.1-0.4	< 0.1

*Above background (C-B ≥ 0.5B) and detection limit (C > DL) and excluding presumably contaminated samples.

Table VI. D-11

ANALYSIS OF THE PRECISION OF FECAL COLIFORM MODEL PREDICTIONS

	Sites		
	Pleasanton	Ft. Huachuca	All Sites and Methods
(C-B,P) Pairs with Detectable* Measured Concentrations	5	2	7
Mean of |OMD|	0.39	1.43	0.69
Largest OMD	+0.88	+1.97	+1.97
Percentage of Discrepancy Factors:			
Below 2	60%	0%	43%
Below 5	80%	50%	71%
Below 10	100%	50%	86%
Geometric Mean of Discrepancy Factors F	2.47	27	4.9

*Above background (C-B ≥ 0.5B) and detection limit (C > DL) and excluding presumably contaminated samples.

Table VI. D-12

ANALYSIS OF THE PRECISION OF COLIPHAGE MODEL PREDICTIONS

	Sites		
	Natural	Seeded f2	
	Pleasanton	Ft. Huachuca	All Sites and Methods
(C-B,P) Pairs with Detectable* Measured	5	15	20
Mean of |OMD|	0.38	0.48	0.46
Largest OMD	-0.52	+ 0.98	+ 0.98
Percentage of Discrepancy Factors:			
Below 2	20%	27%	25%
Below 5	100%	73%	80%
Below 10	100%	100%	100%
Geometric Mean of Discrepancy Factors F	2.42	3.04	2.87

*Above background (C-B ≥ 0.5B) and detection limit (C > DL) and excluding presumably contaminated samples.

Pleasanton or any other site (e.g. an F geometric mean of 2.55 for total coliform at Pleasanton versus standard bacterial plate count values of 2.89 at Pleasanton and 2.70 at all sites). The precision of model predictions of presumptive total coliforms at Fort Huachuca is considerably worse, for assays both on Endo agar (F geometric mean = 5.2) and on Endo broth (F geometric mean = 4.5). At Deer Creek Lake, the model predictions of presumptive total coliforms on Endo broth were consistent one-to-two orders of magnitude underestimates of the net measured aerosol concentrations; the geometric mean of the discrepancy factors was 32. These site differences in model predictive ability may be related to the amount of chlorine in the sprayed wastewater (shown at the bottom of Table VI.D-10). Since model underpredictions of about two orders of magnitude for standard bacterial plate count were observed on the runs at Fort Huachuca using highly chlorinated wastewater (see Table VI.D-2), it is plausible to attribute model underpredictions of one-to-two orders of magnitude for the fragile total coliforms at Deer Creek Lake to the slightly chlorinated wastewater (0.1-0.4 mg total chlorine per liter).

As with total coliform, the analyses of fecal coliform (Table VI.D-11), coliphage (Table VI.D-12), and fecal streptococci (Table VI.D-13) show better precision for the model predictions at Pleasanton than at Fort Huachuca, although the distinction for coliphage is slight. The model predictions at Pleasanton for coliphage (F geometric mean = 2.42) and fecal coliform (F geometric mean = 2.47) tended to give precise estimates of the net measured aerosol concentrations. While subject to uncertainty due to the small number of data pairs, the precision analyses of the pathogenic microorganism model predictions in Table VI.D-14 suggest that model predictions of *Clostridium perfringens* and *Pseudomonas* have less precision than do predictions at Pleasanton of the other microorganism groups.

Table VI. D-13
ANALYSIS OF THE PRECISION OF FECAL STREPTOCOCCI MODEL PREDICTIONS

	Sites		
	Pleasanton	Ft. Huachuca	All Sites and Methods
(C-B,P) Pairs with Detectable* Measured Concentrations	8	2	10
Mean of |OMD|	0.62	0.97	0.69
Largest OMD	-1.33	+ 1.28	-1.33
Percentage of Discrepancy Factors:			
Below 2	25%	0%	20%
Below 5	63%	50%	60%
Below 10	88%	50%	80%
Geometric Mean of Discrepancy Factors F	4.2	9.2	4.9

*Above background (C-B $\geq$ 0.5B) and detection limit (C > DL) and excluding presumably contaminated samples.

A summary of the precision of microbiological dispersion model predictions using all methods at all sites (excluding the Deer Creek Lake predictions for total coliform because of the probable chlorination effect) is presented in Table VI.D-15. Of the five microorganism groups evaluated at sampling sites in addition to Pleasanton, the most precise model predictions were obtained for standard bacterial plate count and coliphage, for which the discrepancies averaged less than a factor of three. However, the geometric means of the discrepancies averaged less than a factor of five for the other three microorganism groups (total coliform, fecal coliform, and fecal streptococci). Considering the imprecision of microorganism aerosol measurements, the predictions of the microbiological dispersion model may have sufficient precision to replace direct measurement in many applications.

Table VI. D-14
ANALYSIS OF THE PRECISION OF PATHOGENIC MICROORGANISM MODEL PREDICTIONS AT PLEASANTON

	Pseudomonas	*Clostridium Perfringens*	Mycobacteria
(C-B,P) Pairs with Detectable* Measured Concentrations	4	2	4
Mean of |OMD|	0.77	0.88	0.47
Largest OMD	-1.01	-1.45	+1.01
Percentage of Discrepancy Factors:			
Below 2	0%	50%	50%
Below 5	25%	50%	75%
Below 10	75%	50%	75%
Geometric Mean of Discrepancy Factors F	5.9	7.5	2.9

*Above background (C-B $\geq$ 0.5B) and detection limit (C > DL) and excluding presumably contaminated samples.

Table VI. D-15

SUMMARY OF MODEL PREDICTION PRECISION FOR ALL SITES AND METHODS

Microorganism Group	(C-B,P) Pairs with Detectable Measured Concentrations	Geometric Mean of All Discrepancy Factors F	Percentage of Discrepancy Factors F Below: 2	5	10
Standard Bacterial Plate Count	98	2.7	43%	77%	95%
Coliphage	20	2.9	25%	80%	100%
Mycobacteria*	4	2.9	50%	75%	75%
Total Coliform+	41	4.4	29%	71%	73%
Fecal Coliform	7	4.9	43%	71%	86%
Fecal Streptococci	10	4.9	20%	60%	80%
*Pseudomonas**	4	5.9	0%	25%	75%
*Clostridium Perfringens**	2	7.5	50%	50%	50%

+Excludes Deer Creek Lake pairs because of probable clorination effect.

*All data pairs are from the Pleasanton sampling program.

VII. DISCUSSION OF MICROBIOLOGICAL DISPERSION MODEL

A. Model Components

1. Aerosolization Efficiency E

It has been reported[66] that aerosolization efficiency depends upon spray nozzle type, spray arc height, spray pressure, and wind velocity. Nozzle type, spray height, and spray pressures are operating conditions that differ from one spray irrigation site to another. Thus, the aerosolization efficiency values given in Table VI.C-2 can be considered characteristic only of the Pleasanton site, and not necessarily characteristic of other spray irrigation sites.

The median aerosolization efficiency obtained for the Rainbird impact sprayers at Pleasanton over the 17 runs during Phase II was 0.33 percent. This agrees very well with the aerosolization efficiencies found for Rainbird impact sprayers at Ft. Huachuca, Arizona (median of 0.29 percent over three runs)[67] at Deer Creek Lake, Ohio (median of 0.47 percent over four runs)[68], and at other sites[69]. All of these studies estimated aerosolization efficiency using water-soluble fluorescent dyes and diffusion modeling.

It is clear from Table VI.C-2 that an order-of-magnitude variation in aerosolization efficiency may occur at a given site. The aerosolization efficiency regression equation (5) associates 80 percent of this variation with changes in meteorological conditions. Equation (5) indicates that the aerosolization efficiency at Pleasanton increases with increasing air temperature, increasing wind velocity, and increasing solar radiation. It agrees with the previously identified relationship of aerosolization efficiency to wind velocity[70] (perhaps due to shearing forces), and implies that aerosolization efficiency is also influenced by other meteorological factors that affect the evaporative capability of the air. Fairly high correlations typically occur among solar radiation, air temperature, wind velocity, and relative humidity. Thus it is difficult to identify through regression the precise combination of these meteorological factors that affect evaporative capability. In summary, an order-of-magnitude variation in aerosolization efficiency may occur at a given site, apparently as a result of variation in atmospheric conditions that influence shearing forces, and evaporative capability.

The aerosolization efficiencies for the two dye runs made during Phase I at Pleasanton[71] were 0.6 percent and 0.5 percent. The equation (5) (p. 142) predictions of aerosolization efficiency for these runs' atmospheric conditions were 1.1 percent and 0.9 percent. This verifies our inference (cf. Section VI.C.3) that equation (5) generally predicts the aerosolization efficiency at Pleasanton to within a factor of two.

Equation (5) may be insufficient to predict the aerosolization efficiency at spray irrigation sites other than Pleasanton because aerosolization efficiency depends upon the operating conditions.[72] At some sites such as Ft. Huachuca and Deer Creek Lake the operating conditions were similar enough to warrant use of equation (5). However, at sites with different operating conditions, assuming that the effects of operating conditions are relatively independent of the effects of meteorological conditions, reasonable aerosolization efficiency estimates can still be obtained. A previous study of spray equipment and operating conditions[73], or a limited dye aerosol sampling program at a site, can be utilized to estimate the site aerosolization efficiency under one set of meteorological conditions. The constant in equation (5) can then be adjusted so that aerosolization efficiencies for a site can be predicted under other meteorological conditions.

It should be noted that the solar radiation measurements at Pleasanton were made with a vertical Belfort Pyrheliograph, which is a short wave instrument. Solar radiation measurements vary considerably depending upon the type of recording instrument used. Thus in making aerosolization efficiency predictions using equation (5), the solar radiation, r, used in the equation should be from a vertical short wave instrument (e.g., vertical Eppley).

2. Impact Factor I

The initial, supposedly rapid, microbiological die-off of wastewater aerosols is seldom quantified separately from the aerosolization efficiency effect in the literature. Results reported by Sorber *et al*[74] can be converted to a net impact factor I = 0.12, for total aerobic bacteria over eight aerosol runs using Andersen samplers. Since this is approximately the 25th percentile of the standard bacterial plate count I distribution in Table VI.C-6, satisfactory agreement is indicated.

The individual impact factor estimates for a microorganism group during a run contain considerable uncertainty. With the exception of total coliform, the majority of individual impact factor estimates were less than twice their standard errors. Frequently, in fact, the standard error exceeded the impact factor estimate. This uncertainty should be kept in mind when interpreting the empirical distributions of impact factors given in Table VI.C-6. However, it is likely that both the central tendency and dispersion of the true I distributions are well represented by the I estimate distributions.

The middle ranges (i.e., from the 25th to the 75th percentile) of the impact factor distributions given in Table VI.C-6 suggest how well various microorganism groups survive the initial impact of aerosolization. The viability of the putative wastewater indicator microorganism groups (fecal coliform, total coliform, standard bacterial plate count, and coliphage) was substantially reduced through aerosol impact. Generally only 6 to 60 percent of these microorganisms in the sprayed wastewater survived the initial seconds of aerosolization.

The pathogenic bacteria and enteroviruses studied appear to survive aerosol formation and initial contact with the atmospheric environment much better than the usual indicator organisms. The impact factor estimates obtained at Pleasanton for *Pseudomonas*, fecal streptococci, *Clostridium perfringens*, mycobacteria, and the enteroviruses were unexpectedly high, usually in excess of 1.0. A discussion is given in Section VII.C.1 of possible explanations for these large impact values, which superficially suggest survival above 100 percent.

The impact factor values for most of the microorganisms studied exhibited variation over several orders of magnitude between their 10th and 90th percentiles. The literature generally implicates ultraviolet solar radiation as a factor in microorganism die-off. There is also some evidence[75] that aerosolized bacteria are reduced mainly at middle relative humidities (40-60 percent), and that high temperatures (>27°C) may also reduce microbiological aerosols. Thus, variation in atmospheric conditions is suggested as a probable cause of the variation in impact factor values within a microorganism group.

The preliminary analysis (see Appendix G), of the association of impact factor values with pertinent meteorological variables during the Pre-Fair runs, is also relevant. This analysis suggests that the impact factors for total coliform, fecal coliform, and coliphage are reduced at low and middle relative humidities. The total coliform and coliphage impact factors appear to be further reduced for the combination of high solar radiation and high wind velocity, and for temperature difference between wastewater and air. The standard bacterial plate count impact factor may be lowered with strong solar radiation. Reductions in the impact factors for the pathogenic bacteria (fecal streptococci, *Pseudomonas*, and *Clostridium perfringens*) seem to occur primarily for the combination of high wind velocities with low relative humidities. Because there were fairly high correlations among solar radiation, temperature, relative humidity, and wind velocity during Pre-Fair runs, it is not clear whether the identified meteorological factor or a highly correlated alternative was actually associated with an impact factor reduction.

As the model prediction examples illustrate, selection of an appropriate impact factor value is a key step in making a model prediction for Pleasanton or elsewhere. To maximize predictive ability, selection of the I value should be based on the relationship of impact factor estimates for a microorganism group in Table VI.C-4 to the relevant atmospheric conditions occurring during their respective aerosol runs.

Such a direct analysis is needed to identify the nature of the relationship and to establish its

strength. Unfortunately, the proper technique, a biased multiple regression analysis, was beyond the scope and time frame of the current research effort. Hence, this analysis was not conducted.

Lacking this impact factor regression relationship, judicious application of the qualitative results of the preliminary analysis of the Pre-Fair data must be substituted. These results provide some basis for predicting whether the impact factor for a microorganism group under a specific set of atmospheric conditions is likely to be below, near, or above the median value obtained at Pleasanton. Thus, these associations of impact factors with atmospheric conditions can be used in making microbiological dispersion model predictions to select an appropriate percentile, presented in Table VI.C-6 as the microorganism I value. For making model predictions, selecting I values below the 25th percentile or above the 75th percentile is not recommended, both because of the uncertainty in the I estimates and because of the weak qualitative associations with atmospheric conditions. Suppose, for example, that a prediction of the total coliform concentration is to be made for a hot, sunny, dry, and windy summer afternoon, which might be the most hostile impact factor case. The 40th percentile I = .13 might be used to obtain a typical total coliform aerosol concentration, while the 25th percentile I = .06 might be appropriate for calculating a "best case" aerosol concentration. Conversely, under very favorable atmospheric conditions, the 60th percentile I = .23 might yield a typical total coliform aerosol concentration, while the 75th percentile I = .55 might give a "worst case" aerosol concentration.

3. Viability Decay Rate

The measurement of a sizable reduction in the microbiological aerosol concentration over a large aerosol age (distance) span downwind from the wastewater source is required to calculate an accurate viability decay rate. In field studies, this requirement dictates the simultaneous operation of several high-volume aerosol samplers, which are expensive and difficult to operate[76]. For this reason, viability decay rates based on field studies have seldom been reported in the literature. A net viability decay rate equivalent to $\lambda = -0.06\ s^{-1}$ was calculated for total aerobic bacteria over eight aerosol runs by Sorber, *et al*[77]. Since this value is around the 15th percentile of the standard bacterial plate count λ distribution in Table VI.C-10, it suggests slightly more rapid decay than do the viability decay rate values presented herein.

The individual viability decay rate estimates in Table VI.C-7 contain substantial uncertainty. Both the relative magnitude of the standard errors of the λ estimates and the frequency of non-negative decay rate estimates (denoted as $\lambda = X$) attest to this uncertainty in the individual estimates. These uncertainties, which reflect state-of-the-art sampling and analytical limitations, should be recognized in interpreting the λ distributions presented in Table VI.C-10.

For hardy microorganisms experiencing slow viability decay, very large sampling distances and very low detection limits are needed to quantify the viability decay rate. Because of their relatively low wastewater concentrations at Pleasanton, the hardy pathogenic bacteria were infrequently detected in the aerosol samples taken far downwind of the spray line. For this reason, only values in the lower (rapid decay) portions of the viability decay rate distributions of the pathogenic bacteria could be quantified in Table VI.C-10.

For all microorganism groups, the upper portion of the λ distribution, which represents the slower die-off rates, cannot be quantified based on the Pleasanton study. Table VI.C-10 suggests that $-0.01s^{-1}$ to $-0.02s^{-1}$ was the lowest viability decay rate which could be detected at Pleasanton. For the hardier and partially analyzed microorganisms, such as mycobacteria, *Pseudomonas,* and fecal streptococci, perhaps $-0.06s^{-1}$ was the lowest detectable viability decay rate.

Values for the unquantified percentiles in Table VI.C-10 can be estimated by interpolating between the highest quantified percentile value and the logical upper limit, $\lambda = 0$ (no viability decay). This interpolation procedure was employed in the prediction examples to estimate the 40th, 60th and 75th percentile λ values for mycobacteria, enteroviruses, and fecal streptococci in Tables VI.C-11, 12, and 13. Even at the siz-

able aerosol ages of these examples, the exponential die-off factor, $e^{\lambda a}$, for these slowly decaying microorganisms makes only a slight reduction in the predicted aerosol concentration. Thus, the added uncertainty in P, introduced by using an interpolated value for the viability decay rate, is slight.

Perusal of Table VI.C-10 indicates that the indicator microorganism groups, especially total coliform and fecal coliform, experienced more consistent and rapid die-off with aerosol age than did the pathogenic bacteria evaluated. Based on their viability decay rates, the microorganism groups seem to fall into three categories. Total coliform and fecal coliform were similar in that they exhibited the most rapid decay and their decay rates could most frequently be estimated. Furthermore, the viability decay rate estimates for total and fecal coliform were also more reliable than for the other microorganism groups. Viability decay appeared to occur on about 50 percent of the runs for a second category of microorganisms (coliphage, *Clostridium perfringens*, and standard bacterial plate count); their rates of decay were also slower than the coliform decay rates. Decay with aerosol age could seldom be detected for the third category (mycobacteria, *Pseudomonas*, and fecal streptococci).

The viability decay rate distributions also reflect substantial variation in microbiological die-off with aerosol age from one run to another. Die-off that increases with high solar radiation, low-to-middle relative humidity, and high temperature has often been suggested[78,79,80]. Thus, the different meteorological conditions during runs are presumed to cause the variation in die-off rates. Our preliminary analysis of the association of viability decay rates with meteorological variables, based on the Pre-Fair Pleasanton data, confirms these general relationships. For most microorganism groups, rapid viability decay does seem to be associated with summer daytime atmospheric conditions as identified through meteorological variables such as high temperature, middle relative humidities and high solar radiation. However, there is also much variation in the viability decay rates of a microorganism group under apparently similar sets of atmospheric conditions.

Selecting a proper decay rate value is crucial to making an accurate model prediction at substantial distances from the microbiological aerosol source. A direct analysis of the relationship of microorganism λ estimates to atmospheric conditions is needed, both to discriminate between the "rapid decay" and "insignificant decay" sets of atmospheric conditions, and to develop a regression relationship to predict the decay rate for the rapid decay conditions. Because of its complexity, such an analysis was beyond the scope and time frame of the present research effort.

Lacking such discrimination and regression relationships, the only basis for selecting a decay rate λ for use in model prediction are the general findings discussed above. Consideration of general atmospheric conditions on a daytime-nighttime scale is recommended as the basis for selecting a viability decay rate value for any microorganism group from the Table VI.C-10 distributions. Because the actual relationships of atmospheric conditions to decay rates are unknown, it is suggested that the decay rates used in predictive model calculations be restricted to the middle range (i.e., from the 25th to the 75th percentiles). Perhaps the 40th percentile might be taken as a typical summer daytime value, while the 60th percentile might reflect typical nighttime conditions.

B. Validity of the Model and its Predictions

The preliminary assessment in Section VI.C.7 of factors affecting Pre-Fair microbiological aerosol levels provided justification for establishing the multiplicative form of the microbiological dispersion model, as given in equation (3). The reasonable physical interpretations that can be given to each model factor and parameter enhance the usefulness of the equation (3) form for expressing the microbiological dispersion model.

In using the microbiological dispersion model for predicting microorganism aerosol concentrations from spray irrigation systems, three major assumptions are made: that the multiplicative form of the model is valid; that microorganism die-off depends only on factors such as atmospheric conditions which are independent of the spray site; and that the sampling, shipping, and assay methods of the Pleasanton study are employed. If these model assumptions are valid, the microbiological dispersion model should be applicable to

any spray irrigation site over the range of meteorological conditions occurring during the Pleasanton aerosol sampling runs. The extremes of meteorological conditions on the Pleasanton aerosol runs (see Table VI.B-1) are summarized in Table VII.B-1.

Table VII.B-1
METEOROLOGICAL CONDITIONS OF PLEASANTON AEROSOL RUNS

	Range of Values	
	Low	High
Dry Aerosol Runs		
Temperature, °C	7	34
Solar Radiation, W/m^2	<20	930
Relative Humidity, %	5	80
Wind velocity, m/s	1.2	8.5
Microbiological Aerosol Runs		
Temperature, °C	8	37
Solar Radiation, W/m^2	<20	900
Relative Humidity, %	5	86
Wind Velocity, m/s	0.5	7.2

At the present state of model development, predictions of the model have an important shortcoming. The procedure suggested above for selecting the I and λ parameter percentiles as a function of atmospheric conditions does not have adequate statistical justification. As discussed above, the existing Pleasanton data have not been analyzed to determine the relationships of the individual I and λ estimates for each microorganism group to the aerosol run atmospheric conditions. Sophisticated regression techniques, such as biased regression, appear necessary to elucidate such relationships. The complexity of this analysis precluded its conduct within the scope and time frame of this research effort. However, since this analysis is necessary to achieve the full potential usefulness of the microbiological dispersion model, it is recommended as a priority research area.

The effect of atmospheric conditions on an individual model parameter (D, E, I, or λ) is sometimes stronger that its net effect on the predicted concentration P. This happens because the atmospheric condition effects on D, E, I, and λ tend to vary in opposite directions that partially cancel out in the resulting prediction. Comparison of the daytime and nighttime cases in Table VI.C-13 illustrates this characteristic. While the summer midday atmospheric conditions reduce the microbiological model factors I and $e^{\lambda a}$ and the diffusion concentration D well below their nighttime levels, they simultaneously elevate the aerosolization efficiency E. Thus, when projecting the effect of a change in a single meteorological variable on the downwind microbiological aerosol concentration, its influence on all of the model parameters should be taken into account.

The accuracy and precision of the predictions of the microbiological dispersion model were examined in the model evaluation. Enough field data were available to provide a thorough model evaluation at realistic field sampling distances ($\leq$200 meters from the source boundary) under a variety of sampling and analytical procedures for standard bacterial plate count, and useful evaluations for total coliform and coliphage. However, the predictive ability of the model remains untested for fecal coliform, fecal streptococci, *Pseudomonas, Clostridium perfringens,* and mycobacteria at sites other than Pleasanton.

The predictions of the microbiological dispersion model were quite accurate over the field data evaluated. The predictions tended to be slightly less than the net measured aerosol concentrations, averaging 64

percent of the net measured value for standard bacterial plate count, and 73 percent for both total coliform and coliphage (see Table VI.D-8). No significant bias could be detected for total coliform and coliphage, but the prediction underestimates for standard bacterial plate count and fecal coliform do indicate a slight bias in their predictions.

The microbiological dispersion model predictions have satisfactory precision, considering the sizable sampling and analytical uncertainty present in microorganism aerosol concentration determinations. The preponderance of the model predictions (e.g. 95 percent of standard bacterial plate count predictions, 73 percent of total coliform predictions, and 100 percent of coliphage predictions) were within one order of magnitude of the net measured values (see Table VII.D-15). Most model predictions (e.g., 77 percent for standard bacterial plate count, 71 percent for total coliform, and 80 percent for coliphage) were within a factor of five of their net measured value. The geometric mean of the discrepancy factors was less than three for standard bacterial plate count, coliphage, and mycobacteria and less than five for total coliform, fecal coliform, and fecal streptococci.

It should be recognized that the model accuracy and precision statistics were based largely on field data obtained within 50 meters of the edge of the wetted spray area. Over these distances, the viability decay model factor $e^{\lambda a}$ has little effect on the computed model prediction P. Thus, the model evaluation has primarily evaluated the appropriateness of the E and I model parameters, with minimal attention given to λ. Since the viability decay rates obtained at Pleasanton varied widely, it is to be expected that both the accuracy and precision of the model predictions will deteriorate with distance from the wastewater aerosol source.

When the wastewater that is aerosolized contains residual levels of total chlorine, the microbiological dispersion model tends to underpredict the net measured aerosol concentration. Table VI.D-2 suggests underprediction of standard bacterial plate count aerosol concentrations by one and one half to three orders of magnitude at a total residual chlorine concentration of 6 mg/l in the sprayed wastewater. The Deer Creek Lake wastewater that was sprayed contained total residual chlorine concentrations in the range of 0.1 mg/l to 0.4 mg/l. The model predictions of presumptive total coliforms on Endo broth and of standard bacterial plate count were both below the net measured aerosol concentrations at Deer Creek Lake, by one to two orders of magnitude for total coliform (Table VI.D-10), and by nearly a factor of two for standard bacterial plate count (Table VI.D-3). The degree of model underprediction for chlorinated wastewater aerosols appears to depend both on the extent of residual chlorination and on the fragility of the microorganism group. Unless sufficient data are available to adjust for this chlorination effect, the microbiological dispersion model should not be used to predict microorganism aerosol concentrations of sprayed wastewater containing residual chlorine.

Considering the imprecision and cost of measuring microorganism aerosol concentrations by field sampling, the predictions of the microbiological dispersion model do appear to be a preferable alternative when the sprayed wastewater does not contain residual chlorine.

C. Microbiological Inferences Derived from the Model

1. Interpretation of Impact Factors Exceeding One

Frequent impact factor values exceeding 1.0 were not anticipated for any microorganism group. Occasional I values above 1.0 are to be expected based on the large I standard errors presented in Table VI.C-4. However, about 50 percent of the values obtained as impact factor estimates for fecal streptococci, *Clostridium perfringens* and mycobacteria exceeded 1.0. Nearly all of the enterovirus and *Pseudomonas* impact estimates exceeded this value. Thus, impact factors larger than one apparently characterize these pathogens under many atmospheric conditions with the measurement methodologies employed at Pleasanton.

The facile interpretation of the I values exceeding 1.0 is that substantial net growth rather than die-off of the microorganisms occurred in the hostile aerosol environment during the initial seconds from aerosol

formation to sampling at 50 meters downwind. However, this facile interpretation is inconsistent with known microbiological behavior. Thus, one or several more subtle phenomena must be responsible for these high observed I values.

Five possible explanations for the consistent pathogen I values above 1.0 are offered:

- *Survival Hypotheses.* After aerosol sample collection, microorganisms may have survived in the supportive BHI collection fluid at undiminished concentrations over the typical 16- to 24-hour holding time required for sample storage and shipment to the analytical laboratory. However, the survival of human pathogens in the wastewater samples which provide the baseline for comparison with the aerosol sampling over the same holding time may have been reduced because the wastewater presents a relatively hostile environment. The preliminary liquid collection media study conducted in the laboratory showed that, when poliovirus 1 and f2 bacteriophage were inoculated in wastewater, slight reductions in concentration occurred after 24 hours at the holding temperature of 4°C. However, in the BHI medium, stable levels of seeded pathogenic bacteria, poliovirus, and bacteriophage were maintained for several days at 4°C. Thus, at least for coliphage and the enteroviruses, a slight elevation (probably less than a factor of two) of the impact factor might be attributable to differential survival in the wastewater and aerosol collection media.

- *Masking Hypothesis.* The assay procedures may have consistently underestimated the wastewater concentrations of the pathogens due to the masking effect of chemical constituents or of the numerous other microorganisms present in the wastewater. Conversely, the assay procedures may have more accurately estimated the pathogen aerosol concentrations, because of the lower bacterial concentrations present in BHI collection medium, the selective decimation of the masking bacteria in the aerosol state, or the selective exclusion of inhibitory chemicals through aerosolization.

- *Mechanical Splitting Hypothesis.* Microorganisms that tend to exist in grouped form in the wastewater (e.g., the long chains of fecal streptococci and the clumps of mycobacteria aggregates with wastewater solids) may have been mechanically split into individual viable organisms prior to the aerosol assay. The potential for mechanical splitting exists both during aerosolization, through impact and shear forces at the spray head, during collection, through the rapid recirculation of the BHI fluid within the high volume aerosol sampler.

- *Regrowth Hypothesis.* Regrowth of aerosol-sampled pathogenic microorganisms in the supportive BHI medium could occur under opportune circumstances. Aerosol sample regrowth would be much more rapid than the corresponding regrowth of these microorganisms in the wastewater environment. An opportunity for regrowth of pathogenic bacteria did occur occasionally when the sample shipping temperature rose higher than the 4°C specified in the sampling protocol. However this hypothesis is not valid for enteroviruses.

- *Aerosolization Efficiency Hypothesis.* The aerosolization efficiency E may have frequently been underestimated. If the proportion of sprayed microorganisms that were aerosolized exceeded the proportion of fluorescent dye aerosolized because the dye is not an adequate physical model of microorganism aerosolization, E would have consistently been underestimated. In addition, occasional underestimation of E due to equation (5) uncertainty is also expected. Given the equation (5) standard error of 0.194, the predicted E is expected to be low by at least a factor of two on 7 percent of the run estimates. Underestimation of E for either reason will result in a compensating overestimation of the impact factor I values for all microorganism groups for the affected runs.

The available evidence suggests that the phenomena represented by all five hypotheses may have

had an effect on some of the impact factors calculated from the Pleasanton data. The survival hypothesis may always be operative in wastewater assay, but the collection media study data indicate that its magnitude is insufficient to alone account for the large impact factor estimates. The occurrence of masking in the wastewater assay is also quite probable; presumably the size of the masking effect could vary considerably from one sample to another, depending on the concentrations of the masking microorganisms or chemicals that may be present. Mechanical splitting is a likely mechanism of variable magnitude for the microorganisms that persist in grouped form. Except for enteroviruses, the regrowth hypothesis is also plausible when the Pleasanton sampling and shipping protocols were violated. However, all detected violations were carefully noted and their occurrence was too infrequent to explain very many of the high I values. For example, the pathogen assay laboratory received only 3 of the 55 microbiological run sample shipments at elevated temperatures (8°C to 9°C). Occasional underestimation of E due to equation (5) uncertainty did occur, but also with insufficient frequency. Biased underestimation of E, because dye is an inadequate physical model of aerosolization, is plausible, but unsubstantiated. In summary, the survival, masking, and mechanical splitting hypotheses appear to be the most likely explanations of consistently obtaining impact factors above 1.0.

Regardless of which hypotheses are correct, the higher than expected impact factor estimates apparently do reflect real phenomena associated with the current state-of-the-art for wastewater and aerosol sampling and assay for pathogens. Therefore, to satisfy the third model assumption, the high impact factor values presented in Table VI.C-4 should be used without adjustment when predicting pathogen aerosol concentrations with the microbiological dispersion model.

2. Relative Aerosol Survival Hardiness of Microorganism Groups

Comparison of the microorganism groups, with respect to their impact factor values and their viability decay rates, provides an indication of the relative survivability of these groups through the wastewater aerosolization process. A relative hardiness measure was constructed as the sum of separate rankings for initial survival (the median impact factor in Table VI.C-6) and for survival with age [the percentage of very low (unquantified or below $0.01 s^{-1}$) decay rates] for each microorganism. The resultant ranking of microorganism groups is presented in Table VII.C-1, with microorganisms having a similar hardiness ranking being clustered together.

It can be inferred from Table VII.C-1 that the commonly used indicators of wastewater microorganisms (total coliform, fecal coliform, coliphage, and standard bacterial plate count) do not survive wastewater aerosolization nearly as well as do the pathogens studied. The evaluated pathogenic bacteria and enteroviruses both better survive the initial shock of aerosolization and more frequently resist aerosol age decay. Therefore, the common "microbiological wastewater indicators", especially total coliform and fecal coliform, are actually very poor indicators of the pathogenic aerosol hazard posed by wastewater spray irrigation. Fecal streptococci appear to be a more acceptable wastewater aerosol indicator. Fecal streptococci are generally present in the wastewater, are readily assayed, and survive wastewater aerosolization well.

D. Model Applications

The microbiological dispersion model should prove valuable in many applications, especially after development of a reliable procedure for selection of the I and λ parameters, and evaluation of model predictions using this selection procedure. The Pleasanton sampling program has demonstrated that, with present microbiological aerosol sampling and assay methods, it is generally impractical to sample wastewater aerosols for microorganisms beyond 100 or 200 meters from their source. Thus, a reliable modeling technique, such as the microbiological dispersion model, is essential to estimate the level of human exposure to pathogens from wastewater aerosols. The microbiological dispersion model could be used to calculate the pathogen exposure levels of plant workers and neighboring residents at existing and candidate spray irrigation sites[81] as part of

an evaluation of the potential public health risk. With limited aerosol sampling data, the microbiological dispersion model could also be used to calculate distant downwind concentrations emanating from other microbiological aerosol sources, such as the aeration basins of sewage treatment plants and cooling towers that reuse municipal wastewater.

Table VII.C-1
AEROSOL SURVIVAL HARDINESS OF MICROORGANISM GROUPS

Microorganism Group	Initial Survival: Median Impact Factor Value (I)	Survival with Age: Percentage of Runs with Low Decay Rate ($\lambda = X$ or $\lambda > -0.01 s^{-1}$)
Total Coliform	0.16	32%
Fecal Coliform	0.13	38%
Coliphage	0.34	49%
Std. Bacterial Plate Count	0.21	67%
Clostridium perfringens	1.2	55%
Mycobacteria	0.89	88%
Fecal Streptococci	1.7	77%
Pseudomonas	14	77%
Enteroviruses	40*	—

*Based on only two special virus aerosol runs

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16. ABSTRACT The purpose of this study was to determine the extent that individuals near spray irrigation sites are exposed to microorganisms in wastewater aerosols. This report reviews a monitoring effort of a spray irrigation site utilizing unchlorinated secondarily-treated wastewater from biofiltration treatment processes. Objectives included an in-depth pathogen screen of wastewater, establishing the relationship between pathogen levels and traditional indicator organisms, monitoring microorganisms in air within 600 meters of the spray source, and development/validation of a microbiological dispersion model for predicting aerosol pathogen concentrations. Effluent was monitored for microbiological, chemical, and physical characteristics and extensive microorganism and dye aerosol samples were collected (77 aerosol runs). Enteroviruses were detected in air, but at a very low density. Conclusions: There is considerable underestimation of pathogen aerosol levels when using traditional indicators to predict human exposures. A microbiological dispersion model may be used with minimal monitoring to estimate exposure. There is little correlation between wastewater levels of traditional indicators and pathogens. Aerosols containing microorganisms are generated by spray irrigation of wastewater; they do survive aerosolization and can be transported to nearby populations. Until dose-response relationships are developed, neither the levels of aerosolized microorganisms that constitute a hazard nor the degree of required wastewater disinfection can be specified.

17. KEY WORDS AND DOCUMENT ANALYSIS

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