



Project Summary

Health Effects of Sewage Aerosols: Additional Serological Surveys and Search for *Legionella pneumophila* In Sewage

R. Northrop, C. Becker, R. Cordell, M. Sulita, N. Altman, R. Anderson, and J. Kusek

Antibody levels to *Legionella pneumophila*, serogroup 1 and Hepatitis A virus (HAV) were determined for 433 persons living within a 1.6 km radius of an activated sludge plant. Sera of children 6 to 13 years of age were also tested for antibody to Norwalk virus. The antibody prevalence rates for *L. pneumophila*, HAV and Norwalk virus were 23.2%, 31.5% and 7.1%, respectively. The prevalence of *L. pneumophila* antibody was higher than expected. Seroconversions were not observed except for a 4-fold rise in HAV antibody in one pair of sera, suggesting new infections did not occur in the 8-month study period by any of these agents.

Using an index of exposure for the number of viable organisms generated at the plant to which participants were exposed, the average index for persons with antibody to *L. pneumophila*, or HAV, was not statistically different from the averages of those without antibody.

In addition, there was no association between how close people lived to the plant, or how long they lived in the study area and being seropositive. These findings augment but still did not prove the previous findings that

sewage aerosols emitted from this plant had no overt health effects.

In search of *L. pneumophila* in sewage and aerosols, guinea pigs were inoculated with the particulates of sewage and air samples collected at the plant. The inoculum was adjusted to contain not more than 10^6 organisms by standard plate count. In one experiment 4 animals were inoculated with undiluted sewage. *L. pneumophila* was identified in spleen cells by direct fluorescent antibody (DFA) 6-7 days later. After 10-fold dilution of the sample none of the 4 animals had evidence of infection. In a second experiment using undiluted inocula, 2 of 4 guinea pigs developed infection as determined by DFA. Infections were not detected in animals inoculated with the respective aerosol samples. These findings suggested that viable *L. pneumophila* or a related agent occurs in sewage.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back.).

Introduction

Objectives of Study

This study is an addendum to a previous study on the health effects of sewage aerosols carried out in the vicinity of the North Side Sewage Treatment Works (NSSTW) in Skokie, Illinois. At the time of the original study, serum samples were collected from 433 persons in the study population. The sera were initially tested for antibody to several enteroviruses in an attempt to discover whether there was a relationship between the level of exposure to sewage aerosols and infection with these enteroviruses. In addition, attempts were made to detect Legionnaires' Disease Bacteria (LDB) in sewage and sewage aerosols.

The purpose of this addendum was to determine: 1) whether there was a relationship between exposure to sewage aerosols and prevalence of antibodies to *Legionella pneumophila*, Hepatitis A virus and Norwalk virus; 2) to determine if there were bactericidal substances in sewage which would have precluded the occurrence of LDB; 3) to evaluate if the reported methodologies for recovering LDB from environmental samples could be applied to sewage; and 4) to attempt to isolate and identify LDB in sewage and aerosols from the NSSTW.

Study Design

An area within a 1.6-kilometer radius of the NSSTW was designated as the study area, which was estimated to consist of approximately 16,000 persons.

The design and conduct of this main study has been described in detail (EPA-600/1-79-019). Of the 16,000 possible residents in the study area a stratified random sample of 2,378 (approximately 15%) were recruited for a questionnaire survey. Of these, 318 persons voluntarily gave two blood samples, one at the beginning and a second at the end of the eight month study period. An additional 115 people gave one blood sample either at the beginning or at the end of the study. Sera of these 751 bloods were tested for presence of antibody to *Legionella pneumophila* or Hepatitis A virus. Blood was not collected from children under six years of age and only blood samples from children six to twelve years of age were tested for antibody to Norwalk virus.

The presence of antibody in the first or only blood collected was used to calculate prevalence rates of immune indi-

viduals. A four-fold rise in antibody between paired sera was taken as evidence of recent infection and was used to calculate incidence rates of infection. Rates were then evaluated with respect to the demographic characteristics, income, residence, occupation, underlying health conditions, and exposure index to viable aerosol particles documented from the questionnaire, health or environmental surveys previously described. From these data sets a dose-response relationship between different levels of exposure to sewage plant emissions and illness and infection rates was investigated. Standard techniques such as regression and analysis of variance were used to relate the health and environmental data.

Isolation of *Legionella pneumophila* was also attempted two years after the sera were collected. Grab samples of sewage influent in the aeration basin of the NSSTW were obtained. Upwind and downwind air samples were collected during the time the influent traveled through the basin. Following suitable preparation in the laboratory, the particulates of sewage and air samples were inoculated intra-peritoneally into separate groups of guinea pigs. It was necessary to determine whether or not guinea pigs would survive the insult of resident organisms in these samples and subsequently display evidence of infection by the slow growing *Legionella pneumophila*. Evidence of infection would be based on a rise in rectal temperature and/or death, and demonstration of *Legionella pneumophila* antigen in peritoneal cells and splenic tissue by direct fluorescent antibody techniques. Preliminary and parallel experiments were conducted to address the other objectives described above and to facilitate interpretation of the results.

Background and Literature Review

Legionella pneumophila

An epidemic of pneumonia at the Legionnaires' convention in Philadelphia in the summer of 1976 led to the discovery of the Legionnaires' Disease Bacterium now called *Legionella pneumophila*. Investigations of this and other epidemics caused by this bacterium have produced evidence implicating an environmental source. Isolation of the bacterium from the environment during or following the epidemic was possible

in a few cases. Some serological studies of populations exposed to *Legionella pneumophila* in these epidemics were done with varying results. The bacterium has been found in nonepidemic-related habitats such as lakes, streams and soils. It has also been isolated from residual water in cooling towers of air conditioning units in association with disease outbreaks, suggesting that disease in man results from exposure to aerosols containing the agent. It was deemed useful to study a population of healthy individuals living around an activated sludge plant with a defined environmental exposure to sewage aerosol in a non-epidemic situation.

Hepatitis A

Although the Hepatitis A virus has not yet been officially classified, evidence indicates that it is most likely an enterovirus of the picorna- or parvovirus type. While enteroviruses have been infrequently identified in sewage aerosols to date, they have been found in abundance in sewage. Primary and trickling filter treatments have only a modest effect on virus levels in sewage. While activated sludge treatment removes 90 to 98% of the enteroviruses there is concern that a large number of potentially infectious virus particles may be aerosolized in the process, creating a health hazard for sewage treatment plant workers and persons residing nearby. The respirable particles may become trapped in the nasopharynx of exposed persons, an appropriate site for an enterovirus to initiate an infection. Thus, the danger of Hepatitis A virus infection occurring through inhalation of the virus into the nose or mouth is a possibility.

Little, if any, work has been done concerning the occurrence of Hepatitis A virus in sewage aerosols. Although a study was done in Israel which suggested a population living near a wastewater irrigation system experienced two to four times the incidence of infectious hepatitis compared to a control population, the interpretation of these findings awaits reanalysis. It was thus felt that since sera and environmental data were available from the original NSSTW study, a serological screening for antibody prevalence in the study population, as related to exposure indices would be important. Serological analysis would be an appropriate indication of experience with the Hepatitis A virus since most infections are subclinical and are not manifested as overt disease.

Norwalk Virus

Norwalk virus is one of the viral agents recently shown to be associated with epidemics of gastroenteritis in older children and adults. Infections, either clinical or subclinical, are relatively infrequent (<5%) in children before age 10 years while 50-60% of adults have serological evidence of such infections. The virus is a small, parvovirus-like particle, apparently spread by the fecal-oral route and shed to maximal amounts in feces during episodes of diarrhea.

Since the Norwalk virus occurs in stools, it may be present in sewage and possibly aerosols for spread to exposed persons around sewage treatment works. Sera of children 12 years of age and less in the study population were tested to determine if the prevalence of a 5-10% antibody would be found as expected from previous studies.

Rotaviruses, which are Reovirus-like viruses, have also been associated with epidemics of diarrhea in infants and children less than 3 years of age. Sero-epidemiological studies have shown that over 70% of children in the 3-5 year old age group have Rotavirus antibody. There was initial interest in determining Rotavirus antibody prevalence in the study population aged 12 years and less, but since blood was not collected from children under 6 years of age, it was decided to withhold those determinations until the Norwalk virus antibody data were evaluated.

Conclusions

1. The prevalence of antibody of *Legionella pneumophila*, serogroup 1, in the study population was higher than expected (23.2%), but there was no association between total viable particle exposure index or length of residency in the study area to implicate aerosols from the sewage treatment plant as a source of exposure.
2. New cases (incidence) of *Legionella pneumophila*, based on a 4-fold antibody rise over the 8-month observation period, were not detected.
3. Prevalence of antibody to Hepatitis A virus was found to be 31.5% which was an expected rate for that study population. Personal exposure indices or length of residency in the study area were not associated with having antibody.
4. One new infection with Hepatitis A virus was detected serologically

suggesting a two-to-three fold higher incidence rate than expected, but the single case was considered a spurious finding.

5. Norwalk virus antibody in the 6-12 year old age group occurred at an expected prevalence rate (7.1%) suggesting no unusual occurrence of infection by this gastroenteritis virus in the population.
6. In two experiments, pathogenic *Legionella pneumophila* was tentatively recovered from grab samples of sewage entering the aeration basin of an activated sludge plant.
7. A liquid medium containing the particulates captured from approximately 200 M³ of air collected 50 meters upwind or downwind from the sewage aeration basins did not contain viable, pathogenic *Legionella pneumophila* when inoculated into guinea pigs

Recommendations

1. Prospective studies of populations newly exposed to sewage aerosols would provide more definitive information on the risk of infection by *Legionella pneumophila*, Hepatitis A or any of the gastroenteritis viruses.
2. Since the epidemiology of Legionnaires' Disease is poorly understood, it would contribute to this understanding to determine the occurrence of *Legionella pneumophila* in sewage. Further studies are needed to determine the source of the organism, i.e., man or the environment; seasonal variation in its occurrence; and conditions that enhance its pathogenicity in man.

The parent study, entitled *Health Effects of Aerosols Emitted from an Activated Sludge Plant*, Grant No 805003, is available as EPA-600/1-79-019 from the National Technical Information Service, Order No. PB 299583.

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The complete report, entitled "Health Effects of Sewage Aerosols: Additional Serological Surveys and Search for *Legionella pneumophila* in Sewage," (Order No. PB 81-179 830; Cost. \$6 50, subject to change) will be available only from:

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