BNL 50787

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AN ASSESSMENT OF THE OCCURRENCE OF HUMAN VIRUSES IN LONG ISLAND AQUATIC SYSTEMS

James M. Vaughn
Edward F. Landry

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DEPARTMENT OF ENERGY AND ENVIRONMENT

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ASSOCIATED UNIVERSITIES, INC.

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UNITED STATES DEPARTMENT OF ENERGY

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BNL 50787
UC-11
(Environmental Control Technology and
Earth Sciences — TID-4500)

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UPTON, NEW YORK 11973

NOTICE

Printed in the United States of America
Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161
Price: Printed Copy $7.25; Microfiche $3.00

April 1978 360 copies

This document was prepared by Brookhaven National Laboratory under a contract with the Nassau-Suffolk Regional Planning Board pursuant to Section 208, Federal Water Pollution Control Act Amendments of 1972 (PL92-500). This Project has been financed in part with Federal Funds from the United States Environmental Protection Agency under grant number P002103-01-0. The contents do not necessarily reflect the views and policies of the United States Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Acknowledgment

The authors wish to acknowledge the invaluable participation of the following members of the Brookhaven National Laboratory Virology Group in the technical conduct of this project as well as in the preparation of this manuscript.

M.Z. Thomas, M.A.

Cheryl Ann Beckwith

Lois J. Baranosky

Marilyn Dahl

Neva Delihas

Wayne Penello

Mary Purpura

Thomas Vicale

The authors further acknowledge the efforts of the members of the Nassau-Suffolk "208" Technical and Citizens Advisory Committees whose foresight and perserverance enable the undertaking of this study and the cooperation and assistance of the officials of the Nassau and Suffolk County Departments of Health and Environmental Control, members of the State Depaetment of Environmental Conservation, the U.S. Geological Survey, the Suffolk County Water Authority, the study site treatment plant operators, and Mr. Butler Flowers and Staff. A special thanks is extended to Mr. David Wiremius and Mr. Aldo Andreoli for their participation and assistance throughout the program.

Abstract

A virus survey was conducted in Nassau and Suffolk Counties under the auspices of the federally-funded "208" program from June 1976 to June 1977. The survey involved the concentration, enumeration, and identification of human enteroviruses from selected aquatic systems on Long Island including embayments, lakes, creeks, public drinking water supplies, groundwater influenced by wastewater recharge, sanitary landfills, and stormwater recharge basins; and the effluents from secondary and tertiary sewage treatment plants.

Enteroviruses were isolated from all systems studied except the public water supply wells. As expected, viruses were most often encountered in the chlorinated effluents of sewage treatment plants. On two separate occasions, wild type Poliovirus was isolated from one of these plants.

The limited sampling conducted at each site (1 per month) obviated any extensive interpretation of the data for the purpose of identifying the precise hazard posed by enteric viruses in Long Island waters. Among tentative conclusions were: support for the continued study of recharge of groundwater aquifers via the application of properly treated domestic wastewater to recharge basins; caution regarding placement of private septic systems in saturated zones near surface water bodies; the discharge of sewage effluents into embayments; and the identification of those areas requiring further virological study.

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I. INTRODUCTION AND OVERVIEW

Growing public concern over the fate of dwindling natural aquatic resources has resulted in a nationwide commitment for the reevaluation of water management practices, especially those related to domestic wastewater disposal. Efforts to evaluate the relevance of various treatment schemes have often been stymied by the lack of adequate information with which to predict the movements and ultimate fates of the potentially pathogenic biological organisms commonly associated with human fecal material. Among these organisms much attention has been given to the final disposition of human viruses. The major human virus groups known to occur in sewage include:

1) Enteroviruses - transient members of the human alimentary tract consisting of over 100 species including Polioviruses, Coxsackieviruses, and ECHO viruses; 2) Adenoviruses - upper respiratory viruses which are able to withstand the acidity of the human gut and may be shed in the feces; 3) Hepatitis virus; and 4) Reoviruses. While only Hepatitis virus and Poliovirus infections have been conclusively proven as being transmitted by the water route (i.e. sewage pollution of drinking water, shellfish beds, recreational waters, etc.), studies have indicated the likelihood of similar transmission of some or all of the species mentioned above. A listing of the viruses which may be water-borne and the diseases associated with them is presented in Table 1.

Reports of human virus isolations from diverse aquatic systems (e.g. rivers, bays, estuaries, treatment plant effluents, etc.), which have appeared on occasion in the literature, have underscored the need for more extensive virus monitoring programs. Such a need was realized by the

Table 1

Human Viruses Commonly Found In Sewage and Diseases Associated With These Viruses.

Group	Subgroup	No. of Serotypes	Type Nucleic Acid	Diseases
Enterovirus	Poliovirus	3	RNA	Mild-Severe Gastroenteritis Abortive Poliomyelitis Aseptic Meningitis Paralylic Poliomyelitis
	Coxsackie-virus			
	A	24	RNA	Summer Minor Illness Herpangina Aseptic Meningitis Common Cold Hand, Foot and Mouth Disease Infant Diarrhea
	B	6	RNA	Aseptic Meningitis Common Cold Pleurodynia Neonatal Disease Sudden Infant Death Syndrome Myocarditis Pericarditis
	Echovirus	34	RNA	Aseptic Meningitis Mild Paralysis Febrile Illness Conjunctivitis Boston Exanthem Disease Infant Diarrhea Vaginitis and Cervicities Pericarditis and Myocarditis
Hepatitis				
	A		RNA?	Infectious (viral) Hepatitis
	B		DNA?	Serum Hepatitis
Adenovirus		31	DNA	Acute Respiratory Disease Pharyngoconjunctival Fever Primary Atpical Pneumonia Epidemic Keratoconjunctivitis (shipyard eye) Intussusception Febrile catarrh
Reovirus		6	RNA	?

Nassau-Suffolk advisory committees which included a virus study as part of their federally-funded "208" program. The study, initiated in June of 1976, was designed to survey the occurence of human enteric viruses in a number of routinely monitored aquatic systems. Enteroviruses were specifically chosen as a model system because of their predominance in sewage-associated systems and the relative ease of their isolation and identification in the laboratory.

The ultimate goal of the virus survey was to provide previously unavailable background information on the presence of these unique organisms in various Long Island aquatic resources. Information generated from the study would then be utilized by those involved in water management planning decisions. In this regard, the virus data were not meant to stand alone, but in conjunction with existing physical and chemical information. In addition, virus results would be used to define trends in specific treatment systems and to delineate areas for future study.

II. LITERATURE REVIEW - HUMAN VIRUSES IN AQUATIC SYSTEMS

Because of the great diversity among the kinds of samples taken during the 208 virus study, it will be necessary to review each as a separate unit (e.g. surface waters, sewage treatment plant effluents, drinking water, etc.).

A. Drinking Water

The likely presence of virus in drinking water has been a difficult, often perplexing problem to evaluate. Among questions facing environmental scientists in this area are those concerning: 1) the minimum infective dose necessary for the establishment of an infection; 2) the documented transmission of virus diseases via the water route and 3) the confirmed occurrence of virus in public drinking water supplies.

While many disagree on the question of how many virus particles are necessary for the establishment of infection in humans, laboratory experiments in tissue culture cells have indicated that one virus is sufficient to produce an infection in human cells (Plotkin and Katz, 1967; Katz and Plotkin, 1967). The infection may or may not lead to disease depending upon a wide range of host-related factors. Hypothetical calculations done by Gerba, Wallis and Melnick (1975a) suggested that where one infectious unit is present per 50 gallons of finished water, a community utilizing 50 million gallons per day (GPD) would have a minimum of 600 of its residents exposed to possible infection each day (assuming 0.2% use of water for drinking purposes, and an infection rate of 30%).

A second problem in the assessment of virus drinking water relates to the lack of epidemiological evidence on the transmission of disease by the water route. To date, the only documentation for water transmission of human disease by sewage-borne viruses is that pertaining to the Hepatitis type A virus, and possibly Poliovirus. In 1955, a sewage-contaminated municipal water supply was blamed for over 30,000 cases of infectious hepatitis in New Delhi (Viswanathan, 1957). Between 1961 and 1970, over 30 outbreaks of water-borne hepatitis were recorded in the United States with a majority being caused by sewage contamination of private or semi-public water supplies (Sobsey, 1975; Taylor et al., 1966). Sobsey (1975) pointed out that the lack of epidemiological evidence for water-borne transmission of other enteric diseases does not mean that transmission of such disease is impossible. Goldfield (1976) suggested that usual epidemiological procedures could not be used to determine water-borne transmission of most

enteric diseases citing a number of reasons including: prior immunity of individuals leading to subclinical infection; the broad spectrum of disease syndromes common to many virus types; and the secondary spread of disease by person-to-person contact obscuring the role of water. Sobsey (1975) concluded that alternative investigative approaches must be utilized to determine the transmission of water-borne enteric disease.

The third problem in evaluating the virus assessment in drinking water centers on controversial isolations and difficult virus testing procedures. As mentioned previously Hepatitis virus was responsible for a massive disease outbreak in New Delhi in 1955. In 1964, Coin et al., isolated enteric virus in 18% of the drinking water samples analyzed in Paris. Among the isolates he identified were Poliovirus types 1 and 3, Coxsackie virus and ECHO virus, with an average concentration of 1 plague-forming unit (PFU) per 300 l (liters) of water. Enteric viruses were also found in 10 l volumes of drinking water in South Africa (Nupin, Bateman and McKinney, 1974). In the United States, enteric virus isolations from drinking water have been sporadic and sometimes questionable. In 1970, Poliovirus type 2 was isolated from an unchlorinated drinking water well in southeast Michigan (Mack, 1973). After chlorine treatment was initiated, the virus could not be detected. Also in 1970, a national controversy arose when the Northeast Water Supply Research Laboratory reported the isolation of enteric virus from the finished drinking water supplies of two Massachusetts communities (Potable Water Senate Hearings). Almost immediately, the Water Supply Research Laboratory of the EPA National Environmental Research Center initiated studies in order to confirm these findings and evaluate the isolation methods used. The investigators concluded that techniques used

by the Northeast Lab required excessive manipulations and were therefore subject to the possibility of extraneous viral contamination. A similar situation recently occurred when Hoehn (1975) reported the presence of Poliovirus in the Virginia's Occoquan Reservoir. Subsequent studies by Akin and Jakubowski (1976) of the Environmental Protection Agency failed to confirm the presence of virus in the system and again raised the question of possible laboratory contamination.

Extensive studies of drinking-water systems in communities located in Ohio, Indiana and Missouri have shown these water supplies to be free of enteric virus (Akin, et. al., 1975a). In these studies, the authors sampled large volumes of drinking water (1900 l) using sensitive virus concentration techniques designed to recover 3-5 PFU per 380 l. In the absence of positive findings, the authors concluded that good conventional treatment was adequate for virus removal from public drinking water supplies.

B-1. Surface Waters

The possible transmission of diseases of viral etiology in surface waters (lakes, streams, bays, estuaries and coastal waters) has been the topic of numerous studies. Concern has been amplified by the recent interest in the conservation of the aquatic environment for both recreational and economic purposes. The latter is of particular importance in an area such as Long Island where coastal waters and embayments serve as important shellfish-growing areas.

The virus hazard has been created by the release of sewage material either directly into the larger water masses, or indirectly via waste-water contamination of their tributary streams and rivers.

Human viruses have been isolated from almost all types of surface water.

Simkova and Wallnerova (1973a) isolated Coxsackie virus from waters of the Danube River. Nestor and Costin (1976) reported similar findings in sewage-contaminated river waters in Roumania. Human enteric virus have been isolated in estuaries (Metcalk and Stiles, 1967; Vaughn and Metcalk, 1975), as well as in seawater and coastal marine sediments (DeFlora, DeRenzi and Badolati, 1975). In the latter study, the concentration of viruses isolated from marine waters ranged from 0.1 PFU per 100 milliliters (ml) in moderately polluted waters, to 40 PFU/100 ml in heavily polluted waters near sewage outfalls. The authors found that viruses readily adsorbed to marine sediments and could be released into the water column by simple mechanical shaking similar to the agitation occurring in natural waters.

The survival capacity of enteric viruses in marine environments is quite unpredictable, even though seawater has been shown to contain antiviral properties. Seawater constituents such as organic matter, particulates and heavy metal ions have been shown to be antagonistic to the action of nonspecific antiviral components, ultimately resulting in the extension of virus survival (Vaughn, 1974). Initial virus inactivation studies were conducted using Poliovirus type 3 in Baltic and North Sea waters by Lycke, et al. (1965). The authors found that marine waters had a virus inactivating capacity (VIC) capable of inactivating 99% of the virus in 8 days at 23 C. Since the inactivating agent or agents were not heat-labile or filterable, the authors suspected that marine bacterium might have been involved in the virus inactivation process. In a similar study, Shuval, Thompson, Fattal, Cymbalista and Wiener (1971) found antiviral activity in Mediterranean and Red Sea waters. Heating and filter sterilization reduced the inactivating capacities of the water leading the authors to also conclude that marine bacteria might play a role in viral inactivation.

Laboratory studies by Metcalf and Stiles (1968) and Vaughn and Metcalf (1975) demonstrated that inactivation of Coxsackie type B3, ECHOvirus type 6 and Poliovirus type 1 was dependent primarily upon water temperature, autoclaved and ultraviolet light-sterilized waters showing similar VIC. Follow-up studies in the field indicated, however, that viruses could be inactivated at an even faster rate in natural environments, suggesting that factors other than temperature were involved. By using a flow-through system, Akin et al. (1976) found that autoclaving and filtration had little effect on the virus inactivating capacity of waters from the Gulf of Mexico. They found water temperature to be the most important factor in virus survival but cited the very complex nature of virus inactivation in an aquatic environment. Temperature was again found to be an important factor in a study by Lo et al. (1976). The study revealed that while Poliovirus could survive 6 weeks at 25 C, survival could be extended to 40 weeks by reducing the temperature to 4 C. Their field studies indicated that while virus were more sensitive to inactivation in natural environments than in laboratory environments, water temperature still played an important role in survival rate. Poliovirus was shown to survive 27 days during the summer months at temperatures of 21 to 26 C, but were still viable for periods of up to 65 days during the winter (0-12 C). The authors demonstrated that survival rate varied greatly with the type of virus being studied. ECHOvirus type 6 was more stable than Poliovirus in both field and laboratory studies. Coxsackievirus type 5 was the most stable, capable of surviving up to 53 weeks at 4 C in laboratory experiments, and over 80 days in field studies.

The survival of enteric virus in non-marine aquatic environments have not been extensively studied. Simkova and Wallnerova (1973b) found that

Coxsackie virus A4 could survive for 45 days at a temperature of 22 C and up to 154 days at 4 C in Danube River water. Using membrane dialysis chambers, O'Brien and Newman (1977) observed inactivation rates of Poliovirus types 1 and 3, and Coxsackievirus types B1 and A13 in the Rio Grande River. They found inactivation to be a function of both water temperature and the virus type. All virus were more readily inactivated at 23-27 C than at 4 C, with Polio 1 and Coxsackie B1 showing greater stability than Polio 3 and Coxsackie A13. Lycke et al. (1965) found river and lake waters to be devoid of inactivating capacity for Poliovirus type 1, but Hermann et al. (1974) demonstrated that Polio type 1 and Coxsackie type A9 could be inactivated by water from a Wisconsin Lake. The viruses were inactivated more rapidly in natural lake water than in sterilized lake water.

The mechanism for the viricidal action of marine and other surface waters remains complicated. The role of powerful oxidants, sunlight, salinity, metals, detrital material and marine organisms have been suggested as contributing to viricidal capacities of natural waters (Won and Ross, 1973), but there is considerable conflicting evidence concerning the effect of heat-labile filterable agents or toxins. All studies seem to agree that water temperature appears to be of primary importance with greatest viral inactivation occurring at higher temperatures. Recent studies by O'Brien and Newman (1977) have indicated viral inactivation at temperatures lower than 37 C might be due to damage of the nucleic acid core of the virus. They found that inactivated virus was still capable of adsorbing to host cells even after exposure to river water indicating no major alteration of external structures. They theorized that inactivation resulted as a consequence of an exposure of the viral nucleic acid to some inactivating agent in the water, damage to the nucleic acid likely resulting in an inability

for the virus to replicate, rendering it functionally "dead". While nucleic acid degradation appears to be the primary mechanism for viral inactivation at temperatures of 25 C and below, it appears that oxidation of the viral protein coat is the most likely mechanism for inactivation at temperatures of 37 C and above (Lund, 1973).

B-2. Shellfish

There has been increasing concern over the likelihood of human virus carriage by shellfish. While there is little epidemiological evidence for the transmission of enteric disease from the consumption of sewage-contaminated shellfish, (with the notable exception of infectious hepatitis), the potential for infection cannot be ignored. Fugate, Cliver and Hatch (1975) outlined a number of reasons why a potential health hazard exists: 1) shellfish raising waters are continually being subjected to high levels of pollution from sewage sources; 2) shellfish, being filter feeders, are able to efficiently concentrate viruses from the surrounding waters; 3) a majority of viruses are concentrated in the digestive organ of the mollusk which is consumed along with all the other parts of the animal; 4) shellfish are frequently consumed raw or with minimal cooking which may not be sufficient to inactivate all of the viruses within them.

The occurrence of human virus (i.e. enterovirus) in various shellfish species is well documented. Morris, Mearns and Kim (1976), while studying the presence of virus in the California mussel found that 18 of the 39 samples tested contained virus. The mussels had been taken from beds located near outfalls which were discharging primary and secondary treated sewage effluent. Viral enumerations revealed concentrations ranging from 25 to 1475 PFU/kg of meat. Fugate et al., (1975) found virus in 2 of 17 oyster samples in Texas and in 1 of 24 samples taken from the Louisiana

Gulf Coast. Oysters had been taken from areas which met the approved coliform standard. Virus isolates were identified as ECHO virus type 4 and Poliovirus type 1 from the Texas oysters, and Poliovirus type 3 from the Louisiana oysters.

In 1968, Metcalf and Stiles isolated Poliovirus, Coxsackie B-3 and Reovirus from shellfish growing in a sewage polluted estuary in New Hampshire. Coxsackie type A was isolated from 7 to 70 oyster samples and 2 out of 10 mussel samples found in a French market (Denis, 1973). Serological assays in suckling mice identified the majority of the French isolates as being Coxsackie virus type A16.

Although many enteric virus isolates have been found in shellfish, there is no epidemiological evidence to indicate that consumption of contaminated shellfish would lead to infection. There is, however, well-documented evidence for the shellfish-mediated transmission of disease by Hepatitis virus. The first reported shellfish-related outbreak occurred in Sweden in 1955 resulting in 629 cases of infectious hepatitis (Roos, 1956). Since then, outbreaks have occurred in 1961 in New Jersey, Mississippi and Alabama; in Philadelphia and Connecticut in 1963; in North Carolina in 1964; in New Jersey in 1966; and in Rhode Island and Massachusetts in 1971 (Portnoy et al., 1975). An outbreak occurred in October and November 1973 (Portnoy et al., 1975) affecting two hundred and sixty-three individuals from Houston and 15 from Calhoun, Georgia, who were infected with hepatitis following the consumption of raw oysters from Louisiana Bay. After eliminating the possibility of contamination during transportation and storage, investigation concluded that the oysters were contaminated prior to, or at the time of harvesting. The area from which the oysters were harvested had been closed 6 weeks earlier due to contamination by polluted flood waters from the

Mississippi Valey. On September 1, the area was recertified by means of a coliform standard. The authors concluded that the hepatitis virus had been retained within the oysters for periods as long as 6 weeks. More recently, Mahoney et al. (1974) detected the presence of Australia antigen (Au), indicative of the presence of type B Hepatitis virus, in Maine clams. The clams were taken from waters known to be contaminated with untreated sewage from a local hospital. It was found that the antigen could be transmitted to previously uninfected clams and they concluded that shellfish could act both as a vector and a reservoir for Au antigen and type B Hepatitis virus.

Shellfish obtain their food through a filter-feeding process in which they selectively ingest small particles of organic matter from large volumes of seawater. Food particles become attached to the mucus secretions of the shellfish and are directed by ciliary action to the mouth region where they are either swept into the mouth, or rejected and passed out as pseudofeces. Since viruses are often attached to small particles of organic material, they readily gain entrance to the inner portion of shellfish.

Di Girolamo, et al. (1977) proposed a mechanism for the attachment of virus to shellfish mucus during feeding. Utilizing a number of enteric viruses in seeded laboratory experiments, they found that virus particles became ionically bound to secretions. The binding sites were found to be the sulfate radicals in the mucopolysaccharides of the shell mucus. The uptake of virus particles by shellfish occurs very rapidly resulting in the accumulation of large numbers of virus in the digestive glands of the animal. Liu, Seraichekas and Murphy (1966a) found 70% of the poliovirus seeded into seawater tanks were accumulated in species of the Northern Quahaug in 48 hours. Di Girolamo, Liston and Matches (1975) reported a

similar rate of uptake in the West Coast oyster with 80 to 90% of the seeded viruses being accumulated within 24 hours. Liu, Seraichekas and Murphy (1966b) found that maximum efficiency of virus uptake occurred when virus concentrations in the surrounding water were at low levels. Hamblet et al. (1969) reported that oysters subjected to low turbidity water accumulated three times as many poliovirus as oysters in high turbidity seawater.

Although high titers of virus can be accumulated within shellfish in a relatively short period of time, the animal's filtering system can work to remove virus when placed in clean water through a process called depuration. Laboratory studies have shown that contaminated shellfish, when placed in fresh running seawater, can be rendered virus-free. Depuration rates have been found to be dependent on the temperature as well as the salinity of the seawater (Liu, Seraichekas and Murphy, 1967). Studies have found removal of virus occurring in 48 hours at 18 C. Reducing the temperature to 13 C resulted in an increase in the depuration time. Little or no depuration occurred at 8 C, a temperature at which the shellfish ceased pumping. The authors also demonstrated that a reduction of 50% in the salinity of the water in the oyster tanks was sufficient to halt the virus depuration process. Studies conducted in an estuarine environment by Vaughn and Metcalf (1975) showed that complete virus removal from seeded oysters required a period of 21-30 days in summer (17-22 C) and 60-80 days during winter months (-1-12 C). These results tended to confirm those of several earlier studies. Hamblet et al. (1969) concluded that under controlled environmental conditions, oysters can effectively eliminate virus irrespective of turbidity levels. The optimal conditions for depuration were judged to be: continuously flowing virus-free seawater of either high

or low turbidity; a temperature optimum of 20 C; and a salinity of greater than 18 parts per thousand (ppt).

In addition to determination of uptake and depuration rates, the question of virus survival within the shellfish has also been addressed. Morris et al. (1976) calculated that enteric viruses could survive in mussel tissue three to six times longer than coliform bacteria. Hedstrom and Lycke (1964) found Poliovirus to be more stable in oyster tissue than in the surrounding waters. Di Girolamo et al. (1970) went one step further in testing the survival of Poliovirus in shellfish during various food preparation procedures. They found a marked stability during refrigeration for periods up to 30 days. Studies with heat processing showed surprising results. The authors were unable to inactivate all of the shellfish-bound Poliovirus after frying or stewing for 8 minutes, baking for 30 minutes, or steaming for 30 minutes. They concluded that none of the procedures were of sufficient duration to generate enough internal heat to bring about total virus inactivation. Later studies conducted by the same authors concluded that total virus inactivation required a 30-minute exposure to temperatures in excess of 70 C.

C. Sanitary Landfills

Sanitary landfills contain a mass of heterogeneous solid waste materials including those generated by households such as animal (pet) feces and fecally soiled disposable diapers. Since fecal material is known to contain potential human pathogens, the possibility exists that such organisms may be collected and passed via the landfill leachates to groundwater aquifers (Pohland and Engelbrecht, 1976).

While a number of studies have investigated landfills for pathogenic bacteria, few have concerned themselves with the fate of human enteric

virus in landfill leachate and leachate-contaminated groundwater. Peterson (1971) examined raw municipal solid wastes and found human enteric virus in 4 of 12 samples in concentrations of 192 to 684 PFU per 200 g of solid waste. The viral isolates were identified as Poliovirus types 1, 2 and 3. Among the waste items most commonly present in the municipal waste were disposable diapers. Further studies by Peterson (1974) demonstrated that enteric viruses could be found in 10% of the soiled disposable diapers analyzed.

The potential hazard of enteric viruses in sanitary landfills depends upon the amount of virus in the landfill, their survival in the landfill environment, and the ability of the viruses to pass through the landfill into the surrounding environment (Engelbrecht et al. 1974). As with most microorganisms, the fate of enteric virus in landfill environments is contingent upon a number of factors including temperature, pH, moisture, duration of storage and the presence of chemical and biological antagonists. A majority of survival studies conducted thus far has dealt with the survival of virus in landfill leachate. Peterson (1971) failed to recover virus after seeding solid waste with Poliovirus in a sanitary landfill. Cooper et al. (1975) reported sporadic recovery of seeded Poliovirus for periods of up to 20 weeks from the leachates of simulated sanitary landfills whose chemical and physical properties were similar to those of natural sanitary landfill leachates. The authors felt that the sporadic occurrence was due to the irregular distribution of the fill, and the non-uniform flow of water over the fill. They were able to show, however, that the leachate had no detrimental effect on Poliovirus over a 48-hour period. Sobsey et al. (1975) was unable to recover Poliovirus or ECHO virus in a simulated sanitary landfill seeded with high concentrations of each virus. They

conceded that their lysimeters might not have been operating for a sufficient amount of time to allow viruses to have traveled the length of the refuse column. Engelbrecht et al. (1974) studied the stability of Poliovirus in landfill leachates at various temperatures and pH. They found that naturally occurring leachate (22 C) at a pH of 5.3 was more viricidal than a pH of 7.0. Additional studies on the effect of temperature showed an almost immediate viral inactivation at 55 C. Similar work by Sobsey et al. (1975) showed 95% virus inactivation in 2 weeks at 20 C, 6 days at 37 C, and 27 days at 4 C.

From the above, it can be determined that the rate of viral inactivation will vary greatly depending on the type of leachate studied. In an effort to determine which specific chemical characteristics were responsible for viral inactivation in leachates. Engelbrecht and Amirhor (1975) fractionated a landfill leachate by ultrafiltration and tested the various subfractions for virus inactivating capacity. They observed that most of the inactivation was found in a 500 molecular weight (MW) permeate. Chemical analysis of the permeate revealed that it contained high concentrations of short chain fatty acids as well as iron (120-190 milligram per liter, mg/l) and zinc (30-48 mg/l).

Little information is available on the passage of viruses from landfills leachates to groundwater aquifers. Existing data on removal of virus in sewage material via adsorption to soil columns cannot be extrapolated to a virus-in leachate situation (Pohland and Engelbrecht, 1976). A single study conducted by Novello (1974) showed an 80% or less retention of Poliovirus in landfill leachate by soil. To date, no follow-up studies have been reported.

D. Storm Water Recharge Basins

There is little or no information available concerning the presence of human viruses in storm water recharge basins, or the groundwater beneath them. It can be speculated that the most likely source of viruses in such basins would be from surface run off (provided such waters would have access to basins). Viruses on entering basins would be subjected to the same removal systems discussed for sewage recharge basins in section E-2 (i.e., adsorption to soil, etc.).

The presence of virus in groundwater beneath stormwater recharge basins could also be indicative of other pollution sources. Such a condition might occur in an area where the basins are in the midst of a heavily developed area making use of septic tanks. In such an area, viruses might enter the groundwater through septic leachates with no direct involvement of the basin.

E-1. Sewage Treatment Plants

The occurrence of virus in human domestic wastewater is well-documented. Poliovirus was first found in raw sewage by Levaditi (1940) and by Paul, Trask and Gard (1940). Melnick (1954) found Poliovirus in secondary treated sewage effluents. Although outbreaks of poliomyelitis were reported shortly thereafter (Little, 1954), the evidence incriminating the outbreak as water borne was circumstantial. Other enteric viruses have been isolated from sewage, such as Coxsackie (Clarke, Knowles, Shimade, Rhodes, Ritchie and Donahue, 1951) and ECHOvirus (Kelly and Sanderson, 1957). More recently, Shuval (1970) found high concentrations of enteric virus in raw sewage ranging from 5 to 11,000 PFU per liter. In a two-year study of Israeli wastewaters, Buras (1976) found that viruses were present throughout the year in both raw sewage and secondarily treated effluents. The highest concentrations reported were during the summer months, with average concentrations of 28,000 PFU per 100 ml in raw influent and 20,000 PFU per 100 ml in the treated effluent. There was considerably less virus found during the winter months. During a period of epidemic poliomyelitis, seven strains of poliovirus 1, 2 and 3 were recovered from Madrid wastewaters (Olivares, 1974). Studies have demonstrated the high number of solid-associated virus in sludge (Wellings, Lewis and Mountain, 1976; Cliver, 1975) which are removed by primary settling during the initial phases of wastewater treatment. Although the raw sludge is rich in nutrients, it cannot be utilized in this form due to the high concentrations and long-term survival of both bacterial and viral pathogens. Investigations have shown that viruses associated with sludge solids are still capable of causing infection (Moore, Sagik and Malina, 1975). A common treatment for raw sludge is anaerobic digestion. Studies have elucidated the mechanism

of viral inactivation during anaerobic digestion. Using raw sludge seeded with different serotypes of Poliovirus, Ward and Ashley (1976) found that virus could be recovered intact from digested sludge, but the concentration varied with temperature and time. They observed a 90% reduction in viral infectivity in one day at 28 C, but required a digestion period of 5 days for the same reduction at 4 C. Since raw sludge exhibited no viricidal activity, they concluded in a later study that the viricidal agent was a product of the digestion process (Ward, Ashley and Moseley, 1976). Fractionation of the digested sludge indicated that the antiviral activity was associated with the liquid portion of the sludge. When this fraction was added to raw sludge viral inactivation occurred. Analysis of the liquid indicated the agent responsible for the inactivation was ammonia (Ward and Ashley, 1977). Inactivation was found to occur only when the pH of the digested sludge was 8.0 or higher where the ammonia would be in the uncharged state. Inactivation was observed for several viruses belonging to the Picornavirus family (Polio, ECHO, Coxsackie) while Reovirus was resistant to the effects of ammonia. The mechanism of inactivation appeared to be cleavage of the major capsid proteins followed by destruction of the viral RNA.

A number of studies have been conducted which were designed to determine the efficiency of virus inactivation at each step of the wastewater treatment process.

1. Primary Settling

The data on virus removal by primary settling is confusing and incomplete. After seeding Poliovirus in raw influent, Clarke et al. (1961) reported that virus failed to settle out within 3 to 6 hours after seeding. Only 40 to 79% of the virus had settled after 24 hours even though 75% of

the solids had settled. Berg (1973b) pointed out that there was no way of measuring those viruses imbedded and adsorbed within the fecal material. Presumably, a large portion of these viruses would settle out with the solids.

2. Storage

Long-term storage has been suggested as a simple method for destroying virus. The survival of virus, however, is directly related to the water quality and the temperature (Berg, 1973b). In his study, Berg reported that 99% inactivation of Polio 1 and ECHO 12 required 60 days of storage at 10 C, and 30 days at 30 C. ECHOvirus type 7 required twice as long for the same inactivation. Survival was found to be longer in clean water than moderately or grossly polluted water (Clarke and Chang, 1959). Because of the lengthy detention times and the large storage facilities required, this method of virus removal is not considered to be practical.

3. Biological Treatment

Several methods of biological treatment have been utilized to remove virus from wastewater including: trickling filters, stabilization ponds and activated sludge. Trickling filters show erratic virus removal rates. Shuval (1970) reported 16 to 100% recovery of virus from wastewater passed through trickling filters. Results from experiments utilizing stabilization ponds to remove virus were equally varied and erratic. In a series of repeated experiments, Shuval (1970) reported that virus removal ranged from 0 to 96% in ponds with a 20 day retention time.

Activated sludge appears to be the best method of biological treatment available for virus removal or inactivation. In laboratory studies, Berg (1971) reported that 96 to 99% of Coxsackie type A9 was removed after a 6 to 8.5 hour treatment period. When Polio 1 was seeded into activated

sludge, 88 to 94% was removed within 7.5 hours. Similar studies conducted at treatment plants yielded reductions of 53 - 71%.

4. Chemical and Physical Treatment

Coagulation appears to be the most effective chemical procedure for removal of viruses from wastewater. The reaction involves a metal cation-protein interaction forming a metal-virus complex which aggregates to form a precipitate (Clarke and Chang, 1959). Aluminum sulfate, calcium hydroxide and polyelectrolytes have been most often used in the coagulation process. Chang et al. (1958) obtained virus reduction up to 99% using 60 to 100 mg/l of alum. By using 10 mg/l of alum as a coagulant, Thorup et al. (1970) removed 85 to 90% of seeded poliovirus type 1. Virus is generally not inactivated by coagulation but precipitated in the sludge. A number of investigators have isolated viable virus from alum sludge and expressed concern over the disposal of such sludge (Gerg, 1973b). Lime $\left[Ca(OH)_2\right]$ is an effective coagulant at concentrations of 400 to 500 mg/l. When the higher concentrations of lime were used, a pH of 11.1 resulted. This pH level was sufficient to destroy or inactivate 90 to 99% of the virus during a 90-minute contact period (Gerg, 1973b). Effective virus removal was also reported using $FeCl_3$ as a coagulant (Chang et al., 1958). Cationic polyelectrolytes have been found to be more efficient in virus removal than nonionic or anionic polyelectrolytes (Berg, 1973a). In deionized water up to 99 percent removal of virus was observed with these polyelectrolytes.

5. Disinfection

Since the majority of sewage treatment plants cannot effectively remove all the potentially harmful microorganisms during biological, physical and chemical treatment, a terminal disinfection stage is necessary for wastewaters. However, due to the complex nature of the effluent wastewaters,

there is ample evidence to indicate that routine disinfection used in most treatments is not sufficient to destroy viruses. There is no one agent which can effectively disinfect all types of wastewaters due to the varying quality of the effluents.

Chlorine is widely used as a terminal disinfectant. The viricidal effectiveness of chlorine is dependent upon pH, retention time, temperature, chlorine concentration and overall quality of the water being treated. There is some disagreement in the literature as to which form of chlorine is the most effective viricidal agent. Between acidic and neutral pH levels chlorine hydrolyzes to yield hypochlorous acid (HOCl), while under alkaline conditions, it exists as hypochlorite ion (OCl^-). Kott, Nupen and Ross (1975) indicated that hypochlorous acid at pH 6.0 was a more effective viridiidal agent against Poliovirus. Clarke and Kabler had previously reported (1954) that the hypochlorous acid form at a residual concentration of 1 mg/l was sufficient to inactivate 99.6% of Coxsackie type A2 in 100 sec at 27 C while similar concentrations of hypochlorite ion required 3.5 minutes to attain the same level of inactivation. Temperature reduction was shown to lengthen the inactivation time but the same difference was noted with HOCl inactivating 99.6% in 7 minutes and OCl^- in 30 minutes. Other investigators have found OCl^- to be a more effective viricidal agent (Scarpino, et al., 1972). The effectiveness of chlorine as a disinfecting agent is complicated by its reactions with other wastewater components leading to the formation of a variety of compounds. The presence of ammonia, for example, results in the production of chloramines. Not only are chloramines less efficient in their viral inactivating capacity, but at concentration of 0.06 mg/l are toxic to fish and other aquatic life. Species within the Enterovirus group have varying sensitivities to chlorine

TABLE 2

TIME TO INACTIVATE 99.99 PERCENT OF TWENTY-FIVE HUMAN ENTERIC VIRUSES WITH 0.5 MG/L FREE CHLORINE IN POTOMAC WATER (pH 7.8 and 2 C)

	Virus	Minutes
1	Reo 1	2.7
2	3	<4.0
3	2	4.2
4	Adeno 3	<4.3
5	Cox B2	6.5
6	Cox A9	6.8
7	Cox B4	7.0
8	ECHO 7	7.1
9	ECHO 5	8.0
10	Cox B1	8.5
11	ECHO 9	12.0
12	Adeno 7a	12.5
13	ECHO 8	13.0
14	ECHO 11	14.0
15	Polio 1	16.2
16	ECHO 29	20.0
17	Adeno 12	23.5
18	ECHO 1	27.0
19	Polio 3	30.0
20	Cox B3	35.0
21	Cox A5	35.3
22	Cox B5	39.5
23	Polio 2	40.0
24	ECHO 12	>60.0
25	Cox A6	>120.0

as seen in Table 2 (Liu and McGowan, 1973). Reovirus type 1 was inactivated in 2.7 minutes, but Coxsackie type A6 virus required over 120 minutes of contact time to reach the same level of inactivation. The inactivation time among members of the same group can vary significantly.

Shuval (1970) reported that viruses were more resistant to the effects of chlorine than bacteria. Chlorine concentrations of 40 mg/l were required to inactive 99.9% of Poliovirus in sewage in 10 minutes. To reach the same level of inactivation only 9 mg/l chlorine was needed to inactivate coliform organisms. Kott, et al., (1975) also demonstrated Poliovirus to be more resistant to chlorination treatment than E. coli.

Other agents have been used to disinfect wastewaters. Ozone appears to have excellent potential value as a terminal disinfecting agent particularly in waters containing organics (Berg, 1973b). It has been shown to inactivate Polio type 3 and Coxsackie B3 in 10 minutes. Iodine has also been used in small water supplies. While being slower in virus inactivation than HOCl, I_2 has the advantage of not forming amines and may be useful in wastewaters containing ammonia (Berg, 1973a). Studies of the use of bromine have yielded results comparable to those for I_2. While all above methods have shown promise in experimental or small scale operations, none have proven to be totally reliable in sewage disinfection processes.

Efforts to produce virus-free sewage effluents have thus far met with little success (Sproul 1974). The major problem appears to rest not with the type disinfectant used, but the quality of the water being disinfected (Berg 1971).

E-2. Sewage Effluent Recharge

Most effluents from wastewater treatment plants contain populations of enteric viruses. The presence of these viruses constitutes a potential threat to wastewater recharge procedures should viruses be carried through soil and contaminate the ground-water aquifers. The fate of viruses in soil, their adsorption, movement and survival, should be carefully studied in order to determine if potential health hazards exist.

A number of field studies have been discussed in the literature which indicated that viruses can be effectively removed from sewage effluents by percolation through soil. At the Santee Water Reclamation Project, chlorinated sewage effluent percolated through 400 feet of sand and gravel was used to supply waters for a recreational lake (Merrell and Ward, 1968). Out of 128 samplings, 2 showed positive viral isolations. After seeding treated wastewater with high concentrations of Polio type 3, no virus could be found after passage through 200 feet of sand reclamation bed. It should be noted that the authors used swabs and gauze pads as water sampling devices. These methods do not represent very effective means of recovering viruses under field conditions. Gilbert, et al., (1976b) found that percolation through 60-90 cm of fine loamey sand was sufficient to remove 99.99% of the viruses found in secondary sewage effluents. Sand filtration was also found to be sufficient to remove over 99% of total coliforms, fecal coliforms, and fecal streptococci (Gilbert, et al., 1976a).

A number of studies have detected the presence of virus in groundwater following the recharge of sewage effluents through sand basins. Hori, et al., (1970) studying the fate of Polio virus type 2 recharged through Oahu Island soils found instances of viral contamination of groundwater despite the good removal characteristics of the soil. The authors

concluded that the possibility of groundwater contamination existed if the underlying soil was interrupted by fissures and fractures which would result in channeling of the percolating waters. In a study of the rapid infiltration of viruses through silty sand and fine gravel, Schaub and Sorber (1977) demonstrated the sporadic occurrence of enterovirus in groundwater. Laboratory experiments confirmed the poor removal qualities of the test soil used in their field experiments.

The probable mechanism of virus removal during percolation through sand or soils is adsorption rather than filtration or sieving (Drewry and Eliassen, 1968). The adsorption process is strongly influenced by a number of factors including the pH of the recharged water, the chemical composition of the soil, the moisture content of the soil, and the rate of recharge (Gerba, et al., 1975). Since viruses are electrically charged colloidal particles consisting of an inner core of nucleic acid surrounded by a protein coat, the pH and ionic strength of the surrounding medium greatly influence the ability of the virus to adsorb to soil particles. Drewry and Eliassen (1968) demonstrated this pH dependence in a study of the ability of bacteriophage to adsorb to different types of soils. They found that maximum adsorption occurred when pH values were below the isoelectric point of the virus particle. Under these conditions, the virus would be positively charged soil.

The ionic strength of the adsorbing environment was also found to be an important factor in the attachment of virus particles. Wellings, et al., (1975) studied the ability of a cypress dome to remove enteric viruses present in treated sewage effluent. No isolations were observed during the first five months, however, 3 isolations of virus from groundwater were later reported following a period of heavy rainfall. The authors

concluded that the rainfall resulted in an increase in the water/soil ratio which acted to desorb the viruses allowing them to move vertically towards the aquifer. A similar desorption effect was seen when deionized water was added to 250 cm calcareous sand columns used to recharge sewage effluent (Lance, et al., 1976). The virus, which had been previously adsorbed to the top cm of the soil column, moved down the column readsorbing at a lower level. Desorption was minimized by drying the columns one day between applications of the sewage, or by addition of cations to the effluent. The investigators concluded that desorption was due to a reduction in the ionic strength of the soil. In a similar study Duboise, et al., (1976) reported that a specific conductance of 700-800 micro-ohms per cm (Mohns/cm) was necessary for maximum retention of virus to soil. The addition of distilled water to simulate rainfall diluted the ionic capacity of the soil and freed the virus.

Robeck, et al., (1962) reported that the rate of recharge was important in the removal of Polio virus type 1 in a sand recharge basin. At recharge rates of 0.6 to 1.2 $\ell/min/m^2$, 99% of the virus was removed during passage through sand columns. At higher flow rates of (38 to 76 $\ell/min/m^2$), viruses were commonly found in the sand column effluent. Gilcreas and Kelly (1955) reported similar results using Coxsackie A5. A flow rate of 7.5 $\ell/min/m^2$ allowed removal of 99% of the virus while a 75 $\ell/min/m^2$ recharge rate resulted in the removal of only 10% of the virus.

Clean dry sand has been shown to have little or no capability for removing virus (Berg, 1973a). Moistened sand showed a better removal efficiency (Nestor and Costin, 1971). Drewry and Eliassen (1968) reported that soils with a high clay and silt content (composed of .5 to 1% organic matter) were effective in removing viruses. Clay particles were found to possess a

larger surface area than sand which provided numerous sites for viral adsorption (Bitton, 1975).

Although viruses are readily adsorbed to soils during the process of recharge, they can remain viable for significant periods of time. Moore, et al., (1975) found that the Poliovirus adsorbed to organic and inorganic particulates was still infective. Schaub and Sagik (1975) reported that clay-adsorbed virus retained its infectivity in tissue culture monolayers and in mice. Bagdasaryan (1964) studied the survival of enteric viruses in soil and concluded that survival was dependent on the pH of the soil, its moisture content, the nature of the soil and its temperature. Sandy soil at a pH of 7.5 provided the best conditions for virus survival, with Polio type 1 surviving for 170 days at 3-10 C. Wellings, et al. (1975) reported isolating Poliovirus in a groundwater well below a recharge basin 28 days after application of sewage effluent was terminated. Duboise, et al., (1976) found Poliovirus capable of surviving 84 days in soil at 3C. Increasing the temperature to 20 C resulted in a 99% inactivation rate in 84 days. A similar study by Tierney, et al. (1977) detected Poliovirus after 96 days in irrigated soils during the winter. Summer survival in soil was significantly shorter, lasting only 11 days.

F. Septic Systems

Little Information is available on the fate of human enteric viruses in septic systems. Since large numbers of human viruses can be shed in feces, there is little doubt of their presence in the system. What is needed is more information concerning the amount of virus removed during the initial stages of settling, the mechanisms and rate of viral inactivation during this period, and the ultimate fate of viruses discharged in septic tank effluents.

Due to the lack of scientific study, the answers to the first two questions have not been determined. One can, however, speculate based upon similar processes occurring during the initial stages of conventional wastewater treatment. As found in primary settling, some viruses will be removed with the solids, the survival depending upon the presence of non-specific viricidal agents present in the sludge, or the toxic metabolic by-products of the resident microbial population.

The fate of viruses discharged in septic tank effluents has been the subject of a few laboratory studies, but little or no data has been gathered from field trials. The studies have concerned themselves with the removal of viruses from effluents by percolation through soils, a subject which has been reviewed in Section E-2. Sproul (1975) reported that viruses discharged from leaking septic tanks could be efficiently removed from the leachates depending on the type of subsoil, the flow rate, and the overall quality of the effluent itself. He recommended using soils which contain a high percentage of silt or clay, with a minimum depth of within 5 to 10 feet of the fractured ledge. The recommended flow rate for such a system was 0.4 to 0.7 GPD/ft^2. Citing the number of viral isolations from groundwater following recharge, Sproul warned that the soil adsorbing layer should be free from fissures or fractures that would lead to channeling and possible contamination of the groundwater. Green and Cliver (1974) reported on laboratory studies involving the removal of virus by sand columns. Using Poliovirus type 1 seeded into septic tank effluent, they found that a majority of the viruses were removed in 60 cm sand columns. The sand columns were the most efficient when the column was unsaturated. Drewry and Eliassen (1968) found soil to be an effective virus adsorbing medium except where channeling occurred. Their recommendations for an ideal

subsurface soil percolation system included a minimum infiltration rate of 2.54 cm per hour with a loading rate of 3 GPD/ft^2, and a high adsorptive soil containing 1 to 2 % silt or clay and 0.5 to 1% organic matter. They concluded that incorporation of the above criteria, in addition to placement of the septic system 100-150 ft from the nearest well would be sufficient to avoid viral contamination of groundwater used for domestic purposes.

III. METHODS AND MATERIALS

A. Sample Site Selection

Sites for virus sampling were chosen by a sub-group of the Technical Advisory Committee which represented a diversity of professional disciplines. The sites selected and the frequency with which they were sampled are described below and reviewed in Table 3.

1. Sites located in Nassau County

a) Meadowbrook Hospital - the site included a 1,000,000 gallon per day (GPD) capacity secondary sewage treatment plant (trickling filter) which services the hospital complex of the Nassau County Medical Center and the Nassau County Jail. Chlorinated effluent was discharged into a series of recharge basins which were located approximately 34 ft. above the groundwater table. Sampling at this site consisted of a 25 gallon sewage effluent sample and a 100 gallon groundwater sample taken from an observation well which was located within 10 lateral feet (downflow) of the primary recharge basin (sample designated as "Meadowbrook Well"). Samples were taken on a monthly basis.

b) Oyster Bay STP - the site consisted of a secondary sewage treatment facility (trickling filter) which disinfected its effluents via chlorination. Treated effluent was discharged directly into Oyster Bay. Twenty-five gallon samples were taken on a monthly basis from this site.

c) Oyster Bay Waters and Shellfish - water (100 gal.) and shellfish (oyster) samples were taken from areas of Oyster Bay which had been designated as "open" or "closed" to shellfishing by the New York State Department of Environmental Conservation on the basis of coliform

TABLE 3

SITES CHOSEN FOR VIRAL ANALYSES

	SITE	LOCATION (COUNTY)	TYPE SAMPLE	FREQUENCY
1.	Meadowbrook Hospital	Nassau	Chlorinated sewage effluent	Monthly
2.	Meadowbrook Well	"	Groundwater from observation well located down flow from sewage effluent recharge basin	"
3.	Oyster Bay STP (a)	"	Chlorinated secondary sewage effluent	"
4.	Oyster Bay - closed waters	"	Bay water (area closed to shellfishing)	Monthly - June - Sept., March - May Bi-monthly - Oct - Feb
5.	Oyster Bay - open waters	"	Bay water (area open to shellfishing)	"
6.	Oyster Bay - closed waters	"	Oysters from closed area	"
7.	Oyster Bay - open oysters	"	Oysters from open area	"
8.	No. Massapequa recharge basin	"	Water from observation well located within recharge basin receiving storm water run-off	Monthly
9.	Bayport Well	Suffolk	SCWA[b] well water	Monthly
10.	Oakdale Well	"	SCWA well water	"
11.	Stony Brook STP	"	Chlorinated secondary sewage effluent	"
12.	Stony Brook Well	Suffolk	Groundwater from observation well located down flow from sewage effluent recharge basin	Monthly
13.	Penataquit Creek	"	Salt water creek receiving fresh water runoff	"

	SITE	LOCATION (COUNTY)	TYPE SAMPLE	FREQUENCY
14.	Great South Bay - open waters	"	Bay water (area open to shellfishing)	Monthly - June - Sept., March - May Bi-monthly - Oct - Feb
15.	Great South Bay - open clams	"	Clams from open area	"
16.	Great South Bay - closed waters	"	Bay water (area closed to shellfishing)	"
17.	Great South Bay - closed clams	"	Clams from closed area	"
18.	Babylon Well	"	Groundwater from observation well located down flow from sanitary landfill site	Monthly
19.	Sunrise STP	"	Chlorinated secondary sewage effluent	"
20.	Sunrise Well	"	Groundwater from observation well located down flow from sewage effluent leaching pools	"
21.	Parkland III STP	"	Chlorinated sewage effluent	"
22.	Parkland III Well	"	Groundwater from observation well located down flow from recharge basin receiving sewage effluent	"
23.	Lake Ronkonkoma	"	Lake Water	Monthly - June - Sept Bi-monthly - Oct. - May
24.	SCHD[(c)] Experimental wastewater septic system (located at Brookhaven National Laboratory) - Influent	Suffolk	Raw wastewater	Monthly
25.	SCHD experimental septic system - effluent	"	Treated non-chlorinated effluent	"

(a) Sewage Treatment Plant
(b) Suffolk County Water Authority
(c) Suffolk County Health Department

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APPENDIX TO TABLE 3

All "well" samples listed were collected from observation wells which had been driven a few feet below the upper surface of the groundwater aquifer.

data. The sites were sampled monthly from June - September, and again from March - May, and on a bi-monthly basis from October - February.

d) North Massapequa - the site was chosen as a representative stormwater recharge basin located in a thickly settled area. Sampling consisted of 100-gallon volumes collected monthly from a U.S. Geological Survey observation well located in the bottom of the basin.

2. Sites located in Suffolk County

a) Bayport Well, Oakdale Well - both sites were Suffolk County Water Authority public drinking water supply wells located in the hamlets of Bayport and Oakdale respectively. Samples, taken at monthly intervals, consisted of 100 gallons each. Both areas had been in service for a number of years with neither having any past problems with high coliform counts.

b) Stony Brook - this site included a 300,000 GPD capacity secondary sewage treatment plant (contact stabilization) and a series of recharge basins into which chlorinated effluent was discharged. At monthly intervals, 25 gallon treated effluent samples were taken along with 100 gallons of water taken from an observation well located some 8 lateral feet downflow from the nearest recharge basin. The basins themselves were some 80 feet above the water table.

c) Penataquit Creek - the creek located in the hamlet of Bay Shore, is a tributary to Great South Bay. In making its way to the bay, the water passes through a residential area and by a hospital. The actual sampling point was the Town of Islip boathouse located well below the above areas.

d) Great South Bay Waters and Shellfish - located within

the town of Islip, sites for collection of water (100 gal.) and shellfish (clam) samples included areas designated as "open" and "closed" to shellfishing by the New York State Department of Environmental Conservation. Samples were taken monthly from June - September, and March - May, and on a bi-monthly basis from October - February.

e) Babylon - the sampling area was a groundwater observation well located within ¼ mile down groundwate flow from the Babylon landfill site. The landfill includes regular rubbish disposal and pits for scavenger-waste disposal, and is located approximately 75 feet above the glacial aquifer. Monthly samples of 100 gallons each were taken from U.S.G.S. observation well.

f) Sunrise Garden Apartments - this site, located in the hamlet of Sayville included a secondary sewage treatment facility (contact stabilization) with a 38,000 GPD measured operating capacity. Chlorinated effluent from the plant was discharged to a leaching field which was located some 5-8 feet above the groundwater table. Twenty-five gallon effluent samples were taken at monthly intervals, along with 100 gallon volumes from an observation well which was sunk within 10 feet downflow from the leaching field.

g) Parkland III - the site consisted of a 260,000 GPD capacity tertiary treatment plant (extended aeration, denitrification, gravity, sand filtration) with recharge basins located some 18 feet above the water table. Twenty-five gallon samples of the chlorinated effluent were taken on a monthly basis along with a 100-gallon volume from an observation well located some 50 lateral yards down water flow from the recharge basins.

h) Lake Ronkonkoma - the site constitutes the largest fresh water lake on Long Island. The area is thickly settled by both residential and commercial concerns, and contains several public bathing areas. Virus sampling was confined to an area within the Islip Town Beach. One-hundred-gallon volumes were taken from a depth of 5 feet (approximately 10-15 feet off shore) on a monthly basis from June - September, and bi-monthly from October - May.

i) Septic System - the site, located at the Brookhaven National Laboratory, contains an experimental septic system which has been constructed by the Suffolk County Health Department in collaboration with William F. Cosulich Associates. The subsurface systems consists of a tile field which has been constructed over an aerobic soil zone. Below this is an anaerobic soil zone - the principle function of which is de-nitrification. Final effluent from the system is collected in a sampling shaft. Samples of this systems raw influent (1 gallon) and final effluent (100 gallon - undisinfected water) were collected for viral analysis on a monthly basis.

B. Sample Collection

Sample volumes of 100-125 gallons each were taken from public water supply wells, groundwater wells near recharge basins and sanitary landfills, embayments, lakes and streams. Twenty-five gallon samples were usually collected from wastewater treatment plants while 1-gallon samples were taken when raw influent was required.

All samples (with exception of raw influents) were collected in plastic 55 gallon tanks (Plast-i-cube, Greif Brothers Corp.). Between sample collections, tanks were thoroughly rinsed with tap water, sanitized with

0.12 N hydrochloric acid (30 min.), and rinsed once again with tap water. Upon arrival at each site, tanks were initially rinsed with 10-20 gallons of sample water before filling with sample material. Brookhaven National Laboratory pumping equipment (i.e. impeller pumps, hosing) was also rinsed with 20-30 gallons of sample water prior to collection. Brookhaven National Laboratory equipment was routinely used for all sampling, with the exception of waters from Oyster Bay (open and closed). In these instances, water samples were taken by means of pumps available on oyster boats belonging to Flowers Inc. of Bayville. Before collection of thses samples, water from the designated area was rinsed through the pumping system for 5-10 minutes.

The above precautions were taken in order to obviate any chance of virus cross-contamination between samples.

Great South Bay clam samples were collected by tonging, while oysters from Oyster Bay were obtained by dredging. Shellfish samples were stored in ice during transport to the laboratory where processing was carried out immediately whenever possible.

C. Virus Concentration Procedures

1. Water Samples

Viruses in large volume water samples were initially concentrated by means of an Aquella Virus Concentrator (Carborundum Corporation). Appropriate sample volumes were pumped through a series of prefilters to remove debris. Sample pH was then adjusted to 3.5 and aluminum chloride was added to a final concentration of 0.0005 M . The water then flowed through virus concentrating filters, where virus was adsorbed to the surface of the filters. Elution of adsorbed virus was carried out with

0.1 M glycine at pH 11.5. Samples were neutralized to pH 7.5. The concentration procedure routinely yielded a final volume of 4 l which was further concentrated in the laboratory. The procedure involved the formation of an aluminum hydroxide floc to which virus particles became adsorbed. After concentration of the floc by centrifugation, viral particles were eluted with 0.1 M glycine (pH 11.5) to a final volume of approximately 50 ml. After the addition of 10% fetal calf serum to each reconcentrate, samples were stored at -72 C until needed.

2. Shellfish Samples

Shellfish (clams and oysters) were shucked and placed in 100 g aliquots. Following homogenization, samples were acidified causing formation of a virus-containing precipitate which could be centrifuged and collected. Viruses were eluted from the precipitate with a glycine-saline solution, then separated from the rest of the precipitate by centrifugation. Virus-laden supernates were then filtered through a series of 47 millimeter (mm) membrane filters (0.8, 0.45 and 0.22 micrometer (um) porosity respectively) and concentrated by ultrafiltration to a final volume of 5 ml. Processed samples were frozen at -72 C while awaiting tissue culture assay.

D. Isolation and Identification

Virus enumerations from field samples were carried on monolayers of Buffalo Green Monkey Kidney Cells (BGM)- Micro-biological Associates). Quintuplicate 0.5 ml sample volumes were placed on prepared cell sheets and incubated for one hour to facilitate virus attachment. After decanting excess sample material, cells were covered with a 4 ml neutral red agar overlay media and incubated at 36 C under 5% CO_2 for a period of eight days. Daily readings were taken to determine the presence of viruses which

appeared as "plaques" (clearings in the normally red stained background indicating cell death as a result of virus infection). After eight days each plaque was "picked" and the isolated viruses were enriched for one week on a monolayer of BGM cells. Isolates were identified in microtiter plates by serum neutralization techniques using enterovirus typing pools made available by the National Institute of Allergy and Infectious Disease.

E. Poliovirus T-Marker Studies

Isolates identified as being members of the Poliovirus group were further tested to determine whether they were vaccine strain or wild type virus. This was accomplished through T-marker tests which differentiate between vaccine strain and wild type based upon the latter's ability to grow at 40 C. T-marker tests were carried out in response to a request by health officials who realized the public health significance of the isolation of non-vaccine Polioviruses from Long Island aquatic systems.

F. Coliform Studies

In order to correlate virus data with a recognizable biological pollution indicator, total and fecal coliform numbers were determined from all sites tested for virus. Coliform enumerations derived from standard "most probable numbers" methods were carried out by the staff of the New York State Department of Environmental Conservation Microbiology Section (Stony Brook), under the direction of Mr. James Redman.

G. Other Chemical and Physical Tests

pH determinations were carried out on all samples with the exception of shellfish. Residual chlorine and turbidity measurements were made on sewage treatment plant effluents (turbidity monitoring began in February 1977).

IV. RESULTS

A. Limitation of Study

Before proceeding with the presentation of the results of the "208" Virus Study, it is appropriate to discuss the major limiting factors which were inherent in the program.

The use of virus concentrating units, such as the Carborundum concentrators used in our study, has greatly facilitated the isolation and enumeration of human enteric viruses from large volumes of water. However, because of the variables involved, (e.g., turbidity, salinity, presence of organics, etc.) it is not reasonable to expect a 100% efficiency of concentration. Similarly, methods for virus extraction from shellfish do not release all of the virus particles bound up within the tissues of the animal. Budgetary considerations required our use of a single host cell type (BGM) which has been shown to be sensitive to a great variety of enteroviruses (Dahling, et al., 1974), but not to all known members of the group. Extending the range would have required the use of additional cell strains, an action which would have involved considerable expense.

As a result of the above limitations, the viral enumerations reported in the following pages must be construed as being representative of the minimum numbers of virus in each sample. It is likely that in most cases there were more than we were able to report. Samples in which no viruses were detected have been labeled NI (no isolates) in the tables rather than with a zero. The NI designation refers to the inability to detect viruses within the constraints of our testing systems, but cannot preclude the possibility of viral presence in very low concentrations.

Isolate identification procedures utilize serum pools which can accurately identify 42 members of the Enterovirus group. These include those species most often encountered in domestic wastewater. As there are over 100 known Enteroviruses, it was impossible to identify all isolates derived from the 208 study. Untypable isolates will be designated by a "U" in the tabulated listings of virus identification.

B. Results of Field Samplings

1. Public water supply wells

All samples from both the Bayport and Oakdale drinking water installations yielded no positive virus results (Tables 4 and 5). Corresponding coliform counts were at the lowest limits of detection (Figures 1 and 2).

Drinking water sample reconcentrates were assayed on tissue culture more extensively than any other sample type. The resulting data are therefore more representative of the entire sample volume.

With the exception of Bayport's August and September readings, pH values showed little fluctuation (Table 26) and were not considered as contributing to any loss of virus.

2. Surface waters

a. Lake Ronkonkoma

Viruses were recovered from the lake on two occasions, September and March. Isolations occurred at times when coliform numbers were not at particularly appreciable levels (Table 6, Figure 3).

The area from which the samples were taken is used extensively during summer months as a bathing beach. With this in mind, it would not be unreasonable to expect a certain level of enteric viruses to be present in the near shore waters in early September, the likely viral source being

TABLE 4

Coliform and Virus Isolation

Bayport Well

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
June 1976	<2	<2	ni
July 1976	<3	<3	ni
August 1976	<3	<3	ni
September 1976	<3	<3	ni
October 1976	<3	<3	ni
November 1976	<3	<3	ni
December 1976	<3	<3	ni
January 1977	<3	<3	ni
February 1977	<2	<2	ni
March 1977	<3	nt	ni
April 1977	<3	nt	ni
May 1977	<3	<3	ni

ni = No Isolates

nt = Not Tested

TABLE 5

Coliform and Virus Isolation

Oakdale Well

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
June 1976	<2	<2	ni
July 1976	<2	<2	ni
August 1976	<3	<3	ni
September 1976	<3	<3	ni
October 1976	<3	<3	ni
November 1976	<3	<3	ni
December 1976	<3	<3	ni
January 1977	<3	<3	ni
February 1977	<2	<2	ni
March 1977	<3	nt	ni
April 1977	<3	nt	ni
May 1977	<3	<3	ni

ni = No Isolates

nt = Not Tested

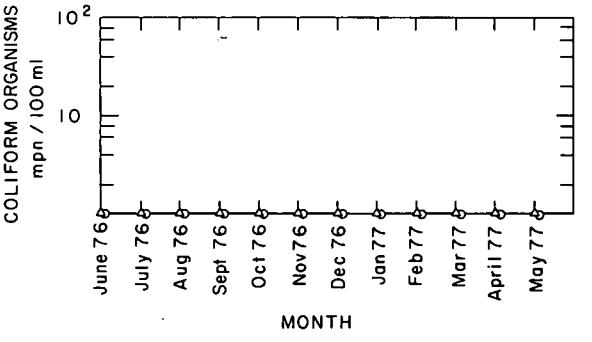

Figure 1. Total and fecal coliform counts (per 100 ml), Bayport Well. 0 - total coliform; Δ fecal coliform.

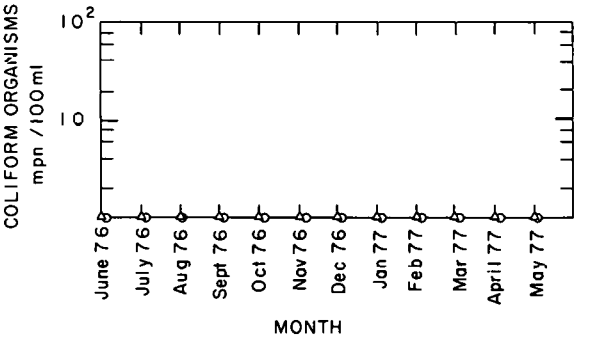

Figure 2. Total and fecal coliform counts (per 100 ml), Oakdale Well. 0 - total coliform; Δ - fecal coliform.

TABLE 6

Coliform and Virus Isolation

Lake Ronkonkoma

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
July 1976	230	230	ni
August 1976	2,300	930	ni
September 1976	43	43	2.3
October 1976	nt	nt	nt
November 1976	930	930	ni
December 1976	nt	nt	nt
January 1977	14	9	ni
February 1977	nt	nt	nt
March 1977	7	nt	6.5
April 1977	nt	nt	nt
May 1977	150	75	ni

ni = No Isolates

nt = Not Tested

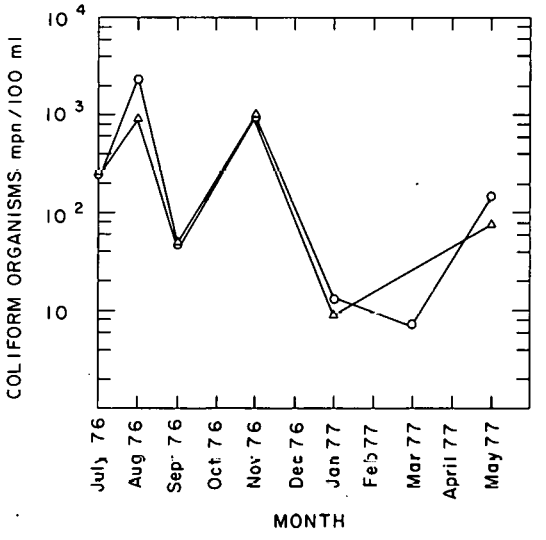

Figure 3. Total and fecal coliform counts (per 100 ml), Lake Ronkonkoma. O - total coliform; Δ - fecal coliform.

the bathers themselves, especially young children. Sampling problems arising from the presence of algal blooms during July and August may have inhibited the isolation of viruses whose presence could also have been linked to bathers. September isolates were confirmed but could not be specifically identified as they were insensitive to our typing antisera (Table 7).

Isolations made in March cannot easily be linked to bathers unless it is proven that viruses can over-winter in lake bottoms. Since there are supposedly no direct sewage discharges into the lake, the source possibilities are logically narrowed to runoff and contamination from the septic systems (via seepage or overflow) of homes situated around or near the lake. The latter possibility is strengthened by the isolation of a vaccine strain of Poliovirus type 2 (Table 7) normally shed by young children who have recently been immunized against poliomyelitis. The remainder of the confirmed March isolates could not be identified.

b. Penataquit Creek

Viral isolations were made in the creek in June and July during periods when total and fecal coliform counts were moderately high (Table 8, Figure 4), but not when total coliform counts reached their highest point in August. It is possible that the August counts were representative of a non-human fecal source (i.e., ducks, seagulls, etc.).

The major sources of contamination in the creek likely occurred from points above our sampling area, rather than from the bay. The consistently high coliform counts suggested a fairly constant source of pollutants, such as runoff, and leakage from the septic systems located along the banks of the creek.

The likelihood that contamination arose from a number of sources

TABLE 7

Virus Isolate Identifications

Lake Ronkonkoma

Date	Identifications Include
Sept. 7, 1976	U*
March 9, 1977	Poliovirus Type 2 (Vaccine strain)
	U*

*U - Identity Unknown

TABLE 8

Coliform and Virus Isolation

Penataquit Creek

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
June 1976	43,000	43,000	25.0
July 1976	1,100	460	8.0
August 1976	230,000	9,300	ni
September 1976	nt	nt	nt
October 1976	9,300	2,300	ni
November 1976	1,500	390	ni
December 1976	930	93	ni
January 1977	9,300	4,300	ni
February 1977	9,300	nt	ni
March 1977	15,000	nt	ni
April 1977	4,300	4,300	ni
May 1977	4,300	430	ni

nt = Not Tested ni = No Isolates

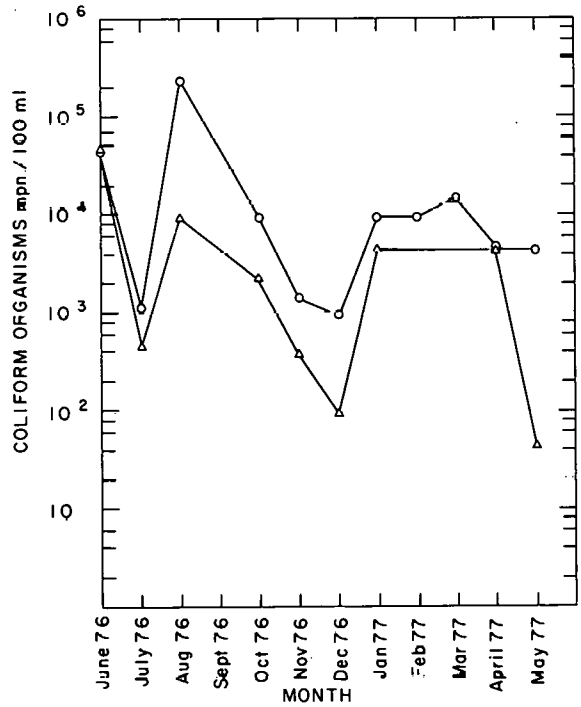

Figure 4. Total and fecal coliform counts (per 100 ml), Penataquit Creek. 0 - total coliform; Δ - fecal coliform.

rather than a single one was further supported by the wide variety of isolates identified from the two samples (Table 9). No Poliovirus species were recovered from any creek samples.

c. Great South Bay - waters and shellfish

i. Open area

Virus recoveries in water occurred twice during the summer. and once in the spring (Table 10). With the exception of the July sample, virus isolations were made during times when coliform counts were at their maximum (Figure 5). Isolations from clam samples occurred in April, when recoveries were also made in the water column, and in June (Table 11). Some moderate correlation was noted between virus isolations and coliform counts (Figure 6) during these months. Difficulties in obtaining shellfish severely limited the total number of samples taken during the study period.

The possible sources of pollution to this region include land runoff, leakage from domestic septic systems located along the bay, and the discharges of previously contaminated tributary rivers and creeks (Note: Penataquit Creek, which was previously shown to contain virus during summer months, empties into Great South Bay at a point just north of where "closed" and "open" water sampling was carried out).

With the exception of the July sample, most of the water and shellfish isolates could not be specifically typed (Table 12). The Poliovirus type 2 isolate occurring in July was later shown to be a vaccine strain.

ii. Closed area

The area sampled was located within one mile in-shore from the "open" area, and was therefore closer to those potential pollution sources previously discussed.

TABLE 9

Virus Isolate Identifications

Penataquit Creek

Date	Identifications Include
June 29, 1976	ECHOvirus Type 6
	U*
	ECHOvirus Type 2
	Coxsackievirus Type A-9
	U*
	U*
	ECHOvirus Type 15
	Coxsackievirus Type B-3
July 15, 1976	ECHOvirus Type 25
	ECHOvirus Type 32

U* - Identity Unknown

TABLE 10

Coliform and Virus Isolation

Great South Bay, Open Shellfish Waters - Islip

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
July 1976	4	4	8.0
August 1976	460	4	1.2
September 1976	93	<3	ni
October 1976	nt	nt	nt
November 1976	43	nt	ni
December 1976	nt	nt	nt
January 1977	nt	nt	nt
February 1977	93	nt	ni
March 1977,	23	<3	ni
April 1977	150	15	2.9
May 1977	nt	nt	nt
June 1977	93	<3	ni

ni = No Isolates

nt = Not Tested

TABLE 11

Coliform and Virus Isolation

Great South Bay Open Shellfish - Clams

Month	Total Coliform/100 g	Fecal Coliform/100 g	Virus PFU/g
September 1976	<20	<20	ni
October 1976	nt	nt	nt
November 1976	nt	nt	nt
December 1976	nt	nt	nt
January 1977	nt	nt	nt
February 1977	20	nt	ni
March 1977	<20	<20	ni
April 1977	170	130	0.3
May 1977	nt	nt	nt
June 1977	70	<20	0.1

ni = No Isolates

nt = Not Tested

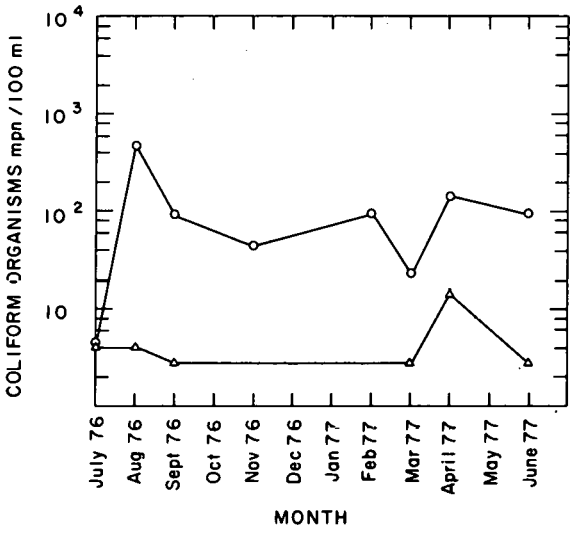

Figure 5. Total and fecal coliform counts (per 100 ml), Great South Bay, Open Shellfish Waters - Islip. O - total coliform; Δ - fecal coliform.

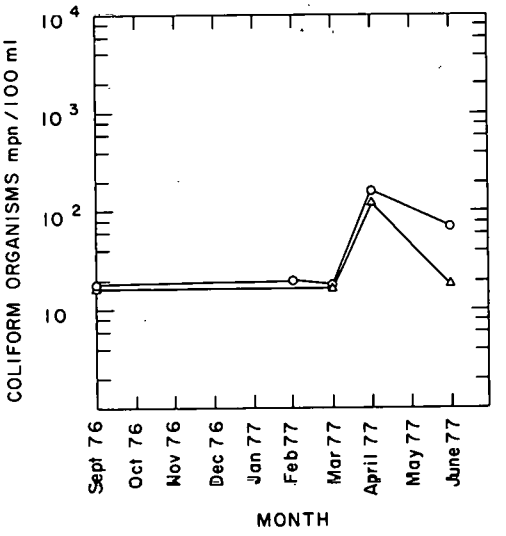

Figure 6. Total and fecal coliform counts (per 100 ml), Great South Bay, Open Shellfish - Clams. 0 - total coliform; Δ - fecal coliform.

TABLE 12

Virus Isolate Identfications

Great South Bay

"Open" Water and Shellfish

Date	Sample Type	Identifications Include
July 7, 1976	Water	U* U* Poliovirus Type 2 (Vaccine Strain) ECHOvirus Type 22 ECHOvirus Type 11
August 18, 1976	Water	U*
April 25, 1977	Water	U*
April 25, 1977	Shellfish	U*
June 2, 1977	Shellfish	U*

U* - Identity Unknown

Viral isolations from closed waters and shellfish were made in July (1976) and June (1977), with an additional isolation made in water alone in February (Tables 13 and 14). In general, viral isolation did not correlate well with coliform counts (Figures 7 and 8), with the exception of the July clam sample.

Isolate identifications, shown in Table 15, included several Polio and ECHO virus types. Of particular interest were the June 2 samples in which Poliovirus type 1 was isolated from both shellfish and the overlying water column.

Extrapolation of data collected from Penataquit Creek suggests that this and other local creeks were contributing to the "viral pollution" observed in this immediate region of the bay.

Based on the limited information collected, there was apparently little virological difference between the waters and shellfish of the "open" and "closed" areas. It must be noted, however, that the distance between the sites was not sufficient to expect any meaningful virus removal from the water column. A significant difference may have been seen, had the "open" testing site been located several miles from the "closed" area.

d. Oyster Bay - waters and shellfish

i. Open area

Virus isolations from "open" waters and shellfish were infrequent (Tables 16 and 17). Corresponding coliform counts also tended to be quite low with the exception of some of the summer readings (Figures 9 and 10). Virus isolates which were recovered in July and March, could not be identified using typing pools (Table 18).

The study area in question, which had been open to shellfishing for

TABLE 13

Coliform and Virus Isolation

Great South Bay, Closed Shellfish Waters - Islip

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
June 1976	430	75	4.0
August 1976	110	23	ni
September 1976	93	4	ni
October 1976	nt	nt	nt
November 1976	2,300	43	ni
December 1976	nt	nt	nt
January 1977	nt	nt	nt
February 1977	150	nt	4.4
March 1977	45	15	ni
April 1977	2,400	460	ni
May 1977	nt	nt	nt
June 1977	23	4	1.1

ni = No Isolates

nt = Not Tested

TABLE 14

Coliform and Virus Isolation

Great South Bay Closed Shellfish - Clams

Month	Total Coliform/100 g	Fecal Coliform/100 g	Virus PFU/g
July 1976	16,000	16,000	0.16
August 1976	20	<20	ni
September 1976	1,300	<20	ni
October 1976	nt	nt	nt
November 1976	nt	nt	nt
December 1976	nt	nt	nt
January 1977	nt	nt	nt
February 1977	<20	nt	ni
March 1977	50	<20	ni
April 1977	630	20	ni
May 1977	nt	nt	nt
June 1977	220	20	0.1

ni = No Isolates

nt = Not Tested

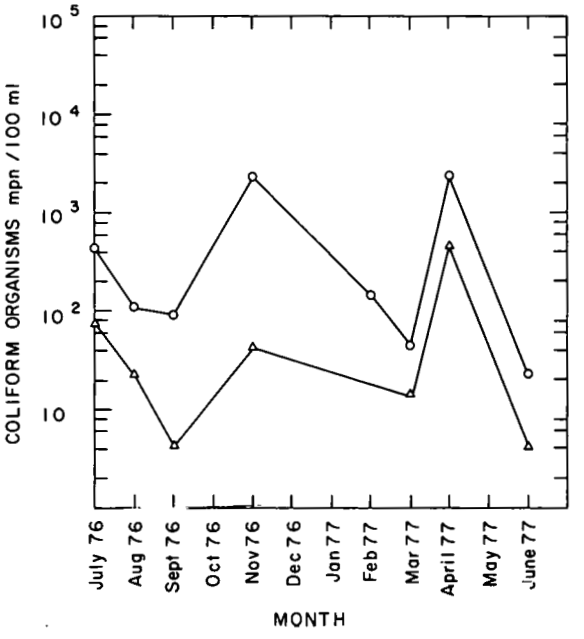

Figure 7. Total and fecal coliform counts (per 100 ml), Great South Bay, Closed Shellfish Waters - Islip. 0 - total coliform; Δ - fecal coliform.

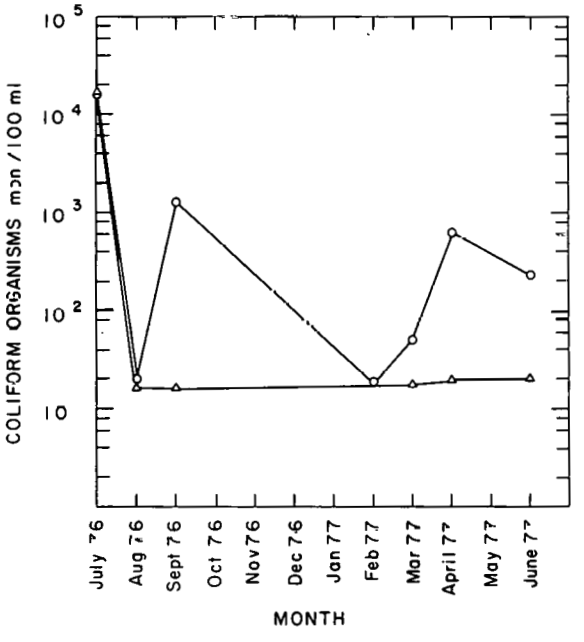

Figure 8. Total and fecal coliform counts (per 100 ml), Great South Bay, Closed Shellfish - Clams. 0 - total coliform; Δ - fecal coliform.

TABLE 15

Virus Isolate Identifications

Great South Bay

"Closed" Waters and Shellfish

Date	Sample Type	Identifications Include
July 7, 1976	Water	U*
July 29, 1976	Shellfish	ECHOvirus Type 20 ECHOvirus Type 23
February 28, 1977	Water	Poliovirus Type 2 (Vaccine strain)
June 2, 1977	Water	Poliovirus Type 1 (Vaccine strain)
June 2, 1977	Shellfish	Poliovirus Type 1 (Vaccine strain)

U* - Identity unknown

TABLE 16

Coliform and Virus Isolation

Oyster Bay, Open Shellfish Waters

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
July 1976	1,100	9	2.8
August 1976	230	93	ni
September 1976	930	43	ni
October 1976	nt	nt	nt
November 1976	23	23	ni
December 1976	nt	nt	nt
January 1977	nt	nt	nt
February 1977	23	nt	ni
March 1977	4	nt	ni
April 1977	< 3	< 3	ni
May 1977	nt	nt	nt
June 1977	15	< 3	ni

ni = No Isolates

nt = Not Tested

TABLE 17

Coliform and Virus Isolation

Oyster Bay, Open Area - Oysters

Month	Total Coliform/100 g	Fecal Coliform/100 g	Virus PFU/g
July 1976	80	20	ni
August 1976	2,400	<20	ni
September 1976	1,100	60	ni
October 1976	nt	nt	nt
November 1976	<20	<20	20
December 1976	nt	nt	nt
January 1977	nt	nt	nt
February 1977	<20	nt	ni
March 1977	50	nt	0.48
April 1977	70	<20	ni
May 1977	nt	nt	nt
June 1977	210	<20	ni

ni = No Isolates

nt = Not Tested

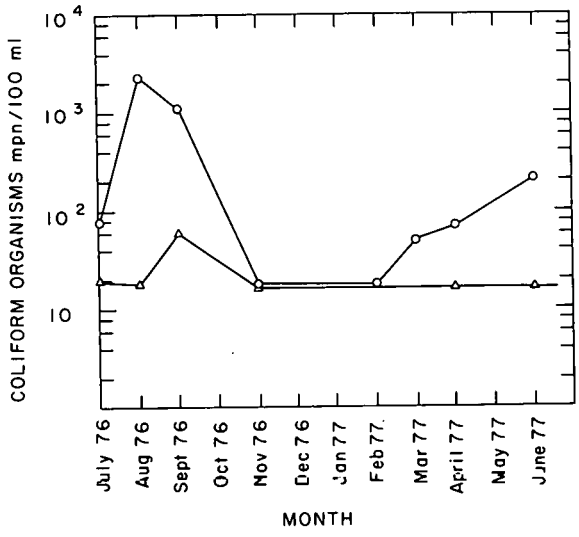

Figure 9. Total and fecal coliform counts (per 100 ml), Oyster Bay, Open Shellfish Waters. 0 - total coliform; Δ - fecal coliform.

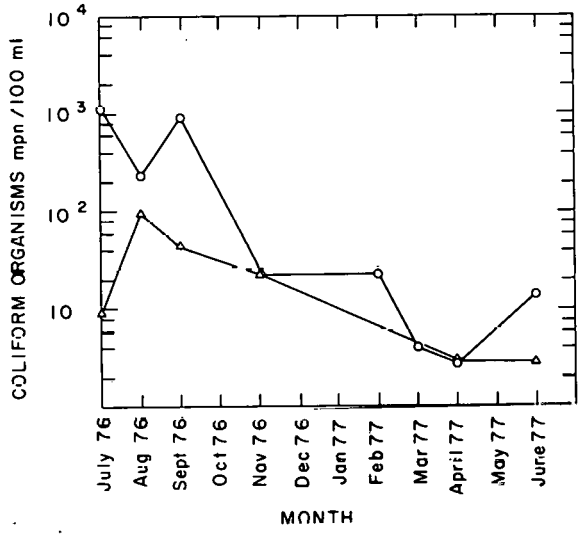

Figure 10. Total and fecal coliform counts (per 100 ml), Oyster Bay, Open Area - Oysters. 0 - total coliform; Δ - fecal coliform.

TABLE 18

Virus Isolate Identifications

Oyster Bay

"Open" Water and Shellfish

Date	Sample Type	Identifications Include
July 20, 1976	Water	U*
March 30, 1977	Shellfish	U*

U* - Identity Unknown

many years, was located several miles from the nearest major pollution source, a secondary effluent outfall. The relative infrequency with which viruses were isolated was probably related to the viricidal properties of the waters, in conjunction with the distance required to reach the "open" area.

ii. Closed area

The bay area studied had been closed to shellfishing for several years. The surrounding banks were extensively developed with single family dwellings.

Viral isolations were not made from any of the water samples tested (Table 19). With the exception of the June 1977 sample, coliform counts in this area were quite low (Figure 11). These findings were difficult to reconcile with shellfish data from the same area which revealed a number of virus isolations and high coliform counts in three of the eight times the area was sampled (Table 20, Figure 12). There are two possible explanations for this discrepancy: 1) the waters in the "closed" area contained heavy concentrations of suspended material (i.e., algae, detritis). It has been shown that such conditions, especially when in a marine or estuarine environment, can severely limit the efficiencies of virus concentration methods, and 2) in extremely turbid estuarine waters, human viruses will not usually remain in a free state. Studies have shown that viruses in the water column readily bind to particulates which later become sedimented. A number of workers have shown greater numbers of virus in sediments than in the surrounding waters. If this was occurring in the "closed" area of Oyster Bay, viruses would be difficult to find in water samples but would still be available for uptake by shellfish.

Little value would be obtained from any attempt to compare data from "open" and "closed" areas based upon so few sampling events.

TABLE 19

Coliform and Virus Isolation

Oyster Bay, Closed Shellfish Waters

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
July 1976	15	15	ni
August 1976	4	<3	ni
September 1976	23	9	ni
October 1976	nt	nt	nt
November 1976	9	9	ni
December 1976	nt	nt	nt
January 1977	nt	nt	nt
February 1977	93	nt	ni
March 1977	<20	nt	ni
April 1977	9	4	ni
May 1977	nt	nt	nt
June 1977	2,400	2,400	ni

ni = No Isolates

nt = Not Tested

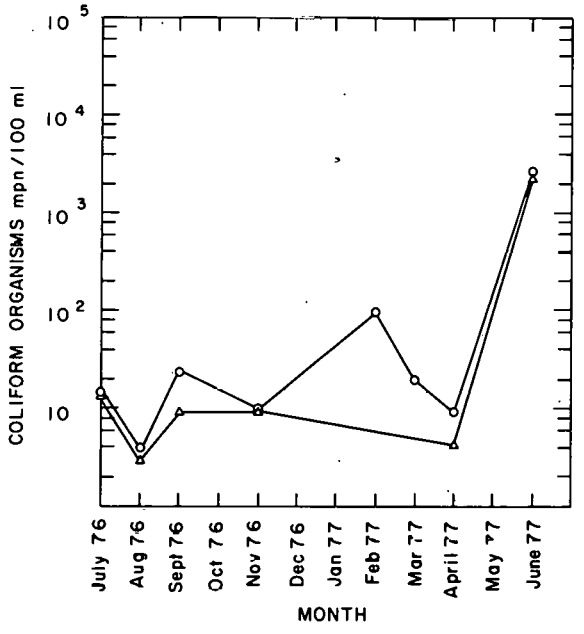

Figure 11. Total and fecal coliform counts (per 100 ml), Oyster Bay, Closed Shellfish Waters. 0 - total coliform; Δ - fecal coliform.

TABLE 20

Coliform and Virus Isolation

Oyster Bay, Closed Area - Oysters

Month	Total Coliform/100 g	Fecal Coliform/100 g	Virus PFU/g
July 1976	50	20	0.48
August 1976	5,400	270	ni
September 1976	1,400	90	ni
October 1976	nt	nt	nt
November 1976	<20	<20	0.08
December 1976	nt	nt	nt
January 1977	nt	nt	nt
February 1977	<20	nt	ni
March 1977	70	nt	ni
April 1977	<20	<20	0.2
May 1977	nt	nt	nt
June 1977	1,300	220	ni

ni = No Isolates

nt = Not Tested

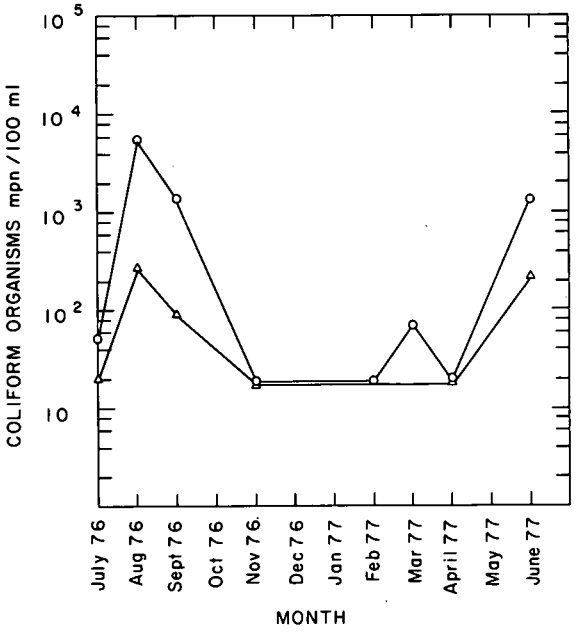

Figure 12. Total and fecal coliform counts (per 100 ml), Oyster Bay, Closed Area - Oysters. O - total coliform; Δ - fecal coliform.

TABLE 21

Virus Isolate Identifications

Oyster Bay

"Closed" Area, Shellfish

Date	Identifications Include
July 27, 1976	ECHOvirus Type 15 ECHOvirus Type 2 U*
November 22, 1976	Coxsackievirus Type B-3
April 27, 1977	U*

U* - Identity Unknown

3. Landfill site

Groundwater samples taken near the Babylon landfill yielded a single positive result (Table 22) during the month of November. Coliform counts during the entire sampling program tended to be quite low (Figure 13). The likely virus source was the scavanger waste pits located at the landfill site. Since no tests were performed on the scavenger waste, it is not possible to comment on removal rates.

Water samples from the Babylon site had a light orange color, and gave off a "chemical smell". It is possible that the extremely poor quality of the water inhibited additional virus isolations.

The only confirmed isolate identification was a Coxsackievirus type B-3 (Table 23).

4. Storm water recharge basin

Viruses were recovered from groundwater beneath the North Massapequa storm water recharge basin during the month of August (Table 24). At no time during the entire sampling period (July '76-May '77) were coliform counts higher than 4 per 100 ml (Figure 14). The pH values (Table 25) for water beneath the basin were among the lowest recorded of any of the areas studied. A contributing factor to the low pH may have been rainfall which tends to be acidic in this region.

Since little is known about the virological make up of storm water runoff, it would be presumptuous to identify this as the sole source of virus contamination. The diversity of viral species isolated (Table 26) suggests a recent human fecal source, lending some credibility to the theory of septic tank seepage from homes surrounding the basin. Further testing would have to be conducted before either or both possibilities could be dismissed.

TABLE 22

Coliform and Virus Isolation

Babylon Well

Month	Total Coliform	Fecal Coliform	Virus PFU/gal
August 1976	2	<2	ni
September 1976	<3	<3	ni
October 1976	23	<3	ni
November 1976	<3	<3	ni
December 1976	<3	<3	3.6
January 1977	<3	<3	ni
February 1977	<3	nt	ni
March 1977	<3	nt	ni
April 1977	<3	<3	ni
May 1977	79	<2	ni

ni = No Isolates

nt = Not Tested

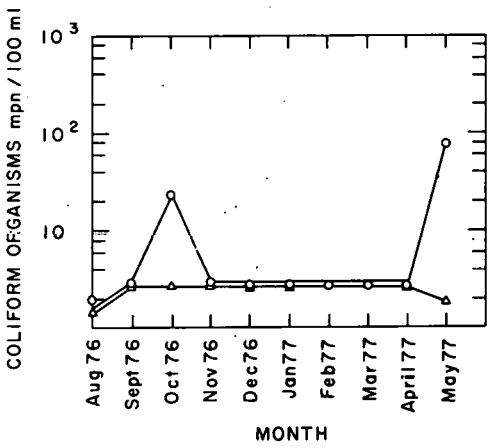

Figure 13. Total and fecal coliform counts (per 100 ml), Babylon Well. O - total coliform; Δ - fecal coliform.

TABLE 23

Virus Isolate Identifications

Babylon Well

Date	Identifications Include
November 17, 1976	Coxsackievirus Type B-3
	U*

U* - Identity Unknown

TABLE 24

Coliform and Virus Isolation

No. Massapequa Well

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
July 1976	4	<3	ni
August 1976	4	<3	4.0
September 1976	<3	<3	ni
October 1976	<3	<3	ni
November 1976	<3	<3	ni
December 1976	nt	nt	nt
January 1977	<3	<3	ni
February 1977	<3	nt	ni
March 1977	<3	nt	ni
April 1977	<3	<3	ni
May 1977	2	<2	ni

ni = No Isolates

nt = Not Tested

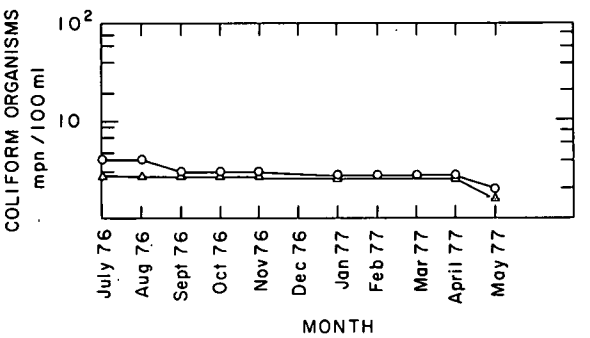

Figure 14. Total and fecal coliform counts (per 100 ml), North Massapequa Well. 0 - total coliform; Δ - fecal coliform.

TABLE 25

Ambient pH Values Of Water From Various Sites

Site	June 1976	July 1976	Aug 1976	Sept 1976	Oct 1976	Nov 1976	Dec 1976	Jan 1977	Feb 1977	Mar 1977	April 1977	May 1977	June 1977
Meadowbrook STP	7.1	6.3	7.1	7.2	7.2	8.2	7.5	7.4	7.4	7.1	7.2	nt	6.8
Oyster Bay STP	6.8	7.4	7.1	6.2	7.1	7.1	7.0	7.2	nt	7.0	7.1	7.4	nt
Parkland STP	nt	7.7	7.2	8.1	7.2	7.2	6.8	7.3	7.3	7.3	7.1	nt	6.2
Stony Brook STP	6.9	7.5	6.5	7.2	6.8	6.6	6.8	6.9	7.3	7.3	nt	7.1	nt
Sunrise STP	nt	nt	7.0	7.4	7.3	7.2	7.0	7.8	7.2	7.2	7.5	7.0	nt
Meadowbrook Well	nt	nt	6.2	6.2	6.1	6.3	6.2	6.3	6.2	6.3	6.4	nt	nt
Parkland Well	nt	nt	7.0	7.0	7.0	7.6	6.7	7.0	6.9	7.0	7.0	nt	6.8
Stony Brook Well	nt	nt	nt	6.6	6.4	6.6	6.4	6.4	6.4	6.6	nt	6.4	nt
Sunrise Well	nt	nt	nt	6.7	6.6	7.0	nt	6.7	7.1	6.8	7.0	6.7	nt
Great South Bay Closed Shellfish H_2O	nt	8.0	8.6	8.1	nt	7.8	nt	nt	7.9	7.5	7.2	nt	7.5
Great South Bay Open Shellfish H_2O	nt	8.2	8.2	8.1	nt	7.8	nt	nt	8.0	7.8	7.3	nt	7.6
Oyster Bay Closed Shellfish H_2O	nt	7.7	7.4	7.5	nt	8.1	nt	nt	6.9	8.3	8.1	nt	7.4
Oyster Bay Open Shellfish H_2O	nt	7.9	7.9	7.6	nt	7.9	nt	nt	8.3	8.1	8.0	nt	7.9
Lake Ronkonkoma	nt	10.0	7.6	7.7	nt	7.2	nt	6.8	nt	6.7	nt	6.6	nt
Pertaquit Creek	6.6	7.5	6.4	6.8	nt	7.1	7.1	7.0	6.6	6.6	6.6	6.7	nt
Bayport Well	6.0	6.2	8.8	8.9	6.6	7.1	7.4	6.8	6.9	7.2	6.7	6.8	nt
Oakdale Well	5.8	6.3	6.1	6.2	6.3	6.1	5.7	6.1	6.6	6.5	6.3	6.4	nt
No. Massapequa Well	nt	6.0	5.7	5.6	5.5	5.3	nt	5.0	5.0	4.5	4.7	4.5	nt
Babylon Well	nt	nt	7.3	7.1	7.1	7.2	6.9	6.9	7.0	7.1	7.2	7.0	nt
SCHD Exper. Site (effluent)	nt	7.0	6.1	6.2	6.5	6.0	6.0	6.2	6.2	6.3	6.0	5.7	nt

TABLE 26

Virus Isolate Identifications

North Massapequa Well

Date	Identifications Include
August 4, 1977	ECHOvirus Type 23 ECHOvirus Type 11 Coxsackievirus Type A-16

5. Sewage treatment plants

a. Discharge to surface waters - Oyster Bay

The secondarily treated, chlorinated effluent discharged from the Oyster Bay sewage treatment plant (STP) was found to contain significant numbers of viruses on four sampling occasions (Table 27). As is typical with sewage effluent, a wide variety of virus species was isolated (Table 28). There was little correlation between viral numbers isolated and corresponding coliform counts (Figure 15).

Viruses were isolated in summer, early fall, late winter, and spring. Isolations were not made when residual chlorine levels were in excess of 1.0 parts per million (ppm) (Table 29, Figure 16), but there is insufficient information to conclude that use of such residuals would consistently result in virus-free effluents. (Note: chlorine residual readings presented in all STP samples taken on the day that virus sampling occurred. They should not be interpreted as being the levels that existed throughout the month.)

Beginning in March, effluent turbidity levels were monitored in order to investigate a previously proposed relationship between virus occurrence and high turbidities in STP effluents. Isolations were made in March and April when turbidity levels were 20 and 24 Nephelometric Trubidity Units (NTU) respectively (Table 30). No isolations were recorded in May when the turbidity level was 10 NTU. While these data do not contradict the theory of a virus-turbidity relationship, they do not of themselves represent a confirmation. This could only be established by more intensive sampling and comparison.

TABLE 27

Coliform and Virus Isolation

Oyster Bay STP

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
June 19768	4,300,000	390,000	nt
July 1976	2,300,000	430,000	227.0
August 1976	23	<3	ni
September 1976	430	43	67.2
October 1976	43	<3	ni
November 1976	9	<3	ni
December 1976	430	<3	ni
January 1977	39	<3	ni
February 1977	13	<2	ni
March 1977	150	nt	2636.4
April 1977	2,300	nt	216.4
May 1977	23	<3	ni

* Unchlorinated

ni = No Isolates

nt = Not Tested

TABLE 28

Virus Isolate Identifications

Oyster Bay

STP

Date	Identifications Include
July 12, 1976	ECHOvirus Type 25 ECHOvirus Type 14 Coxsackievirus Type A-16 Coxsackievirus Type B-3 ECHOvirus Type 17 ECHOvirus Type 27 Coxsackievirus Type B-6 ECHOvirus Type 11 ECHOvirus Type 13 Coxsackievirus Type A-7 Coxsackievirus Type B-4
September 21, 1976	ECHOvirus Type 5 ECHOvirus Type 25 Coxsackievirus Type B-2 ECHOvirus Type 17 ECHOvirus Type 11 Coxsackievirus Type B-5 ECHOvirus Type 6 Poliovirus Type 3 (Vaccine strain) ECHOvirus Type 12
March 8, 1977	Coxsackievirus Type B-3 ECHOvirus Type 11 Poliovirus Type 2 (Vaccine strain)
April 5, 1977	U* ECHOvirus Type 6 Coxsackievirus Type B-3 Coxsackievirus Type A-17

U* - Identity Unknown

TABLE 29

Residual Chlorine Values (ppm) For Sewage Treatment Plant Effluents

Site	Month												
	June 1976	July 1976	Aug 1976	Sept 1976	Oct 1976	Nov 1976	Dec 1976	Jan 1977	Feb 1977	Mar 1977	Apr 1977	May 1977	June 1977
Meadowbrook STP	1.0	2.0	2.0	1.5	1.5	1.0	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	nt
Oyster Bay STP	nt	nt	1.0	nt	2.0	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	nt
Parkland STP	nt	1.5	2.0	2.0	2.0	<0.2	<0.2	<0.2	<0.2	<0.2	nt	nt	<0.2
Stony Brook STP	1.5	3.0	3.0	2.0	2.0	<0.2	<0.2	<0.2	<0.2	<0.2	nt	<0.2	nt
Sunrise STP	nt	nt	2.0	2.0	2.0	nt	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	nt

TABLE 30

Turbidity Values (NTU) for Sewage Treatment Plant Effluents

Site	Month			
	March 1977	April 1977	May 1977	June 1977
Meadowbrook STP	19	10	14	nt
Oyster Bay STP	20	24	10	nt
Parkland STP	37	9.5	nt	20
Stony Brook STP	10	nt	25	nt
Sunrise STP	25	6.8	19	nt

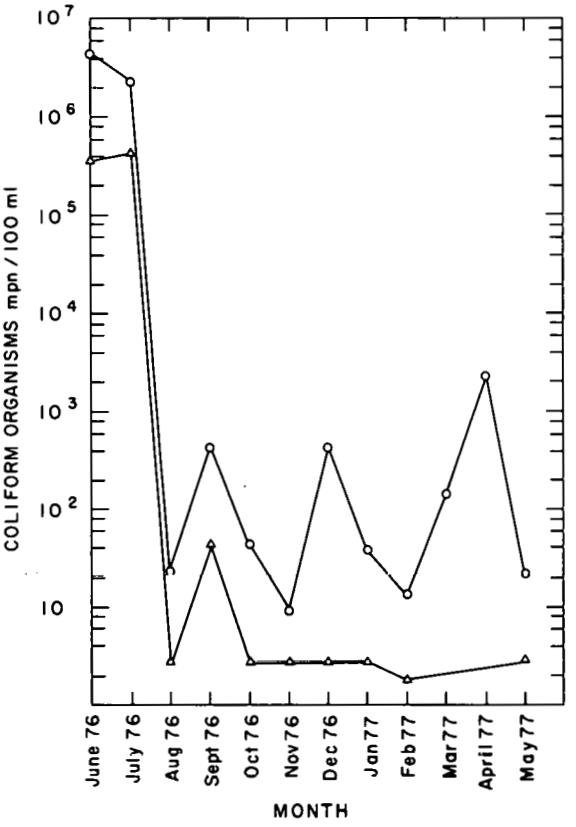

Figure 15. Total and fecal coliform counts (per 100 ml), Oyster Bay STP. O - total coliform; Δ - fecal coliform.

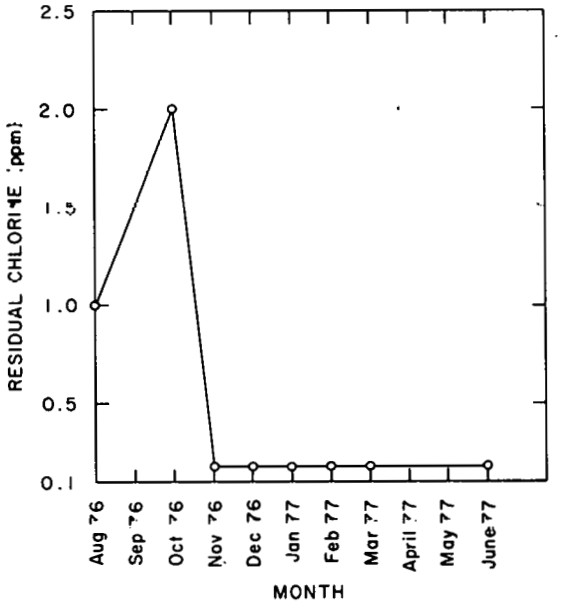

Figure 16. Residual chlorine (ppm), Oyster Bay STP.

b. Discharge to leaching fields - Sunrise Garden Apartments

With two exceptions, which cannot be accounted for, significant numbers of viruses were routinely isolated in chlorinated treated wastewater (Table 31). At the same time, extremely high coliform counts were also recorded (Figures 17 and 18). The high levels of virus and bacteria are indicative of grossly inadequate treatment procedures which resulted in effluents of such poor quality that chlorine residuals as high as 2.0 ppm (Figure 19) were unable to affect any appreciable disinfection. The net result was an effluent that often resembled (microbially) the product of a primary treatment plant. It was impossible to identify all isolates from each sample, and is likely that many more virus species would have been identified than indicated in Table 32.

Despite the high virus numbers entering the leaching fields, only two samples from the groundwater observation well yielded positive results (Table 33). This unexpected finding indicated the extraordinary virus adsorbing capacity of the soil. It is probable that a majority of the viruses in the effluents were bound to small particles. The particles were then removed during horizontal passage through the soil by a sieving action. Reductions were also noted in coliform numbers (exceptions occurred in February and April). Precise determinations of virus and bacterial removal could not be made due to a lack of information concerning effluent residence time in the leaching fields, and the soil characteristics of the area.

TABLE 31

Coliform and Virus Isolation

Sunrise STP

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
August 1976	93,000	43,000	1440.0
September 1976	24,000,000	4,600,000	1900.0
October 1976	2,400,000	9,300	854.2
November 1976	4,600,000	43,000	ni
December 1976	2,400,000	23,000	1232.0
January 1977	230,000	4,300	10.8
February 1977	110,000,000	930,000	ni
March 1977	2,400,000	nt	990.0
April 1977	930,000	43,000	4000.0
May 1977	9,300,000	2,300,000	120.0

ni = No Isolates

nt = Not Tested

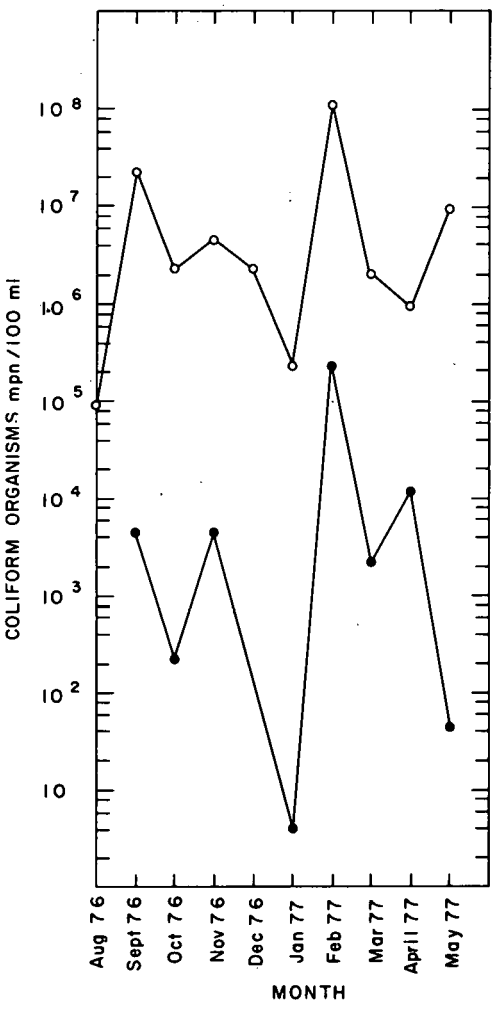

Figure 17. Total coliform counts (per 100 ml), Sunrise STP and Sunrise Well. O - total coliform - STP; ● - total coliform - Well.

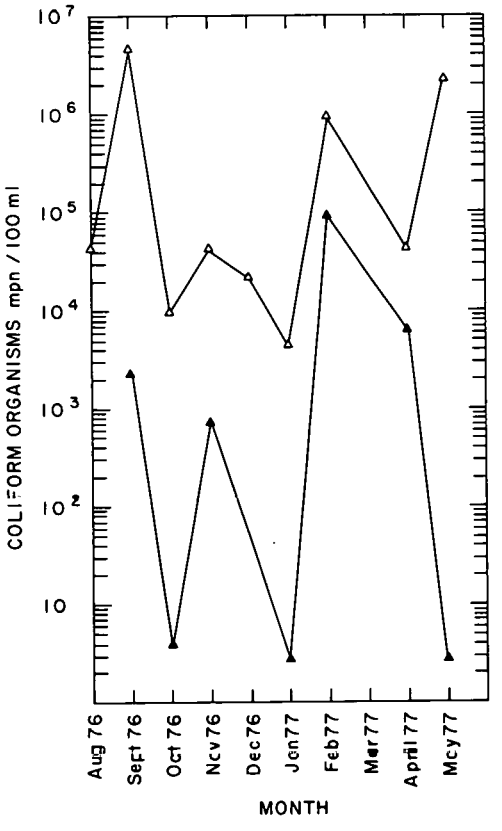

Figure 18. Fecal coliform counts (per 100 ml), Sunrise STP and Sunrise Well. Δ - fecal coliform - STP; ▲ - fecal coliform - Well.

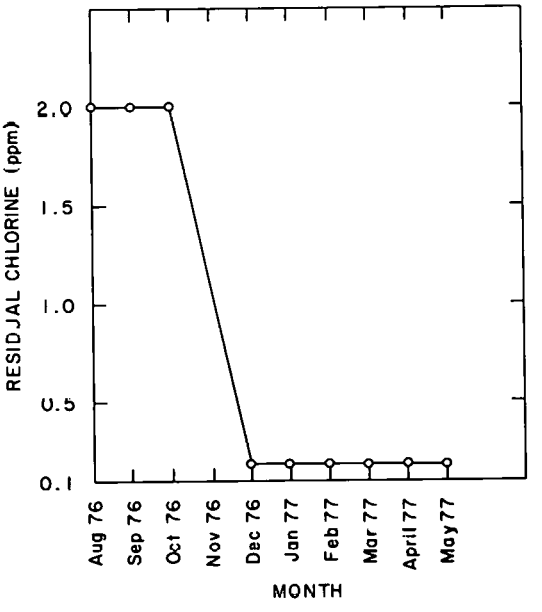

Figure 19. Residual chlorine (ppm), Sunrise STP.

TABLE 32

Virus Isolate Identifications

Sunrise

STP and Observation Well

Date	Sample Type	Identifications Include
August 13, 1976	Effluent	Coxsackievirus Type B-3 ECHOvirus Type 6 ECHOvirus Type 7 ECHOvirus Type 21 Coxsackievirus Type B-4
September 22, 1976	Effluent	ECHOvirus Type 6 Coxsackievirus Type B-6 Poliovirus Type 2 (Vaccine strain) Coxsackievirus Type B-2 ECHOvirus Type 7
October 19, 1976	Effluent	Coxsackievirus Type A-16 ECHOvirus Type 15 U* ECHOvirus Type 31
December 15, 1976	Effluent	ECHOvirus Type 31 ECHOvirus Type 24 ECHOvirus Type 25 Coxsackievirus Type B-3
January 18, 1977	Effluent	Coxsackievirus Type B-3 ECHOvirus Type 24
January 18, 19-7	Observation Well	U*
March 22, 1977	Effluent	Poliovirus Type 2 (Vaccine strain) Poliovirus Type 1 (Vaccine strain) ECHOvirus Type 6 Coxsackievirus Type B-4
April 20, 1977	Effluent	U* ECHOvirus Type 2 Coxsackievirus Type B-3 Poliovirus Type 3 (Vaccine strain)
May 16, 1977	Effluent	U*
May 16, 1977	Observation Well	U*

U* - Identity Unknown

TABLE 33

Coliform and Virus Isolation

Sunrise Well

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
September 1976	4,300	2,300	ni
October 1976	230	4	ni
November 1976	4,300	750	ni
December 1976	nt	nt	nt
January 1977	4	<3	3.8
February 1977	230,000	93,000	ni
March 1977	2,300	nt	ni
April 1977	12,000	6,400	ni
May 1977	43	<3	5.7

ni = No Isolates

nt = Not Tested

c. Sewage treatment plants with groundwater recharge basins

i. Meadowbrook STP

Viruses were isolated in chlorinated effluents on three occasions (Table 34). Of interest was the isolation of virus during periods when coliform counts were extremely low (September and February), and the absence of virus isolates during months when coliform counts were unusually high (Figures 20 and 21), the exception being the sample from June 1976. In all likelihood, human viruses were present during those periods of high coliform densities (August and January), but their adsorption to virus concentrating filters may have been inhibited (other workers have noted similar difficulties when using the virus concentrator in grossly contaminated waters. The process responsible has not as yet been determined). Viruses were recovered from effluents with chlorine residuals as high as 1.5 ppm (Table 29, Figure 22). Isolate correlation with turbidity levels could not be made (Table 30). Isolate identifications (Table 35) included a wide variety of enterovirus species. Among the isolates obtained from the September 1976 sample were Coxsackievirus types B-3 and B-4. The same virus species had been reported during that period as having been isolated from numerous patients suffering from a variety of clinical symptoms by Dr. Wayne Klein, Chief of Virology Service, Nassau County Medical Center (Meadowbrook Hospital).

Small numbers of viruses were found on three occasions in the observation well (Table 36), indicating vertical movement of virus particles through the basin. The likelihood of horizontal movement of viruses cannot be commented upon due to the location of the observation well. The well

TABLE 34

Coliform and Virus Isolation

Meadowbrook STP

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
June 1976	430,000	23,000	80.0
July 1976	23,000	9,300	ni
August 1976	750,000	43,000	ni
September 1976	<3	<3	6.4
October 1976	230	<3	ni
November 1976	230	<3	ni
December 1976	2,300	43	ni
January 1977	11,000,000	2,400,000	ni
February 1977	49	11	100.0
March 1977	9,300	nt	ni
April 1977	9,300	nt	ni
May 1977	2,300	4	ni

ni = No Isolates

nt = Not Tested

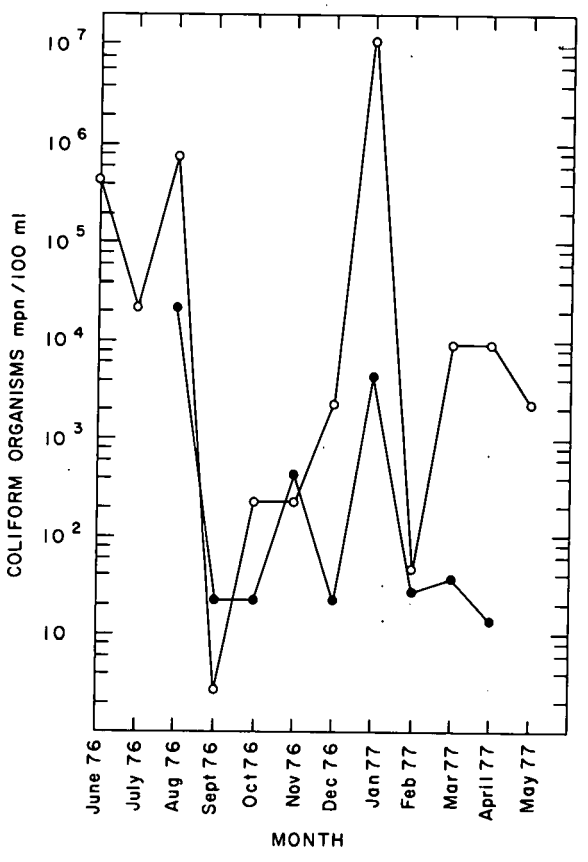

Figure 20. Total coliform counts (per 100 ml), Meadowbrook STP and Meadowbrook Well. 0 - total coliform - STP; ● - total coliform - Well.

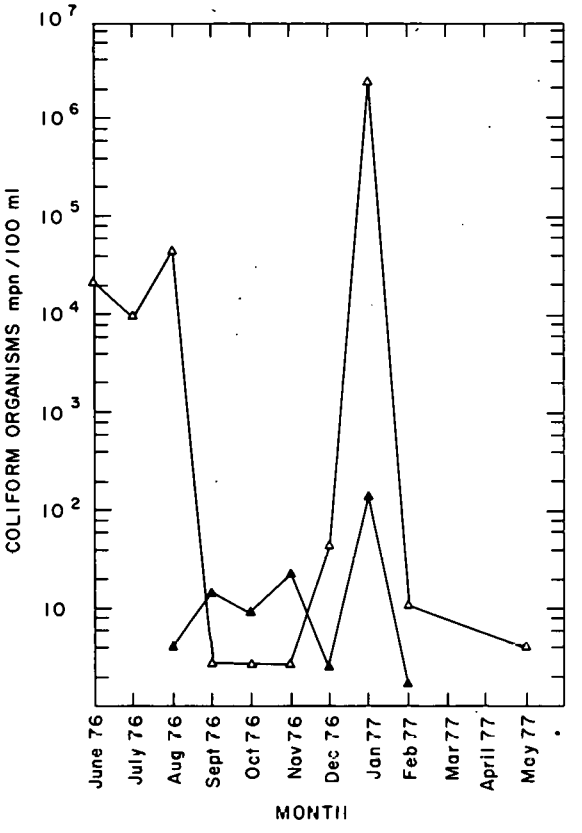

Figure 21. Fecal coliform counts (per 100 ml), Meadowbrook STP and Meadowbrook Well. Δ - fecal coliform - STP; ▲ - fecal coliform - Well.

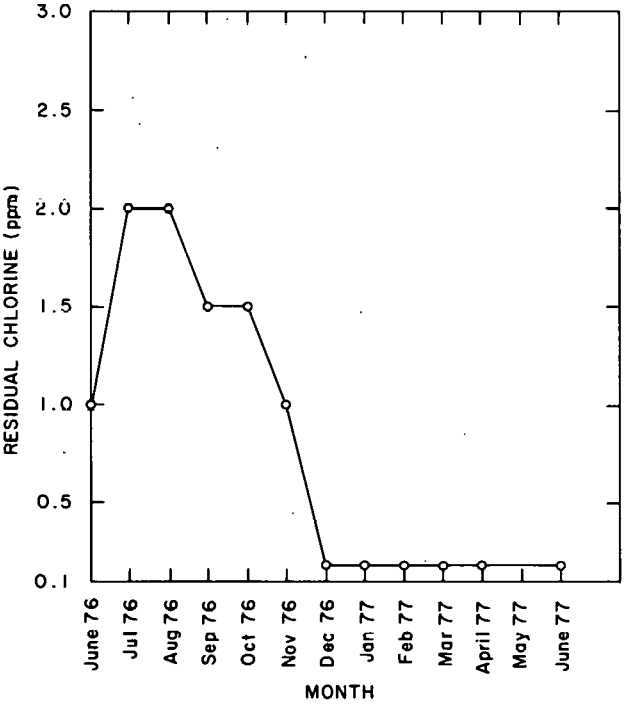

Figure 22. Residual chlorine (ppm), Meadowbrook STP.

TABLE 35

Virus Isolate Identifications

Meadowbrook

STP and Observation Well

Date	Sample Type	Identifications Include
June 22, 1976	Effluent	ECHOvirus Type 13 ECHOvirus Type 21 Coxsackievirus Type B-3
August 17, 1976	Observation Well	ECHOvirus Type 12 U*
September 13, 1976	Effluent	Coxsackievirus Type B-4 Coxsackievirus Type B-3 ECHOvirus Type 6 Poliovirus Type 1 (Vaccine strain)
September 13, 1976	Observation Well	U*
February 2, 1977	Effluent	Coxsackievirus Type B-4 ECHOvirus Type 30 U*
April 5, 1977	Observation Well	U*

U* - Identity Unknown

TABLE 36

Coliform and Virus Isolation

Meadowbrook Well

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
August 1976	23,000	4	1.3
September 1976	23	15	3.6
October 1976	23	9	ni
November 1976	430	23	ni
December 1976	23	<3	ni
January 1977	4,300	150	ni
February 1977	27	<2	ni
March 1977	39	nt	ni
April 1977	15	nt	2.4
May 1977	nt	nt	ni

ni = No Isolates

nt = Not Tested

had been sunk within 8 ft. of the bank of the basin. At such close proximity, it is likely that the well drew from the dome of recharged water that extended outward from beneath the basin. Well samples were therefore not representative of groundwater that had undergone any appreciable horizontal flow. Viral isolations showed little relationship to coliform counts in the well water samples.

ii. Stony Brook STP

Human viruses were isolated from chlorinated effluents during winter and spring months (Table 37). On three occasions there were correlations with unusually high coliform counts (Figures 23 and 24). Most of the isolations occurred when chlorine residuals were less than 0.2 ppm (Table 29, Figure 25). The sporadic nature of the coliform and virus levels suggests a temporary breakdown in treatment or disinfection processes. Such breakdowns were known to occur at this and other plants studied.

Well samples yielded no virus isolations, indicating the inability of viruses to penetrate the 80 ft. from basin bottom to groundwater aquifer (Table 38). Coliform counts were also substantially reduced during soil percolation (with the obvious exception of the December sample). The results for this month do not fit the trends observed over the year and cannot be readily explained.

Overall, the results were viewed as supporting the practice of the recharge of properly treated sewage effluents through basins located at reasonable distances (e.g., 80 ft.) above groundwater aquifers.

iii. Parkland III STP

The Parkland III plant, which was the only tertiary treatment system sampled during the study, experienced a number of operating problems during

TABLE 37

Coliform and Virus Isolation

Stony Brook STP

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
June 1976	7,500	3,900	ni
July 1976	2,300	150	ni
August 1976	9	<3	ni
September 1976	9,300	<30	ni
October 1976	4	<3	ni
November 1976	11,000,000	nt	84.4
December 1976	2,400,000	430,000	369.6
January 1977	2,300	23	ni
February 1977	9,300	430	ni
March 1977	4,300	nt	32.4
April 1977	930,000	240,000	23.2
May 1977	240,000	240,000	ni

ni = No Isolates

nt = Not Tested

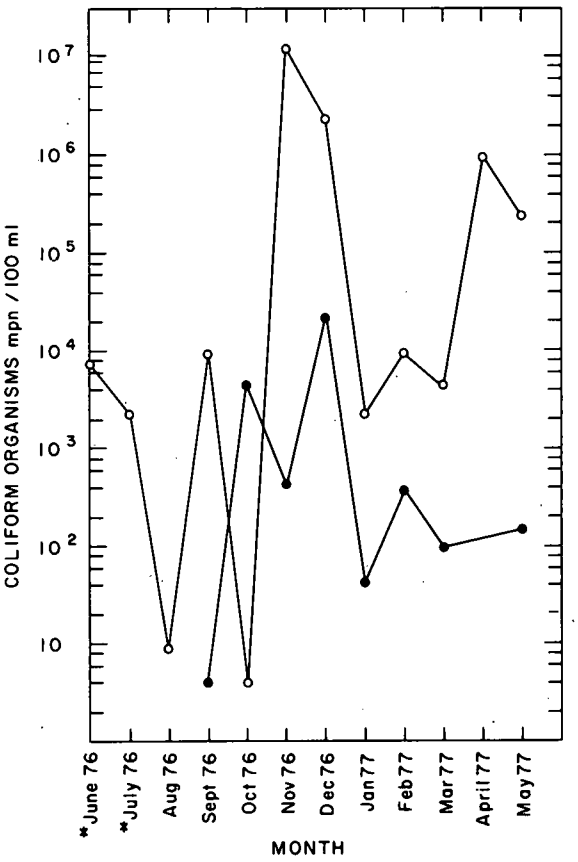

Figure 23. Total coliform counts (per 100 ml), Stony Brook STP and Stony Brook Well. 0 - total coliform - STP; ● - total coliform - Well.

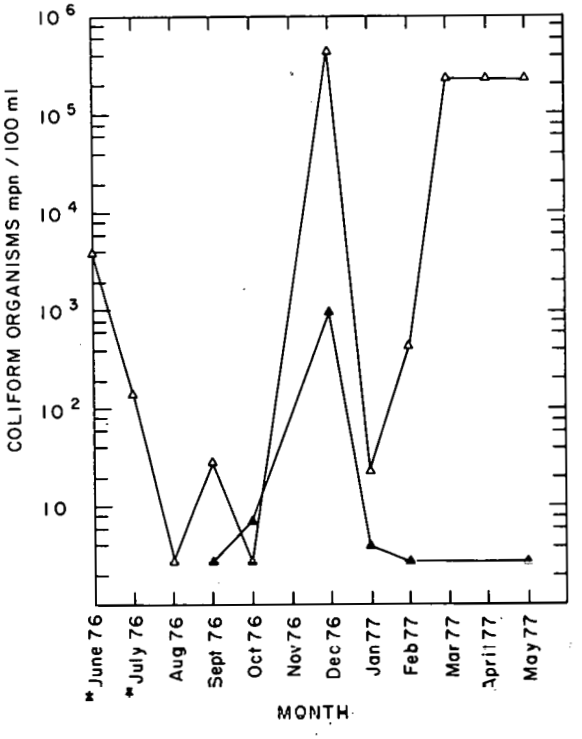

Figure 24. Fecal coliform counts (per 100 ml), Stony Brook STP and Stony Brook Well. Δ - fecal coliform - STP; ▲ - fecal coliform - Well.

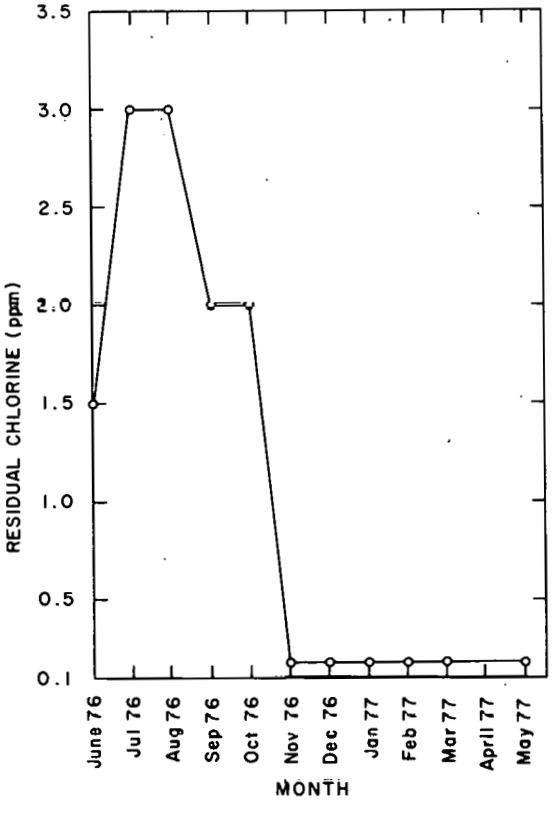

Figure 25. Residual chlorine (ppm), Stony Brook STP.

TABLE 38

Coliform and Virus Isolation

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
September 1976	4	<3	ni
October 1976	4,300	7	ni
November 1976	430	nt	ni
December 1976	23,000	930	ni
January 1977	43	4	ni
February 1977	390	<3	ni
March 1977	93	nt	ni
April 1977	nt	nt	ni
May 1977	150	<3	ni

ni = No Isolates

nt = Not Tested

TABLE 39

Virus Isolate Identifications

Stonybrook

STP

Date	Identifications Include
November 9, 1976	ECHOvirus Type 2 ECHOvirus Type 21 U* Coxsackievirus Type A-16 Coxsackievirus Type B-3
December 13, 1976	Coxsackievirus Type B-3 Poliovirus Type 1 (Vaccine strain)
March 16, 1977	ECHOvirus Type 6
April 12, 1977	U*

U* - Identity Unknown

the study period. As a result, monthly coliform counts were quite high, and viruses were isolated on six different occasions (Table 40, Figures 26 and 27). Viral and bacterial numbers were lowest when chlorine residuals were above 1 ppm (Table 29, Figure 28). At residuals below 0.2 ppm, the microbial quality of the effluent often resembled that of primary treated sewage. The highest virus count occurred in March when turbidity was at a high of 37 NTU (Table 30). As noted with a previously discussed STP effluent, the isolations of additional virus during the months of December, January, and April were probably inhibited by the presence of excessive numbers of coliform bacteria.

Isolate identifications included the broad range of enteric viruses commonly associated with municipal wastewater (Table 41). As of this writing, three Poliovirus isolates recovered from effluent samples during February, March, and April have been tentatively identified as being wild type (non-vaccine) strains. Final confirmation of these isolations will be made with the assistance of the Center for Disease Control (C.D.C.) Atlanta, Ga.

Comparatively low numbers of viruses were isolated from the observation well on three occasions (Table 42). The well was situated a sufficient distance from the basins to be representative of some horizontal flow. The high virus and coliform numbers occurring in improperly treated effluents represented a never-intended stress to the removal capacities of the recharge system. Despite the loading, the system appeared to have removed a significant number of organisms. It is not known how far the viruses could have moved through the aquifer, but they would likely have been subject to the same removal mechanisms that occur

TABLE 40

Coliform and Virus Isolation

Parkland III STP

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
July 1976	430	3	ni
August 1976	4	<3	ni
September 1976	75,000	430	6.8
October 1976	930	15	ni
November 1976	430,000	430	ni
December 1976	930,000	4,300	22.0
January 1977	11,000,000	23,000	94.7
February 1977	23,000	230	315.5
March 1977	230,000	nt	1070.7
April 1977	2,400,000	93,000	94.0
May 1977	nt	nt	nt
June 1977	2,400	2,400	ni

ni = No Isolates

nt = Not Tested

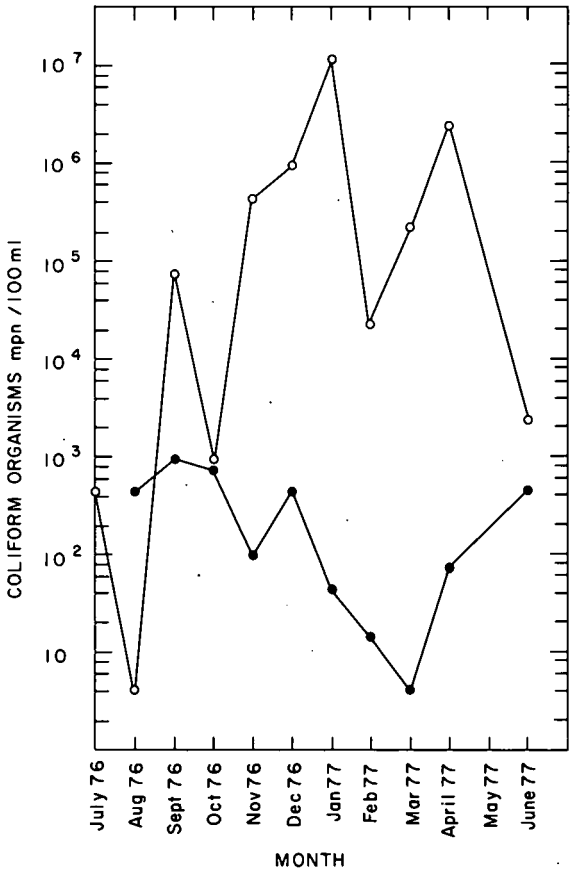

Figure 26. Total coliform counts (per 100 ml), Parkland III STP and Parkland III Well. 0 - total coliform - STP; ● - total coliform - Well.

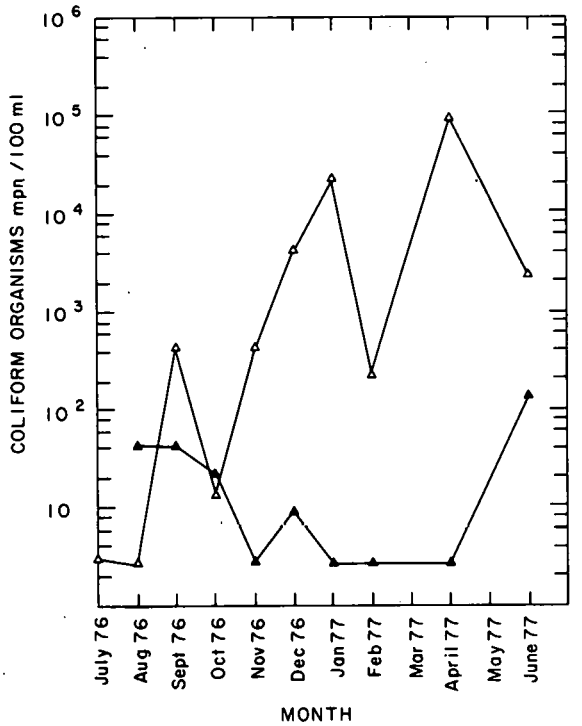

Figure 27. Fecal coliform counts (per 100 ml), Parkland III STP and Parkland III Well. Δ - fecal coliform - STP; ▲ - fecal coliform - Well.

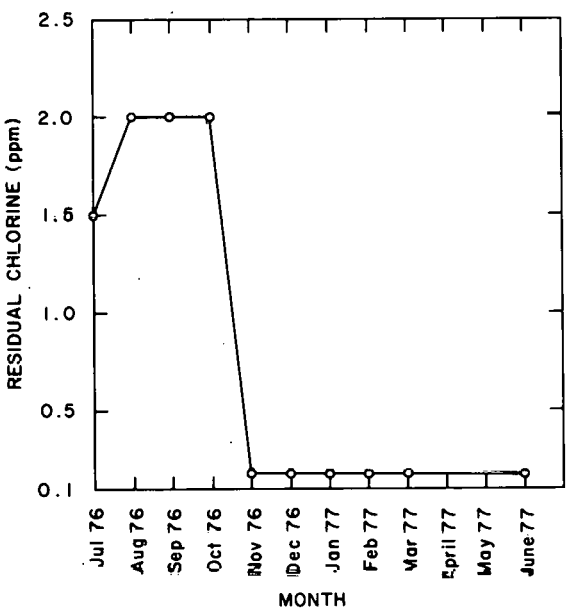

Figure 28. Residual chlorine (ppm), Parkland III STP.

TABLE 41

Virus Isolate Identifications

Parkland III

STP and Observation Well

Date	Sample Type	Identifications Include
August 17, 1976	Observation Well	ECHOvirus Type 6
September 6, 1976	Effluent	ECHOvirus Type 9
December 14, 1976	Effluent	U*
December 14, 1976	Observation Well	ECHOvirus Type 21 ECHOvirus Type 24
January 18, 1977	Effluent	Poliovirus Type 3 (Vaccine strain) U*
February 8, 1977	Effluent	Coxsackievirus Type B-3 Poliovirus Type 3 (Non-vaccine strain) Poliovirus Type 2 (Vaccine strain)
February 8, 1977	Observation Well	U* ECHOvirus Type 25 U*
March 15, 1977	Effluent	U* Poliovirus Type 2 ECHOvirus Type 13 ECHOvirus Type 25 Poliovirus Type 3 (Vaccine strain) Coxsackievirus Type A-16
April 13, 1977	Effluent	U* Poliovirus Type 3 (Non-vaccine strain) ECHOvirus Type 32

U* - Identity Unknown

TABLE 42

Coliform and Virus Isolation

Parkland III Well

Month	Total Coliform/100 ml	Fecal Coliform/100 ml	Virus PFU/gal
August 1976	430	43	3.7
September 1976	930	43	ni
October 1976	750	23	ni
November 1976	93	<3	ni
December 1976	430	9	1.6
January 1977	43	<3	ni
February 1977	15	<3	10.6
March 1977	4	nt	ni
April 1977	75	<3	ni
May 1977	nt	nt	nt
June 1977	460	150	ni

ni = No Isolates

nt = Not Tested

during vertical penetration through basins.

Because of the prevalence of low quality effluents, it was not possible to adequately assess the recharge system's ability to perform under normal plant operating conditions.

6. Experimental septic system

The routine isolation of high concentrations of human viruses and coliform bacteria from raw septic tank influent was expected (Table 43). No unusual species were noted among the many isolates identified (Table 44).

Results from tests of the system's undisinfected effluents were nothing less than remarkable. Viruses were isolated on a single occasion in the very beginning of the study (Table 45). Effluent coliform counts were often similar to those found in drinking water (Figure 29). There was little evidence of any major system failure, and removal efficiency did not appear to be affected by seasonal change.

Simple adsorptive processes cannot account for the tremendous removal rates observed. Further elucidation of the mechanisms involved must await additional study.

TABLE 43

Total and Fecal Coliform/100 ml and Virus PFU/gal - SCHD Influent

Month	Total Coliform	Fecal Coliform	Virus PFU/gal
July 1976	23,000	2,300	5,400.0
August 1976	110,000,000	110,000,000	600.0
September 1976	11,000,000	11,000,000	10,000.0
October 1976	24,000,000	2,400,000	2,730.0
November 1976	4,600,000	4,600,000	1,800.0
December 1976	24,000,000	4,600,000	8,880.0
January 1977	2,100,000	43,000	1,660.0
February 1977	930,000	930,000	ni
March 1977	11,000,000	nt	672.0
April 1977	11,000,000	4,600,000	ni
May 1977	7,500,000	2,300,000	ni

ni = No Isolates

nt = Not Tested

TABLE 44

Virus Isolate Identifications

SCHD Septic System

Influent and Effluent

Date	Sample Type	Identifications Include
July 8, 1976	Influent	U* ECHOvirus Type 21 Poliovirus Type 2 (Vaccine strain) Coxsackievirus Type A-16 ECHOvirus Type 25 Poliovirus Type 3 (Vaccine strain)
July 8, 1976	Effluent	U*
August 2, 1976	Influent	ECHOvirus Type 23 U* ECHOvirus Type 11
September 14, 1976	Influent	ECHOvirus Type 11 Coxsackievirus Type B-3 Coxsackievirus Type B-5 ECHOvirus Type 2 Coxsackievirus Type A-16 ECHOvirus Type 23 U*
October 5, 1976	Influent	Poliovirus Type 1 (Vaccine strain) ECHOvirus Type 21 ECHOvirus Type 12 ECHOvirus Type 24 U*
November 2, 1976	Influent	Coxsackievirus Type A-16 U*
December 7, 1976	Influent	Poliovirus Type 2 (Vaccine strain) ECHOvirus Type 2 Coxsackievirus Type A-16 U*
January 10, 1977	Influent	ECHOvirus Type 11 Coxsackievirus Type B-3 U*
March 14, 1977	Influent	Coxsackievirus Type B-3 ECHOvirus Type 2 ECHOvirus Type 25 ECHOvirus Type 14 U*

U* - Identity Unknown

TABLE 45

Total and Fecal Coliform/100 ml and Virus PFU/gal - SCHD Effluent

Month	Total Coliform	Fecal Coliform	Virus PFU/gal
July 1976	93	<3	10.0
August 1976	23	<3	ni
September 1976	230	<3	ni
October 1976	43	<3	ni
November 1976	28	4	ni
December 1976	<3	<3	ni
January 1977	<3	<3	ni
February 1977	<3	<3	ni
March 1977	<3	nt	ni
April 1977	<3	<3	ni
May 1977	4	4	ni

ni = No Isolates

nt = Not Tested

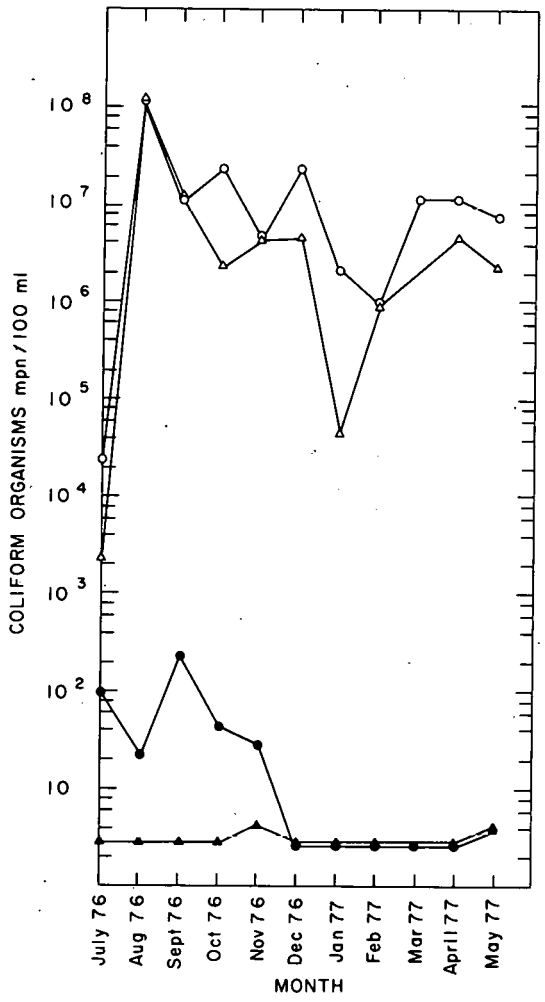

Figure 29. Total and fecal coliform counts (per 100 ml) - SCHD, experimental septic system influent and effluent. O - total coliform - influent; Δ - fecal coliform - influent; ● - total coliform - effluent; ▲ - fecal coliform - effluent.

V. DISCUSSION

A. Introduction

There is as yet no established standard for viral pollution levels in aquatic systems. The reasons for this include the difficulty of sampling, the nonexistence of a single standard method for enumeration and identification, and the lack of concise epidemiological information concerning the waterborne transmission potentials of the virus groups involved. In isolating members of the Enterovirus group in Long Island aquatic systems, we do not stress their significance as disease causing agents, but rather as indices of recent contact with human fecal material.

The study described herein does not represent the "definitive" statement on pollution in the areas studied. Such a determination would be obviated by the low sample numbers, and the brief duration of the program. In addition, there was no information gathered on non-Enterovirus species which may also be found in sewage polluted systems (e.g., Adenovirus, Reovirus, Rotavirus (Reo-like), Norwalk-agents).

The conclusions presented on the following pages were developed with the above restrictions in mind, but based primarily upon the results obtained from the study. As this rationale exists throughout the report, the dangers of out-of-context interpretation by the reader cannot be underestimated.

B. Discussion of Results from Field Samples

1. Public Water Supplies

Reliable technology for the study of virus in drinking water has only been recently developed. Methods now exist which enable specialized laboratories to sample volumes of water ranging from 380 ℓ to 1900 ℓ

(Sobsey et al., 1973; Farrah et al., 1976). The techniques have recently been approved and included in the 14th edition of Standard Methods for Examination of Water and Waste Water (1976).

Several studies have dealt with the sensitivities of the new methods. Hill et al., (1976) reported that 3-5 PFU per 380 ℓ could be recovered when 1900 ℓ of sample water were tested, with overall recovery efficiencies ranging from 28 to 42%, with an average of 35%. The methods have been successfully tested for a number of viruses including Polio, Coxsackie, ECHO, Reo and Adenovirus.

Few virus isolations would be expected in public water supplies due to a number of factors including: the inability of human viruses to reproduce outside of their host; the natural physical, biological and chemical processes that inactivate virus in aquatic environments; and the efficiency of virus removal and inactivation by conventional drinking-water treatment plants (Akin and Jakubowski, 1976). Evidence for the presence of virus in drinking water is sketchy and incomplete. A few reports have cited viral isolations from drinking-water. After subsequent testing, the findings could not be confirmed and were judged to be the result of contamination. To avoid similar errors in the future, Akin and Jakubowski (1976) proposed a set of guidelines for sampling finished water:

1. Personnel directly involved in sample collecting and handling should routinely have throat and rectal swabs collected. They should be processed if a virus-positive water sample is found.

2. Aseptic technique in a closed system should be used for sample collecting and processing.

3. When samples are to be stored prior to testing, they should be

placed in ultralow temperature freezers that contain no other type of virus sample.

4. Samples should be processed in isolation facilities where no other type of virus sample is handled.

5. Multiple barriers to air contamination should exist, i.e., separate isolation facility, laminar flow hoods, etc.

6. All isolates must be confirmed as being viral in nature.

Few existing laboratories can meet all the above recommendations, particularly in regard to a separate isolation facility. The Brookhaven National Laboratory Virology facility was able to adopt a majority of the Environmental Protection Agency's recommendations for studying the drinking water samples from Bayport and Oakdale.

The study wells were specifically chosen because of their relatively shallow depths and vulnerability to contaminants because of their location. The water quality met appropriate drinking water standards in all cases.

Based upon our data which showed no virus isolations from any drinking water samples, it may be concluded that virus and bacterial-free water should result from the adequate treatment of groundwater from public supply wells that have been located considerable distances from possible pollution sources such as contaminated rivers or streams, or heavily developed (housing) areas where leaching from closely packed septic systems may adversely affect water quality in the surrounding area. In light of the above findings, it is tentatively recommended that such measures be taken whenever possible for public water supplies on Long Island. As the systems studied (Bayport, Oakdale) may not represent the "average" water supply well on Long Island, it is further recommended that additional virological studies be carried

out on a variety of public water supply systems in order to lend support and further justify the above conclusions.

2. Surface Waters

a. Lake Ronkonkoma

A review of the available literature reveals no previous report of isolation of human viruses in lake water in the United States. The major reason for this would appear to be the lack of virus studies actually carried out on this particular type of aquatic resource.

Isolation of human viruses in Lake Ronkonkoma samples on two out of seven occasions (28.5%) could theoretically be attributed entirely to the influence of bathers. The theory is easily applied to the occurrence of viruses at a bathing area in early September, a time when the area was still in use. Survival of human viruses in lake water would appear to be extensive (Hermann et al., 1974), especially if they become trapped within the sediments. It is possible (but not proven) that such viruses could survive for periods of up to six months, which would explain the isolations in March of 1977. There were, however, an insufficient number of measurements taken between September and March to totally support or refute this theory.

It is impractical to ignore the possibility of other sources contributing to viral presence in the lake. As previously mentioned, the March isolations could reflect the passage of feces - contaminated liquid from domestic septic systems to the water of the lake. Information gathered at other sites concerning the likelihood of horizontal migration of virus particles through soils would suggest that septic systems would have to be located adjacent to the shores of the lake for

this type of movement to occur with any predictable frequency. In order to adequately assess the likelihood of this particular type of source, it would be necessary to test the waters in those areas where septic systems are known to occur.

Standards now exist regarding the placement of septic systems near surface waters. It is recommended that further study be carried out to assess the adequacy of these standards in preventing the passage of viral contaminants into these waters.

b. Penataquit Creek

Numerous studies have demonstrated the presence of human viruses in the tributary waters leading to embayments (Metcalf and Stiles, 1967; Vaughn and Metcalf, 1975). In most instances, pollutants have been traced to direct discharge of treated or untreated sewage into the rivers, creeks, or streams under study. The likelihood of the passage of infectious viruses through tributaries and into embayments of estuarine regions has been established. In the case of Penataquit Creek, the two positive samples out of a total of eleven taken (18.1%) could not be traced to a regular discharge of sewage effluent. While leakage from septic systems along the creek bank, run-off from streets, and the discharge from large boats located in the creek may be suspect, the periodicity of virus isolation (and that of bacteria) suggests a more intermittent source.

Penataquit Creek exerts an influence on the water quality of the nearby region of Great South Bay. An improvement in the quality of this and other creek waters would likely result in a corresponding improvement in the immediate area of the bay.

c. Marine Embayments and their Shellfish

(Author's note: Over the past several months much debate has been centered on the adequacy of the coliform index to accurately identify the potential hazards posed by sewage-borne pathogens to commercial shellfish beds. It is not the intent of the 208 virus study to pursue this question, and the present report contains insufficient information to properly address the problem. Caution is therefore advised regarding any correlation of viral and bacterial data which could not be supported by the authors or by Brookhaven Laboratory.)

Numerous investigators have described the isolation of human viruses from shellfish and shellfish growing waters (see Literature Review - Section II). In most of the cases described, the source of viral pollution was the discharge of primary or secondary sewage treatment plants.

Although a definite correlation between viral numbers in Penataquit Creek and those in Great South Bay could not be established, the creek obviously represents one of the sources of contamination to the bay. The transmission from creek to bay was probably in effect during the entire year (even though we were unable to isolate them from the creek at all times), with the actual virus concentrations fluctuating with the season. Viruses were isolated from "closed" waters in 37.5% of the samples tested while being found in 28.5% of all "closed" clam samples. The "open" area yielded positive results in 37.5% of the water and shellfish samples. These results do not conflict with established survival patterns for marine waters which show extended survival in water, shellfish and sediments (Vaughn and Metcalf, 1975; Akin et al., 1975b; DeFlora et al., 1975).

The installation of septic systems along the immediate shoreline has

been curtailed by state and county regulation, which should prevent further movement of viruses to near shore areas of the bay. The role of storm-water run-off as a source of human viruses has been suggested but not proven in the area studied. Additional studies would be necessary to define both the extent of the pollution contributed by run-off, and the likely measures for control.

The "open" and "closed" areas studied in Oyster Bay were probably influenced by separate sources of pollutants. Results showed that 12.5% of all water and shellfish samples taken at the open site contained species of human viruses. Likely sources of viral contamination to this region include overland run-off, septic tank leaching, and the nearby (1-2 miles) discharge of treated sewage effluent from the Oyster Bay sewage treatment plant. While the major viral source could not be determined within the confines of this study, it should be noted that previous work by one of the authors (Vaughn and Metcalf, 1974; Metcalf, Vaughn and Stiles, 1972), conducted in a similar bay system receiving discharges from secondary treatment plants, indicated the presence of human viruses in shellfish beds that were located 7-8 miles from the nearest outfall.

The "closed" site was located several miles west of the "open" area discussed above. Microbial contamination at this site was probably influenced slightly by the sewage outfall, the more likely sources being from overland run-off and septic tank seepage from the numerous older homes surrounding the area. The results of sampling in this area yielded no virus isolates in the water column, yet 37.5% of the shellfish tested did contain viruses. The likely reason for this discrepancy, previously discussed in the Results section, was the heavy turbidity of the water. This

finding raises some interesting questions concerning the accuracy of the sole use of water samples to predict the viral quality of shellfish residing in especially turbid environments.

As the sources of viral pollution in these areas cannot be specifically identified without further study, it is impractical to offer concrete suggestions concerning their control.

3. Babylon Landfill

The banning of the open burning of trash, and the demise of the "town dump" have popularized the use of sanitary landfills for the disposal of trash items. Certain precautions should be taken to prevent the passage of viruses through the landfill and into the groundwater aquifer. Such precautions could include the use of impermeable membranes beneath the fill to prevent leaching, or the use of "filtering systems", such as artificial peat bogs, to polish the leachates before percolation to groundwater aquifers.

Investigators have previously isolated human viruses in solid wastes (Peterson, 1974), but few have reported similar isolations in landfill leachates. To date no reports have described isolations in leachate-contaminated groundwater. Correlation of the results of our study with those of previous studies was complicated by the presence of scavenger waste pits on the Babylon landfill, a practice which is apparently not often used in other parts of the country. The presence of so obvious a source of human viruses tended to diminish the likelihood of other potential sources such as disposable diapers.

Virus isolations were made in 10% of the groundwater samples tested. Because neither the scavenger waste, nor the landfill leachate was tested,

little can be concluded concerning the virus removing capacity of the landfill itself (significant removal could have actually occurred during movement through the groundwater aquifer between the landfill and the observation well).

While the greatest threat to groundwater pollution by landfill leachates is likely chemical rather than biological in nature, the possible movement of potentially harmful microbes through landfills (especially those which mix domestic sewage or sludge with fill) cannot be ignored. Studies to define procedures (e.g. those precautions mentioned above) for the abatement of biological pollutants in leachates would be indicated.

4. Storm Water Recharge Basins

Little is known about the occurrence, transmission and survival of human viruses in storm water, and questions concerning their passage through storm water recharge basins are moot. The isolation of viruses from the groundwater beneath the North Massapequa recharge basin provides more questions than answers. Since the storm waters entering the basin were not tested, it is not certain that viruses were ever present within them. The only alternative viral source noted was possible leaking or overflow from septic systems located around the basin. Again, there is not sufficient information to make this conclusion.

Additional testing of groundwater and the storm water run-off entering the basin over a period of time would likely provide information regarding viral source, or at the very least provide additional data with which to determine the significance of the single isolation that was encountered.

Should storm water be identified as the virus source, it would be most

interesting to determine the effect of the low pH of waters beneath the basin on the removal of viruses during percolation through the soil (as previously mentioned, pH levels between 3.0 and 5.5 tend to enhance virus adsorption to many surfaces).

5. Sewage Treatment Plants

Currently practiced sewage treatment methods cannot guarantee the removal of all human viruses. Isolation of virus in treated effluents is therefore not surprising. The results of tests carried out on a number of sewage treatment plant effluents indicated that efforts could be made to minimize the number of viruses in treated wastewater (i.e. carrying out standard treatment practices in a properly designed plant). Three of the plants released significant virus numbers in less than 50% of their effluent samples tested (Stony Brook STP - 36.3%, Oyster Bay STP - 36.3%, Meadowbrook STP - 25.0%). The Parkland III plant showed a slightly higher frequency with 54.5% of samples taken yielding positive results. Least effective at removing viruses (and bacteria) was the Sunrise Garden Apartments plant which showed an 80% frequency of virus isolation.

The Oyster Bay facility was the only plant studied which was discharging treated effluents into surface waters. While the virus removing efficiency of this plant was among the highest of those studied, significant numbers of virus particles were periodically released into areas of the bay which are now closed. It can be calculated, given the survival capacities of viruses in such systems, that even infrequent discharges of viruses and other microbial pollutants can eventually affect the water quality of the entire bay area. Such a risk should not be ignored and more effective virus-removing methods, or alternative means of effluent disposal should be considered.

The increasing demand for potable water to supply domestic and commercial needs has prompted a search for methods to supplement fresh water reserves. Among methods proposed are several dealing with the recharge of groundwater aquifers with renovated wastewater, including: spray irrigation; land application; well injection; and percolation through recharge basins. Inherent in any scheme of wastewater reuse is the potential hazard posed by the pathogenic microorganisms commonly found in sewage. The success of many recharge methods may depend largely upon their ability to successfully remove these organisms. An important facet of the 208 virus study was the monitoring of several groundwater recharge sites in order to qualitatively assess their ability to remove human viruses (Note: quantitative assessment would require more elaborate programs than those conducted for 208). While being unable to define all the necessary conditions, it was hoped that the program of monthly viral analysis would be able to indicate the likelihood of returning virus-free waters to groundwater aquifers.

While not usually listed among recharge methods, the use of subsurface leaching fields associated with sewage treatment plants will eventually result in the return of water to the aquifer. It is recommended that effluents of similarly low quality to those found at the Sunrise plant not be used for such purposes. However, the information gathered at this site may be useful as an index of the efficiency of such recharge systems under "worst possible conditions". Viruses were isolated from 80% of the STP effluent samples taken, while only a 22.2% frequency was noted in the groundwater observation well. The data suggested that this type of disposal of low volume effluents in fairly isolated areas would be practical, providing the effluents were of adequate quality.

Among treatment plants discharging into recharge basins, the best results were obtained from the Stonybrook site where no viruses could be isolated from the 8 samples tested. Parkland III yielded positive results in 20% of samples taken, and Meadowbrook showed an isolation frequency of 25.0%. Previously cited studies have demonstrated an inability of effluent borne viruses to penetrate appreciable distances through soil columns depending on soil composition and effluent application rates. The apparent inability to recover significant numbers of virus at the Stonybrook site was likely a result of the soil depth from the bottom of the recharge basin to the aquifer, which measured some 80 ft.

The 34 ft. soil layer from basin to aquifer at the Meadowbrook site seemed to be a less efficient virus remover. This conclusion does not account for differences in effluent qualities, and soil characteristics. Studies of the latter may have indicated the presence of small fissures which would have allowed rapid virus infiltration by channeling. Had the observation well been located further down-flow, rather than within the dome of recharged water, some estimate of virus removal during horizontal flow would have been possible. In the absence of this information, it can only be assumed that removal rates through the aquifer would be similar to those encountered during percolation through the recharge basin. Based upon this, it is calculated that viral penetration in the aquifer would not be significant after the first 100-200 ft. of travel. Confirmation of this hypothesis would require an additional study of the site which would include the installation of a second observation well 150 ft. down groundwater flow from the recharge basin.

The microbial quality of effluents discharged from the Parkland III plant did not resemble those of a properly operated tertiary treatment system. In spite of this, encouraging removal rates were noted in observation well waters. Based upon these data, it is conceivable that the recharge of properly treated effluents would contribute no significant virus numbers to the aquifer. The premise could be confirmed with a study similar to that just completed.

On the basis of viral information derived from this and other ongoing and recently completed studies, the following general guidelines concerning the recharge of domestic sewage treatment plant effluents on Long Island are presented for consideration:

1. The overall microbial quality of effluents to be recharged should, at the very least, conform to standards prescribed for secondary effluents, including a suggested fecal coliform count of no greater (and preferably less) than the EPA recommended 200 per 100 milliliters (geometric mean). Properly treated secondary effluents with chlorine residuals of 1.5 - 2.5 ppm (15 min. contact time) should contain reasonably low numbers of viruses that should be removed during percolation.

2. Recharge basins should be located in areas where groundwater aquifers are at a significant depth. Because of differences in soil characteristics, an exact figure cannot be indicated. Depths to groundwater of 60-100 feet would appear to be adequate for the removal of a majority of virus particles. Shallower recharge zones might be acceptable to a minimum of approximately 30 feet. Construction of recharge basins with distances to groundwater of less than 30 feet. Construction of recharge basins with distances to groundwater of less than 30 feet would have to be carefully scrutinized. Alternative treatment methods may modify the above condiserations.

3. Recharge basins should not be constructed in areas abutting lakes, rivers, creeks, streams or coastal waters where saturated soil conditions would facilitate the movement of viruses.

4. Consideration should be given to the siting of recharge operations with respect to their proximity to public water supply wells.

5. A series of additional monitoring wells should be constructed at each recharge site in order to routinely monitor the quality of the recharged water and its effect on aquifer quality.

6. Experimental Septic System

A major portion of Suffolk County is unsewered, and is likely to remain so for some time, necessitating the use of septic tanks. In an effort to find a more efficient septic system, the Suffolk County Health Department constructed an experimental subsurface system on the grounds of Brookhaven National Laboratory, which treated a portion of raw wastes originating from the Laboratory's apartment complex.

Results from the testing of this system which was part of the 208 virus program, indicated it to be most promising for the treatment of small volumes of raw wastewater. In spite of the large number of viruses and bacteria entering the system, undisinfected effluents consistently revealed significant removals of both. The removal mechanisms involved could not be determined within the confines of the 208 program, but it is hoped that this research may be conducted in the future.

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