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Human Viruses in the Aquatic Environment:

A Status Report With Emphasis on the EPA Research Program

Report To Congress

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HUMAN VIRUSES IN THE AQUATIC ENVIRONMENT: A STATUS REPORT WITH EMPHASIS ON THE EPA RESEARCH PROGRAM

REPORT TO CONGRESS

Prepared by the
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Office of Drinking Water
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ABSTRACT

Enteric viruses enter water through fecal wastes from infected individuals. These viruses abound in urban sewage due to the continual occurrence of viral infections, usually in children and often without symptoms. Viruses that infect humans are incapable of multiplying in the environment, but are rather resistant and may survive for extended periods. The health concern over their waterborne transmission stems primarily from three factors: (1) much virus-laden waste ultimately contaminates surface waters, the source for most drinking water, (2) only a few virus particles may be required to produce an infectious dose, and (3) the enteric viruses can produce a variety of serious diseases. Since water is consumed by all, even a low level of viral contamination may significantly contribute to the disease burden of a population.

In comparison to the traditional coliform bacteria indicators of water quality, enteric virus levels in wastewater are extremely low. Waste and water treatment processes further reduce the levels. Although viral detection methods have been dramatically improved over the past 15 years, this technology is not adequate to assess the occurrence of the extremely low levels possibly present in drinking water. Likewise, the role of drinking water in maintaining the background level of viral disease is difficult to evaluate epidemiologically because the more common person-to-person spread of disease is continually occurring.

Viruses have been isolated from many surface water streams used as drinking water sources and it now appears that full conventional treatment may be required (and will be adequate) to produce reasonable assurance of a virologically safe drinking water. Even so, it is of particular importance that we remain diligent to the possible risk of the contamination of drinking water supplies by viruses and other pathogenic microorganisms. Since its inception, the U.S. Environmental Protection Agency has supported and is continuing to support studies designed to fully evaluate the role of water in viral disease transmission.

PREFACE

The Safe Drinking Water Act, as amended (42 U.S.C. §300f et seq.), states very succinctly [Section 1442 (a)(7)] that the Administrator of the U.S. Environmental Protection Agency "shall carry out a study of virus contamination." In a broad interpretation of this wording, the study of the sources of viral contamination would require the tracking of viruses from the infected individual, through the sewage system, the natural aquatic environment and ultimately to the contamination of a glass of drinking water. The development of the capability to conduct such a study began about 15 years ago as the awareness of a potential virus-in-water health question began to surface. This report will not review the development of this capability, but rather will use the data thereby obtained to put into focus the preventive-health questions and answers that relate to the subject as they are currently perceived.

The report is divided into twelve sections that represent key areas of interest and activity in the virus-in-water field. It concludes with a discussion of the limitations of the current state of knowledge and recommends nine specific areas for further research effort. Reference to the older scientific literature is made frequently. However, the report focuses on recent findings obtained from the ongoing research activity and the drinking water survey of the EPA. The research program of EPA is carried out through the support (both in-house and grants/contracts) of projects by three laboratories within the Environmental Research Center-Cincinnati: Health Effects Research Laboratory, Municipal Environmental Research Laboratory and Environmental Monitoring & Support Laboratory. It is hoped that this report accurately conveys the state of knowledge regarding this potential health hazard and has clearly revealed the studies engaged in by this Agency in response to the mandate of the Act and the health needs of the American public.

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GENERAL OVERVIEW

Human viruses enter the aquatic environment primarily through sewage discharges. Some viruses are excreted in large numbers in the feces of infected individuals. These viruses are grouped under the heading of "enteric viruses" and about 100 such virus types have been identified. These are the ones of primary concern in the waterborne transmission of viral disease. Although enteric viruses frequently produce asymptomatic infections, they are capable of producing paralysis, heart anomalies, infection of the brain and eyes, upper respiratory infections, gastroenteritis and many less serious symptoms. Children under the age of 10 years appear to be the most susceptible to infection. In the aquatic environment, the viruses are unable to multiply but may remain viable for an extended period of time, especially at low temperatures, thereby posing a health risk to users of these waters.

The occurrence of viruses in the environment and the related health risk have been a subject of EPA research programs for several years. Outbreaks of viral disease caused by the ingestion of sewage-contaminated waters have been well-documented by epidemiological studies. However, the role of "acceptable" drinking water in spreading and maintaining the endemic level of viral disease has not been elucidated. One major objective of EPA-funded work as well as work supported by others has been the development of methods for the recovery of low numbers of viruses from large water samples. Fairly sensitive methods have been developed in the past few years and these have been used to quantitate the number of viruses in environmental samples from various locations. In addition, these methods have been used to evaluate the effectiveness of sewage and water treatment processes, including disinfection, for viral inactivation and removal.

Even though the amount of data available from these studies is very limited, an estimate of the level of contamination that could occur in drinking water can be made. However, viral exposure levels predicted from such models are highly speculative in that the accuracy of the data and assumptions concerning the effectiveness of water treatment are open to question. Many virologists feel that the actual environmental concentrations of viruses are much higher than those currently reported because of known and suspected limitations of the virus-recovery methods. There is also a fairly widespread view that water treatment procedures are not continually applied in all plants. It is also felt that the procedures are not as effective in removing or inactivating naturally-occurring viruses as they are in removing the laboratory strains used in treatment-efficiency studies. Therefore, it is possible that viruses may be present in some treated drinking waters at concentrations near or below the level detectable with current methodology.

The EPA has applied state-of-the-art virus recovery methods to drinking water samples collected from community systems in many locations. A total of 225 samples with a maximum volume of 1,900 liters/sample has been

collected from 56 systems in the past six years. No confirmed viral isolations have been made from these samples. Other laboratories have conducted viral testing of water supplies, and viral isolations from two treated supplies in the United States have been reported in the technical literature. More testing and evaluation is needed to confirm the occurrence of viruses in such relatively small volumes of a few hundred liters of treated drinking water. In addition to the techniques that must be developed or improved to obtain an accurate indication of the occurrence of viruses in water, the actual number of viruses needed to produce infection is currently under investigation.

Conventional water treatment (coagulation-sedimentation, filtration and disinfection) is believed by most authorities to be capable of producing virologically-safe drinking water. However, the adequacy of disinfection (the final water treatment to defend against microbial-disease transmission) to assure complete inactivation of naturally-occurring viruses has been questioned in recent years. Based upon the information presently available, it is recommended that research should be initiated or continued in nine areas in order to more fully evaluate the possible health risk from viruses which might be in drinking water. These recommendations are:

1. The development of improved recovery methods with emphasis on increased sensitivity for the total number and types of viruses that may be present in water and wastewaters.

2. The further evaluation of the disinfection capability of chlorine and other disinfectants on natural viruses under field conditions and new viruses implicated in waterborne disease outbreaks (e.g. gastroenteritis virus).

3. The development of practical methods to remove/inactivate all detectable viruses from treated sewage and sludge.

4. The further evaluation of the viral contamination of surface and ground water as one of the factors to be considered in the land application of wastewater and sludge.

5. The development of a broader data base for estimating the minimum infective dose for ingested viruses.

6. The development of methods for the laboratory cultivation of hepatitis A virus and agents of acute viral gastroenteritis.

7. The evaluation of the role of lower animals as reservoirs of viruses that may infect humans.

8. The development of epidemiological approaches to determine the extent of endemic waterborne viral transmission.

9. The elucidation of specific factors and mechanisms responsible for viral inactivation and destruction in natural waters and soils.

HISTORICAL PERSPECTIVE

The field of environmental virology came into being in the 1950's largely as a result of the tremendous expansion of virological research made economically and technically feasible by the development of animal cell culture techniques. This led to initiation of research which showed that:

1. Enteroviruses were more resistant to chlorine than the coliform bacteria group used to indicate the sanitary quality of water.

2. Enteroviruses had high survival capabilities under various environmental conditions including wastewater treatment processes and were not efficiently removed by these processes.

3. The minimum infectious dose for enteroviruses was very low (on the order of one plaque-forming unit under certain experimental conditions). This was four to six orders of magnitude lower than the number of enteric bacteria needed to initiate infection.

These findings, coupled with epidemiological studies incriminating water as a vehicle for transmission of infectious hepatitis, led to increased concern about possible spread of other viral diseases by drinking water. As a result, efforts to isolate viruses from water and consequent efforts to improve these methods were initiated.

By 1965, interest in the subject was widespread and considerable information had been developed. At an international symposium convened that year, reports on various aspects of the waterborne virus question were presented. The symposium proceedings (Berg, 1967) were widely distributed and undoubtedly played a key role in enhancing interest in environmental virological research. The divergence of opinion on the seriousness of the problem of the spread of viral disease by drinking water was brought out in the first and last paragraphs of the conference summary statement as follows:

> We have been told that the number of proved outbreaks of waterborne diseases does not pose a serious public health problem. But, we know also that detection of infections resulting from waterborne transmission would be difficult if any of the infections were inapparent, even if subsequent contact transmission should produce a high disease rate.
>
> Finally, we must decide upon the direction of our future efforts. To this end, I would make this final admonition: knowing that viruses are present in our sewage and in our rivers, that viruses have been demonstrated in the water supply of a major city,* that it is possible for waterborne transmission to occur without being readily detected, I would say that if we are to err in the direction we take, we must err on the side of safety. We must do sufficient research to be certain

*Paris, France

that there is no important waterborne transmission, and should we find instead that there is a danger, then, at least we can do something about it.

Despite much additional research and subsequent major national and international conferences on the subject (Snoeyink, 1971; Malina and Sagik, 1974; APHA, 1976), the controversies conveyed by the above statements have not been resolved and are still with us today. The importance of the so called "focal infection" (caused by ingestion of water containing extremely low levels of virus) in the spread of enteric viral disease in a community has been neither proven nor disproven. This is mainly because epidemiological research methods are not sufficiently sensitive to determine whether or not waterborne "focal infections" occur. How far we must go in research "to be certain that there is no important waterborne transmission. . ." has not been determined. Despite technological improvements which have significantly increased the sensitivity of virus detection methods, calculations based on the number of viruses which might be expected in conventionally treated drinking water, if treatment is truly as effective as indicated by virus-seeded studies, indicate that these methods must be more sensitive by many more orders of magnitude in order to detect the viruses at the levels in which they may be present. In the few instances when viruses have been found in treated drinking water, the validity of the results has been challenged, in some cases on direct evidence, e.g. virus contamination problems with controls, and in other cases on what may be called circumstantial evidence, e.g. presence of high free-chlorine residuals or only a few viruses found in the source water.

It is against this background that this report has been prepared. The concerns and controversies described above are further delineated and expanded upon in the body of this report. The objective has been to place the problem in perspective, based on the information currently available, and to point out areas in which more information is needed to resolve the present dilemma.

INTRODUCTION

The persistent fear of death and debilitation from the ravages of infectious agents of past generations was apparently allayed in the United States with the control of the remaining dreaded disease--paralytic poliomyelitis--in the mid-1950's by the widespread administration of the newly-developed vaccine. The lack of concern over the epidemic return of this viral disease is reflected in the increasing number of children who are not receiving the protective vaccine. In 1976, 23 years after the introduction of a vaccine, 38 percent of the children in this country between the ages of 1 to 4 years had not received a primary vaccination (MMWR, 1977). Yet untreatable infections with numerous viral agents remain a major cause of morbidity in this country. Few communities escape the gastroenteritis and upper respiratory infections that sweep through the country in autumn or early winter each year. The actual number of cases that occurs is difficult to quantitate because reporting of cases by medical personnel to the health agencies is not required. In addition, many affected people do not seek medical treatment during the relatively short-term illness. Nonetheless, these outbreaks, thought to be primarily of viral etiology, are a significant drain on the health of the nation and these outbreaks are among the most common diseases experienced in the U.S. today (Dingle, et al., 1953). In addition, viral diseases not uncommon in this country, e.g. aseptic meningitis, myocarditis, hepatitis and influenza, may cause death or long-term illness.

The transmission of the common viral infections is thought to be primarily from person to person through direct contact. This mechanism would appear to explain the spread of the vast majority of the respiratory infections in which the primary portals of exit of the virus are through the nose and mouth. However, approximately 100 viruses have been identified that infect the lining of the stomach and intestine as well as the pharynx. The primary portal of exit for these enteric viruses is the intestinal tract. Thus, the virus is excreted in the feces of infected individuals with viral concentrations reported to be as high as 10^7 and 10^8 infectious units per gram (Sabin, 1956). These enteric viruses abound in sewage from larger communities where the likelihood of some degree of viral transmission is occurring continually. The isolation of infectious virus from sewage was first reported in the early 1940's and since that time numerous studies have found a wide variety of viruses present in sewage. These viruses are rather resistant to inactivation in wastewater and surface water, and depending upon temperature and other factors, may survive for days to months in this environment (Akin, et al., 1971). Since most domestic wastes are discharged directly or indirectly into the surface water systems that supply much of the country's drinking water, viral contamination of these waters is continuous. Thus, the waterborne transmission of the enteric viruses could significantly augment the direct person-to-person spread of these viral infections.

SOURCES OF ENTERIC VIRUSES

Man is thought to be the only important reservoir for members of the human enteric viruses. However, viruses that appear to be human viral serotypes have been isolated from the feces of domestic animals (Grew, et al., 1970; Graves and Oppenheimer, 1975). It is assumed that these animals are only passive shedders of viruses ingested via grossly contaminated food or water and that the viruses do not multiply in these hosts. An exception to this view may be the situation that appears to exist with the newly identified rotaviruses. Serologically related rotaviruses have been isolated from the feces of children, calves, piglets, mice and foals having acute gastroenteritis (Woode, et al., 1976). The role of the animals in the natural transmission of rotavirus disease in man has not been explored. In fact, the role of domestic and wild animals in the transmission of enteric viral disease to man has not been extensively studied and further work in this area is needed. Nonetheless, domestic waste from humans would appear to be the primary source of viruses in surface water as indicated by the close association of virus isolations and domestic pollution sources (Berg, et al., 1971).

Enteric viral infections are common in children, especially those below five years of age. The high frequency of occurrence is obscured by the fact that only a small percent of those infected manifest serious disease. Prior to the introduction of the poliovirus vaccine, it has been estimated that only one of every 1,000 children infected with the wild virus contracted paralytic disease (Melnick and Ledinko, 1951). A similar pathogenic ratio probably exists for many of the other enteric viruses. Therefore, an indication of the prevalence of enteric viruses in a community must be determined from viral excretion data. The findings of a recent study conducted in Seattle, Washington, indicated a fecal isolation rate of enteroviruses (other than poliovirus) of two to four percent among family members selected for the study (Cooney, et al., 1972). Poliovirus isolates were not considered in the evaluation because they were assumed to be primarily of vaccine origin. Gelfand, et al. (1957) in earlier work in Louisiana found that as many as 16 percent of the healthy children included in their study were excreting viruses other than polioviruses during the summer months. They also found excretion rates to be inversely related to socio-economic status. Interestingly, Chin, et al. (1967) were able to demonstrate the presence of vaccine strain poliovirus in sewage when as few as 0.3 percent of the local population had received the live-virus vaccine shortly prior to the examination of the sewage. The Seattle study also found children less than one year of age to have an average of 1.5 enteric viral infections per year which dropped to 0.58 for those two to five years of age and considerably lower for those over five.

VIRUSES IN SEWAGE AND SEWAGE EFFLUENTS

Some enteric viruses enter surface waters directly from infected individuals through swimming and other water activities. Viruses may also enter surface water directly from waste discharges of individual dwellings located along the water's edge. However, most human enteric viruses that are present in surface water are introduced through the discharges of urban sewerage systems. Sewage from these areas normally contains a wide variety of enteric virus types that vary in concentration according to the time of day and the season of the year. Wild viruses are shed in peak numbers in the late summer or early fall. However, this seasonal peak becomes much less pronounced in the semi-tropical region of the country. In recent years, the predominant enteric viruses isolated from sewage have been the vaccine-derived polioviruses and these viruses are found year-round without a dominant summer-fall peak.

The number of enteric viruses isolated from sewage samples is very much dependent upon the methodology used to recover the viruses. Concentration methods, of which there are a large variety, are generally used and the recovery efficiency may vary substantially. A few investigators have attempted to quantitate virus in sewage by direct inoculation of the unconcentrated sample into the cell culture assay system. Buras (1976) used such a technique on sewage samples from Haifa, Israel, and reported the surprisingly high number of 10^6 viral units/liter during midsummer. The weekly average for eleven consecutive months dropped to 174,000 units/liter. Fannin, et al. (1977) reported up to 440 viral units/liter in Chicago sewage with the direct inoculation procedure. EPA virologists found 1,450 viral units/liter in a sample of Cincinnati sewage; however, other samples were negative for virus by the same technique (Akin, unpublished data). Grinstein, et al. (1970) found only two of 76 one-ml samples positive for virus in Houston, Texas, sewage. Most investigators in this country have concluded that viral concentration procedures are required to quantify the virus level in sewage because of the relatively low numbers that may be isolated during a particular sampling period.

Using various concentration procedures, virus levels in sewage from U.S. urban areas have been reported up to approximately 6,000 units per liter (Vaughn, 1977a). More common, however, are recoveries ranging from near 100 to 400 virus units per liter (Safferman and Morris, 1976; Grinstein, et al., 1970; Wellings, et al., 1974). Viral recoveries from sewage in other countries (e.g. Israel, India, South Africa) have generally been higher than those reported in U.S. studies (Buras, 1976; Rao, et al., 1972; Nupen, 1970). This finding may reflect a less dilute sewage due to water conservation practices and possibly to a higher prevalence of enteric viral infections in these countries. As has been previously stated, a wide variety of virus concentration/recovery methods are used. Since this is an active area of research and experimentation, no standardized methodology is utilized. Therefore, a true comparison of the currently available sewage viral concentration data from one study area to another is not possible.

Two factors have been identified that may further influence the accuracy of the data; one factor may inflate the viral count and the other may result in a substantial underestimation of the viral number. Most quantitative virology is based on the counting of plaques produced in a cell monolayer overlaid with semi-solid medium containing a vital stain. The plaques are circumscribed areas produced by the death of a group of cells within the monolayer. Several independent investigators, including Brashear, Gerba and Riggs, have recently reported the inability to confirm a viral causation for all of the plaques that appear on some cell types when incubated with environmental samples (personal communications). Therefore, the reporting of unconfirmed plaques as viral isolates may give inflated numbers. On the other hand, it is known that viruses in the aquatic environment are normally associated with particulate matter and concentration procedures that remove particulates without prior virus elution may result in an underestimation of the number of viruses present. In many of the reports it is impossible to determine the consideration given to these two sources of error. In addition, most studies have found polioviruses to be the most frequent type of viruses isolated. The vast majority of these have vaccine-like markers and are considered to be of vaccine origin (Horstmann, et al., 1973). They are generally assumed to be non-pathogenic. However, of the 142 cases of poliomyelitis reported in this country in the last eight years, 44 have been "vaccine associated" (MMWR, 1977). This observation suggests that the vaccine virus may be pathogenic for a small susceptible portion of the population. Therefore, the maximum (or average) number of pathogenic viruses that occur in raw sewage from U.S. urban areas is not known. However, from the reports that are available from field studies and with reasonable allowances for the known variables, it would seem extremely unlikely that the total concentration of pathogenic viruses would ever exceed 10,000 virus units/liter of raw sewage and would most often contain less than 1,000 virus units/liter.

Of possibly more importance to evaluating the drinking water hazard than the amount of virus present in raw sewage are the following considerations: (1) the efficiency of sewage treatment in virus removal, (2) the portion of wastewaters that receive treatment, and finally, (3) the amount of wastewater that constitutes the drinking water sources. A study conducted in the early 1960's (currently being updated by EPA) indicated that for the 155 U.S. cities studied, municipal wastewater constituted a maximum of 18 percent of the surface water supplies for these cities (FWPCA, 1966). Within the next few years, it is anticipated that legislation and public interest will result in at least secondary treatment for practically all municipal wastewater entering a surface stream. By adding a reasonable safety factor, we may assume that, in the near future, surface water entering treatment plants will be composed of no more than 25 percent wastewater and that this will have been subjected to at least secondary treatment. Therefore, the efficiency of secondary treatment in the removal of viruses from sewage becomes of primary importance in assessing the waterborne transmission of viruses.

The term "secondary treatment" is generally applied to the treatment of primary settled sewage by one of three biologically active processes: (1) trickling filtration, (2) activated sludge digestion, or (3) oxidation pond stabilization. The viral removal/inactivation potential of each treatment has been studied at the laboratory and pilot scale level, as well as under full-scale field conditions. Meaningful viral-removal-efficiency studies can only be conducted by comparing the decrease in virus levels in the effluent with time after adding a known level of viruses before treatment. These studies should be based on actual detention times of the wastewater in the treatment system. This information is difficult to obtain with natural systems and such studies are usually done with high levels of seeded virus using theoretical detention times based on plant design and specification. Such studies have yielded highly variable viral removal efficiencies, ranging from near zero to 99 percent (Berg, 1973a). This variability may be due to: (1) the actual fluctuation in viral removal efficiency of the treatment process, (2) a failure to collect time-phased samples before, during and after the treatment process when there is substantial variation in the viral load entering the plant, and (3) the variation in efficiency of the virus recovery methods used. The addition of a disinfection step to the treatment may also yield inconsistent results. This is most likely due to the failure to add sufficient disinfectant to produce a viricidal residual for a desired contact time (Olivieri, et al., 1971). In addition, the presence of protective protein and particulate matter may physically interfere with the ability of a disinfectant to produce a virus-free effluent.

The contribution of viruses to surface water by effluents from sewage treatment plants can possibly best be determined by focusing on the number of natural viruses found in these effluents rather than on a theoretical percent removal by the treatment process. In a recent study conducted by the Sanitation Districts of Los Angeles County (California), viruses were recovered from 27 to 60 twenty-gallon samples of activated sludge effluent. The positive samples contained from 0.4 to 136 viral units/liter (Miele, 1977). Sagik and Sorber (1977a) have studied the viral content of secondary effluents from three systems in two Texas communities. Means of 5 and 179 viral units/liter were determined for two trickling filter systems and a mean of 3 viral units/liter for the oxidation pond system. Heyward (Personal communication) has recovered a mean of 165 and 269 units/liter from activated sludge and trickling filter effluents respectively in a community in Washington state.

The observations from these current studies have yielded virus levels consistent with previously reported findings from unchlorinated secondary effluents (Wellings, et al., 1974; Metcalf, et al., 1974). Therefore, it would appear from the available data that the virus level in secondary effluents in U.S. systems would not be expected to exceed 1,000 viral units/liter. If a maximum of 10,000 units/liter were present in raw sewage, as was suggested earlier in this report, then the typical viral reduction by secondary treatment may be considered to be on the order of 90

percent. Disinfection and/or tertiary treatment may reduce the virus level by one to four additional orders of magnitude. However, these additional treatment steps will most likely not be routinely applied to wastewaters that are discharged directly into surface water streams.

VIRUSES IN SURFACE WATERS AND TREATMENT PLANT INTAKES

There are no known specific viricides in fresh surface waters, and at cool temperatures, viruses have been found to survive for many days and perhaps months before their infectivity is destroyed by oxidative and other catabolic forces (Akin, et al., 1971; Herrmann, et al., 1974). To further extend the maximum viral pollution concept, we will assume that no viral inactivation occurs in wastewater from the point of discharge into a stream until it enters the intake of a downstream treatment plant. Based on data from an earlier study (FWPCA, 1966), an estimate was made that water treatment plants that use the worst polluted surface waters would have no more than 25 percent of their water composed of wastewater effluents. By using this figure, the maximum virus level at a treatment plant intake would be on the order of 250 viral units/liter (or 95,000 units/380 liters, a typical sample volume equivalent to 100 gallons). This "worst case" number is truly hypothetical in that it represents the combination of extreme conditions at each step along the way from raw sewage to a surface water treatment plant intake. The few studies that have been conducted to determine virus levels at intakes have found considerably fewer viruses than this estimated maximum number. Berg (1973b) reported the isolation of 6 and 38 viral units/380 liters at two intakes in the Midwest. Brashear (unpublished data) found two of fourteen 380-liter samples positive for virus at an intake on the Ohio River. The two positive samples yielded a total of only one and two viral units. A current EPA-funded study has reported the isolation of a maximum of 38 viral units/380 liters at an intake on the Missouri River. Viruses were isolated from 27 of 42 190-liter samples (O'Conner, et al., 1977). A preliminary joint study conducted by EPA and a contractor in December, 1975, at the same site had yielded between 9 and 86 viral units/380 liters (Akin, unpublished data). Even though these data do not represent extensive sampling, they do show the viral concentrations recovered from relatively heavily polluted surface waters. The maximum viral concentration observed was about three orders of magnitude lower than the predicted "worst case" number. More virus sampling of surface water should be conducted at plant intakes in order to better quantitate the true maximum virus challenge to the water treatment technology employed by the typical plant.

THEORETICAL CONCENTRATIONS OF VIRUSES IN TREATED DRINKING WATER

Few persons knowledgeable in public health and sanitation would deny the occurrence of waterborne transmission viral disease. Waterborne outbreaks of infectious hepatitis and gastroenteritis have been reported. Such outbreaks are reported to the USPHS, Center for Disease Control, by state or local health personnel and are formally investigated by a joint effort of CDC and EPA at the request of the appropriate state health department. Most of these outbreaks have occurred with untreated water supplies and with treated water supplies that have had obvious deficiencies in the system (Craun and McCabe, 1973; CDC, 1976). The answer to these problems lies in the application of established sanitary engineering principles using "fail-safe" systems that insure uniform and uninterrupted water treatment. Also, a vigorous effort to encourage compliance with sanitary codes designed to prevent recontamination of treated water in the distribution system could substantially reduce the number of outbreaks. An important question that remains is whether drinking waters are virologically safe from the endemic spread of infectious disease when they are derived from unprotected surface water sources that have been subjected to "good" conventional water treatment processes.

Numerous studies conducted over the past 15 to 20 years have shown that conventional water treatment can typically remove or inactivate six to eight orders of magnitude of virus (Robeck, et al., 1962; Chang, 1968; Sobsey, 1975). This overall level of reduction represents a summarization of the reductions derived from studies of the individual treatment steps using laboratory cultured virus inoculum. Applying the larger reduction figure (10^8), Sproul (1976) has calculated that the treatment of a source water containing 300 viral units/380 liters would result in a finished water that contained one infectious unit of virus per 120 million liters. If the same treatment conditions are uniformly applied to a source water containing the maximum theoretical number of 95,000 viral units/380 liters, then one infectious unit would be present in about 0.4 million liters of finished drinking water.

DISINFECTION OF DRINKING WATER

Studies conducted in the 1950's demonstrated that poliovirus and other enteroviruses were inherently much more resistant to chlorine than the coliform bacteria used to indicate the sanitary quality of drinking water (Clarke and Kabler, 1954; Clarke, et al., 1956). Investigations conducted during this period also demonstrated considerable differences in chlorine inactivation rates among various enteric virus strains and types (Kelly and Sanderson, 1958). Other observations discussed elsewhere in this report have shown their generally high survival under certain environmental conditions including sewage treatment processes and their apparently low minimum infectious dose level (about 1 viable unit). These findings have raised the level of interest and concern about the possibility of hazards posed by the viruses in drinking water. Since disinfection is considered the last line of defense against disease transmission by drinking water, much effort has been expended in assessing the efficiency of disinfection and factors which may interfere with efficient disinfection of drinking water. The main interfering factors include the inherent or native differences in viral resistance to disinfection and those conferred on viruses by their association with particulate matter.

Inherent Virus Disinfection Resistance

Liu, et al. (1971, 1973) conducted a broad investigation of the free-chlorine resistance of 25 human enterovirus types in chlorine demand-free water and in Potomac River water at a pH of 7.8. Included were three reoviruses, three adenoviruses, three polioviruses, eight coxsackieviruses, and eight echoviruses. They found that the time required for 99.99 percent inactivation at 2° C and 0.5 mg/l free residual chlorine ranged from 2.7 minutes for reovirus 1 to more than 120 minutes for coxsackie A6 virus.

More recently EPA has funded research to intensively examine the disinfection resistance of six of the 25 enteroviruses used in Liu's investigation, including two of which were very sensitive, two which were very resistant and two which were intermediate in resistance (Engelbrecht, et al., 1978). The resistance of all six of these viruses has been examined under chlorine demand-free conditions at pH 6.0 and 10.0 and three of these viruses have been studied at the pH used by Liu, et al. (1973), i.e., 7.8. The disinfection criteria and experimental conditions were similar, consisting of the time required for 99.99 percent inactivation at free chlorine levels of 0.5 mg/l.

The results of the studies conducted at pH 7.8 indicated that the time required for 99.99 percent inactivation (4.5 to 6.7 minutes) was much shorter than indicated by the results of the Liu et al. (1973) study (8.0 - 39.5 minutes). The resistance of the six enteroviruses to hypochlorous acid (HOCl) at pH 6 was found to vary by a factor of five in the time required for a 99.99 percent reduction (1.4 to 6.8 minutes). The time

required for a 99.99 percent reduction of these same viruses by the hypochlorite ion (OCl^-) at pH 10.0 ranged from 23.6-193 minutes, or 10 to 150 times that required for equivalent reduction at pH 6.0. Interestingly, the virus type (echo 1) that showed the greatest sensitivity at pH 6.0 was the one most resistant at pH 10.0. This result indicated that changing the pH had different effects on the chlorine resistance of different viruses in addition to the alteration of the chlorine species present. Further indication of this phenomenon has been shown in chlorine dioxide (ClO_2) disinfection studies. In contrast to chlorine, ClO_2 does not react with water to hydrolyze to form other compounds. The ClO_2 molecule remains intact in the same form over a wide pH range (Benarde, et al., 1965). Yet the rate at which ClO_2 inactivates poliovirus 1 increases as the pH is increased from 4.5 to 9.0. Poliovirus 1 was inactivated over three times faster at pH 9.0 as at pH 7.0 (Cronier, et al., 1977). Previously, Benarde, et al. (1965) had shown a similar effect in E. coli disinfection studies using chlorine dioxide. Thus it appears that both E. coli and poliovirus 1 are much more sensitive to ClO_2 at slightly alkaline pH's than at neutral or acid pH's. Morris (1970) pointed out the possibility that pH changes might alter the ionic charge and interfacial potential at the microbial surface, resulting in changes in sensitivity of the microorganism to germicidal action. He pointed out the need for such information in attempting to develop systematic knowledge of disinfection and disinfection mechanisms.

Scarpino, et al. (1972) obtained unusual disinfection results which may have been caused by changes of this nature. They reported a reversal in the viricidal efficiency of hypochlorous acid and hypochlorite ion. Their results showed that hypochlorite ion was seven times as effective as hypochlorous acid against poliovirus 1 although companion studies conducted with E. coli gave conventional results; hypochlorous acid was 50 times as effective as hypochlorite ion (hypochlorous acid has been well established in the literature as a much stronger disinfectant than hypochlorite ion). In discussing these results, they speculated that components of a borate buffer system used in one of their studies may have had a major effect on $HOCL \rightleftharpoons OCl^-$ equilibrium, may have suppressed ionization, or may have resulted in the formation of previously undescribed viricidal forms. Subsequent studies indicated that the presence of potassium chloride in the pH 10.0 buffer was responsible for the enhanced disinfection rate shown by hypochlorite (Scarpino, unpublished data). More recently, Engelbrecht, et al. (1978) have confirmed these results and also have shown that the presence of potassium chloride also accelerated virus inactivation by hypochlorous acid.

The results of two recent studies indicate that viruses may develop increased resistance to chlorine. Studies by Bates, et al. (1977) showed that progeny of poliovirus 1 repeatedly cycled through chlorine disinfection were somewhat more resistant to chlorine than the original parent stock virus. The resistant progeny required about 2.5 times the length of exposure required by the parent stock for inactivation to the same level.

The existence of extremely chlorine resistant polio 1 viruses in the environment has also been reported recently (Shaffer, et al., 1977). At free chlorine levels of about 0.5 mg/l and pH 7.1, these isolates showed an initial decline in number of about 90 percent in 2 minutes with virtually no decrease in titer after 30 minutes exposure. These findings are contrary to the results of many previous studies by others. Isolates of these viruses have been obtained for confirmatory studies and further investigation in two extramural research projects currently being funded by EPA.

Concern has also been expressed that the inherent disinfectant resistance of viruses grown from a human host (usually in epithelial cells in the digestive tract) may differ from that of viruses adapted to replication in laboratory cell culture systems. Use of cell-culture-grown viruses in "seeded" field studies to determine the efficacy of virus removal and inactivation by water treatment processes has also been criticized (Sproul, et al., 1969; see discussion by Shuval at the end of Sproul's article). In efforts to provide information on both of these points of controversy, EPA has recently funded a laboratory study designed to compare disinfection resistance of naturally-occurring and cell-culture-grown viruses and a field study designed to determine the efficiency of water treatment processes for removal and inactivation of naturally-occurring enteroviruses.

Aggregation or clumping of viruses has long been considered to be a factor involved in increased viral resistance to disinfection. Until recently, however, no evidence of this has been available. Through a series of EPA research grants at the University of North Carolina, evidence of this phenomenon and information on the factors involved in virus aggregation and deaggregation have been obtained (Sharp, et al., 1976; Floyd, et al., 1976; Floyd and Sharp, 1977; Young and Sharp, 1977). Although the effects are complex and appear to be different with different viruses, some general information has been developed from these studies as follows:

1. Although differences in halogen resistance of up to 300-fold between aggregated and dispersed viruses have been shown, the differences thus far shown are differences in rates of inactivation. No evidence of complete resistance by the aggregates to inactivation has been found.

2. The type and concentration of metallic cations and pH are important determinants with regard to aggregation. Aggregation does not appear to be related to virus isoelectric point characteristics.

3. Induced aggregates of some viruses are quite stable although those of other viruses can be dispersed easily. Natural virus aggregates appear to be more stable than induced aggregates.

Additional studies to improve the state of our knowledge in this area are currently in progress.

While disinfection information on a large number of enteroviruses is available, data on several viruses including infectious hepatitis A virus and gastroenteritis virus(es), each of which have been implicated in waterborne disease outbreaks, are not available because of technological problems associated with conducting such research on these particular viruses. Disinfection studies on infectious hepatitis A virus under EPA funding are now in progress.

Protective Effects of Particulate Matter

For disinfection to be effective, contact must occur between the disinfectant and the microorganism. Because source waters may contain a variety of inorganic and organic particulates, some of which may originate as fecal wastes, and because of the tendency of microorganisms, particularly viruses, to adsorb to various kinds of particles, removal of particulate matter (usually measured by light scattering) has been considered very important during water treatment. Although the major reason for removal of particulate matter (turbidity) has been the concern that it may interfere with disinfection, until recently little direct evidence of such interference had been found. The reduction of the Public Health Service standard for turbidity from 5 Nephelometric Turbidity Units (NTU) to 1 NTU specified in the National Interim Primary Drinking Water Regulations has brought the need for information in this area into sharp focus.

Previously, laboratory studies had established that enteric bacteria and enteroviruses ingested by aquatic nematodes found in some water supplies were protected against very high doses of chlorine (Chang, et al., 1960). In another instance, persistance of coliforms in a water supply system containing a substantial level of residual chlorine was attributed to ingestion and survival of the coliforms in small crustaceans present in the water (Tracy, et al., 1966). More recent studies indicate that virus adsorbed onto surfaces of particles, such as clay, remained exposed to the disinfectant and inactivation rates were affected only slightly, if at all, when compared to the inactivation rates of free virus particles (Symons and Hoff, 1975; Stagg, et al., 1977). Viruses precipitated with aluminum phosphate also showed inactivation rates similar to those of free viruses (Hoff, 1977). Boardman (1976) reported that poliovirus associated with kaolin, calcium carbonate and alum was inactivated at a slower rate than free virus particles. The effect was greatest when the particles (calcium carbonate and alum) were formed in the presence of virus.

Disinfection studies on viruses associated with organic material presents a different picture. Moffa and Smith (1974) showed that cell-associated poliovirus was more resistant to inactivation by chlorine dioxide than freely suspended virus. Other research in this area also indicates that this type of virus-particulate complex is much more resistant to inactivation than freely suspended virus (Hoff, 1977). Virus associated with cell debris could be detected after exposure to more than 1.5 mg/l of HOCl for nearly an hour. Under similar conditions, free virus

was not detectable after two minutes exposure. While it is not known how well such complexes simulate viruses as they exist in natural waters, it is likely that this model is more representative of natural conditions than a model employing freely suspended virus. Studies showing that coliforms associated with the solids in sewage effluents are very well protected from inactivation (Hoff, 1977) provide indirect evidence that viruses present in such solids also would be protected. Additional studies of this type using chlorine dioxide and ozone as disinfectants are in progress. It is significant that cell-associated viruses, while extremely well protected against inactivation, are still able to initiate infection of cell cultures (Hoff, 1977). Other recent studies have also shown that enteroviruses associated with both inorganic and organic solids remain infective for cell cultures (Moore, et al., 1974) and animals (Schaub and Sagik, 1975).

As indicated above, it is not known at present the state in which enteroviruses exist in natural waters. Data indicate that viruses intimately associated with certain types of particulates are much more likely to survive disinfection than free viruses. Additional research to further refine our knowledge in this area is needed. The data also indicate that in addition to the final barrier, disinfection, water treatment unit processes which remove particulates constitute important barriers for preventing virus passage through these water treatment processes.

TESTING OF DRINKING WATER FOR VIRUSES

Even though the development of methods for recovering viruses from water has advanced markedly during the past few years, no practical method exists for the sampling of extremely large volumes of drinking water. Farrah, et al., (1977) have speculated that the sampling of 100,000 liters of water was possible with a viral recovery procedure tested in their laboratory. However, investigators who have routinely conducted environmental virus testing have not exceeded sample volumes of 1,900 liters (500 gal). Obviously, these sample volumes are insufficient to recover viruses if the observed viral level in source waters and the predicted reductions through treatment accurately reflect the real world situation. Concern for the validity of this conclusion has been a major force in the initiation of field studies and surveys for human viruses in treated waters from relatively small sample volumes of 380 to 1,900 liters.

The EPA began studies in July, 1972, using virus concentration methods tested in its laboratories. These studies have continued to the present time and have incorporated improvements in the methodology as they have been developed. The EPA results obtained with the methods that were used prior to the development (by numerous investigators), testing (Hill, et al., 1976), and publication (APHA, 1976a) of a tentative standard method have been reported (Clarke, et al., 1975; Akin, et al., 1975). Currently, this standardized method is being used by EPA to test up to 1,900-liter samples of drinking water from sites throughout the country.

In the current EPA study, 119 drinking water samples have been collected for viral analysis from 42 communities. A listing of the sites is shown in Table 1. Also shown are the total number of samples collected at each site, the mean sample volume and the type of treatment the drinking water received. Data on the fecal coliform content of the source water are given so as to provide some indication as to the degree of domestic pollution of the raw water supply. The sampling sites fell into two major categories: (1) sites that utilized surface waters that contained significant domestic wastewater and invariably treated the water with the full conventional processes, and (2) sites that used surface waters from supposedly protected watersheds that were marginally treated, if treated at all. Arrangements were made through state health departments and the local water utilities for the sampling. A mobile laboratory was taken to each site; the sample was collected and partially processed on site before returning to the Cincinnati laboratory.

Table 1. Community Drinking Water Systems Sampled for Viruses During the Current EPA Field Study*

No.	Site	No. Collected	Mean Sample Volume (liter)	Treatment**	Fecal Coliforms in Source Water Counts per 100 ml	
					Range	Geo. Mean
1	Chester, IL	10	1851	C	180-13000	1429
2	Indianapolis, IN	16	1832	C	1-2000	90
3	Cape Giardeau, MO	8	1522	C	430-8000	1345
4	Glasgow, MO	10	1885	C	400-5100	1154
5	Kansas City, MO	3	1893	C	240-490	343
6	Lexington, MO	12	1904	C	2300-16000	5980
7	St. Joseph, MO	4	1893	C	670-2600	1434
8	Hueston Woods, OH	1	984	C	13	13
9	Rocky Fork, OH	1	1249	D	240	240
10	Stonelick, OH	1	1893	N	ND	ND
11	Altoona (Blair Gap), PA	1	1094	D	60	60
12	Bradford, PA	1	307	D	2	2
13	Central City, PA	1	613	D	2	2
14	Curwensville, PA	1	1325	D	0	0
15	Derry, PA	1	606	D	6	6
16	Emporium, PA	1	257	D	0	0
17	Johnstown, PA	1	678	D	4	4
18	Lock Haven, PA	1	632	D	1	1
19	Nanty Glo, PA	1	428	D	5	5
20	Reynoldsville, PA	1	363	D	25	25
21	Sheffield, PA	1	212	D	28	28
22	Shomokin, PA	1	379	D	ND	ND
23	Unionville, PA	1	1893	D	3	3
24	Williamsport, PA	1	424	D	6	6
25	Wilpen, PA	1	602	D	32	32
26	Alburg, VT	1	1718	D	9	9
27	Barre, VT	1	791	D	11	11
28	Coventry, VT	1	1893	N	0	0
29	Island Pond, VT	1	1893	D	0	0
30	Montgomery Center, VT	1	1893	D	26	26
31	Montpelier, VT	1	1211	D	100	100
32	Orleans, VT	1	1893	D	19	19
33	Rutland, VT	1	844	D	8	8
34	Troy, VT	1	1893	N	4	4
35	Fairfax, VA	8	379	C	0-5	1

No.	Site	No. Collected	Mean Sample Volume (liter)	Treatment**	Fecal Coliforms in Source Water Counts per 100 ml Range	Geo. Mean
36	Cedar Grove, WV	4	954	C	290-870	522
37	Clarksburg, WV	2	1578	C	0-48	7
38	Fayetteville, WV	2	1730	C	62-160	99
39	Keyser, WV	1	939	C	19	19
40	Milton, WV	2	1578	C	63-690	209
41	Ripley, WV	5	1673	C	170-3400	409
42	Teas Valley, WV	5	1893	C	0-4800	37

* No viruses were isolated from any of these samples

** C = Conventional treatment (coagulation/sedimentation, filtration and disinfection; except at Hueston Woods, Ohio, which used slow sand filtration and disinfection)

D = Disinfection only

N = No treatment

ND = Not Done

The viral recovery procedure used was essentially that of the tentative standard method (APHA, 1976a) and is outlined in Figure 1. As can be seen in Table 1, the 1,900-liter sample volume was not always achieved due to the plugging of the viral adsorbents by the particulates present in the water. In order to evaluate the recovery efficiency of the method with samples from various water supplies, an extremely low level of poliovirus vaccine was seeded periodically into a water sample and the concentrate assayed in cell cultures by the plaque technique. The results of the positive-control tests are shown in Table 2. The recoveries ranged from 0 to 70 percent and reflected the limitations of the method. Ill-defined changes in water quality that interfere with viral adsorption and elution are thought to be responsible for the low and variable recoveries experienced within systems as well as between systems (Sobsey, 1978).

The field studies conducted by EPA comprise the most extensive sampling of drinking water supplies that has been reported. In addition to these studies, a joint EPA-grantee longitudinal study is currently examining 1,900 liters of finished water per week from a plant that utilizes the Missouri River as its source water. The incoming water is highly polluted with domestic waste, as indicated by the fecal coliform densities and the isolation of human enteric viruses (O'Conner, et al., 1977). The Carborundum Company, through its leasing of a viral sampling service, has also claimed wide experience in the testing of various types of waters including finished waters (Shaffer, et al., 1977). However, their specific findings are considered confidential by the lessee and are not reported in detail in the literature. Some yet unpublished studies are also being conducted by the Epidemiology Research Center of the State of Florida (EPA funded), the Fairfax County, Virginia, Water Authority and the University of Maryland.

Figure 1. Recovery Procedure Used to Isolate Viruses From Drinking Water Samples

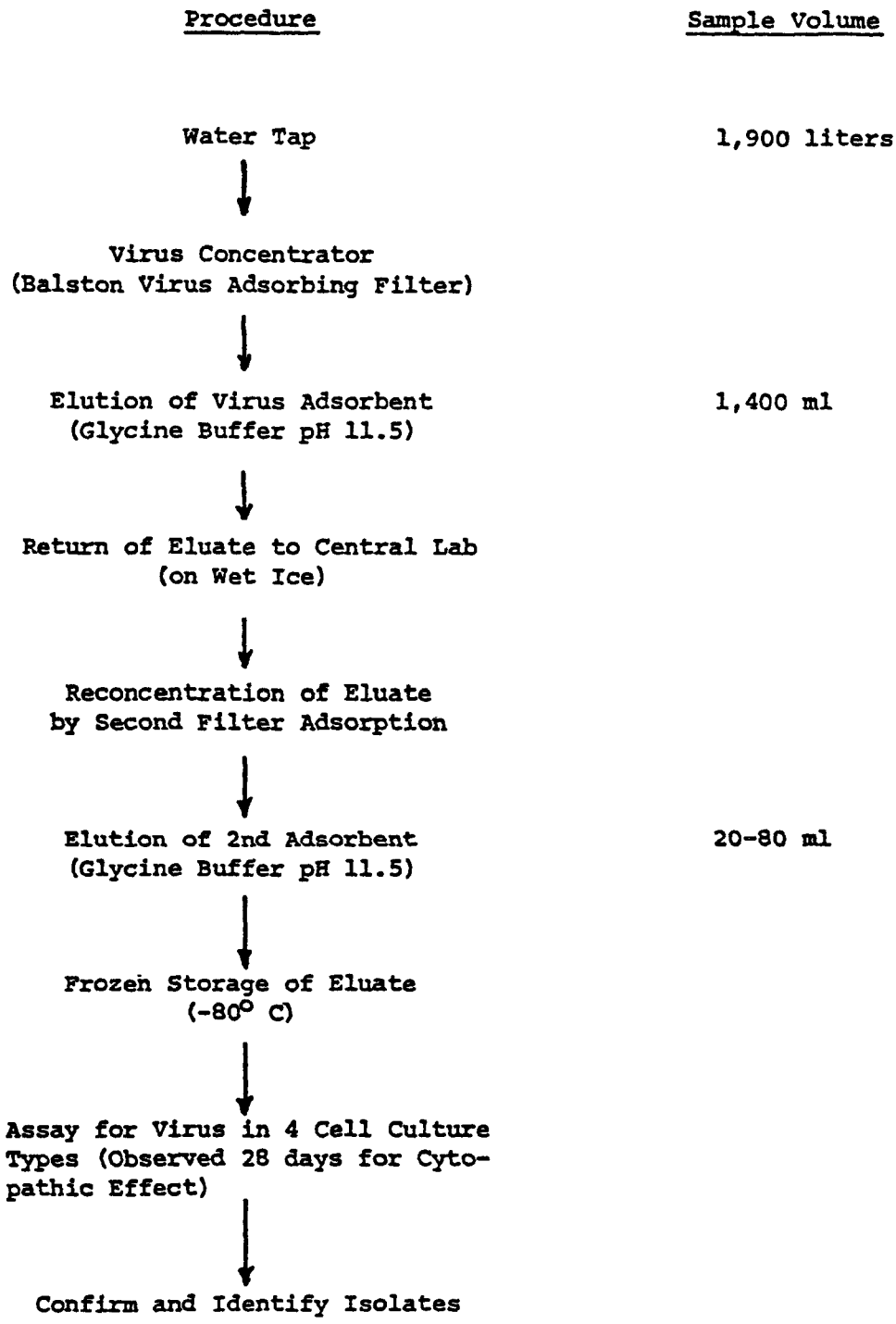

Table 2. Results of Positive-Control Seed-Study with Poliovirus Vaccine and Treated Drinking Water from Six Communities

SITE	SAMPLE VOLUME (Liters)	TOTAL VIRUS INPUT (PFU)	VIRUS RECOVERED PFU	VIRUS RECOVERED %
Indianapolis, IN	1,900	37	3	8
Indianapolis, IN	1,900	78	0	0
Indianapolis, IN	1,900	43	1	2
Indianapolis, IN	1,900	41	4	10
Indianapolis, IN	1,900	61	1	2
Lexington, MO	1,900	20	5	25
Lexington, MO	1,900	10	7	70
Glasgow, MO	1,817	82	6	8
Chester, IL	1,900	75	25	33
Ripley, WV	1,900	106	3	3
Cedar Grove, WV	1,518	106	0	0

PFU = plaque forming unit

RESULTS OF VIRAL TESTING OF DRINKING WATER SYSTEMS

Since 1972, EPA has collected 255 drinking water samples from water supply systems in 56 communities. An additional 50 samples have been collected by an EPA-grantee in the ongoing longitudinal study. No confirmed virus isolations have been made from any of these samples and the Agency is aware of only two studies in which viral isolations from a treated water supply have been claimed. An echovirus was reported to have been isolated from a chlorinated supply in Dade County, Florida (Wellings, et al., 1975). In an EPA-funded survey, poliovirus was reported to have been isolated from four water samples collected in the summer of 1975 from a water supply in Fairfax, Virginia, that utilized full conventional treatment (Hoehn, et al., 1977). The latter finding created considerable concern because the source water quality and the extent of treatment applied would have suggested the great improbability of virus recovery in the volumes of water tested. The authenticity of the isolations, however, has been questioned (Akin and Jakubowski, 1977), but no obvious source of sample contamination was evident. A subsequent test of the same water supply a year later was conducted by EPA (in-house) and the laboratory that reported the positive finding (Carborundum Company). Eight 380-liter finished water samples were concurrently collected by each laboratory during July, 1976. The concentrated samples were split into equal volumes and half of each sample was assayed for viruses by each laboratory. An additional 34 samples were collected between June and September, 1976, and assayed only by the Carborundum laboratory.

A single viral isolate (identified as poliovirus 1) was made from a sample collected on September 2nd by the Carborundom team and assayed in their laboratory (Hoehn and Randall, 1977). No other viruses were isolated from the 50 380-liter finished water samples. However, six units of poliovirus 1 were isolated from a rectal swab taken from a Carborundum field-team member on the day that the positive water sample was collected. The periodic collection of rectal specimens from all personnel directly involved with the study had been initiated during the 1976 sampling to identify possible viral contamination sources. The possibility that the water sample could have been contaminated by the worker cannot be ruled out; however, no direct contact or mechanism of transmission was evident.

The previously mentioned findings raised serious questions as to the differences in viral recovery sensitivity of methodologies used by the two laboratories and to the environmental quality controls of the Carborundum procedures. Therefore, an intensive double-blind seeded study involving both laboratories and sponsored by the EPA Office of Drinking Water was planned and is currently underway.

VIRUSES IN GROUND WATER

Once considered a reliable source of microbiologically-safe drinking water that did not require treatment, the purity of ground water is now being questioned. The rapidly moving trend to the land application of domestic wastewaters has made the penetration of viruses through soil into aquifers a distinct health concern. Studies have shown that viruses can be isolated from ground waters when domestic waste is applied to or released into certain types of overlying soil (Mack, et al., 1972; Wellings, et al., 1972; Vaughn and Landry, 1977). In an effort to better quantitate and define this potential hazard, EPA has funded studies in this area. Vaughn (1977b) has shown that viruses in a tertiary-treated effluent can penetrate 5.3 meters of a sandy Long Island, New York, soil from a recharge basin and can be isolated from the underlying ground water. A seeded study conducted with high concentrations of poliovirus introduced into the recharge basin has shown that the virus can penetrate the soil and enter the upper aquifer (7.6 meters below the surface) within 24 hours. Studies by Sagik and Sorber (1977b) in Texas have shown that the spray irrigation of secondary-treated wastewater may transmit viruses at least through 1.4 meters of clay soil. Gilbert, et al. (1976) conducted similar studies at a recharge site in Arizona and were unable to recover viruses from about 50 water samples collected 6 to 9 meters below the surface at a seven-year-old recharge site. The findings of these two studies indicate that the virus-soil interactions are complex and many variables may be involved in the viral contamination of ground water from wastewater recharge sites.

RISK OF VIRAL EXPOSURE FROM DRINKING WATER

Assumptions have been made in this report that suggest that one tissue-culture-infectious unit (TCIU)/0.4 million liters would be the maximum number of viruses that most likely could be transmitted through drinking water under currently accepted treatment and sanitary standards. At this concentration, the residents of a typical city of 100,000 population would be exposed to about 110 TCIU of viruses each day in the water used for domestic purposes based on U.S. average community use of 640 liters/person/day and 69 percent residential use (Murray and Reeves, 1977). Only a portion of the total volume used would be ingested in an "uncooked" condition whereby the virus could remain in an infectious form. Based on information compiled by Lippy (unpublished data) it is estimated that an "average" person drinks about one liter of tap water/day. At this consumption rate and under the conditions outlined above, one TCIU of virus would be ingested with water by a single individual in the typical city about every four days. The number of cases of clinical disease that would result from the infections produced by this level of ingestion is unknown, but would most likely be several orders of magnitude lower than the ingestion frequency. Factors important in the manifestation of clinical disease from viral ingestion include: (1) the immune state of the consumer as a result of previous exposure to the virus, (2) the number of viral particles required to produce infection, and (3) the virulence of the virus (i.e., the disease:infection ratio).

The hypothetical level of exposure to viruses in drinking water would appear to be low. However, this assessment has not been based on rigorous data in many areas. Even though the model used the maximum detected virus levels and allowed the virus environmental survival advantages (to approximate a "worst case" situation), the actual numbers of viruses present most assuredly are underestimated due to the inefficiency of available virus recovery methodology. We experienced a rather low mean recovery of 15 percent from our positive controls using poliovirus (Table 2). Most method-development studies have been conducted with laboratory-cultured vaccine poliovirus because it is a hardy virus that is safely and easily enumerated in cell culture. Very little recovery-efficiency data are available for the approximately 100 enteric viruses remaining to be studied. However, limitations in the concentration procedure exist with some of the adenoviruses, and limitations in cell culture assay systems for recovering some of the coxsackie A viruses, the hepatitis A virus and the gastroenteritis agents have long been recognized. The efficiency of the entire recovery system for the complete spectrum of the wild enteric viruses in the state that they exist in nature is not known. Therefore, it seems quite possible that no more than one percent of the total number of viral units present in environmental samples is actually enumerated with currently available methods. Also, the viral removal efficiencies by sewage and water treatment processes are influenced by meterological conditions and are subject to human, equipment and process failure and, therefore, optimum treatment conditions are not always maintained. The awareness of these limitations coupled with the unexpected and as yet

unexplained finding of poliovirus in relatively small volumes of treated finished water in Virginia (Hoehn, et al., 1977) warn against the drawing of premature conclusions as to the magnitude of the risk.

Basic to determining the true risk of infection from ingested viruses is an understanding of the minimum number of TCIU required to produce infection in a susceptible person. A widely quoted study has shown that only one TCIU could be required (Katz and Plotkin, 1967). However, this study was conducted in premature infants who were exposed to poliovaccine virus via gavage and may not reflect the actual risk in nature. More extensive studies are currently being funded by EPA. Investigators at the University of Wisconsin are using a wild porcine enterovirus and piglets to study the minimum infectious and pathogenic dose of viruses in this animal model system. It has been assumed that a low dose of wild virus may result in asymptomatic infection in a susceptible host, whereas, a larger dose may result in overt disease. However, such a dose-response relationship has not been quantitated.

Other investigators at the University of Wisconsin are conducting a study in humans under DHEW safeguards established for the protection of human subjects. The study involves the feeding of low doses of oral (Sabin) poliovaccine to 3- to 6-month old infants in order to determine the minimum number of particles that must be ingested to produce an immunologic response with the protective vaccine. The full dose of vaccine is given 10 days later so that infants not responding to the low dose will be protected against any subsequent natural exposure to the wild pathogenic poliovir-uses. These virus feeding studies will hopefully provide definitive evidence as to the likelihood of producing an immunologic response by the natural ingestion of extremely low numbers of waterborne virus particles.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

The existence of an appreciable health risk from the waterborne transmission of enteric viruses through drinking waters in this country is not apparent. This may be because the risk is truly minimal or it may only reflect the difficulty in documenting the endemic spread of low levels of infectious viruses through water supply systems. The finding of viruses at intakes to water treatment plants with rather inefficient virus recovery methods coupled with the likelihood that water treatment processes are not uniformly applied and are not as efficient in removing naturally-occurring viruses as has been indicated with seeded studies suggest that the latter may be true. Many questions that bear directly on the subject remain unanswered. It has long been hypothesized that low levels of viruses are transmitted through drinking water and thereby give rise to sporadic asymptomatic infections in those people ingesting this water. These cases in turn give rise to person-to-person spread of large numbers of viruses that result in cases of overt disease. An adequate approach for testing this hypothesis has not been devised.

Virus recovery methods have been improved but remain relatively inefficient in recovering all viruses from wastewaters and natural waters (Sobsey, 1978; Williams and Jakubowski, 1978). Reported recoveries with laboratory-cultured seeded viruses typically range from 0 to 70 percent. In addition, some investigators believe that a major portion of the natural viral population is firmly attached to or deeply embedded in particulates and is not recovered at all by currently available methods. This methodology question must continue to be addressed. The embedded particles may be protected from water disinfection. Therefore, laboratory studies with seeded viruses that do not represent the physical state of natural viruses may yield a false viricidal efficiency of such disinfectants. In addition, recent findings indicate that some viruses may not be removed by adsorption-sedimentation-treatment procedures nearly as readily as the standard testing virus, poliovirus 1 (Farrah, et al., 1978):

The viral agents of infectious hepatitis and acute gastroenteritis (diseases that can be spread by water) cannot be recovered from contaminated water due to lack of laboratory culture systems. This may also be true of other viruses. Therefore, significantly more viruses may be present in all types of surface waters than have been reported. Ground water may also be contaminated with viruses; factors important in the prevention of viral penetration through soil into the aquifer at wastewater recharge sites must be defined. More basic studies that deal with the molecular and the physico-chemical levels of virus interactions need to be conducted so as to better understand the mechanism of viral attachment and adsorption to particulates as well as the viral-inactivating factors present in soils and the aquatic environment. The minimum infectious dose of enteric viruses when ingested with food and water has not been satisfactorily evaluated and, therefore, needs further work.

Two recent reports have recommended areas that need further research (APHA, 1976b; NAS, 1977). Based upon the information presently available, it is recommended that research should be initiated or continued in nine areas in order to more fully evaluate the possible health risk from viruses which might be in drinking water. These recommendations are:

1. The development of improved recovery methods with emphasis on increased sensitivity for the total number and types of viruses that may be present in water and wastewaters.

2. The further evaluation of the disinfection capability of chlorine and other disinfectants on natural viruses under field conditions and new viruses implicated in waterborne disease outbreaks (e.g., gastroenteritis virus).

3. The development of practical methods to remove/inactivate all detectable viruses from treated sewage and sludge.

4. The further evaluation of the viral contamination of ground water as one of the factors to be considered in the land application of wastewater and sludge.

5. The development of a broader data base for estimating the minimum infective dose for ingested viruses.

6. The development of methods for the laboratory cultivation of hepatitis A virus and agents of acute oral gastroenteritis.

7. The evaluation of the role of lower animals as reservoirs of viruses that may infect humans.

8. The development of epidemiological approaches to determine the extent of endemic waterborne viral transmissions.

9. The elucidation of specific factors and mechanisms responsible for viral inactivation and destruction in natural waters and soils.

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APPENDIX I

Virus-in-Water Related Research Projects Funded by the Environmental Research Center-Cincinnati During FY 77 & 78

Title	Activity Type	Supporting Lab*
Occurrence of Viruses in Drinking Water Supplies.	In-house	HERL
Evaluate Methods for Concentrating and Recovering Viruses from Drinking Water.	In-house	HERL
Improve Methods for Isolation, Identification and Recovery of Human and Other Animal Viruses from Surface Waters.	In-house	HERL
Increase Isolation Sensitivity of In Vitro Systems for Detecting Enteric Viruses.	In-house	HERL
Evaluate Tentative Standard Method for Selected Enteric Viruses.	Grant	HERL
Identification, Isolation and Characterization Hepatitis A Agent.	Grant	HERL
Isolate, Cultivate, Characterize Etiologic Agent(s) of Non-Bacterial Gastroenteritis (Norwalk, Hawaii Agents).	Grant	HERL
Determine Minimal Oral Infectious Dose and Oral Pathogenic Dose of Enteroviruses in a Natural Animal Host.	Grant	HERL
Human Health Hazards of Viruses in Drinking and Recreational Waters.	Grant	HERL
Longitudinal Study of Viruses and Coliforms in Raw and Treated Water.	Grant	HERL
Factors Affecting the Adsorption, Transport and Infectivity of Animal Virus in Soil-Water Systems.	Grant	HERL
Investigation of Potential Virus Survival and Movement at a Land Reclamation Site Utilizing Sewage Sludge.	Contract	HERL
Fate of Human Viruses in Groundwater Recharge Systems.	Grant	HERL
Human Enteric Virus Survival in Soil Following Irrigation with Sewage Plant Effluents.	Grant	HERL

Title	Activity Type	Supporting Lab*
Effect of Virus Particle Aggregation on the Disinfection of Water Supplies.	Grant	MERL
Virus Sensitivity to Chlorine Disinfection of Water Supplies.	Grant	MERL
Inactivation of Naturally Occurring Entero-viruses.	Grant	MERL
Effect of Particulates on Disinfection of Enteroviruses in Water by Chlorine Dioxide.	Grant	MERL
Effect of Particulates on Ozone Disinfection of Bacteria and Viruses in Water.	Grant	MERL
Removal of Virus from Public Water Supplies.	Grant	MERL
Effect of Turbidity on Disinfection by Chlorine.	In-house	MERL
Methodology for Concentration, Recovery, and Identification of Viruses from Ambient Waters and Wastewaters.	In-house	EMSL
Development of Methods for Quantitation of Adsorbed Viruses in Waste and Other Water.	Grant	EMSL
Quantitative Methods for Virus in Water.	Grant	EMSL
Identification and Detection of Water-borne Viruses by Immunoenzymatic Methods.	Grant	EMSL
Development of Field Virus Concentration Technology.	Grant	EMSL
Development of Methods for the Detection and Inactivation of Viruses in Various Waters.	Grant	EMSL

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* HERL - Health Effects Research Laboratory

MERL - Municipal Environmental Research Laboratory

EMSL - Environmental Monitoring & Support Laboratory

APPENDIX II

GLOSSARY

Asymptomatic - Presenting no subjective evidence of disease.

Double Blind Experiment - As used in this report, a study in which EPA (Cincinnati) and the Carborundum Company exchanged and analyzed viral samples, the virus identity and concentrations of which were only known to a third party.

Elution - The removal of virus from material by washing with a liquid.

Enterovirus - A virus that infects cells of the intestinal tract.

Etiology - The causal relationship between a virus and the specific disease.

Gavage - The introduction of material into the stomach by a tube.

Infectious Units - Either a single virus particle or a stable viral clump that is infectious for a living host system.

Longitudinal Study - Research occurring over a period of time.

Plaques - Small, clear, circular areas on a lawn of growing cells (monolayer) which result from the virus-induced deaths of groups of cells. The number of plaques indicates the concentration of virus particles.

Progeny - Viral descendents.

Recharge Basin - An underground basin in which water is deliberately added to restore water capacity.

Seeded Virus Study - An experiment in which a known concentration of virus is added to some medium such as water.

Titer - The concentration of viruses in a given volume of liquid.

Vaccine-like Markers - As used in this report, proteins on the coat of a strain of poliovirus which react with various types of antibodies in a manner similar to those of the specific poliovirus used in the preparation of live (Sabin) vaccines. The markers could also pertain to growth at a specific temperature.

Wild Viruses - Viruses recovered from the environment or from an infected individual.