

# Human viruses in water, wastewater and soil

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Report of a  
WHO Scientific Group

Technical Report Series

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International Reference Centre  
for Community Water Supply



World Health Organization

241.1-79H4

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World Health Organization, Geneva 1979

ISBN 92 4 120639 X

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PRINTED IN SWITZERLAND

79/4491 — 8000 — La Concorde

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**WHO SCIENTIFIC GROUP ON HUMAN VIRUSES IN WATER,  
WASTEWATER AND SOIL**

*Geneva, 23-27 October 1978*

*Members \**

- Dr G. A. Bagdasarjan, Chief, Laboratory of Sanitary Virology, A.N. Sysin Institute of General and Municipal Hygiene, Moscow, USSR
- Dr G. Berg, Biological Methods Branch, Environmental Protection Agency, Cincinnati, OH, USA
- Dr Hou Yunte, Assistant Fellow, Institute of Virology, Chinese Academy of Medical Sciences, Beijing (Peking), China
- Dr Kuo Ren, Associate Fellow, Institute of Medical Biology, Chinese Academy of Medical Sciences, Kunming Shi, China
- Dr E. Lund, Department of Veterinary Virology and Immunology, Royal Veterinary and Agricultural University of Copenhagen, Denmark
- Dr J. L. Melnick, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, TX, USA (*Chairman*)
- Dr V. Chalapati Rao, Virologist, National Environmental Engineering Research Institute, Nagpur, India (*Vice-Chairman*)
- Professor H. I. Shuval, Environmental Health Laboratory, The Hebrew University-Hadassah Medical School, Jerusalem, Israel
- Dr J. S. Slade, Virologist, Thames Water Authority, London, England (*Rapporteur*)
- Dr Sng Ewe Hui, Department of Pathology, Ministry of Health, Singapore

*Secretariat*

- Dr P. Brès, Chief Medical Officer, Virus Diseases, WHO, Geneva, Switzerland (*Secretary*)

---

\* Unable to attend: Dr S. G. Drozdov, Director, Institute of Poliomyelitis and Virus Encephalitides, Moscow, USSR.

# **HUMAN VIRUSES IN WATER, WASTEWATER AND SOIL**

## **REPORT OF A WHO SCIENTIFIC GROUP**

A WHO Scientific Group on Human Viruses in Water, Wastewater and Soil met in Geneva from 23 to 27 October 1978. Dr P. Brès opened the meeting on behalf of the Director-General. Dr J. L. Melnick was elected Chairman, Dr V. C. Rao Vice-Chairman, and Dr J. S. Slade Rapporteur.

### **1. INTRODUCTION**

Increasing attention is being paid to the contamination of water and soil by viruses. This problem, as a factor in the spread of viral diseases, has far-reaching implications which have yet to be fully appreciated by the medical and public health professions.

Most of the rivers that serve as sources of drinking-water carry varying amounts of wastewater, which sometimes reach a proportion of 50% and more during periods of low flow. Extremely rapid urbanization in developing countries has raised critical problems of water supply and waste disposal. In many parts of both the developing and the developed world increasing demands on available water resources due to the growth of the world's population and the concurrent expansion of industrial needs make the recycling of domestic wastewater inevitable.

Extensive practical knowledge of the monitoring and treatment of bacterial contamination of waters is available, but there is only limited experience with regard to viral contamination. Present water treatment procedures may not always be sufficient to prevent viruses from reaching community water supplies. One major problem is the development of adequate methods to ensure that viruses pathogenic to man are eliminated from heavily contaminated and reclaimed waters.

Thirty years have passed since the first studies on the presence of human enteric viruses in water were begun in earnest, but the public health significance of such contamination has yet to be evaluated

(1, 2). This has been due in part to the lack of suitable methods for the detection of enteric viruses. Studies have shown that these viruses easily survive present sewage treatment methods and many can persist for several months in natural waters. Few reports of viral contamination of water in developing areas have been published, but one can assume that where sanitation is less advanced contamination of water is common and viruses must be abundantly present.

WHO, through its Environmental Health Programme, has already given attention to water pollution control in developing countries (3), the reuse of effluents (4) and the disposal of community wastewater (5). In addition, the Organization is concerned with the public health significance of viral contamination of food—e.g., shellfish (6, 6a). The Proceedings of the International Conference on Viruses in Water, held in Mexico City in 1974 under the auspices of the American Public Health Association and the Pan American Health Organization, provided a most useful comprehensive review of the situation (7). In 1975 the WHO Regional Office for Europe convened a Working Group on Bacteriological and Virological Examination of Water, which resulted in the compilation of a manual of procedures—shortly to be published (8)—that will serve virologists and public health laboratory personnel who have to deal with the problem.

The present report makes an assessment of the public health importance of viruses in water, wastewater and soil, and of the nature of risks for exposed persons; it refers to the methods available for monitoring viruses in different situations, and identifies priority areas for further research. The growing importance of these problems over the next decade or two should be of interest to all those responsible for public health and economic planning, whether in the developing or the developed countries.

## 2. HUMAN VIRUSES IN POLLUTED WATER

More than 100 different virus types are known to be excreted in human faeces (Table 1). More than 1 000 000 infectious virus particles may be excreted per gram of faeces by infected persons, regardless of whether or not they manifest illness. Concentrations as high as 100 000 infectious virus particles per litre have been detected in raw sewage. These viruses may survive for several months in wastewater, tapwater, soil and shellfish. Furthermore, they may resist conven-



Table 1. Human enteric viruses that may be present in water

Virus group	No. of types	Disease caused
Enteroviruses:		
Poliovirus	3	Paralysis, meningitis, fever
Echovirus	34	Meningitis, respiratory disease, rash, diarrhoea, fever
Coxsackievirus A	24	Herpangina, respiratory disease, meningitis, fever
Coxsackievirus B	6	Myocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory disease, pleurodynia
New enteroviruses	4	Meningitis, encephalitis, respiratory disease, acute haemorrhagic conjunctivitis, fever
Hepatitis type A (probably an enterovirus)	1	Infectious hepatitis
Gastroenteritis virus (Norwalk type agents)	2	Epidemic vomiting and diarrhoea, fever
Rotavirus (Reoviridae family)	?	Epidemic vomiting and diarrhoea, chiefly of children
Reovirus	3	Not clearly established
Adenovirus	> 30	Respiratory disease, eye infections
Parvovirus (adeno-associated virus)	3	Associated with respiratory disease in children, but etiology not clearly established

Note: Other viruses which, because of their stability, might contaminate water are the following:

(1) SV40-like papovaviruses, which appear in the urine. The JC subtype is associated with progressive multifocal leukoencephalopathy.

(2) Creutzfeld-Jakob (C-J) disease virus. Like scrapie virus, the C-J virus resists heat and formaldehyde. It causes a spongiform encephalopathy, characterized by severe progressive dementia and ataxia.

tional water and wastewater treatment procedures, including chlorination, and may be found far from the original source of contamination.

## 2.1 Enteroviruses

Polioviruses, group A and B coxsackieviruses, and echoviruses, are different species of the genus *Enterovirus* (family, Picornaviridae). The term "enteric virus"—an epidemiological concept—is applied to any viruses disseminated by the faecal route. They multiply primarily in the alimentary tract and are excreted in substantial amounts in the faeces for varying periods of time, with a mean shedding period of up to 50 days. The best studied of these enteroviruses are the polioviruses

and the least studied are the many serotypes of group A coxsackieviruses, which are usually isolated in suckling mice. Numerous studies have readily demonstrated the presence of enteroviruses in sewage, in effluents from sewage treatment plants and in contaminated streams. Although shellfish have not been formally implicated to date in the transmission of enteroviruses, clams and oysters grown in contaminated water do acquire and harbour enteric viruses such as hepatitis A virus. These viruses can persist in raw or insufficiently cooked shellfish.

Polioviruses can cause serious nervous system disease. Actually clinically manifest disease occurs only in between 1 in 100 and 1 in a little over 1000 cases of infection, depending chiefly on the virulence of the virus and the age of the host. At present in countries in which live poliovirus vaccine is widely used the excreted polioviruses are usually vaccine-derived and their pathogenicity is low, although reversion to neurovirulence during human passage may occur. The other enteroviruses can also cause nervous system disease, usually of a transient nature (aseptic meningitis) but on occasion clinically similar to typical paralytic poliomyelitis. Some enteroviruses, such as enterovirus type 71, have given rise to large outbreaks of central nervous system disease characterized by encephalitis, or paralysis, or both, with many fatalities. Group B coxsackieviruses also have the potential for causing significant types of disease. These include epidemic pleurodynia (Bornholm disease), pericarditis (chiefly in older persons), serious and often fatal myocarditis in infants, and congenital defects (chiefly cardiac) in infants born of mothers experiencing infection during pregnancy. In addition, family studies suggest that mild respiratory disease may be the result of certain enterovirus infections. In summary, enteroviruses can cause serious disease, but fortunately, under most circumstances, do so in only a very small proportion of infections.

This infrequent association with severe disease may well help to explain why reports of the waterborne spread of enteroviruses have been so few. A very important principle, best exemplified by the polioviruses, is that the severity of the outcome of infection in a nonimmune host is directly related to host age. In developing countries, in which wild polioviruses are prevalent, infections are typically acquired very early in life, when the risk of serious disease is lowest. Most older children and adults are thus immune. In these areas waterborne spread undoubtedly occurs and may be a significant factor in the process of natural immunization. As sanitation

has improved in some of these areas, paralytic poliomyelitis has increased, presumably because infections are delayed to an older age, but virus spread is not totally prevented. A similar trend should evolve (perhaps is already evolving) with respect to disease caused by the other enteroviruses. However, the populations most vulnerable to such diseases are those of developed countries. Data on the prevalence of immunity to the nonpoliomyelitis enteroviruses are fragmentary, but several studies suggest that urban populations are frequently exposed to these agents.

## **2.2 Hepatitis A virus**

The agent of type A viral hepatitis has recently been characterized and may soon be classified as belonging to the genus *Enterovirus*. This virus is excreted in faeces over a relatively extended period and, on the basis of many well-studied outbreaks, the conclusion has been reached that it is often spread via water. Further, the large 1955–1956 outbreak in Delhi, India, caused by gross sewage contamination of the water supply, provided fortuitous evidence of an important characteristic of the responsible agent—namely, its ability to withstand levels of residual chlorine (greatly raised to combat the emergency) which apparently were adequate to kill most of the other enteric pathogens that must also have been present. Numerous other waterborne outbreaks of viral hepatitis A have been reported (9).

## **2.3 Gastroenteritis viruses of the Norwalk type**

These viruses have recently been recognized and show a strong resemblance to the enteroviruses, with which they may soon be classified. There appear to be at least two different serotypes in this group. These viruses have been identified as the cause of outbreaks but laboratory methods for their isolation are not yet available.

## **2.4 Reoviruses and rotaviruses**

Reoviruses have often been recovered from contaminated surface waters. Although highly infectious, little is known about their ability to cause disease. Rotaviruses, a recently discovered group of the Reoviridae, have been found to be the major pathogen of nonbacterial infantile diarrhoea throughout the world. The virus has been detected in approximately 40% of infants with diarrhoea. During the

peak of winter outbreaks in temperate climates, the virus has been observed in specimens from 80–90% of infants and young children hospitalized with diarrhoea. Large numbers of particles ( $10^9$  per gram of faeces) may be excreted by infected individuals. However, assay methods for detecting human rotaviruses in water are not yet available.

## **2.5 Adenoviruses**

Commonly thought of as respiratory viruses, adenoviruses almost invariably infect the alimentary tract and are abundantly shed in faeces. While infections restricted to the alimentary tract cause only mild symptoms, or none at all, overt disease commonly results when other sites, respiratory and conjunctival, are also infected. Faecal shedding of adenoviruses is extremely common among young children.

Although efforts to detect viruses in sewage-polluted water have commonly employed methods selected for the detection of enteroviruses rather than of adenoviruses, the latter have been discovered in a number of such studies. However, the only well-documented instances of the waterborne spread of adenoviruses have been the epidemics of pharyngoconjunctival fever associated with swimming-pools.

## **2.6 Parvoviruses**

The adeno-associated viruses (AAV) were the first parvoviruses of human origin to be recognized. The available evidence suggests that these viruses are very stable agents. AAV, together with adenoviruses, have been recovered from faeces, and hence are almost certainly present in contaminated water. However, data on the frequency of excretion are lacking. Seroepidemiological studies indicate that antibodies to AAV, especially types 2 and 3, are widely prevalent in young children and that infection may be associated with childhood respiratory disease. There is as yet no adequate evaluation of the impact of AAV on human health.

# **3. THE MINIMUM INFECTIVE DOSE OF INGESTED VIRUSES**

To assess the hazards caused by viruses present in water and soil it is important to consider the minimum infective dose for man when

these viruses are ingested. Obviously, a number of factors as well as the actual concentration of virus determine whether a particular human host will become infected. These factors include the type of virus involved, the route of penetration, and the susceptibility of the host.

Reported data show that for a range of viruses of human origin, including the enteroviruses, doses as low as a single infectious unit are capable of inducing infection in man (10-16). Circumstances in which a common source of an outbreak is not recognized may occur more frequently than is ordinarily thought. The following hypothetical example of the consequences of a low concentration of viruses in a drinking-water supply may illustrate this eventuality. In a city of 1 000 000 population consuming water treated conventionally but insufficiently to remove all viruses, the expected concentration of virus might be 1 infectious unit per 20 litres of drinking-water; this situation could give rise to the following circumstances. Assuming each person drinks about 1 litre of water daily, then each day an average of 50 000 persons would ingest at least 1 infectious virus particle in their water. Conservatively, because of immunity and other host resistance factors, one can assume that only 1% of those exposed would become infected—i.e., 500 persons per day, or 182 500 ( $500 \times 365$ ) persons per year. Assuming that only 1 in 50 persons infected would become ill, 3650 persons would have obvious clinical disease per year, characterized by a broad range of symptoms caused by the enteric viruses (see Table 1). In addition to this burden of illness, the 182 500 persons could act as carriers who in turn might infect their contacts.

On the basis of these considerations, the Scientific Group concluded that the presence of even a few enteric viruses in a large volume of drinking-water should be prevented, since treatment measures exist to achieve this goal and detection techniques are becoming available which can provide the required level of monitoring.

#### 4. LIMITS OF EPIDEMIOLOGICAL INVESTIGATIONS

Current epidemiological techniques are not sufficiently sensitive to detect low-level transmission of viral diseases through water, for two main reasons:

(1) Most enteric viruses cause such a broad spectrum of disease syndromes that scattered cases of acute illness would probably be too varied in symptomatology to be attributed to a single etiological agent.

(2) Many viruses cause inapparent infections that are difficult to recognize as being waterborne. A person may contract a viral infection by coming into contact with contaminated water, and the virus may actively multiply in the intestines or in the respiratory tract without his developing overt symptoms of the disease. He may suffer only mild gastrointestinal or respiratory distress for a few days, or have no symptoms at all, yet he can act as an effective carrier and transmit the virus by droplet infection or by contaminated fingers to other individuals, who may then develop acute symptoms of the disease.

In recent years about 60% of all documented cases of disease attributable to drinking-water in the USA were caused by unknown or unrecognized agents. In addition, at present no field-tested method exists for the detection in water of the agents of viral hepatitis A and viral gastroenteritis. Nevertheless, there is today ample epidemiological evidence that viral hepatitis A is frequently waterborne and has caused numerous epidemics, some quite massive, in various areas of the world (e.g., China, India, and the USA). While many of these outbreaks have been associated with small untreated water supplies, in a number of cases treated municipal supplies were implicated (9, 17). These difficulties have led to an emphasis on the detection of the readily demonstrable enteroviruses in water as an indication of the possibility of contracting disease from other viruses present in water.

## 5. SOURCES OF INFECTION

Enteric viruses may be spread from one person to another by three main pathways: direct person-to-person contact, via faecally contaminated water, and via contaminated food. All three pathways are considered to be important, but the main concern of the Scientific Group was the second pathway, and also the third insofar as crops may be contaminated by means of the second. The potential routes for the dissemination of waterborne viruses are shown in Fig. 1.

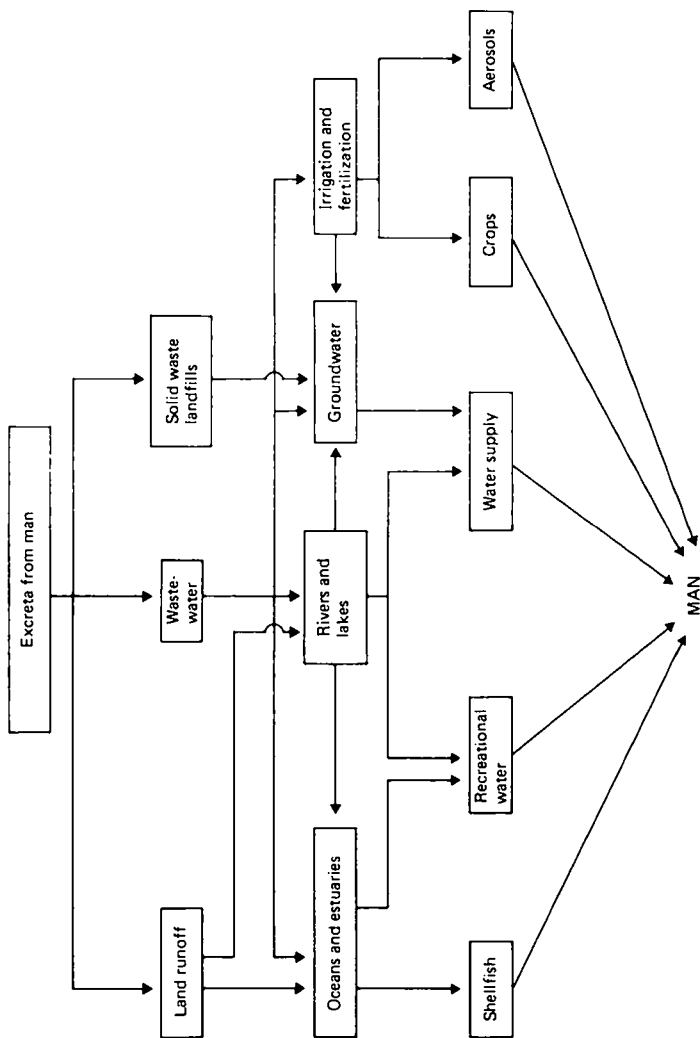


Fig. 1. Transmission routes of human enteric viruses

Source: Reference 2, page 502.

## 5.1 Wastewater

Large numbers of viruses of human origin are normally found in both sewage and wastewater. The number and types present will depend on the extent and nature of virus excretion by the population, the amount of dilution by water uncontaminated by viruses and the ability of the viruses to survive under local conditions. These factors can vary widely, depending on the time of year and the health of the community. Concentrations in wastewater are also dependent on the degree of treatment. Most conventional treatment processes reduce concentration levels but generally significant numbers of viruses survive.

The variability of virus numbers is illustrated by data from India (18), where viruses were recovered from 100% of raw sewage and effluents collected from different treatment plants throughout the year. When sewage from a middle-income group community in Nagpur was monitored over a 6-year period, virus concentrations were found to range from 200 to 11 000 plaque-forming units (PFU) per litre. Observations of seasonal variations showed a sharp increase during the rainy season, which in general coincided with the peak occurrence of reported paralytic poliomyelitis cases. Poliovirus was the predominant virus present in Nagpur sewage, constituting nearly 80% of the isolates; between 60% and 80% of these polioviruses appeared to be wild strains characterized by the temperature marker test (rct) (19). In countries with temperate climates the maximum virus content in sewage has been detected in the warm summer months and the minimum in the cold winter season, though adenoviruses and echoviruses may also be detected in early winter.

## 5.2 Drinking-water

Sources of drinking-water can be heavily contaminated. For example, in Europe enteroviruses at concentrations of up to 300 PFU/l have been isolated from the Rhine, Seine, Marne, and Moselle rivers (20). In Ghana (21) it has been reported that not only rivers and ponds but also well-water contained enteroviruses. Outbreaks of viral hepatitis A occurred in certain rural areas of China during the late 1950s and early 1960s. On epidemiological grounds, the cause of these outbreaks was considered to be the contamination of streams and deep wells, which served as the source of the drinking-water supply (Hou Yunte, personal communication). In 1976 a waterborne



outbreak of viral hepatitis A was observed during the rainy season in a rural area in Liaoning Sheng in the north of China, where, owing to heavy rain, cesspools overflowed and the deep wells became contaminated with sewage. More than 1000 cases were reported in this epidemic.

Despite the fact that methods for concentrating and detecting small numbers of viruses in large volumes of water have not been standardized and the methods currently in use vary considerably in their efficiency of recovery, it is important to note that virologists in a number of different countries have been able to demonstrate the presence of enteric viruses in drinking-water samples taken from public water-supply systems including systems that treat the water by conventionally accepted methods of filtration and disinfection.

Recent work in India has been designed to examine whether or not viruses were present in water treated by conventional procedures and also to evaluate the virological quality of water as delivered to the consumer (18). The subsequent isolation of human enteroviruses in some samples collected from the distribution system indicated the possibility of the introduction of contaminated groundwater through leaks in the distribution pipes at a location not far from the sampling point. In spite of the presence of 0.2–0.8 mg/l of total residual chlorine, the occurrence of viruses in certain samples indicated a lack of adequate contact time with the chlorine. Studies carried out during outbreaks of viral hepatitis A in Yeotmal, Kamptee (small towns near Nagpur), and Bombay resulted in the isolation of other viruses at concentrations of 1–7 PFU in 12–40-l samples of drinking water collected from the distribution system. Routine monitoring of water from taps during interepidemic periods in Nagpur yielded 1–7 PFU from 30–60 l of water (of 50 samples examined 7 contained viruses).

In a study during the 1960s in Paris, enteric viruses were detected in 18% of 200 samples, and the average virus concentration was estimated as 1 infectious unit per 250 l. From South Africa it has been reported (22) that treated drinking-water samples (which did not contain faecal coliforms) contained viruses. In a Romanian study (23), coxsackieviruses were detected in 2 out of 65 drinking-water samples. In this case, treatment consisted of flocculation with aluminium sulfate and lime followed by sand filtration. More recently, in the USA, poliovirus has been detected on several occasions in treated drinking-water containing 1.3–1.7 mg/l total chlorine (24). In Israel enteroviruses have been detected in 5 out of 21 dug wells

supplying untreated drinking-water, of which in some instances the coliform counts were very low or zero (H. I. Shuval, personal communication).

Investigators in the USSR have reported on the isolation of enteric viruses in drinking-water samples taken from the distribution system in Moscow and Kujbišev after the application of conventional, well-operated treatment processes, with the water meeting national bacteriological standards (25, 26). Since the introduction in 1975 of a new requirement that the water should be disinfected so as to achieve a residual of free available chlorine ( $\text{HOCl} + \text{OCl}^-$ ) of 0.3 mg/l after 30 minutes of contact, no viruses have been detected in the water-distribution systems of communities adopting this procedure (G. A. Bagdasarjan, personal communication). Apparently, the previous practice of chlorination to obtain a residual of combined chlorine was not sufficient to inactivate the viruses that had penetrated the filtration stage of the water-treatment plant.

In London, the concentration of enteric viruses detected in the River Thames at the intakes of the city water supply varied from about 100 PFU/l during the winter months to less than 1 PFU/10 l during the summer, with negative results only on rare occasions (J. S. Slade, personal communication). No viruses have, however, been detected after treatment consisting of 2–6 weeks of storage, rapid and then slow sand filtration, followed by final chlorination aimed at achieving at least 0.5 mg/l of free residual chlorine after 1 hour of contact.

### 5.3 Seawater

Many communities discharge their wastes into estuaries, bays, harbours, and other coastal waters. The oceans thus receive both treated and untreated domestic and industrial wastes, including sewage effluents and sludge. When the ocean is used in this manner, there is a constant danger of the pollution of shellfish growing areas, which constitutes a potential threat to public health.

Numerous outbreaks of viral hepatitis A associated with the consumption of raw shellfish grown in sewage-contaminated seawater have been reported (6, 6a). These have confirmed that the virus can survive for sufficient periods in sewage and subsequently in the sea and seafood itself to allow the effective transmission of the disease through the marine environment.

It has been necessary to develop special methods to detect viruses in seawater and sediments, to measure their inactivation and to evaluate the validity of bacterial indicators of viral pollution. Recent advances in methods of virus concentration from marine waters and sediments have made field studies possible. Enteroviruses can now be concentrated from 400-l volumes of turbid seawater with an average efficiency of 50%, and they can also be recovered from marine sediments. The presence of enteric viruses in estuarine water and seawater has been well documented. For example, enteroviruses were found in 14 out of 48 seawater samples obtained at varying distances from a sewage outfall off the coast of Tel Aviv (H. I. Shuval, personal communication). In the USA enteroviruses have been demonstrated in shallow, moderately polluted coastal areas as well as in the vicinity of deep marine sewage outfalls discharging chlorinated, secondarily treated sewage.

Minimal coliform findings in water samples bear no relation to the extent of viral contamination. Detection of enteric viruses in marine water in the absence of faecal coliforms ( $< 1/100$  ml) has been reported in several studies. This implies that a bacterial indicator, such as faecal coliforms, may at times be inappropriate for assessing the presence of viruses in contaminated waters. Techniques for monitoring water quality should include separate indicators for the presence of bacteria and of viruses.

Persistence of enteroviruses in the marine environment has been demonstrated. Viral inactivation in the sea is a relatively slow process, with a 90% reduction in concentration occurring in a matter of a day or so, as compared to the rapid disappearance of coliform bacteria, which usually show the same reduction rate in periods of up to a few hours. Enteric viruses have been reported to survive over 130 days in seawater held in the laboratory. The primary factor involved in virus survival appears to be temperature, with survival being greatly prolonged at low temperatures. Other factors believed to be implicated in viral inactivation are bacterial antagonism, the presence of suspended solids, salinity, pollution, solar radiation, and the type of virus involved. Inactivation in seawater appears to be very unpredictable: for example, water with the same salinity collected from the same site on different days has shown wide variation in inactivation patterns. This variation suggests that natural dying-off occurs randomly, depending on virus type, physiochemical differences within virus populations of the same type, and the physical, biological and chemical composition of the water.

High concentrations of viruses in sediments from sewage-polluted seawater have also been reported in recent field studies. Viruses adsorbed on to such solids remain infectious for both tissue cultures and the human organism and survive longer than do free viruses. Viruses in sediments may still pose a public health problem, as the water/mud interface is not a static system. Sediments can be resuspended easily in response to currents, storms, the movement of boats and of swimmers, dredging, or changes in water quality. Therefore, the sampling of surface water alone may not give a true indication of the potential viral disease hazard.

#### **5.4 Water used for recreation**

Polluted water may cause a health hazard during recreational activities, primarily swimming (especially if the head is immersed); but infection is also possible as a result of wading and boating. Generally, the risk is considered lower than that which would arise from drinking such water. However, swimmers and nonswimmers alike may ingest from 10 to 50 ml of water each time they bathe and thus may swallow viruses if these are present in the water. In addition to ingestion, exposed mucous membranes and breaks in the protective skin barrier may be portals of entry for viruses.

Swimming-pools have been implicated as the source of adenovirus conjunctivitis and pharyngitis, as well as enterovirus meningitis. Coxsackievirus B5 has been isolated from patients who had been swimming in lakes in which this organism was found to be present. Pools containing no free residual chlorine allow the survival and accumulation of viruses, and thus may become a source of infection with the viral diseases prevalent in the community. Properly maintained and disinfected swimming-pools seem to pose little risk of infection, but examples of infections from poorly maintained pools demonstrate that there is a potential hazard.

Swimmers in polluted seawater suffer from significantly higher rates of gastrointestinal disease compared to nonswimmers or to those who swim in unpolluted seawater (27). The most susceptible group was found to be children up to 10 years of age, and it is possible that rotaviruses may play a role as causative agent. Enteric viruses have been found at bathing places in coastal areas in water which met a bacteriological standard of less than 1000 coliforms/100 ml (28).

## 5.5 Soil and crops

As populations grow and water resources to meet expanding urban and agricultural demand become depleted there is an increasing worldwide interest in the reuse of wastewater for irrigation in both arid zones and areas of normal humidity.

In addition, in some countries the disposal of wastewater by direct application to land is being considered as a means of reducing pollution loads in heavily contaminated rivers and lakes. The direct use of human excreta (nightsoil) for soil fertilization has been widely practised in parts of Asia for centuries, and more recently sludge from modern wastewater treatment plants has been used as a soil conditioner or has been spread on land as an inexpensive means of disposal.

Although there is extensive literature on the transmission of pathogenic bacteria, helminths and protozoans through the spreading of wastewater, sludge and nightsoil on to land, concern about hazards from viruses caused by this practice has only recently been raised and available information remains limited. The possible deposition of significant concentrations of viruses on the soil might result in several health hazards:

- (1) direct virus infection of farm workers and their contacts;
- (2) virus contamination of crops used for human consumption;
- (3) virus contamination of drinking-water sources as a result of surface run-off or infiltration into groundwater;
- (4) dissemination of viruses by insect or animal vectors in contact with contaminated soil;
- (5) where application by wastewater sprinkler-irrigation is practised, virus dissemination by aerosol may occur, with consequent risks of infection through the respiratory tracts of farm workers, residents of adjacent areas or travellers in the vicinity.

The concentration of enteric viruses in fresh human faeces may be as high as  $10^5$ - $10^8$  PFU/g (J. L. Melnick, personal communication). The number of enteric viruses in wastewater may vary according to environmental factors such as weather and season, as well as local endemic and epidemic conditions. Concentration in wastewater is also a function of the degree of treatment, but research to date indicates that conventional treatment is only partially effective in

removing viruses. Concentrations ranging from 1 to 100 PFU/ml have been detected (29, 30), and higher numbers have been found in raw primary sludge ranging from 10 to 1000 TCID<sub>50</sub> (tissue culture 50% infective dose)/ml (31). Even well-digested sludge remains a potential source of contamination.

Factors that affect the survival of enteric viruses in soil include pH level, ionic concentration, moisture content, temperature, exposure to sunlight and the presence of organic matter. Viruses readily adsorb to soil particles, and this has been reported to prolong their survival. However, these viruses remain as infectious to humans as free viruses. Enteric viruses in loamy and sandy-loamy soil have considerable stability, with survival times of up to 170 days (32). Poliovirus has been detected in soil irrigated with infected sewage sludge and effluent after 96 days in winter and 11 days in summer, and on the surface of mature vegetables 23 days after irrigation had ceased (33). Poliovirus has also been recovered in soil and on the surface of crops 8 days after irrigation with experimentally infected sewage (34). These reported periods of virus survival are not necessarily maximum values since other enteric virus types may be even more resistant. In addition, some viruses may be difficult to recover by the methods used in these studies.

Virus survival on crops is shorter than in the soil since the viruses on crop surfaces are directly exposed to detrimental environmental factors such as sunlight and desiccation. However, more prolonged survival can be expected in the moist or more protected parts of plants, such as within the folds of leafy vegetables, in deep stem areas and on rough cracked surfaces of edible roots. Other studies have indicated that human viruses can penetrate damaged roots and, under certain conditions, enter the stem and leafy parts of edible plants (35-37). While evidence of this phenomenon is still tenuous, its possible role in crop contamination should not be overlooked. Once crops are harvested, enteric viruses can survive for prolonged periods during commercial and household storage at low temperature. For example, polioviruses and coxsackieviruses applied to the surface of vegetables can survive for more than 2 months under refrigeration (38). The risk of human infection associated with virus-contaminated crops is greatest in the case of fruits and vegetables generally consumed raw. However, there is also a possibility that vegetables consumed after thorough cooking might become infected by contact with kitchen surfaces, utensils and hands contaminated by raw crops.

## 5.6 Groundwater

The application of wastewater on to land, whether for agricultural irrigation or as a method of treatment and disposal, poses the possible risk of virus contamination of groundwater. The factors that influence the movement of viruses in soil have recently been evaluated. The rate of application, the soil composition and structure, and the pH level, organic content and ionic strength of the effluent are also relevant.

Wastewater application rates are one of the critical factors in the possible penetration of viruses into groundwater. While normal rates of water application for agricultural irrigation are about  $1 \text{ m}^3$  of water/ $1 \text{ m}^2$  of land/year, hydraulic loading rates for effluent disposal on land can be as high as  $100 \text{ m}^3/\text{m}^2/\text{year}$ . With higher rates of loading, virus removal declines, but in one case—even with flow rates as high as  $10 \text{ m}^3/\text{m}^2/\text{day}$ —99.9% removal was obtained after travel through 2.5 m of sandy-loamy soil.

Soil composition and structure affect virus movement, since viruses are readily adsorbed on to clays under appropriate conditions, and the higher the clay content of the soil the greater is the expected removal. Similarly, sandy loams and soils containing organic matter are also favourable for virus removal. Soils containing sand or sand and gravel mixtures do not achieve good removal, while fissured limestone aquifers under shallow soil allow viruses to travel for great distances and can present serious groundwater pollution problems.

Despite very high application rates of effluent to basins of loamy sand, averaging  $90 \text{ m}^3/\text{m}^2/\text{year}$ , no viruses were detected in wells 6 m deep, 6 m from the edge of the infiltration basin, indicating a virus removal of at least 99.9% (39). Travel of viruses has been studied at a wastewater reclamation project near St Petersburg, FL, USA, where chlorinated secondary effluent was applied by a sprinkler-irrigation system to a sandy soil containing little or no silt or clay (40). Polioviruses and echoviruses were detected in underdrains 1.5 m below the surface, demonstrating that viruses survive aeration and sunlight during spraying as well as percolation through 1.5 m of soil. Although at first no viruses were detected in wells 3 m and 6 m below the surface, they were detected after heavy rainfall, indicating that viruses were migrating through the soil. The same authors also failed to detect faecal coliform bacteria in well-water samples containing viruses.

A low pH favours adsorption while a high pH can result in the elution of adsorbed viruses. High concentrations of soluble organic matter in the wastewater may compete with viruses for adsorption sites on the soil particles, resulting in decreased virus adsorption or even in the liberation of already adsorbed viruses, while the presence of high concentrations of cations usually enhances virus retention. Viruses retained near the soil surface may be eluted and washed down to lower strata by heavy rainfall.

Since the factors influencing the movement of viruses in soil are still not fully elucidated, and since effluent and soil conditions vary so extensively, caution should be exercised in the vicinity of wastewater irrigation or land disposal sites with regard to wells supplying drinking-water. Careful study of local conditions is required. Reasonable safety measures should include the siting of such wells at a suitable distance away and the routine virological monitoring of water quality.

## 5.7 Aerosols

Many processes involving water and wastewater can lead to the formation of virus-contaminated aerosols, which may subsequently infect humans by the respiratory route. Although aerosols may be formed naturally by waves and waterfalls, the most significant sources are probably associated with wastewater treatment and spray-irrigation.

Humans may be infected by aerosols containing pathogenic bacteria or viruses mainly by inhalation of particles measuring 0.2–2  $\mu\text{m}$ , which penetrate the alveoli. However, larger droplets, in the 2–5  $\mu\text{m}$  range or more, which are trapped in the upper respiratory tract, are removed by ciliary action and may find their way into the digestive tract by ingestion. A retrospective study of possible health risks associated with sprinkler-irrigation with wastewater was carried out in Israel (41). In 77 agricultural settlements practising sprinkler-irrigation with oxidation pond effluent after 3–7 days' detention time, the incidence of typhoid fever, salmonellosis, shigellosis, and infectious hepatitis was from 2 to 4 times higher than in 130 control settlements not practising sewage irrigation. In the first group, clinical influenza rates were double those reported in the controls. However, when laboratory-confirmed cases of influenza were compared, no differences were found. The authors suggest that the increased rates of clinical influenza might be attributable to respiratory infections



associated with enteroviruses spread by sprinkler-irrigation and misreported as influenza. The distance between residential areas and areas irrigated with sewage varied from 300 m to 3000 m. The authors note that although their study suggests that the excess of enteric disease could be associated with aerosol transmission of pathogens, other hypotheses might explain the phenomenon. For example, the pathogens could have been carried into the community on the clothes or bodies of irrigation workers and passed on by direct contact in the communal dining-hall. With the aim of confirming these preliminary findings further investigations are being conducted.

In the process of droplet formation at the surface of aerated liquids, the droplet scavenges organic material and microorganisms, with the result that the aerosol particles may contain a bacterial or virus concentration 100 or more times greater than that of the ambient water (42). This suggests that bubbles formed during aeration processes of sewage treatment such as the activated sludge method may lead to the formation of droplets containing very much higher concentrations of pathogens than the wastewater itself. During sprinkler-irrigation, which is commonly used for wastewater applications to the land, between 0.1% and 1% of the liquid is transformed into aerosol depending on the type of spray device, the pressure and the wind speed. Viruses in seawater become concentrated by rising air bubbles, which, on bursting at the surface, form jet droplets that can be carried considerable distances (43). Thus, seawater in which raw sewage is present may produce an airborne health hazard in adjacent residential or recreational areas, even in cases where bathing, leading to direct exposure, is not practised.

Once formed, aerosols may travel considerable distances: for example, enteric microorganisms contained in aerosols formed by wastewater treatment processes have been detected 1200 m downwind, while microorganisms from sprinkler-irrigation of wastewater from food-processing might be spread as far as 25 km (44). Coliforms were detected at 350 m, salmonellae at 60 m, and enteroviruses at 40–100 m downwind of fields irrigated by wastewater spraying (45). Some 30% of the aerosol particles were in the respirable size range of under 5  $\mu\text{m}$ . The same authors also found that the detection rate of viable organisms increased under conditions of high humidity and low solar irradiation and that night-time values were 10 times higher than those found during the day.

In a study of the occurrence of viruses in aerosol emissions of wastewater treatment facilities, coliform bacteria were much less

stable than coliphages in the airborne state (46). This suggests that the presence of the latter organisms may be a more appropriate indicator of airborne viruses.

Field data on the dispersion of aerosolized viruses by sewage treatment and land disposal systems, and on the associated health risk, are still limited. However, sufficient evidence is available to indicate that a potential health hazard may exist and that steps to reduce this risk may be warranted. In Japan, there is currently a trend to cover wastewater aeration tanks with sheets of light plastic to reduce aerosol spread.

## **6. VIRUS MONITORING**

### **6.1 Purpose**

During an outbreak caused by an enteric virus, sampling should be aimed at determining whether or not the water supply is contributing to the spread of disease. Samples of raw water, fully treated water, and tapwater should be examined.

The last-mentioned is particularly important where the integrity of the distribution system is suspect. The finding of any enteric virus, even of low pathogenicity, in drinking-water is a danger signal, since the water may contain viruses of higher pathogenicity.

In circumstances in which virological facilities can be provided, it is advantageous regularly to monitor effluents and raw-water sources for the presence of viruses. This will provide baseline data against which exceptional circumstances, such as may occur during an epidemic, can be compared. Furthermore, an established system of monitoring will avoid the delay that may be experienced if facilities are only provided in response to epidemic conditions. The monitoring of wastewater may provide an early indication of viral infection in the community, although the presence of a virus in sewage does not necessarily indicate a public health risk and the inevitable delays associated with present methods of virus detection may reduce the value of these data. Nevertheless, the detection of virus types not previously observed or a marked increase in the concentration of a specific virus may indicate the developing of a virus disease in the community not yet apparent from clinical cases.

Although virological standards for potable supplies are not yet in general application, routine monitoring of drinking-water for the presence of viruses can provide an additional degree of public health protection. Such routine monitoring would be particularly justified in the numerous cases in which large urban centres use as their source of raw water heavily polluted rivers that carry a significant flow of sewage. These situations should rightly be considered a form—albeit indirect—of wastewater reuse. Regular virus monitoring should be mandatory in every case of direct reuse of wastewater for potable supplies.

## 6.2 Methods

### 6.2.1 *General considerations*

A number of techniques can be used to detect the presence of enteric viruses. No one method can be applied to all types of virus and the types which are isolated will therefore depend to some extent on the technique used. Most current investigations are limited to those viruses which are easily grown in tissue cultures. Other viruses require more specialized techniques—for example, rotaviruses are usually studied by means of electron microscopy of faecal extracts. Some viruses, such as that of hepatitis A, cannot as yet be easily isolated by any method. The results obtained from a virological examination can therefore represent only a small fraction of the total virus content.

The method most generally used consists essentially of three parts: concentration, culture, and identification. The culture and identification techniques are similar to those used in other fields of virology—i.e., the virus obtained after concentration is inoculated on to living cells (generally a tissue culture, although animals can be used), and the isolated viruses are identified usually by means of specific antisera. It is in the field of concentration that special techniques have been developed. Most concentration techniques are based on one of two principles, either ultrafiltration or adsorption followed by elution. With the ultrafiltration method (now called “reverse osmosis”), the sample, possibly after some initial clarification, is passed through a filter capable of retaining virus-sized particles. The main disadvantages are the lengthy time required and the parallel concentration of substances toxic to cell cultures. With the adsorption/elution method, the pH level and salt content of the

sample are adjusted so that viruses are adsorbed on to a suitable surface from which they can subsequently be eluted into a small volume. Adsorption is promoted by low pH levels and high salt concentrations and elution by high pH levels, or high organic content, or both. The adsorbing surface can be provided by a variety of materials, such as cellulose ester membranes or glass-fibre filters. Particulate suspensions—for example, iron oxide or aluminium hydroxide—can also be used. Continuous flow and multistage processes are available for large volumes.

### 6.2.2 *Water*

The method of choice for any one sample will depend on a number of factors of which the most important are the expected content of viruses and the amount of suspended solids. Samples may range from clean tapwater, in which the virus content is low, to raw sewage, in which viruses may be so numerous that little or no concentration is required. Whatever method is adopted it should be used only after the following limitations have been considered:

(1) Although some methods yield quantitative recovery of known amounts of added viruses, they may not detect all viruses present in field samples nor give the same quantitative recovery for different viruses.

(2) The efficiency of any one method may vary, especially with changes in the quality of the water sampled.

(3) The methods described are supported by varying amounts of data. None has been studied with more than a few virus types and few studies are available that compare the efficiency of one method with that of another under the same conditions.

(4) Some of the techniques require expensive equipment. With methods evolving rapidly there is a risk of obsolescence.

Some of the available methods are summarized in Table 2 and described in more detail in Annex 1.

### 6.2.3 *Sediments*

In natural waters such as rivers large quantities of particulate matter may be present. Most of the viruses in such waters are often associated with these solids, which should therefore always be eluted.

Table 2. Methods for the detection of virus in different types of water and sludges

Method	Type of sample
Direct inoculation without concentration (30, 47).	Wastewater, sludge.
Swab sampling (48, 49).	Wastewater samples (method sensitive but not quantitative).
Filter adsorption/elution methods. Adsorption and precipitation methods employing polyvalent cation salts. Use of preformed alum, aluminium hydroxide iron (III) oxide (50), iron (III) chloride or lime flocs. Flocculation by added salts (51). Hydroextraction (52) and aqueous polymer two-phase separation techniques (53). Soluble alginate (54). Precipitation by low pH (55).	0.2-5.0-l samples where more than 1 infectious unit of virus per litre is expected.
Flat membranes, hollow fibre (56). Flow-through filter adsorption of acidified samples followed by elution at high pH. Single or multistage procedures (57).	Large-volume samples, of 5-400 l or more. In general, single-stage procedures may be used for samples of up to 20 l—i.e., for samples containing relatively large amounts of virus such as are likely to occur in sewage, treated sewage effluents, and polluted surface water.
Filtration through pleated filters followed by elution (58). Organic flocculation followed by elution (59). Filtration through adsorptive filters with positive charge followed by elution (60). Adsorption to glasspowder followed by elution (61).	Groundwaters, less polluted surface waters and highly treated wastewater (multistage procedures).

The degree to which viruses trapped within solids are extracted by current procedures is not known, but it is possible that there are many more viruses within the solids than on the surfaces, and that most or all of them escape detection.

#### 6.2.4 Sludge

The number of viruses in primary sludge and chemical flocs may be so high that concentration procedures are not required even when

the corresponding raw sewage calls for such measures. Primary sludge may contain 10–1000 TCID<sub>50</sub>/ml. However, sludge samples present extreme problems from the point of view of toxicity to cell cultures.

Some suitable techniques are given in Annex 1. They cannot be relied on as truly quantitative methods because their efficiency, which is based on experimental laboratory studies, may not be relevant to field conditions (62–65).

### **6.3 Bacteria and bacteriophages as indicators of enteric viruses**

The observation that there are in general many more indicator bacteria in polluted water than there are viruses has led to the hope that these bacteria would serve to reveal whether or not viruses were present (66). Recent studies have shown, however, that viruses can survive in a wastewater effluent that has been subjected to a disinfection sufficient to destroy all faecal coliforms and streptococci. Moreover, there is evidence that the ratios of faecal coliform bacteria to viruses are greater near sewage outfalls than they are at points distant from the outfalls, demonstrating that the indicator bacteria are more sensitive to adverse environmental conditions than are viruses. Furthermore, several investigators have recovered viruses in water in which no faecal coliforms were detectable. The evidence is now overwhelming that while faecal coliform and other vegetative faecal bacteria serve as an indication of faecal pollution, the absence of these indicators offers no assurance that viruses are also absent. It is emphasized that the coliform tests are still the simplest method for assessing the faecal contamination of water.

Consideration has been given to the possible use of bacteriophages of enteric bacteria as indicators of enteric viruses. The speed and economy of bacteriophage tests compared with those for enteric viruses make such a proposition attractive. There have been some promising reports of their use. For example, it has been reported from the USSR (G. A. Bagdasarjan, personal communication) that *Escherichia coli* phages show higher resistance to physical and chemical factors than do coliform bacteria, and that coliphages are now used as indicators of virus pollution of water and for the evaluation of waste treatment procedures and water-source quality. Some other workers have also advocated the use of phages as indicators but published results are scanty. However, it has been considered that the wide variations in the sensitivity of the many

different types of bacteriophages that might be present in different types of water would make these methods difficult to establish. The Scientific Group was of the opinion that because of its importance this problem should be given further consideration and more studies should be encouraged.

## 7. VIRUS REMOVAL BY TREATMENT PROCESSES

### 7.1 Wastewater

All sewage treatment processes remove or destroy viruses to some degree, but none is likely to remove all the viruses present. Moreover, the efficiency of a given procedure may vary considerably depending on the design of the plant, its location, the skills of the operator, the nature of the effluent (volume and quality of industrial effluent present), and other factors.

Primary sedimentation can remove a significant proportion of viruses (up to 50%) owing to their association with solid matter.

Of the secondary treatment procedures, the activated sludge process is the most effective biological method, removing 60–99% of the viruses present. However, the results from trickling filters and stabilization ponds vary, though well-designed multicellular ponds can remove 80–95% of the viruses.<sup>1</sup>

Chemical coagulation is regarded as one of the most effective single-step treatments. Alum or lime is commonly used, but iron salts have also been employed. Lime is probably the most efficient (90–99% reduction), since it not only removes the viruses physically but also inactivates them by exposing them to a high pH. The filtration of coagulated effluents is an important additional process, slow sand filtration being more effective than rapid sand filtration. Adsorption methods, using clays, coal or activated carbon, can remove viruses to some extent, but the process is not efficient.

The application of wastewater to land can be a valuable tertiary treatment and is being used successfully in a number of countries.

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<sup>1</sup> In the activated sludge process the liquid fraction of sewage is mixed with recycled sludge and aerated by mechanical means. In trickling filters the liquid is trickled through a porous bed of filter material on which microbes grow. In stabilization ponds whole sewage is passed slowly through a series of lagoons. All three processes act by providing suitable conditions (sufficient time, oxygen, etc.) for microbes to break down impurities.

Little evidence is available about the survival of viruses in the soil or run-off water, but a number of studies show clearly that they can survive for long periods in soil and may be eluted by heavy rainfall. Adequate space and the correct type of soil are essential, as are careful management techniques and regulations regarding the agricultural use of such land.

Under circumstances in which some form of disinfection must be applied to render wastewater safe before discharge into the environment, chlorine is widely used for this purpose but it is far from ideal. Its efficacy is reduced by the presence of organic material, inadequate contact time and insufficient dose, while temperatures, pH level and the presence of ammonia also exert an influence. Consequently these factors may render conventional chlorination, in which residuals of combined chlorine are used for up to 2 hours' contact time, completely ineffective with regard to viruses. The less than optimum application of chlorine in the field is emphasized by the frequent isolation of viruses from a number of chlorinated effluents. Unwanted chemicals such as chloroform may also be formed.

The total disinfection of effluents containing solids is most reliably achieved by prolonged storage, heat or penetrating radiation, but these methods are often not practicable. Other methods of disinfection are being sought, but this is not a simple matter and disinfectants must be selected according to need.

## **7.2 Sludge**

If raw sewage contains viruses, the sludge produced by treatment plants is likely also to contain viruses to a greater or lesser extent. Primary sludge and chemical flocculation products contain a higher concentration of viruses than does secondary sludge. Sludge should be handled accordingly and safe disposal can be a problem. In some areas sludge is incinerated but in most places it is spread on land, usually in a stabilized form to reduce odour and transportation problems. The application of inadequately treated sludge to land may give rise to serious public health problems, including the hazard of virus contamination. Such sludge should not come into contact with crops which will be eaten raw—or even cooked, because they are brought into the kitchen raw. It is also important to avoid the disposal of sludge in places or at times of the year when run-off to water-sources may become a problem.



Table 3. Virus removal by sludge treatment processes

Sludge type	Treatment	Result
Raw primary or secondary sludge, or both.	None.	10-100 times higher concentration of virus than in raw sewage.
	Anaerobic digestion at 30-35 °C or 50 °C with an average retention time of 3 weeks or more.	The temperature and retention time are more than sufficient to inactivate enteroviruses, but owing to the method of operation a small percentage will escape, so that digested effluents may contain demonstrable amounts of virus.
Raw or digested sludge.	Drying beds.	Depending on the efficiency of the drying (temperatures and solar irradiation) viruses may be destroyed in a few weeks, but may still be present even after 4 months.
	Composting.	Windrow composting can generate sufficiently high temperatures (60-70 °C) to ensure a virus-free compost, provided mixing or aeration is efficient. Regrowth of enteric bacteria may take place.
	Pasteurization and other heat treatments.	Complete inactivation of viruses may be obtained.
	Irradiation with 2-5 kGy.	A very high degree of virus inactivation may be obtained.
Chemical flocs from alum, iron salt or lime treatments. Lime-stabilized sludges.	None.	A very high concentration of viruses—much higher than in raw sewage—may be found in alum and iron salt flocs. Lime flocs may contain demonstrable amounts of virus but generally less than in raw sewage.

Further information on sludge treatment methods and their likely effect on virus content is presented in Table 3.

### 7.3 Drinking-water

The majority of processes used to treat sources of potable water are capable of reducing virus numbers, but with the possible exception of high-grade disinfection none of them can be relied upon to remove all viruses under all circumstances. Furthermore, their efficacy may vary considerably depending on the design and operation of the plant, differences in water quality, temperature and

other factors. Storage in a reservoir for a period of several weeks or longer is effective, particularly in warm weather, but requires suitable site conditions. Rapid sand filtration or microstraining (in which particulate matter is removed by passing water through a fine stainless-steel gauze (80 000 apertures/in<sup>2</sup>, or about 12 000/cm<sup>2</sup>) on a rotary filter) results in a negligible reduction in virus numbers, though slow sand or biological filtration has been shown to be highly effective. Flocculation processes, generally used in combination with rapid filtration, have been reported to remove between 60% and 99% of viruses depending on the dosage applied and local conditions. However, viruses removed by flocculation are not inactivated and care must be taken with the disposal of the infectious sludge. Lime flocculation, which is often applied to renovated waters, is very effective (99.9% reduction) provided highly alkaline conditions (pH > 11.5) are maintained for at least 1 hour. An advantage of this process is that viruses are both removed and destroyed. Adsorption by activated carbon can remove viruses but the adsorbed viruses may be liberated at a later stage, when organic material competes for available adsorption sites.

It was the opinion of the Scientific Group that all potable water supplies derived from virus-contaminated sources should be disinfected. Other treatment processes alone are not adequate under all conditions. Disinfection processes can destroy viruses with great efficiency when used correctly. The most widely used disinfectants are chlorine and ozone. When using chlorine it is most important to differentiate between free and combined chlorine (i.e., different forms of chloramines for the latter). Free chlorine is a highly effective virucide, whereas combined chlorine is far less active and functions much more slowly although its effect lasts a longer time. There may be more than a hundredfold difference in the virucidal efficiency of the two forms. To be effective, an adequate level of free chlorine must be present in the water for a suitable period of contact. The amount required will depend on the quality of the water, in particular its pH level and ammonia and organic content. It has been found that in water of low turbidity derived from surface sources a chlorine dose sufficient to provide a residual of 0.3–0.5 mg/l of free chlorine after 30–60 minutes' contact time provides a high degree of safety. In circumstances where drinking-water is likely to become contaminated after leaving the treatment plant it is not possible to achieve complete protection, but the maintenance of residual chlorine in the distribution system will provide an additional safeguard.

The Scientific Group was aware of the potential health problems that may be associated with the formation of carcinogenic compounds such as trihalomethanes when water containing organic material is chlorinated, and felt that alternative disinfection techniques for effective virus inactivation should be developed. However, in circumstances where there is a risk of waterborne disease, there should be no hesitation in continuing current water disinfection procedures with chlorine until effective alternatives are available. A promising alternative approach is the treatment of the raw water prior to disinfection by granular activated-carbon filtration to remove the precursors of such carcinogens. This pretreatment may allow for the continued use of chlorine as an effective disinfection procedure.

Ozone has also been shown to be an effective viral disinfectant, preferably for clean water, where residuals of 0.2–0.4 mg/l are maintained for 4 minutes. Ozone has advantages over chlorine for treating water containing ammonia but, unfortunately, it is not possible to maintain a residual in the distribution system. There are also difficulties with dosimetry.

Unfortunately, under the average conditions of operation of many treatment plants it can be expected that viruses from contaminated water-sources may penetrate the drinking-water distribution system. It is noteworthy that viruses have been isolated from treated water supplies on several occasions.

## **8. NATURAL RECYCLING AND INTENTIONAL RECLAMATION OF WATER**

### **8.1 Natural recycling and pollution**

The water cycle that occurs in nature is similar to the one of reuse. This cycle combines the processes of dilution, filtration, adsorption, sedimentation and biological activity which result in the purification of water. Unfortunately, when untreated or partially treated sewage is discharged into surface water which is abstracted downstream for further use, there may not be sufficient time for the various natural purification processes to take place. Such unintentional recycling of polluted water occurs in many areas. In some places, the load of sewage may be so considerable that the natural purification processes cannot function any more.

To ensure the safety of such water it is important that effective decontamination processes should be adopted to remove viruses and other pathogens. When feasible, the destruction of these microorganisms should take place at the source of pollution, where they are concentrated and more easily accessible, rather than after dissemination throughout the environment. In practice this means at the sewage works. Such action would greatly reduce the problems caused by viruses in rivers, lakes and seas. Treatment by land application may also be unsatisfactory. There is accumulating evidence that the capacity of soil to remove viruses is dependent on a number of variables and that following rainfall they may penetrate through the soil and contaminate groundwater. Decontamination of sewage at its source would prevent such an occurrence.

## 8.2 Intentional reclamation of water

A cautious public health approach to water quality management would be that, wherever possible, potable water supplies should be derived from the best-quality water available and that the sources of public water supplies should be as free as possible from chemical and microbial contaminants, whose total removal by the treatment processes at present available is not always assured. However, in view of the increasing demand for water, some countries are seriously considering the intentional recycling of wastewater containing, in its original state, a heavy load of viruses. Such direct reuse, known as "pipe-to-pipe" or "closed loop" systems, obviously carries with it inherent dangers. Nevertheless, in certain extreme cases of water scarcity, direct recycling may be the only available course of action. In such cases a series of advanced wastewater and water treatment processes should be applied to remove pollutants to the greatest extent possible. A typical combination of unit processes that might be suitable for the direct recycling of wastewater is presented in Fig. 2. Each stage of such a treatment system should reduce the content of viruses by 90–99%, thereby assuring a total reduction of initial concentrations by as much as 12 log cycles.<sup>2</sup>

Indirect reuse, in which the treated effluent is supplied to lakes, rivers or groundwater, is often considered safe, because the water undergoes some natural purification and subsequent plant

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<sup>2</sup> A 1 log cycle reduction is a tenfold decrease (90%); a 2 log cycle reduction is a hundredfold decrease (99%); and so on.

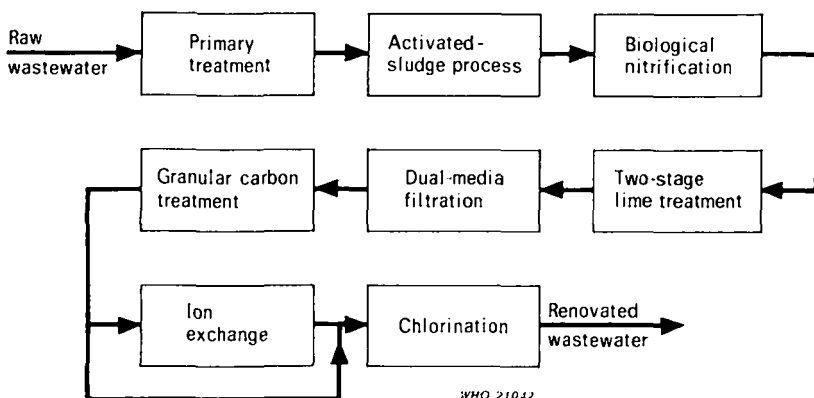


Fig. 2. Treatment system for producing potable water

Source: Reference 4, page 36.

processing. However, such indirect reuse, particularly in the case of heavily polluted rivers carrying 50% or more of effluent, may present essentially the same degree of health risk as that associated with direct reuse.

### 8.3 Permissible limits of viral contamination

One of the problems considered by the Scientific Group was the need to set virological guidelines for the intentional reuse of wastewater in the light of current technology. Because of the special public health risk associated with the direct reuse of wastewater for potable supplies, the Group concluded that no viruses should be detectable in samples of between 100 l and 1000 l of directly reclaimed drinking-water. Furthermore, an adequate residual of disinfectant should be maintained at the tap, and renovated water should be monitored frequently for viruses, using the techniques described in this report. The Group also felt that a similar approach should be applied to many of the instances of indirect wastewater reuse in which large communities obtain their drinking-water from highly polluted rivers carrying a significant percentage of wastewater.

## 9. CONCLUSIONS

While bacterial contamination of water and soils and the associated health risks have been thoroughly studied, attention is now increasingly being focused on the hazards associated with virus contamination of water. The Scientific Group reviewed the current state of knowledge on this subject and concluded that the contamination of water and soil by wastewater and human faeces containing enteric viruses may pose real public health problems. This is also applicable to areas of the world in which the major waterborne bacterial diseases have been brought under control.

There are over 100 different types of enteric viruses, all considered pathogenic to man. Their concentration in wastewater may reach 10 000–100 000/l, and they have the ability to survive for months in water and in soil. In some instances, the ingestion of a single infectious unit can lead to infection in a certain proportion of susceptible humans.

On numerous occasions viral hepatitis A epidemics have been waterborne. Many outbreaks of viral hepatitis A have resulted from eating shellfish grown in sewage-contaminated estuarine and coastal waters. It is also probable that a significant proportion of the reported waterborne gastroenteritis outbreaks of nonbacterial etiology have been associated with waterborne viruses (e.g., rotaviruses).

While the Scientific Group recognized that massive waterborne outbreaks of virus-associated diseases have been detected only on limited occasions, it concluded that the constant exposure of large population groups to even relatively small numbers of enteric viruses in large volumes of water can lead to an endemic state of virus dissemination in the community, which can and should be prevented.

Bacteria used as conventional indicators to evaluate the safety of potable water supplies have been shown to be significantly less resistant than viruses to environmental factors and to water and wastewater treatment processes. As a result, enteric viruses may be present in water that manifests little or no sign of bacterial pollution.

Where surveys have been carried out, viruses have been detected in the drinking-water supply system of a number of cities, despite the fact that these supplies have received conventional water treatment, including filtration and disinfection, which are considered adequate

for protection against bacterial pathogens. Plans for the recycling of wastewater for domestic consumption are being considered in some cities, while many others are drawing their water supply from contaminated surface sources carrying a significant proportion of wastewater. In both situations the risk of viruses penetrating the supply system must be carefully evaluated so that adequate monitoring and treatment can be provided.

Methods for the concentration and enumeration of viruses in large volumes of water have been developed but are not yet standardized. Through the use of such methods large water samples can be monitored for viruses on a routine basis.

Water treatment methods capable of accomplishing effective virus removal and inactivation are now available, so that conventional water treatment plants can be suitably modified to deal with this problem. The formation of carcinogenic compounds when water containing organic material is chlorinated may give rise to a potential health problem. However, in situations in which there is a risk of waterborne communicable disease there should be no hesitation in continuing current water disinfection with chlorine until alternative techniques for effective virus inactivation are developed.

Viruses present in wastewater and sludge applied to land for irrigation, fertilization or disposal purposes can survive in soil for periods of weeks or even months. Edible crops, contaminated either by contact with virus-laden soil or by wastewater sprinkler-irrigation, can harbour viruses for sufficient periods of time to survive harvesting and marketing, and thus their eventual consumption constitutes a potential health risk.

Only limited data are available on the health risks resulting from the dispersion of viruses in aerosols created by sewage treatment and land disposal systems. However, a potential hazard does exist and steps to reduce it may be warranted. Disinfection of effluent prior to land disposal, particularly in the case of sprinkler-irrigation in the vicinity of inhabited areas, could be an effective preventive measure.

## **10. RECOMMENDATIONS**

(1) Wherever possible, drinking-water should be free from human enteric viruses. To ensure that this goal is being achieved, a 100-l to 1000-l sample should be tested by the most sensitive method

available. In all cases of intentional direct wastewater reuse for domestic consumption, this procedure should be considered essential and should be applied at least in large urban areas in which potable supplies are derived from virus-polluted sources, such as surface water containing a significant proportion of wastewater either untreated or insufficiently treated to inactivate viruses. Further consideration should be given to the establishment of recommended virus concentration limits for water for recreational purposes, and wastewater effluent and sludge for agricultural use.

(2) Where virological facilities can be provided, it is desirable to monitor wastewater effluents, raw-water sources and drinking-water for the presence of viruses. This will provide baseline data to evaluate the health risk faced by the population.

(3) In the light of the greater resistance of many enteric viruses to disinfection and other treatment processes compared to that of bacteria utilized as pollution indicators, drinking-water derived from virus-contaminated sources should be treated by methods of proved high efficiency for removing or inactivating viruses and not only bacteria. Particular emphasis should be given in such cases to ensure the effective disinfection of drinking-water with, for example, free available chlorine residuals of 0.5 mg/l maintained for a contact time of 30–60 minutes or an ozone residual of 0.2–0.4 mg/l maintained for 4 minutes.

(4) Because of the ability of viruses to survive for long periods in seawater, it is recommended that coastal bathing and shellfish growing areas should be protected from contamination by wastewater and sludge. Virus monitoring of these areas is a desirable measure.

(5) Control procedures should be instituted in all situations in which wastewater or sludge is used for irrigation or fertilization, to prevent the contamination of vegetables and fruits which are to be eaten raw. (Moreover—even though they may eventually be cooked—contaminated raw vegetables are liable to pollute other food in the kitchen.) Where it is nevertheless planned to irrigate such crops or where sprinkler-irrigation is to be used near populated areas, the effluent should be treated so that it reaches a high microbiological quality approaching that of drinking-water.

(6) Since the factors that influence the movement of viruses in soil are still not fully understood, and since effluent and soil conditions vary so greatly, caution should be exercised if wastewater irrigation or land disposal takes place in the vicinity of wells



supplying drinking-water. Careful study of local conditions is required and the cautious siting of such wells and routine virological monitoring of the water are advised as safety measures.

(7) Further research is necessary into the health risks associated with viruses in water and soil. These studies should include the development and evaluation of methods of detecting viruses and alternative indicators of virus pollution (e.g., phages) and the improvement of treatment methods for the inactivation and removal of viruses from water and wastewater. The dissemination and survival of viruses in the natural environment should also be investigated.

(8) A standard method should be developed for the concentration and detection of viruses in large volumes of drinking-water (e.g., 100–1000 l) based on a full evaluation in different laboratories of present techniques. Such an attempt would facilitate the development of virus-monitoring programmes and would ensure a maximum degree of comparability of results. A laboratory quality-control system should be developed to enable participating laboratories to standardize their procedures.

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## ACKNOWLEDGEMENTS

The Scientific Group wishes to record its appreciation of the contributions made by the following persons: Dr H. Gorchev, Consultant, Environmental Health Criteria and Standards, WHO, Geneva, Switzerland; Dr L. Irving, Fairfield Hospital, Fairfield, New South Wales, Australia; Dr E. Strijak, Environmental Health Technology and Support, WHO, Geneva, Switzerland; and Dr M. J. Suess, WHO Regional Office for Europe, Copenhagen, Denmark.

## Annex 1

### METHODS FOR THE DETECTION OF VIRUSES IN WATER AND SLUDGE

A number of methods for the detection of viruses in water have been reviewed and evaluated (1, 2). These procedures are mainly concerned with the techniques of sample concentration, leaving the virological work to specialized laboratories. Virological examination is dealt with in a manual on the examination of water for pollution control in preparation by the WHO Regional Office for Europe (3), and the 15th edition of the American Public Health Association's *Standard Methods for the Examination of Water and Wastewater* includes a chapter on virus examination (4). These works should be referred to for more detailed descriptions of procedures. A short description and an evaluation of some of the methods are given below.

Water should be dealt with differently according to its expected virus load. As the nature and turbidity of organic matter are known to influence the efficiency of virus concentration methods, these factors should be considered in the choice of the method. In addition to its efficiency, a virus concentration method should be suited to the task in hand and should not involve the use of sophisticated apparatus unless required. In the following paragraphs, several methods are tentatively suggested, along with alternative methods which do not require expensive equipment.

Raw domestic wastewater from urban areas may contain up to  $10^5$  TCID<sub>50</sub>/l, and even  $10^6$  TCID<sub>50</sub>/l may occasionally be found. If at least  $10^4$  TCID<sub>50</sub>/l are present, viruses can be demonstrated by direct inoculation without concentration. However, with most samples a concentration procedure is necessary.

#### **1. Methods for sample volumes of 0.2-5 litres**

##### **1.1 Simple filter adsorption/elution systems**

These techniques are based on the adsorption of viruses to filters, followed by their subsequent elution into a small volume of fluid. Filters made from cellulose derivatives or fibreglass are commonly used. Virus adsorption is often promoted by the addition of salts such as magnesium or aluminium chloride and a lowering of the pH to 3.0

or 3.5. The samples may be of 0.2–5 l taken as dip or swab samples and the expected virus load should be more than 1 infectious unit per litre.

Problems which may be encountered include the possible removal of viruses on particulate matter if the sample is clarified prior to virus adsorption. Certain soluble substances in the water may interfere with the adsorption of virus particles on to the filter. There may also be an incomplete elution of viruses adsorbed on to the filter. Wastewater samples often require prefiltering, but if a prefilter is used it must be eluted in order to obtain quantitative virus recovery. Care should be taken to reduce the time of exposure of viruses to extreme pH levels to avoid inactivation.

The advantages of these techniques are their simplicity and speed. The degree of efficiency obtained with artificially contaminated samples is reported to be satisfactory, but field samples are more difficult to handle, and some viruses may behave differently from those used in the development and testing of the method.

### **1.2 Adsorption and precipitation methods employing polyvalent cation salts**

Viruses may be adsorbed on to preformed alum, aluminium hydroxide, iron oxide or lime flocs, or alternatively salts may be added to the sample to form flocs *in situ*. The size of the sample that can be handled is limited by the bulk of the precipitate, which might clog filters and be too large to handle by centrifugation. Elution from the precipitate is achieved by the use of a buffer at a high pH or with proteinaceous liquids such as beef extract or fetal calf serum at a neutral or elevated pH.

### **1.3 Hydroextraction and aqueous polymer two-phase separation techniques**

In the hydroextraction procedure a dialysis bag containing the sample is placed in a polyethylene glycol (PEG) solution or in a bed of dry PEG. Water and microsolute pass out of the dialysis bag into the PEG but viruses and other macrosolutes are retained and thereby concentrated. This method has been used to concentrate viruses in wastewater samples of 500 ml reduced to a final volume of 5 ml.

A similar degree of concentration has been obtained by the two-phase separation technique. In this process polymers are added to the sample, resulting in the formation of two aqueous phases. Under suitable conditions viruses are concentrated into one low-volume phase.

These methods seem best suited to samples of 0.2–2 l volume. The degree of efficiency is reported to vary from 5% to 100%. If the expected virus load is less than 10 infectious units per litre, then additional steps should be used, such as adding another two-phase separation step and thereby achieving a tenfold increase in the degree of concentration. These methods have proved useful for moderately or grossly polluted water but not for sludge samples. They may also be used as a final step to concentrate eluates from precipitates or filters in large-volume concentration procedures.

Both of these methods are simple and require little technical equipment. However, in the two-phase separation technique, recovery efficiency varies unless the pH level, ionic strength and salt concentration of the samples are carefully controlled. It has been reported that strains of coxsackievirus type B2 and echovirus 6 are inhibited by dextran sulfate 2000 and that phase separation is not always achieved. If the proper conditions cannot be obtained, it is advisable to use hydroextraction instead.

#### **1.4 Soluble alginate filters**

Alginate membrane filters can be made in the laboratory and cast on a filter-paper support. The water sample is filtered through an alginate membrane, which is subsequently dissolved in sodium citrate solution to produce the virus concentrate. The dissolved filter can then be inoculated into cell cultures. Virus losses on or in the filter do not occur because the filter is dissolved. A sample of up to 1 litre may be passed through a 47-mm diameter filter with good virus retention but such a filter is not suitable for larger samples because the filtration rate is too slow.

Pre-filtering of the sample is nearly always necessary because alginate filters clog more easily than do the microporous filters used to concentrate viruses by adsorption. They are also adversely affected by high ionic strengths.

Neither small volumes of unclarified raw waters nor large volumes of highly treated waters can be conveniently processed by this method.



## 2. Methods for large amounts of water (5–400 litres and more)

The examination of large samples of water, which has only been carried out in a few laboratories, requires special techniques and equipment.

### 2.1 Tangential fluid flow ultrafiltration systems

Ultrafiltration is a process of selective molecular separation. Different membrane configurations, such as hollow fibres, flat discs, and cartridges of rolled sheets, are available, but little experience with these configurations has been reported. Only a flat membrane method has so far found general application.

### 2.2 Flow-through filter adsorption/elution systems

Filter adsorption/elution (FAE) procedures are employed for concentrating viruses from water, wastewater and other fluids. These methods are based on the ability of viruses to become reversibly adsorbed to suitable surfaces. Among the filter materials and configurations that have been employed as adsorbents are the following: (a) cellulose nitrate membranes, 0.45  $\mu\text{m}$  porosity, (b) fibreglass cartridge-type depth filter, 0.45–8.0  $\mu\text{m}$  porosity, and (c) fibreglass–asbestos–epoxy filter discs, 0.45–0.65  $\mu\text{m}$  porosity.

In some cases, the water being processed must first be clarified to prevent clogging of the virus adsorbent by suspended matter in the water. For prefiltration (clarification), materials such as a synthetic polyester fibre may be used, or fibreglass that has been treated with Tween 80 or serum, or some similar substance that coats virus-adsorptive sites on the prefilters and thereby prevents virus adsorption. Many of the viruses in water are adsorbed to particulate matter, however, and in these prefiltration systems efforts are not usually made to recover them. A significant proportion of the virus content may therefore be lost. This problem can be partly solved by keeping the prefilter pad over the membrane filter in the same holder and eluting them together. To a large extent, the need for prefiltration has been obviated by the introduction of pleated filters. In these microporous filters, solids are retained without significantly reducing the flow and the associated viruses are subjected to elution procedures.

To enhance virus adsorption, the water is acidified and in some cases a polyvalent cation salt, such as calcium, magnesium, or aluminium chloride, is added. Trivalent aluminium ions may be more efficient than divalent magnesium or calcium ions for increasing virus adsorption. The recent introduction of filters with a net positive charge may obviate the need to use low pH levels and added cations. A method using glasspowder as the virus adsorbent, which has been found useful for river-water and drinking-water samples of 10–30 l, could possibly be employed for larger volumes. However, this method has yet to be tested in different laboratories.

Adsorbed viruses may be eluted from the filters with a small volume of eluent, usually a slightly to moderately alkaline proteinaceous fluid, such as serum, beef extract, or nutrient broth, or a highly alkaline glycine buffer.

Both single and multistage FAE procedures have been developed. In a single-stage process the viruses are adsorbed and eluted once only, while in multistage procedures, this may take place two or more times in succession. In the first stage of a multistage procedure, the water is often treated on a continuous-flow basis, thus making it possible to process larger volumes. At each successive stage of the multistage procedures smaller filters and eluate volumes are employed, thereby achieving greater degrees of concentration than is possible in a single-stage procedure. Organic flocculation may be used in a two-stage process. In general, single-stage FAE procedures can be conveniently used for fluid volumes of up to about 20 l, which makes them suitable for waters that are likely to contain relatively large amounts of viruses, such as sewage, treated sewage effluents, and polluted surface waters. Multistage procedures were developed primarily for processing large volumes of clean water containing relatively small quantities of viruses.

### **3. Elution procedures for samples of fresh and saline waters containing solids**

The most effective eluents currently available are beef extract, serum, and similar fluids that compete with viruses for adsorption sites on the solids. High pH buffers of various compositions are also used but are probably not as effective.

When pleated filters are employed, the viruses adsorbed on to the trapped solids are eluted at the same time as viruses on the filters.

When captured separately on prefilters, solids should be eluted. If the volumes of eluates are large, concentration is necessary, and this can be accomplished by the organic flocculation technique with beef extract, serum and similar substances. Where elution has been achieved by a high pH buffer, further concentration may be accomplished by the FAE procedure or by other processes already described.

#### **4. Methods for sludge samples**

The methods applicable to sludges are similar to those used to recover viruses from solids in water. Thus the viruses may be eluted directly from the sludge sample by means of glycine buffer (pH 7.5-9), or beef extract, or similar organic eluents. Precipitation or flocculation at a low pH level by means of alum or iron(III) chloride followed by elution may give better results on samples containing fewer viruses. Elution at a high pH level cannot be recommended if the sludge contains ammonium ( $\text{NH}_4^+$ ) because of its conversion to ammonia ( $\text{NH}_3$ ), which is virucidal.

The polyelectrolytes employed as sludge thickeners may be used as agents in the recovery of viruses from sludge. If a high molecular polyacrylamide is added to the sludge sample in a concentration of, for example, 100 mg/l, a floc may be obtained which can be filtered through a 1-mm mesh sieve (or through a funnel with a loose cotton plug). The floc may then be eluted with glycine buffer or beef extract, etc. Ultrasonic treatment, possibly in the presence of sodium dodecyl sulfate, can be used to break up the solids. Even in eluates cell toxicity problems may occur, and in such cases passage to new cell cultures may be helpful.

The degree to which viruses trapped within sludge solids are eluted by these procedures is not known.

#### **5. An assessment of current methods**

None of the methods described so far is totally quantitative. More breadth in testing is needed. Relatively few virus types have been studied and only a few environments have been sampled with any given test procedure. The technology is evolving rapidly and comparative tests by workers in different laboratories are needed to achieve standardization and quality control.

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