ABSTRACT

MARK S. R. GRAY. Survival of Hepatitis A Virus, Poliovirus 1 and F-specific Coliphages in Disposable Diapers and Landfill Leachates.

(Under the direction of Dr. Mark D. Sobsey)

Fecally soiled disposable diapers are often placed in solid waste landfills for disposal purposes. However, the public health impact that these disposal methods have on humans that come in contact with fecally soiled disposable diapers or on the environment is not fully known. Fecally soiled disposable diapers and leachates were seeded with hepatitis A virus (HAV), poliovirus 1 and F-specific (F+) coliphages and incubated aerobically and/or anaerobically at 5, 25 and 40 degrees celsius. Samples were taken at specific intervals over a 90-day period and were assayed for surviving viruses. In some cases, viruses survived >90 days (the duration of the experiment) in fecal suspensions and solid waste landfill leachates, especially at low temperatures. In both fecally soiled diapers and leachates all viruses survived longer at lower temperatures, with 1 log₁₀ inactivation in 30 days at 5° celsius under all conditions. At 40° celsius, viruses were reduced to non-detectable levels (>4 log₁₀ reduction) in 10-30 days in diapers and in as little as 3 days in leachates. In some cases, viruses were inactivated more rapidly under anaerobic conditions rather than aerobic conditions. Also, the relative survival of individual viruses within leachate samples was not consistent. HAV had the greatest survival rate in leachate 1 at 5 and 25°C and in leachate 2 at 25°C. Poliovirus survived the best in the other leachate conditions: 40°C in both leachate 1 and 2 and at 5°C in leachate 2. Although virus survival in leachates did not display any general trend according to virus type, comparisons of virus survival in fecal suspensions versus sanitary landfill leachates indicates that viruses survive longer in fecal suspensions by as much as 7-fold. Under most conditions, persistence of F-specific coliphages, HAV and poliovirus was similar, thus suggesting that these phages may be useful enteric virus indicators.

ACKNOWLEDGEMENTS

I would like to thank my academic advisor Dr. Mark Sobsey for taking a chance on me and allowing to do my research for this degree in his laboratory. Also, I would like to thank him for all of his help and support throughout my two long years of research in the Environmental Virology Laboratory. I would like to thank Dr. Ricardo DeLeon for all of his support and guidance during my two years here, and for encouraging me to attend the University of North Carolina at Chapel Hill for my graduate studies. Another necessary thank you goes out to Dr. Fredric Pfaender for taking the time to be one of my committee members and for reading this technical report and attending my oral defense. Many thanks to Doug Wait and Dorothy Thompson for always helping out in the laboratory whenever I needed something. Thank you to Dr. Michael Symons in the Biostatistics Department for helping me with the statistical analysis of my data and showing me that all of my numbers actually did mean something. Another thank you goes out to all of the Dr. Sobsey lab group. I would also like to thank my family and friends for always being there and helping me survive my two years here. Also, a thank you goes out to C.L. Lassiter and Carol Carden for their friendship and help. Mostly, a thank you goes to The Procter & Gamble Company for supporting and funding this research project.

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INTRODUCTION

Diaper-aged infants and children are infected by enteric (gastrointestinal) viruses and can transmit them to others by direct and indirect routes. Fecally soiled disposable diapers are one of many sources for possible transmission. Upon removal of fecally soiled disposable diapers from infants and children, they are disposed of and become part of the solid waste stream. Once in this stream, the fate and transport of fecally soiled diapers is not well documented. It is common to place the solid wastes in which fecally soiled disposable diapers are mixed into solid waste landfills.

From the moment that disposable diapers become contaminated with fecal material through their ultimate deposition in a landfill, there are potential opportunities for fecally soiled diapers to contaminate humans and the environment with viruses. Viruses that may be present in the feces can infect individuals that come in contact with either the fecally contaminated environment, the fecally soiled disposable diapers or the infected infant.

Although fecally contaminated disposable diapers continue to enter the solid waste stream and be deposited in landfills, little is known about the public health consequences of these actions. Therefore, it is important

to learn more about the fate and transport of fecally contaminated disposable diapers in the environment. An important consideration that must be examined is the potential health risks from enteric viruses in fecally contaminated diapers and their interactions with humans that come in contact with them through various routes.

Currently, there are no standards to specifically control the disposal of fecally contaminated disposable diapers. This may be due to the fact that no studies have ever documented viral contamination of groundwater and nearby surface waters from landfill leachates. In contrast, microbial and even viral standards have been placed on wastewaters and sludges that are disposed of into the environment.

Other than for poliovirus, relatively little is known about the survival of some important enteric viruses in fecally soiled diapers under different environmental conditions, including sanitary landfills and leachates. Therefore, the comparative survival of hepatitis A virus (HAV), poliovirus 1 and F-specific (F+) coliphages as indicator viruses was studied under various conditions in both fecally soiled disposable diapers and solid waste landfill leachates.

OBJECTIVES

1. To determine the effect of temperature on the reduction of hepatitis A virus, poliovirus 1 and F-specific coliphages in fecally soiled diapers over time.
2. To determine the effect of temperature on the reduction of hepatitis A virus, poliovirus 1 and F-specific coliphages in solid waste landfill leachates over time.
3. To determine the effect of aerobic and anaerobic conditions upon fecally soiled diapers on virus survival over time.
4. To compare the reduction of hepatitis A virus in fecally soiled diapers and solid waste landfill leachates with that of poliovirus 1 and F-specific coliphages.
5. To determine if there is a difference in virus survival rates between an "older" landfill leachate and a "younger" landfill leachate.
6. To determine whether viruses survive better in fecal suspensions or in solid waste landfill leachates.

REVIEW OF THE LITERATURE

Viruses

Viruses are obligate intracellular parasites that are biologically active with the capability to infect every type of eucaryotic or procaryotic cell. Viruses are able to infect humans, other animals (including invertebrates and protozoa), plants, fungi, actinomycetes, bacteria, blue-green algae and even free living mycoplasmas. They are thought to be the simplest form of life. As non-cellular biological entities, viruses require certain elements for their own replication, and they must depend on a host for these essential components. Viruses range from 20-250 nm in size. They consist of one form of nucleic acid, either DNA or RNA, and a protein coat, also known as a capsid, that surrounds the nucleic acid and serves as protection for the nucleic acid and for binding to host cells. The capsid can be either icosahedral (spherical or cubic), helical or complex. Some viruses have a lipoprotein envelope surrounding the capsid. Viruses are important disease causing agents and important for the study of human infection or the spread of it.

Enteric Viruses

Human enteric viruses are infectious viruses that infect the alimentary tract, are excreted from man and are found in human feces. There are over 100 types of enteric viruses. The Picornaviridae family contains many enteric viruses, including: coxsackie A viruses, coxsackie B viruses, polioviruses, echoviruses, other enteroviruses and until recently, hepatitis A virus. Other enteric virus families are the Reoviridae family with reoviruses, human rotaviruses, the Caliciviridae family including the Norwalk virus, and the Adenoviridae family with the human adenoviruses (Table 1).

Humans are infected with enteric viruses through the fecal-oral routes involving direct and indirect personal contact. Enteric viruses are also spread by contaminated food and water. Once ingested, the viruses are able to infect the alimentary (gastrointestinal) tract, having survived the acidic conditions (low pH) of the stomach. They are shed in the feces and sometimes can be found in quantities as high as 10^{10} viral particles per gram of feces. Most of the enteric viruses, the enteroviruses, hepatitis A virus and caliciviruses are non-enveloped, icosahedral, single-stranded plus sense RNA viruses. Enteric viruses range from 20-100 nm in diameter.

In general, enteric viruses are more resistant to inactivation by environmental factors than are other

viruses, and, hence they tend to survive longer outside the body than most other mammalian viruses. Furthermore, several documented outbreaks of waterborne, foodborne and other environmentally-transmitted viral gastroenteritis and other enteric virus diseases are reported in the U.S. each year (Craun and Levin, 1988, Herwaldt, et al., 1992). Therefore, control of the viruses either before they reach the environment or once in the environment is very important to minimize the risk of environmentally transmitted human enteric virus infections.

TABLE 1

Human Enteric Viruses that may be Present in Feces*

Virus Group	Number of Serotypes	Disease Caused
Enteroviruses:		
Poliovirus	3	Paralysis, meningitis fever
Echovirus	34	Respiratory disease, meningitis, rash, fever
Coxsackievirus A	23	Respiratory disease, herpangina, fever, meningitis
Coxsackievirus B	6	Carditis, meningitis, pleurodynia, fever, respiratory disease
New enteroviruses (types 68-71)	4	Meningitis, fever, respiratory disease, hemorrhagic conjunctivitis, rash, encephalitis
Hepatitis A** (enterovirus 72)	1	Infectious hepatitis
Caliciviridae:		
Norwalk virus	>1	Epidemic vomiting and diarrhea (gastro-enteritis), fever
Hepatitis E virus	1	Infectious hepatitis
Other Norwalk-like viruses	>1	Gastroenteritis
Rotavirus	>4	Gastroenteritis, diarrhea
Reovirus	3	Unknown
Adenovirus	41	Respiratory disease, gastroenteritis, conjunctivitis
Coronaviruses	?	gastroenteritis, necrotizing enterocolitis of newborns

* Modified from Rao, V. C. and Melnick, J. L. (1986).
Environmental Virology.

** Now classified in a separate genus in the Picornaviridae family

Hepatitis A Virus

In 1942, Virchow fed volunteers duodenal contents and was able to transfer hepatitis infections (Ginsberg, 1988). Since that time, hepatitis A virus (HAV) has been classified in the Picornaviridae family. HAV was classified as enterovirus 72 but is now in a separate genus. HAV is 27 nm in diameter with an icosahedral shape. It is a non-enveloped virus that has single-stranded, positive polarity ribonucleic acid of about 7500 nucleotides and a 3' poly-A tract. There are four major polypeptides in the capsids designated VP-1, VP-2, VP-3 and VP-4. The sedimentation coefficient of HAV is 156 to 160S. Its buoyant density in a cesium chloride gradient is 1.33-1.34 grams/cm³.

HAV is one of the most stable enteroviruses within the environment, including in soils, sewage and water (Sobsey et al, 1988). HAV can survive up to 1 hour at 60°C. It is resistant to several disinfectants and organic solvents such as alcohol, diether and Freon^R. Hepatitis A persists at ambient temperatures for several weeks and remains infectious under desiccating conditions. HAV is stable at acidic pH levels such as 3.0 for extended periods of time. To disinfect HAV in drinking waters, a free chlorine concentration of 0.2-0.3 mg/l is needed. Like many other enteric viruses, HAV is quite resistant to typical doses of preformed chloramines. Studies have shown that HAV is persistent in soil, sewage and the water environment.

Hepatitis A virus is spread through the fecal-oral route, and can also be spread through contaminated food and/or water. The most common source of hepatitis infections is close person-to-person contact (Volk et al, 1991). There appears to be one HAV serotype whose antigens react with the same antibody that is worldwide in distribution and endemic in both developing and third world countries.

Once ingested, HAV is able to survive the low pH of the stomach acids, reach the bowels and from there enter the blood stream and reach the liver. The liver is the target organ, where the viruses multiply and necrotize the hepatocyte cells. HAV can be shed in feces at concentrations up to 10^9 infectious particles per gram of feces. Currently, there is no vaccine available for HAV in the U.S., although vaccines are being developed and some are recently licensed in other countries. Once hepatitis A has been acquired through natural infection, a long lasting immunity is developed. Infectious hepatitis caused by the hepatitis A virus has a typical incubation period of 15-40 days. The severity of the disease ranges from an asymptomatic infection with a gastrointestinal illness to an acute infection with jaundice and necrosis of the liver. Viruses are shed in the feces during the incubation period and the acute phase. Virus can also be detected in the blood at the end of the incubation period and briefly during

the acute phase. HAV infections may last several weeks to months. HAV has a common seasonal occurrence in the autumn and the winter. Young children are the target age group for the virus, however most of these infections are asymptomatic. In older children and adults symptomatic illness is more likely. The annual incidence of hepatitis A was >30,000 reported cases in 1991 and the incidence is now increasing. Furthermore, it is estimated that the true incidence of hepatitis A is larger than are reported cases, perhaps by a factor of 10.

Hepatitis A virus was first conclusively isolated in 1973 and it was first cultivated in cell cultures in 1979 (Provost and Hilleman, 1979). Currently, HAV can be cultivated in several different cell lines, however, the main cell line for propagation is a fetal rhesus kidney cell designated FRhK-4. Cytopathogenic strains of HAV have been isolated and they can be detected by several tests including standard plaque techniques as developed by Cromeans in 1987. Radioimmunofocus assays and gene probe assays also have been used.

Poliovirus

Poliovirus is another enteric virus that is a member of the Picornaviridae family. Like HAV, poliovirus is non-enveloped virus with single stranded positive polarity ribonucleic acid surrounded by an icosahedral shaped capsid consisting of four major polypeptides (VP-1, VP-2, VP-3 and

VP-4). Poliovirus has a buoyant density in cesium chloride of 1.33-1.34 g/ml. Characteristic of enteroviruses, polioviruses remain stable in the environment and they are acid stable at a pH of 3.0 for several hours. Poliovirus is relatively resistant at high temperatures for short periods of time, but not as thermostable as HAV.

Poliovirus has a restricted host range in both cell cultures and animals affected by the virus. However, most poliovirus strains can be grown on continuous or primary cell line cultures derived from a variety of monkey or human tissues.

Poliovirus infects individuals through the fecal-oral route by direct or indirect human contact or by contaminated food or water. There are three antigenic serotypes of polio that can be found worldwide. Poliovirus causes poliomyelitis and the infection can manifest in three forms. The abortive form of the disease is the most common form for most people. It is fairly common in young children and may be asymptomatic or it may be characterized by headache, fever, malaise, sore throat and other general illness characteristics. The second form is aseptic meningitis which generally causes meningeal infections. There is stiffness in the back and neck and full recovery usually occurs within one to two weeks. The third form is paralytic poliomyelitis. This form causes damage to the motor neurons in the spinal cord and paralysis occurs. Incubation time

for poliovirus is 3-35 days with a mean of 7-14 days.

As recently as 1958, there were 6000 reported cases of paralytic polio in the United States annually. There are approximately 250,000 to 2 million cases of paralytic poliomyelitis in developing and third world countries (Volk et al, 1991). Due to the use of vaccines, few outbreaks occur in developed countries such as the U.S. The two main vaccine types used are the killed vaccine by Salk and the live vaccine by Sabin. However, live attenuated vaccines have led to high concentrations of vaccine strain polioviruses in fecal wastes and sewage, since the live vaccine allows for fecal shedding of the virus at concentrations as high as 10^6 infectious particle per gram of feces.

Bacteriophages

Bacteriophages are viruses that are able to infect bacteria. Although there are limited ranges of bacteria type that each bacteriophage can infect, there are a vast number of different bacteriophages because of the diversity of bacteria; almost all bacteria are susceptible to at least one bacteriophage.

The potential of using bacteriophages of enteric bacteria as models for the fate of viruses in natural waters and water treatment is great (IAWPRC, 1991). Several different morphological phage types can be found naturally in sewage. One such bacteriophage group is known as male-

specific (F+) coliphages. These phage are able to infect those coliform bacteria that have pili ("male" bacteria) expressed from the F-plasmid. F-specific coliphages in sewage and other environmental samples can be identified through conventional assay techniques using susceptible host cells. One specific host bacterium used is Salmonella typhimurium with an F-plasmid from E. coli K-12 (F' 42 lac::Tn5) inserted into the genome to induce production of pili on the cell membrane (strain WG49). This host does not detect interfering somatic coliphages because it lacks the outer cell membrane receptors for them.

Male-specific coliphages can either have single-stranded RNA or single-stranded DNA for their nucleic acid. The RNA phages belong to the Leviviridae family and they exhibit cubic symmetry and have a diameter of about 25nm. The DNA phages are members of the Inoviridae family, and they are long filamentous phages. These phages are relatively easy and quick to assay in sewage and fecally contaminated water samples. Typical F-specific phage concentrations in raw sewage are 10^2 - 10^4 PFU/ml.

Occurrence, Survival and Fate of Enteric Viruses in the Environment

In many parts of the world untreated or partially treated human wastes are disposed of into the environment. These wastes may contain microorganisms that are potential

health hazards. These microorganisms contaminate the environment directly from sewage, sludge, nightsoil, municipal solid wastes or indirectly through runoff. One major public health concern is associated with human enteric viruses present in domestic drainage and sewage that may survive if introduced into the environment and subsequently cause illness in susceptible persons if they come in contact with and ingest them (Hurst, et al., 1991). Enteric viruses survive present methods of sewage treatment. Conventional primary and secondary treatment reduces enteric viruses by only 90-99.9%. Once they are released into the environment, enteric viruses may persist for several months in natural waters and soils (Hurst, et al., 1980). Enteric viruses have been detected at soil surfaces in fields irrigated with raw sewage and recovered from sewage treatment plant discharges (Hurst, et al., 1980, Tierney, et al., 1977). In many places in the world, untreated or partially treated wastewater is infiltrating through the soil or sand and percolates to underground reservoirs (groundwater) (Lefler, et al., 1974).

Night Soil

Night soil is undecomposed wastes, usually fecal wastes usually from individuals and households, which is collected for treatment and disposal. Night soil is used as a fertilizer for crops and is sometimes treated prior to land application. There appears to be a dearth of information

concerning the survival, fate and public health risks of viruses in night soil. It is thought that some human enteric viruses may survive nightsoil treatment and get redistributed through the resulting slurry. There is no conclusive evidence that enteric viruses are completely inactivated during anaerobic digestion (Gadre, et al., 1986). One study using Salmonella and Ascaris ova noted a higher incidence of ascariidiasis in farmers who used night soil rather than sewage-irrigated water (Cai, et al., 1988).

Sewage

Wastewater can either be disposed of to the environment without treatment or by going through a series of treatments such as primary, secondary or tertiary treatment. Raw sewage has an estimated average density of enteric viruses of approximately 7,000/Liter (Straub, et al., 1992). Primary treatment of raw sewage removes suspended solid particles which are the main components for virus sorption. About 50-60% reductions of virus concentrations occur in this process. Secondary treatment involves biologic treatment of the wastewater. During secondary treatment, approximately 90-99.9% reductions of viruses occurs. Even after being treated, sewage contains viable virus particles. Enteric viruses have been detected in soils and waters where sewage has been released. One study noted that where sewage effluents with viruses were disposed of on land in the winter, it took roughly 4 months to reduce virus populations

to undetectable levels (Tierney, et al., 1977). Adequately treated wastewater has many potentially beneficial uses. One such use is for agricultural purposes by spray irrigation, drip irrigation, overland flow or infiltration basins. In some cases, treated wastewater is used to recharge groundwater by subsurface injection systems in order to replenish aquifers that are being depleted in that area. All of these applications pose a potential risk of releasing enteric viruses into the environment if viruses are not adequately removed or destroyed prior to disposal or land application.

Sludge

Sludge probably contains the highest concentration of viruses due to the fact that viruses are concentrated into sludge during treatment but are not necessarily rendered inactive (Goyal, et al., 1979). Virus reduction in sludge treatment can be ineffective and this signifies a potential public health problem. Old practices included dumping sludges into oceans. Today, this method is hardly used and in the near future will no longer be allowed for the disposal of sludge. One of the best conventional methods to treat sludge is anaerobic or aerobic digestion which reduces virus concentrations by as much as $>3 \log_{10}$. Sludge can be incinerated, although there are concerns about air pollution and residuals such as ashes. Sludge can be disposed of with solid wastes in landfills by several alternatives:

1. Co-disposal which mixes sludge with solid wastes;
2. Top cover which is covering the surface of the solid waste landfills with sludge; and
3. Monofill which is disposing only sludge into a landfill.

Land application of sewage sludge is a common practice. The sludge is spread onto land in a liquid or dry state, primarily for agricultural purposes. Currently, the Environmental Protection Agency (EPA) is setting new standards on sludge treatment and disposal and proposes that sludge can only be disposed by unrestricted land application if there is <1 virus per either 4 grams or 1 gram of total solid dry weight sludge solids. To achieve these standards, sludge must be treated further than conventional digestion. Lime treatment of sludge raises the pH ≥ 11.5 and destroys viruses and other microorganisms. This process also is compatible with agricultural use of the sludge. As an alternative to liming, sludge can be treated with waste ash such as alkaline ash, fly ash or gypsum to raise pH levels and reduce the moisture content. Composting sludges uses aerobic, biological processes to generate heat up to $55-65^{\circ}\text{C}$ and to dry the sludge. For composting, the sludge is placed in mound formations or in wind rows or the process is done in engineered vessels. However, treatment does not always guarantee complete inactivation of viruses. One study recovered viruses from a sludge burial site in Montana 6

months after the last sludge disposal (Straub, et al., 1992). This indicates that enteric viruses can persist in composted sludge unless the composting process is designed and operated efficiently.

Municipal Solid Waste

Potential sources of enteric viruses in municipal solid waste are from soiled disposable diapers, pet feces, food waste, other hygienic household wastes, wastewater sludges and infectious wastes from the health care industry (Sobsey, 1989). There is a concern that domestic solid waste material may contain large amounts of untreated human and animal excrement and that enteric viruses may find their way from sanitary landfill into surface waters or ground water (Cooper, et al., 1974). Due to the heterogenous nature of municipal solid wastes, infectious agents may be present in material with fecal matter and this has an inadequately known impact on the environment (Engelbrecht, et al., 1974). Potential hazards from pathogenic agents reaching sanitary landfills is a function of 3 conditions.

1. The amount and nature of pathogens initially placed in the landfill;
2. The ability of pathogens to survive or retain their infectious properties under landfill environments;
3. The ability of pathogens to move through landfills into the surrounding environment and be a potential hazard to man (Engelbrecht, et al., 1974).

Therefore, an increased concern for the fate of pathogens, especially enteric viruses in landfills from municipal solid

wastes is necessary in order to adequately assess their potential risks to human health.

Landfills

The use of landfills has become more widespread since their development in the 1960's. Before sanitary landfill development, most solid waste refuse was deposited in dumpsights. Due to their unsightliness, overwhelming infestation by vectors such as flies and rats, along with smoke from dump fires and foul odors, the development of sanitary landfills came about. In the U.S. alone, approximately 141-190 tons of municipal solid waste is generated per year and the majority of this waste is landfilled (Sobsey, 1989, U.S. EPA, 1984; 1988a; 1988b). Public health concerns about the fate of enteric organisms in fecal material once in landfills has led to increased research in this area, primarily in the 1970's.

Soiled Disposable Diapers in Landfills

A number of infectious agents may be present in solid wastes from many sources including soiled disposable diapers. The impact of soiled disposable diapers on the microbiology of solid waste landfills and landfill leachates has been a public health concern for more than 2 decades (Sobsey, 1985). Moreover, there has been concern that enteric viruses from fecally soiled diapers will persist and perhaps migrate through landfilled solid wastes. About 0.7-2.7% on a dry weight basis and 0.6-3.1% on a wet weight

basis of municipal solid waste is soiled disposable diapers (Sobsey, 1989). About 2 million tons of soiled disposable diapers are disposed of annually with one-third of these soiled with feces (Pahren, 1987).

Virus Survival in Landfills and Landfill Leachates

There have been a number of studies, both field and laboratory, on the survival and transport of enteric viruses in solid waste landfills and landfill leachates. Most results indicate that virus survival and recovery is low. In one field study, enteric viruses of poliovirus type 1 and poliovirus type 3 were found in only one of 22 leachate samples examined from 21 different landfill sites in the U.S. and Canada (Sobsey, 1978). However, another study using a lysimeter seeded with poliovirus simulating landfill conditions still detected viruses after 76 days (Engelbrecht, et al., 1974). Viruses have been detected in leachates from lysimeters rapidly brought to field capacity. Lab scale municipal solid waste lysimeters with simulated refuse and seeded with either lab or field strains of poliovirus type 1 and echovirus type 7 had no detectable viruses in lysimeter leachates over a 4 month period or in the refuse after termination of the experiment and analysis of lysimeter contents for viruses. One reason for the wide variability in results may be due to detection methods. Viruses may sorb to particles in the lysimeter, landfills or leachates and they may become neither inactivated nor

detectable. No information was found in the published literature concerning hepatitis A virus survival in solid waste landfills or landfill leachates. Most previous studies on viruses in landfills and leachates were done with easily cultured enteroviruses (e.g. poliovirus or cytopathogenic culturable viruses in field samples).

Environmental Factors

Enteric viruses are quite persistent in the environment and even more so than most other mammalian viruses. Enteric virus survival potential is a function of interacting biological and physical factors (Rhodes, et al., 1988). Several factors that influence the survival of enteric viruses in the environment (water, soil, air and surfaces) are:

1. Temperature
2. pH
3. Light and solar radiation
4. Dissolved organics
5. Particulates
6. Drying and desiccation (moisture)
7. Natural microbial activity

Temperature

In general, most microbes survive better at low temperatures and temperature is one of the most important factors for enteric virus survival in the environment. Virus inactivation is temperature dependent, and enteric viruses can remain infectious for many weeks in the environment, especially at low temperatures (Feacham, et

al., 1983). Experiments conducted by Berg, et al., (1978) showed higher virus inactivation at thermophilic conditions over mesophilic conditions. Experiments by Straub, et al., (1992) showed that MS2 inactivation nearly doubled as temperatures increased from 15°C to 27°C in sludge-amended soils. No virus was recovered after 24 hours at 40°C in these same experiments. Other experiments have produced similar results showing more rapid inactivation times for HAV at 35°C and longer survival at 20°C and 5°C (Mbithi, et al., 1991).

In several different experiments with different viruses and various conditions, it was noted that temperature was a major factor for virus inactivation. At room temperature or lower, HAV remains infectious for several weeks, even under desiccating conditions (Caton, 1986, Melnick, 1982, Frosner, 1984, Parry, et al., 1984). Other experiments by Sobsey, et al., (1975) provide results showing that poliovirus inactivation is highly temperature dependent in lysimeter leachates. Field studies conducted by Tierney, et al., (1977) further confirm the temperature-dependency of virus inactivation by detecting poliovirus in soil that had been flooded with spiked, treated wastewater or sewage sludge 96 days after flooding in the winter and only 11 days after flooding in the summer.

Studies by Hurst (1988) showed virus survival at both aerobic and anaerobic conditions in sterile and non-sterile

conditions to be greatest at 1°C (152-588 days) and least at 37°C (1.4-3.5 days) with 23°C (10-33 days) in between the two.

Desiccation (Moisture Content)

The inactivation of viruses in the environment is dependent on desiccating or drying conditions (moisture content). Non-enveloped viruses survive better under drying conditions or in low relative humidity much better than enveloped viruses. By being able to withstand desiccation, enteric viruses have the ability to persist longer in the environment. Lefler, et al., (1974) found that extreme dryness did not eliminate poliovirus type 1 and f₂ bacteriophage for up to 77 days of incubation at 4 to 8°C.

Hepatitis A virus has been found to survive well in dried feces (Mbithi, et al., 1991, McCaustland, et al., 1982). It was also found that HAV survived better at low relative humidity levels than at high or ultrahigh relative humidity levels and that HAV survives better than poliovirus (Mbithi, et al., 1991). Furthermore, once dried on to polystyrene surfaces HAV could still be detected after one month (Mbithi, et al., 1991, Sobsey, et al., 1988).

Despite virus persistence upon drying, some virus inactivation occurs upon drying. For example, more rapid virus inactivation occurs in dried soils than in wet soils. Poliovirus type 1, MS2 bacteriophages and PRD-1 bacteriophages experienced rapid losses of infectivity when

dried to less than a 5% soil moisture concentration (Straub, et al., 1992). Other experiments found that when soil moisture was evaporated to 2.9% (wt/wt), rapid loss of virus infectivity occurred. Enteric viruses survived longer in soil with a 10% moisture content by 3 to 6 times over air-dried soil (Sobsey, et al., 1987, Bagdasaryan, et al., 1964). Since reported results vary greatly, other factors probably play important roles in virus inactivation in soils and on other surfaces.

Soil

Many studies dealing with virus survival in the environment were conducted in soils. Soils influence the survivability of viruses mainly due to sorption. A combination of soil type, soil moisture and soil temperature are important in determining the rate of inactivation (Straub, et al., 1992).

There are three main types of soils with respect to texture or particle size. They are classified as sand, silt and clay. Viruses sorb differently to different soil types and are offered protection from other factors by the soils. One study noted that sludge-amended clay loam soils offered more protection than sludge-amended sandy loam (Straub, et al., 1992). Bacteriophage MS2 survived longer in sea water when associated with kalonite clay than in clay-free seawater (Sobsey, et al., 1987, Gerba, et al., 1975). Poliovirus was protected from inactivation by heat ,

microorganisms and certain salts when associated with estuarine sediments (Sobsey, et al., 1987, LaBelle, et al., 1982).

However, other studies have found that soils don't always enhance survival of viruses, but may lead to more rapid inactivation. One study showed that poliovirus type 1 and reovirus type 3 in aqueous suspensions of eight different soil materials did not always survive longer than in soil-free suspensions (Sobsey, et al., 1987, Sobsey, et al., 1980). HAV, echovirus type 1, poliovirus type 1 and MS2 were studied in miniature soil columns and determined to be greatly influenced by soil type in terms of survival and transport (Hall, 1991).

Microbial Activity

Other microorganisms in the environment can create antagonistic conditions that inactivate viruses in the environment at a more rapid rate. Hurst (1988) reports that virus survival in soil can be greater under sterile conditions than non-sterile conditions. Using different soil types at different temperatures, viruses appeared to be adversely affected to a greater extent by aerobic organisms and there was a greater inactivation rate in all non-sterile soils exposed to different temperatures. In wastewater treatment, biologic activity is one of the most important factors in secondary treatment contributing to the reduction of enteric viruses.

Adsorption and pH

As mentioned earlier, viruses have the ability to sorb to particles (such as soils and solid waste components) and in some cases will be protected from inactivation. Virus adsorption on clays is well documented. By reversing adsorption of bacteriophage T₂ and poliovirus type 1 to clays, Carlson, et al., (1968) found fully infectious viruses. In experiments addressing virus detection in leachate samples, viruses were not detected until a chelating agent (EDTA) was added to the samples to prevent blocking and sorption of virus particles (Sobsey, et al., 1974).

The direct effects of different pH levels on virus persistence indicate that enteric viruses can survive in low pH conditions (pH 3.0) much better than other viruses. However, most enteric viruses survive best near neutral pH (7.0) and within the range of pH 5.0 to pH 9.0, which is typical for most environmental waters. However, some enteric viruses, notably enteroviruses and HAV, can withstand low pH levels such as pH 3.0 for over one hour.

Other Environmental Factors

Sunlight (UV light) and solar radiation inactivates microbes if the UV rays can reach the target sites for inactivation in the organism. Less inactivation occurs in turbid waters. Studies by Battigelli (1992) show that UV disinfection of drinking waters is somewhat effective in

reducing certain enteric viruses in waters of high quality, with doses of 25mWsec/cm² being more effective than 16mWsec/cm² (the current recommended standard) on HAV and coxsackie B5 virus.

Dissolved organics and particulates may either protect enteric viruses from inactivation or promote inactivation of enteric viruses. For example, proteolytic enzymes or heavy metal particles increase inactivation of enteric viruses. Other organics may enhance microbial growth and create antagonistic conditions while other organics and particulates may be protective and absorb UV light (such as humic and fulvic acids and mineral clays).

Enteric Viruses in Institutionalized Settings

Even though sanitation and sanitary conditions in the United States are of high quality, community and more localized outbreaks of gastrointestinal illnesses still occur. Many of these outbreaks are caused by such enteric viruses as rotaviruses and hepatitis A virus. These agents are common causes of outbreaks in institutional settings such as hospitals, schools, nursing homes, mental institutions, family day-care homes and day care centers (Mbithi, et al., 1990, Ansari, et al., 1988, Hadler, et al., 1980).

Rotavirus infections, which mainly infect young children 4 months to three years old, are an important

problem. Rotaviruses account for one-half of all hospitalized cases of diarrhea in children 6 to 24 months of age and feces of infected children may contain 10^9 viral particles per gram.

Enteric Virus Infections in Day-Care Centers

Day-care centers appear to be important in the spread of enteric viruses in the United States. This is a very important public health concern due to the large and increasing number of infants and young children that are cared for in day-care centers. There has been a continual increase in the use of day-care centers, and with this increase, an increase in reports of infectious illnesses among children in day-care centers has been observed (LaBorde, 1991). In day-care centers, infectious enteric diseases are the second most reported illnesses occurring both sporadically and in outbreaks (LaBorde, 1991). Many of these outbreaks have been attributed to enteric viruses such as hepatitis A virus or rotaviruses. Hepatitis A virus has emerged as a common problem in day-care centers during a time of rapid growth of day-care centers in the United States and is a significant health problem in these places (Hadler, et al., 1986). These outbreaks of hepatitis A are quite common throughout the United States, with those in day-care centers reported from more than 25 states in a 10 year period. These outbreaks occur due to hepatitis A virus stability in the environment, its fecal-oral mode of

transmission and the nature of the population at risk. Specific control measures have been advised and/or implemented to help decrease the spread of hepatitis A virus and other enteric pathogens in day-care centers. Until the occurrence of HAV decreases in day-care centers, it is reasonable to state that day-care centers are important in the spread of hepatitis A virus in the United States (Hadler, et al., 1980).

Enteric Virus Survival in Day-Care Centers

Many enteric viruses are able to survive in the environment for extended periods of time and remain infective until such time that a potential host comes in contact with them. As previously noted, many enteric viruses are resistant to heat, drying, disinfection and other environmental antagonists, and this increases the probability of exposure to enteric viruses from environmental sources. Another factor that increases the transmissibility of enteric viruses is the fact that low infectious doses (as little as one or a few cell culture infectious units) are sufficient to cause infection and possibly illness, either symptomatic or asymptomatic. Hepatitis A virus has the capability of surviving up to one month on a contaminated object (fomite) at ambient conditions in the environment. One advantage that enteroviruses have over other microorganisms in terms of transmission via environmental routes is that they can be

transmitted through fecally contaminated hands and objects without having to first multiply in a medium such as food (Laborde, 1991).

Transmission of Enteric Viruses in Day-Care Centers

Transmission of enteric viruses, including hepatitis A virus, in day-care centers has been documented. Studies are finding that transmission of hepatitis in day-care centers is more common than previously suspected. This prevalence may be due to the many different potential pathways for virus transmission. Because many enteric pathogens including viruses are associated with fecal material, contact with feces present a potentially high risk for becoming infected. Diaper changing has been suggested as the highest-risk procedure for transmission of enteropathogens among day-care children and personnel (Butz, et al., 1990, CDCIDS Group, 1984, Hadler, et al., 1980). The spread of hepatitis A is facilitated in children in diapers through contact with other children (LaBorde, 1991). Diaper-aged infants appear to be the predominant individuals for initiating the spread of enteric infections. Person-to-person transmission of enteric disease appears to be highest among children who are mobile but not yet toilet trained (Sobsey, 1989). The rate and extent of transmission in this age group is a function of:

1. Asymptomatic infection rates (causing the presence of the disease agent to go unnoticed initially) and
2. Quality of hygiene and toilet habits of this age group (Hadler, et al., 1980).

Since many infants may have asymptomatic infections, hepatitis A virus or other enteric virus outbreaks ("silent epidemics") might not be observed until a larger outbreak occurs in which other individuals then exhibit frank symptoms of illness. Transmission may occur through many routes. Direct person-to-person contact of infected individuals can lead to the spread of the virus, and is considered the most important pathway in the United States in general and in day-care centers in particular (Hadler, et al., 1986). Fomites are another vehicle for transmission of viruses and other pathogens. Since virus stability in the environment and on objects has been documented, contact with any contaminated surface or object has the potential to lead to infection. Children who have poorer sanitary habits may be more likely to become infected by fomites mainly through oral exploration of contaminated surfaces (LaBorde, 1991). Enteric viruses have been found on the hands of care-givers and on environmental surfaces (Sobsey, 1989). These findings indicate the need to further improve hygienic conditions in day-care centers and other high risk settings.

Target Populations for Enteric Virus Infections in Day-Care Centers

Many outbreaks of enteric viruses, particularly hepatitis A virus outbreaks, have been linked to day-care centers. One study that was conducted in Phoenix, Arizona reported that over a 10-month period, 40% of the reported

cases of hepatitis type A or viral hepatitis of unspecified type occurred in persons closely associated with day-care centers and 44% of patients with hepatitis A were associated either primarily or secondarily with day-care centers (Hadler, et al., 1980). Considering a broader view of enteric infections, infectious diarrhea was 30% more common among children attending day-care centers than among those at home or in family day-care homes (LaBorde, 1991).

Not only were infectious hepatitis A outbreaks associated with day-care centers, they could be linked to specific types of day-care centers. Hepatitis A outbreaks occur primarily in centers that enroll infants and toddlers who are in diapers (Hadler, et al., 1986). These statistics implicate not only day-care centers with young children but particularly those which include diaper-aged infants. When discussing enteric illnesses in general, diaper-aged day-care center children are at the greatest risk of enteric illness, with illness rates as much as 17 times higher in 0-2 year-olds than in 3-5 year-olds (Sobsey, 1989, Sullivan, et al., 1984). LaBorde (1991) states that enteric infections are the highest in children less than three years of age and higher attack rates occur in children 0-12 months. Besides being highly susceptible to enteric illness, diaper-aged infants are very important in the role of transmission of enteric viruses. The primary role of children aged one to two years in outbreaks in day-care

centers has been documented. Day-care centers with diaper-aged children less than two years of age have a 70% risk for the spread of the infection as opposed to a 14% risk for the spread of the infection in day-care centers only with children greater than two years of age (Hadler, et al., 1986).

Diaper-age children increase the spread of infections not only to each other but also to day-care center employees and other people that may come in contact with them. Hadler, et al., (1980) states that younger children transmit enteric infections more frequently than older children, to one another and to employees in the center and adults at home. This may be attributed to diapers, fomites and the ability of viruses to survive and be readily spread via feces in this environment. The spread of HAV from young children to their adult caretakers is not limited to day-care centers; it occurs in orphanages and mental health institutions and may be an important source of sporadic hepatitis in urban communities (Hadler, et al., 1980). In some cases, children and day-care center employees are not affected as drastically in terms of clinical illness as parents or other contacts. In two series of outbreaks, 15% of the clinical cases of HAV occurred in employees of day-care centers, 5-15% in children in day-care centers and the majority of the cases (70-80%) in contacts of children, usually parents and other household contacts (Hadler, et

al., 1986, Hadler, et al., 1980, Storch, et al., 1979).

This information indicates three characteristic features:

1. Children have mild or asymptomatic infections
2. Adults are the major group with clinical hepatitis
3. Persons having contact with one or two year-old children run the highest risk of infection (Hadler, et al., 1986)

This evidence indicates that day-care centers are a major potential public health risk for the spread of hepatitis A infections within day-care centers and into families and the general community. This risk should be studied more carefully and controlled more adequately.

Methods to Control Enteric Infections in Day-Care Centers

Some control measures to minimize the risk of enteric diseases in day-care centers are summarized below.

Measures needed to minimize the risk of enteric disease transmission in day-care centers
(Hadler, et al., 1986)

1. Hygienic standards*
 2. Maintenance of proper facilities
 3. Regular cleaning of environmental surfaces
 4. Efforts to limit contacts between children of different age groups
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* See next page for hygienic standards

Hygienic standards to minimize the risk of diseases in day-care centers (modified from Sobsey, 1989)

1. Frequent handwashing
 2. Diapering hygiene
 3. Disinfection of fomites and surfaces
 4. Food handling hygiene
 5. Sufficient space to prevent over crowding; separation of spaces for different activities (food preparation, diapering, toileting, etc.)
 6. Policies to deal with infected and ill children with respect to physical isolation and removal from the facility.
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Health Risks of Soiled Disposable Diapers to Solid Waste Handlers

Once soiled disposable diapers are discarded in the trash, they no longer are a threat of infection in day-care centers unless there is an opportunity to tamper with the trash. However, soiled diapers are collected and then combined with other wastes to become part of the municipal solid waste stream. Now, contact with soiled disposable diapers poses a potential hazard to those workers in the municipal solid waste activity that may come in contact with them. There are few definitive sources of information dealing with health risks and health problems in municipal solid waste handlers when exposed to soiled disposable diapers. However, it is thought that the risks are minimal. Once deposited in the trash, the fecal material is usually confined and the probability of contact with it is low.

Also, soiled disposable diapers are only one component of a larger solid waste stream which may contain other refuse that poses more of a public health risk than soiled disposable diapers (Sobsey, 1989). This does not eliminate the risks associated with soiled disposable diapers. Fox, et al., (1974) proposes that an index of risk due to diaper-derived enteric virus infections can be monitored by observing increased infections from these viruses in waste handlers. Nevertheless, there are no instances in the literature that indicate that the use of disposable diapers was an occupational health hazard to sanitation workers (Pahren, 1987, Shapiro, et al., 1971).

On a broader scale considering more exposure sources than soiled disposable diapers, the risks that handlers face from general exposure to municipal solid wastes, wastewater and sewage sludge indicates little or no documentable increased risk of enteric illness among those workers or the community in general (Sobsey, 1989). One epidemiological study done on workers and residents at the Upper Ottawa Street Landfill in Hamilton, Ontario, Canada showed no association between landfill exposure and any gastrointestinal, hematologic or genitourinary conditions (Sobsey, 1989, Hertzman, et al., 1987). Other factors may pose public health threats such as dusts and leachates from sanitary landfills. Large numbers of microorganisms, including pathogens, were found in both solid waste and

leachate samples (Pahren, 1987, Donnelly, 1983). Lundholm et al., (1980) found that workers at a compost plant were affected by dust particles containing gram-negative bacteria. No links to enteric illnesses from viruses in dusts at solid waste landfills have been documented. Overall, exposure to soiled disposable diapers by municipal solid waste workers and exposure to municipal solid wastes by workers and the community in general do not appear to cause adverse health effects or an increased risk of illness due to enteric microbial pathogens, including enteric viruses (Sobsey, 1989).

Enteric Viruses on Surfaces and Susceptibility to Disinfectants

One of the most important factors in the transmission of enteric viruses in day-care centers is how the viruses are spread. Enteric viruses can contaminate hands and surfaces in day-care centers and remain viable over extended periods of time. Virus-contaminated hands play an important role in virus transmission by fecally contaminated environmental surfaces and fomites (Mbithi, et al., 1991, Ansari, et al., 1988, Cohen, et al., 1987, Levy, et al., 1975). Viruses on surfaces and fomites not only remain viable, they are transferable and can go from hands to fomite to other hands, thereby infecting persons who come in contact with contaminated surfaces. Enteric viruses have

the capability to persist on environmental surfaces even under drying and desiccating conditions. The viruses that survive under these conditions are not as easily inactivated or disinfected as other states of enteric viruses. Research on the relative efficacy of disinfectants for enteric viruses on surfaces versus in suspension has shown that enteric viruses on surfaces are better able to withstand exposure to some commonly used disinfectants. This highlights the problem of how to control these viruses and prevent their spread via fomites and surfaces.

Transfer of viruses between skin and inanimate surfaces has been documented for several viruses (Springthorpe, et al., 1990, Ansari, et al., Cliver, et al., 1984, Hall, et al., 1980, Pancic, et al., 1980). Surfaces can be classified as nonporous inanimate, porous inanimate and animate. Studies by Ansari et al., (1988) found that a variety of porous and nonporous inanimate surfaces can act as rotavirus donors or recipients in institutional settings. Day-care centers fall into this group of institutional settings. Not only are surfaces a site where rotaviruses may be transferred to or acquired from but rotaviruses can retain their infectivity for several hours on the skin and the transfer of rotaviruses from hands to animate and inanimate objects has been documented to occur after 60 minutes (Ansari, et al., 1988).

In day-care centers, hands are frequently contaminated

and enteric viruses such as rotaviruses are able to remain infectious for several hours and lead to the contamination of surfaces (Sattar, et al., 1986, Springthorpe, et al., 1990). Viruses are able to contaminate surfaces by:

1. Direct contact deposition from the contaminated secretion or excretion of an infected host.
2. Transfer via other animate or inanimate surfaces.
3. Deposition from contaminated fluids in contact with the surface.
4. Deposition from large or small particle aerosols. (Springthorpe, et al., 1990).

Infectious rotaviral particles have been recovered from naturally contaminated and frequently handled surfaces in day-care centers (Keswick, et al., 1983). One experiment documented that at 20, 60, and 260 minutes after rotavirus inoculation, 57, 42.6 and 7.1%, respectively, of the input infectious virus could be recovered from contaminated fingers (Ansari, et al., 1988). Since rotavirus can survive several hours on hands and is readily transferred between contaminated and clean surfaces on contact, the potential for becoming infected is very high (Springthorpe, et al., 1990; Ansari, et al., 1988). Rotaviruses are only one group of the many important enteric viruses in day-care centers or other institutional settings.

Hepatitis A virus is easily transferrable between surfaces and is able to persist in the environment for extended periods of time. Hard environmental surfaces and fomites in day-care centers are potential vehicles for

prolonged periods of time after being contaminated by hepatitis A virus (Mbithi, et al., 1991). The hepatitis A virus is able to survive well in dried feces (McCaustland, et al., 1982). In other studies, when hepatitis A virus was dried on polystyrene surfaces, it could still be detected after one month at both 5°C and 25°C (Sobsey, et al., 1988). Since HAV survives in dried feces and on surfaces and is easily transmitted from one surface to another, HAV has the potential to infect any person who is exposed to this environment.

One commonly used practice is the use of disinfectants to clean up surfaces, objects and hands after contact with fecal material or potential contact with feces when changing diapers or any other similar activity in day-care centers. However, non-enveloped viruses, mainly enteric viruses, differ markedly from one another in their sensitivity to many disinfectants (Springthorpe, et al., 1990), and some are not readily inactivated by many types of commonly used chemical disinfectants (Ansari, et al., 1989, Springthorpe, et al., 1986). Studies have shown that the majority of chemical disinfectants now in use in both institutional and domestic environments do not inactivate HAV and therefore are ineffective in the decontamination of HAV-contaminated hard surfaces (Mbithi, et al., 1990). Rotavirus proved even more resistant to disinfection when dried on nonporous inanimate surfaces (Ansari, et al., 1989, Lloyd-Evans, et

al., 1986). Since many disinfectants must come in contact with contaminated hands and skin, powerful disinfectants are not useable in day-care centers. Studies by Mbithi et al., (1990) discovered that 2.0% glutaraldehyde, QAC with 23.0% HCl and sodium hypochlorite with free chlorine greater than 5,000 ppm worked sufficiently for the inactivation of HAV on surfaces. Of the three disinfectants found to be the best, glutaraldehyde is commonly used for medical instruments and appliances, QAC (with a pH of 0.4) is recommended for toilet bowls, urinals and enamel surfaces and sodium hypochlorite is used for general purpose disinfection. However, chemical disinfection of environmental surfaces must not be considered an effective control measure for inactivating viruses in day-care settings because the preferred disinfectants (glutaraldehyde, acidified QAC and free chlorine) are potentially too hazardous for routine and widespread use. Enteric viruses that contaminate environmental surfaces from fecal sources and persist pose a public health risk and to the extent possible need to be controlled at the source. However, the technology to destroy or inactivate viruses in disposable diapers or at the source (on the child) does not exist at the present time and has not been incorporated into the design and composition of the product. This means that fecally soiled disposable diapers represent one potential source of exposure to human enteric viruses that must be adequately

characterized and properly controlled. This study is intended to better characterize the survival and persistence of some important human enteric viruses and indicator viruses of diaper origin in the environment.

MATERIALS AND METHODS

Disposable Diapers and AGM

Pampers medium disposable diapers for girls manufactured by Procter & Gamble were used. The diapers were from lot number 19IOLHA. Diapers had 16mm diameter disks punched out of them from the rear portion of the diaper. These disks were then weighed and later mixed with a fecal suspension that was already spiked with viruses.

AGM (absorptive gel material) samples were provided by the Procter & Gamble laboratories in Cincinnati, Ohio. AGM is the absorptive material that is found within disposable diapers. Samples of AGM aqueous substance number W2392.01 were weighed out and used in the preliminary experiments.

Sanitary Landfill Leachates

Three different sanitary landfill leachates were provided by Procter & Gamble for use in this study: BFI-Bigfoot Run leachate from a young landfill, Glen Willow landfill leachate from a mid-age landfill and BFI-Laubscher leachate from an old landfill. Only the "older" and "younger" landfill leachates were used for the survival experiments. The two landfill leachates were from midwest

landfills from Ohio and Indiana. Both landfills were constructed in the 1970's (15-20 years old) and were built with typical design features for that period. They both have liners and a leachate collection system. The "young" leachate comes from a landfill area that is <2 years old, while the "older" landfill leachate comes from a collection area that is >15 years old. Physical and chemical analysis of the leachate samples was conducted by Weston-Gulf Coast Laboratories, Inc. at University Park, Illinois (Table 2). When samples were received, the openings to the containers were wrapped with parafilm M (American Can Company, Greenwich, Connecticut) and then immediately stored at 4° celsius.

Table 2. Results from the Physical-Chemical Analysis of Solid Waste Landfill Leachates

Parameters	RESULTS*	
	"young" No. 2	"old" No. 1
Alkalinity	1870	1190
BOD 5 Day	9300	300
COD	8800	500
Hardness	2630	1020
Ammonia, as Nitrogen	73.7	113
TKN	105	120
Total Organic Carbon	2880	175
TOC Test 2	2850	179
pH	5.9	6.2
Phosphate, as P	3.2	0.84
Sulfate	394	25
Specific Conductance	6150	5020
Total Solids	7820	3140
Total Suspended Solids	150	250
Turbidity	1150	3200
Volatile Acids	2570	28.6
Calcium, Total	780	207
Iron, Total	250	55.3
Magnesium, Total	166	123

* All results are in units of mg/L except for turbidity (NTU), specific conductance (umhos) and pH (pH).

Fecal Samples

All fecal samples were obtained from one source (a healthy female infant) and delivered to the lab by Dr. Ricardo De Leon. Samples were collected on Pampers disposable diapers, brought to the laboratory and stored at 4° celsius. Fecal samples were removed from the diaper and weighed. The fecal samples were mixed with phosphate buffered saline (PBS) to achieve a 20% feces wet weight-to-volume concentration.

Determination of Diaper Size, Recovery Procedure and Concentration of Fecal Suspensions

A preliminary study was carried out for determination of basic test procedures and parameters. Three diaper punch sizes, 60, 25.4 and 16 mm in diameter, were studied in preliminary experiments to determine a diaper punch size that would be appropriate for the subsequent research. Replicate punches from the rear portion of a diaper were removed, weighed and tested for absorption of distilled water. Diaper punches were placed in 50ml conical centrifuge tubes and various amounts of distilled water were added to each sample and left for 1 hour. At various intervals within the hour, more water was added in order to determine the maximum amount of liquid absorption that was possible. An appropriate size diaper punch to be used was determined along with an optimal amount of liquid to be added during the experiments.

The initial method tested for recovery of the absorbed liquid was centrifugation. A 50ml conical centrifuge tube with a 16mm diameter diaper punch and distilled water was centrifuged for ten minutes at 5000g. Extraction by syringe treatment was tested as an alternative method. A 60cc syringe was packaged with a small amount of glass wool (approximately .015g) to cover the outlet. A coarse grade porous (fritted) polypropylene support plate (disk; Porex Technologies), 3mm thick and 26mm in diameter, was placed over the glass wool to retain particulates. Next, approximately 0.190 g of glass wool was placed on top of the filter to prevent larger particles from clogging the polypropylene filter. The diaper punch-liquid mixture was poured into the syringe. The liquid phase was separated from the solid phase by physically squeezing it through the filter using the syringe plunger and collecting it in a 50ml conical centrifuge tube.

A workable concentration of the fecal suspension was determined. Various weight-volume ratios of fecal suspension were added to a 16mm diaper punch up to the optimal liquid absorption volume that was previously determined. The fecal suspension plus diaper was mixed and allowed to sit for one hour. After one hour, the samples were placed into a syringe assembly as described above and the liquid phase was separated from the solid phase. The suspension used was the maximum percent concentration of

feces wet weight in PBS that could be filtered with recovery of the majority of the liquid and without clogging the filter.

Preliminary Virus Inoculation and Recovery Experiments

Experiments were done to determine the extent to which test viruses in feces were retained or inactivated by diaper or adsorptive gel material during a short-term (1 hour) contact period. This contact time was chosen because it is long enough to achieve equilibrium or quasi-equilibrium physical adsorption.

Aliquots of 100ml of 20% fecal suspensions were inoculated with hepatitis A virus (HAV) cytopathic, strain HM-175, poliovirus 1, strain LSc, and male-specific coliphages (F+) at known concentrations. Pre-weighed disposable diaper punches were placed in 50ml conical centrifuge tubes. Volumes of 20ml of the feces-virus suspension were added to the tubes. The tubes with the diaper plus virus-feces suspensions were then mixed vigorously by vortexing. Periodically (about every 15 minutes) throughout the period of one hour, each tube was vortexed to insure complete mixing. After one hour, each sample was poured into separate 60cc syringe assemblies for separation of the solid phase from the liquid phase. Additional liquid remaining in the tubes that was not initially recovered from the 50ml conical centrifuge tube was rinsed out with 5ml of PBS, which was added to the

appropriate syringe. The liquid was squeezed out and collected in a different sterile 50ml conical centrifuge tube. After the liquid samples were collected and recovery volumes were determined, they were fluorocarbon-extracted, centrifuged and the supernatant was assayed for HAV and poliovirus. Two separate controls were run with each of the experiments. One control was a fecal suspension with virus that had no diaper material added and was filtered through a syringe assembly just as test samples were filtered. The other control was a fecal suspension with virus that had no diaper material added to it but it was not filtered through a syringe assembly. These same experiments were run using PBS without fecal material as the liquid. In addition, the PBS was spiked with poliovirus and hepatitis A virus and samples were processed using the same experimental procedures as for fecal suspensions.

Preliminary inoculation and recovery experiments were conducted using absorptive gel material (AGM). The desired amount of distilled water to be used was 20ml. Different amounts of AGM were added to 20ml volumes of distilled water in 50ml centrifuge tubes. These samples were then mixed vigorously and then held for one hour. Remaining water in the tubes not absorbed to gel was collected and the volume was determined. An optimum amount of AGM to achieve saturation by liquid but no liquid excess was determined in this way. AGM experiments on adsorption of viruses in fecal

suspensions were then carried out by using the same protocol as that used in the disposable diaper experiments.

Virus Propagation and Assays

Propagation

Poliovirus 1, strain LSc, was propagated and assayed by plaque technique in Buffalo Green Monkey Kidney (BGMK) cells as described by Sobsey et al, (1978). To prepare poliovirus stock, roller bottles containing confluent monolayers of BGMK cells were rinsed with a serum free medium and inoculated with a small volume of stock poliovirus at a multiplicity of infection (MOI) of 0.01 plaque forming units per cell. The cells were incubated at 37°C on a roller apparatus for one hour for virus adsorption to cells. After the 60 minute adsorption period, fresh maintenance medium was added to the roller bottles and the cells were incubated at 37°C until cytopathic effects (CPE) began to appear and destroy the monolayer (approximately 2-4 days). Cells were then frozen at -70°C and thawed at room temperature three successive times to lyse the cells and release the viruses. The cell debris-virus mixture was poured into a 250ml centrifuge bottle and trichlorotrifluoroethane (Freon) was add at a 1:1 ratio. The fluid solution was vortexed until complete mixing had occurred. The mixture was then centrifuged at 5000g for 20 minutes. The supernatant was recovered for use as poliovirus stock, aliquoted and stored at -70°C.

Hepatitis A virus (HAV) cytopathic strain HM-175 was propagated and assayed by plaque technique in fetal rhesus monkey kidney (FRhK-4) cells as described by Cromeans et al, (1987). Stock virus of HAV was prepared by infecting roller bottles containing a confluent monolayer of FRhK-4 cells by rinsing with a serum free medium and inoculating with stock HAV at a MOI of 0.1 plaque forming units (PFU) per cell. The cells were incubated at 37°C on roller racks for 60 minutes to allow for virus adsorption to the cells. After one hour of adsorption, fresh maintenance medium was added to the roller bottles and the cells were incubated at 37°C until cytopathic effects decimated the monolayer of cells. Cells were then frozen at -70°C and thawed three consecutive times to lyse up the cells and release the viruses. The cell debris-virus liquid mixture was then poured into a 250ml centrifuge bottle and trichlorotrifluoroethane (Freon) was added at a 1:1 ratio. The solution was vortexed until the solution was completely mixed. The mixture was centrifuged at 5000g for 20 minutes. The supernatant was recovered for use as HAV virus stock, aliquoted and stored at -70°C.

Male-specific (F+) coliphage stock was prepared from one-liter volumes of raw sewage. After collecting the sewage, its pH was adjusted to approximately 7.2. Sodium chloride (4 M) was added to each liter to a final concentration of 0.3 M. The F+ coliphages were precipitated

and extracted by adding polyethylene glycol (PEG) to a final concentration of 12% and allowing the mixture to stir at 4°C overnight. The following day, the mixture was poured into 500ml centrifuge bottles and spun at 7000g in the Sorvall RC3B centrifuge for 30 minutes. The supernatant was poured off and the pellet was resuspended in approximately 10ml of PBS. An equal volume of chloroform was added to the PBS-pellet sample and the mixture vortexed to insure complete emulsification. The mixture was spun at 4500g for 20 minutes. The supernatant was recovered and used for F+ coliphage stocks.

Assays

Poliovirus samples were assayed in BGMK cells grown to 90-95% confluency in 60 x 15 mm tissue culture dishes at 37°C in 5% CO₂. Samples were diluted serially ten-fold in PBS. The liquid growth medium was aspirated from the cells in the dishes and 0.2ml of a sample dilution were inoculated onto the cells. Replicate plates were inoculated for each sample dilution used. The inoculated dishes were incubated at 37°C for 1 hour, and every 15 minutes the dishes were gently shaken to redistribute the sample over the cells. After one hour of incubation, the cells were overlayed with 7ml of Eagle's MEM with 1.0% agar plus 1.0% neutral red. The agar medium was allowed to solidify and the plates were incubated at 37°C in 5% CO₂ for three days. Plaques were enumerated on the cell layer and poliovirus titer was

calculated and recorded as plaque forming units (PFU) per ml.

Samples were assayed for HAV in 90% confluent FRHK-4 cells in 60 x 15 mm tissue culture dishes grown at 37°C in 5% CO₂. Samples were diluted serially ten-fold in serum free 1XMEM. HAV dilution blanks contained antipolio serum at a dilution of 1:1000. The liquid growth media was aspirated off the cells and 0.2ml of a sample dilution were inoculated onto the cells. Replicate plates were infected for each sample dilution tested. The inoculated dishes were incubated for 60 minutes at 37°C, and every 15 minutes the dishes were gently rocked to redistribute the sample over the cells. After a one hour incubation, the cells were overlayed with 6ml of Eagle's MEM containing 0.5% agarose. After the agarose solidified, the dishes were incubated at 37°C in 5% CO₂. After 7 days, another 0.5% agarose medium overlay containing neutral red was added to the plates and they were returned to 37°C in 5% CO₂. After two to three more days of incubation, plaques were counted on the cell layer. HAV titer was calculated and recorded as PFU/ml.

Prior to coliphage experiments, 0.2ml of host Salmonella typhimurium type WG49 was grown up in a shaker flask (approximately 200rpm) in 20ml S. typhimurium growth broth at 37° celsius for approximately 12 hours. The following day, after the inoculation and recovery experiments were completed, the samples were assayed using a

single agar layer method. Samples were serially diluted ten-fold in 4.5ml dilution blanks of PBS, and then 1.5ml volumes were removed and added to 13.5ml of double strength nutrient rich agar with 0.03% tetrazolium violet. The WG49 host was then added to the mixture in amounts of 0.3ml per sample. The agar medium plus host plus sample was mixed and poured into 100 x 15 mm standard petri dishes and allowed to solidify. The plates were inverted and placed in a 37°C incubator overnight. The following day plaques were enumerated and PFU/ml were determined.

Virus Survival in Disposable Diapers and Sanitary Landfill Leachates

Viruses in a suspension of 20% feces wet weight in phosphate buffered saline (PBS) were inoculated into disposable diaper material and incubated. A 20% fecal suspension of 1720 ml was prepared and inoculated with 36ml of F+ coliphages, 12.6ml of HAV and 4.5 ml of poliovirus. After thoroughly mixing, 20 ml aliquots were placed into separate 50ml conical centrifuge tubes each containing a 16mm diameter, pre-weighed, disposable diaper punch. Samples were mixed by vortexing and incubated at temperatures of 5°, 25° and 40° celsius. Samples for processing and virus assays were removed at 0, 1, 3, 10, 30, 60 and 90 days. Samples were incubated aerobically at all three of the test temperatures and anaerobically at 25° and

40° celsius. On sampling days, a replicate (aliquot) was removed for recovery and processing of the test viruses. The sample was added to a 60cc syringe and separated by the same process as described for the preliminary experiments. Once the liquid phase was obtained, approximately 5ml of the sample was set aside and used for the F+ coliphage assays. The remaining liquid was fluorocarbon-extracted, centrifuged and the supernatant was assayed for HAV and poliovirus.

Viruses inoculated into sanitary landfill leachates were incubated in two different leachate samples: an "older" landfill leachate and a "younger" landfill leachate. Initially, each leachate was aliquoted into 360ml volumes. The leachates were then prepared by bubbling with nitrogen to reduce them and make them anaerobic. Once the samples were reduced to anaerobic conditions, 5ml of poliovirus, 6ml of HAV and 10ml of F+ coliphages were added to each leachate type. The leachate samples were then aliquoted into replicate 8ml samples and placed in 8ml capacity, screw cap polypropylene tubes (Sarstedt). The tubes were placed in anaerobic "glove" bags (Instruments for Research and Industry, Cheltenham, Pennsylvania), filled with nitrogen gas and then sealed to obtain an anaerobic environment. A bag containing samples was incubated at temperatures of 5°, 25° and 40° celsius. Samples were removed from bags for processing and virus recovery at 0, 1, 3, 10, 30, 60 and 90 days. The samples were tested for pH, redox potential and

HPC bacteria plate counts. The remaining sample was supplemented with 0.5 molar EDTA to a final concentration of 0.1 M and mixed occasionally during a 30 minute period. The EDTA-treated samples were serially diluted and stored for virus assays. Preliminary experiments were done to confirm that a previously developed EDTA treatment method would allow for quantitative recovery of test enteric viruses (Sobsey, et al., 1974). Virus assays on a complete time series of samples was performed at the end of the 90 day experimental period.

Statistical Analysis of Data

Statistical analysis of the data was accomplished by using the computer software program Systat (Systat, 1989). Analysis of variance (ANOVA) was used to analyze the data and a P value of $\leq .05$ was considered a significant difference between regression lines. An ANOVA was used to compare the regressions of best fit lines. The slopes were analyzed to determine whether or not they were parallel and the y-intercepts were analyzed to determine whether or not they were coincident.

Separate models for each sample under each different condition with each virus were run. Values were input into Systat in a spreadsheet form and analyzed for significant differences. Survival data was input as $\log N_t/N_0$, and all other variables were input as dummy-dependent-variables with numbers assigned to them for each condition or virus type.

Once the models were input and values were obtained for that specific model, the p values were used for comparison with the other models at different conditions or with different viruses and significant differences were determined. This procedure allowed for individual comparison between each test model with other test models and determined which slopes were parallel and which intercepts were coincident. In the interpretation of these kinetic data, only differences in slopes of regression lines were considered biologically significant. The slope of the regression line is taken as an estimate of the rate of virus inactivation. Differences in y-intercepts of regression lines were recorded, but their biological significance could not be readily interpreted.

RESULTS

PRELIMINARY EXPERIMENTS

Size and Volume Determination of Diaper Punches

The results of preliminary experiments to determine the appropriate size of diaper disk punches from the rear portion of disposable diapers indicated that 16mm diameter disk punches were the appropriate size to use. Fifty ml conical centrifuge tubes were used as containers for absorption experiments. The 25.4 and the 60 mm diameter disk sizes absorbed too much liquid and would not fit conveniently in the 50ml conical centrifuge tubes or the 60cc syringes. The 16mm diameter disks could absorb enough water to reach maximum absorption capacity and still fit in the centrifuge tubes.

<u>Diaper disk diameter</u>	<u>mean weight</u>	<u>mean absorption volume</u>
60 mm	1.8 grams	90-100ml
25.4 mm	0.61 grams	30-40ml
16 mm	0.29 grams	22-25ml

A 60cc syringe was used to recover the liquid that was absorbed by the diaper disks rather than a centrifugation method. When 50ml of distilled water were added to 16mm

disks and centrifuged at 5000g for 20 minutes, only 42-74% of the water was recovered. On the other hand, when a 60cc syringe was used for the recovery of the absorbed water, 69-92% of the liquid was recovered.

Determination of Fecal Suspension Concentration

A 20% fecal suspension was determined to be optimal for the fecal experiments. Various percent concentrations of fecal suspensions (w/v) in different volumes were placed on 16mm diaper disks in 50 ml conical centrifuge tubes. After one hour of absorption, the diaper material-fecal suspension mixtures were added to 60cc syringes and the absorbed liquid was physically removed by squeezing it out of the diaper using the syringe plunger. A 20% fecal suspension was the highest concentration that gave a recovery rate for the liquid of 70% or higher. Only 21% of the liquid was recovered from the 30% fecal suspension. The lower concentrations of 10% and 15% fecal suspensions had 92% and 79% recovery of the absorbed liquid, respectively. Although recovery rates were higher at lower fecal suspension concentrations, they were not the desired suspensions concentrations for the experiments. The highest feces concentration that allowed good recovery of liquid was the desired goal.

Absorbing Gel Material Experiments

Absorbing gel material (AGM), also was used in the preliminary experiments. The amount of AGM to be used was

determined by adding different quantities of AGM to 50ml conical centrifuge tubes, and then adding 20ml volumes of water. The mixture was held for one hour, and after that time, the samples were poured into 60cc syringes and the liquid was squeezed out. AGM amounts of 0.07-0.09 grams were determined to absorb the maximum amount of liquid and allow for the best liquid recovery.

Preliminary Virus Adsorption Experiments

The results of the short-term virus adsorption experiments are summarized in Table 3 and presented in detail in Appendix Tables 1-4. Three separate types of adsorption experiments were run: virus adsorption to diaper material (Appendix Tables 1 and 2), virus adsorption to AGM (Appendix Table 3) and virus adsorption in leachate samples (Appendix Table 4).

Disposable Diapers

Relatively little adsorption to diaper material occurred when the sample medium was mixed with PBS or fecal suspensions. There was 100% recovery of F+ coliphages from the PBS-diaper mixture and a mean F+ coliphage recovery of 84% from fecal suspension-diaper mixture. For HAV, mean recovery from PBS-diaper pulp suspensions was 81% and mean recovery from fecal suspension diaper pulp was 83.5%. Poliovirus had the lowest mean recovery from the PBS-diaper mixture of only a 46%. However, in the fecal suspension poliovirus mean recovery was 92%. These results indicate

that overall, viruses do not readily sorb to the diaper material and are easily eluted or recovered from the sample.

Absorbing Gel Material

Experiments on F+ coliphage, HAV and poliovirus adsorption to AGM saturated with fecal suspension or PBS showed similar results (Appendix Table 3). There was 100% recovery of F+ coliphages from AGM plus PBS and an averaged 78% recovery from AGM-fecal suspensions. On average, 53% of HAV was recovered from the AGM-PBS mixture while 100% was recovered from AGM-fecal suspension. Poliovirus had the lowest mean recovery of only 36% from the AGM and PBS mixture. On the other hand, a mean 97% poliovirus recovery occurred with the AGM-fecal suspension. These results show that viruses generally do not sorb readily to AGM in either PBS or fecal suspensions.

Virus Recovery in Landfill Leachates

As shown by the results in table 3, virus detection was masked or suppressed by leachates 1 and 2 when EDTA was not added to the samples. In leachate 1, virus masking was substantial at values $> 95.5\%$ in all concentrations that were investigated. Masking of viruses occurred in leachate 2 also, but not to the same extent as leachate 1. In leachate 2 virus masking was $>99\%$ at the lowest concentration tested (5×10^4 PFU/ml), 88% at virus concentrations of 5×10^5 PFU/ml and 47% at a virus dilution of 5×10^6 PFU/ml. EDTA added to the leachate samples at a

final concentration of 0.1M prevented virus masking. The concentrations of viruses in the EDTA-treated samples were similar to those of the MEM control samples. This indicated that EDTA had no adverse effect on virus detection, since virus levels in MEM supplemented with EDTA were essentially the same as those in MEM without EDTA.

In experiment 1 of the preliminary EDTA experiments, the leachate samples were supplemented with EDTA before the viruses were added. In experiment 2, EDTA was added to the leachate samples 45 minutes after the viruses were added to the leachates. In both experiments, the addition of EDTA to the leachates before and after the addition of viruses prevented any appreciable loss of virus detectability. In experiment 2, virus concentrations in leachates treated with EDTA were approximately the same as virus concentrations in corresponding MEM control samples.

These results indicate that the addition of 0.1M EDTA to leachate samples overcomes any impediments that the leachates may create in the detection of viruses. In subsequent experiments, leachate samples had 0.1M EDTA added to them at the time of virus assays to maximize virus detectability. The results of these experiments indicate that EDTA treatment prevents or reverses leachate masking of viruses that are still infectious.

Virus Adsorption to Disposable Diapers Suspended in
Landfill Leachates

Results for virus adsorption to diaper pulp material saturated with solid waste landfill leachates can be seen in Table 1 and Appendix Table 4. For HAV, the greatest amount of sorption occurred in leachate 1, with a mean of 41% and the least amount of sorption, 0%, occurred in leachate 2. In the PBS control mean sorption was 4% and in leachate 3 mean sorption was 35%. Leachate 2 had the greatest amount of sorption for poliovirus with a mean of 48% and leachate 1 was similar with a mean sorption rate of 46%. The PBS control had the lowest virus adsorption, with a mean of 17%. Leachate 3 had a mean sorption of 30% for poliovirus. Overall, HAV had 20% mean sorption to diaper pulp when leachates were present and poliovirus sorption was a mean 35%. In general, poor sorption occurred with all three test viruses when exposed to disposable diapers suspended in sanitary landfill leachate medium.

Table 3

Effect of 0.1 M EDTA on Poliovirus Detection in Solid Waste
Landfill Leachates

Sample	Virus Dil'n ^b	Plaque Forming Units Per ml	% of MEM Control Virus Detected
Experiment 1 ^c			
MEM	E4	~5.5 E4	(100)
Control	E5	5.5 E5	(100)
	E6	5.6 E6	(100)
MEM +	E5	6.6 E6	100
EDTA	E6	5.7 E6	100
L1	E4	<2.5 E1	<4.5
	E5	8.8 E3	1.6
	E6	1.2 E5	2.1
L2	E4	2.0 E2	<1.0
	E5	6.9 E4	12
	E6	3.0 E5	53
L1 + EDTA	E5	6.6 E5	100
	E6	5.7 E6	100
L2 + EDTA	E5	6.3 E5	100
	E6	5.6 E6	100
Experiment 2 ^d			
MEM	E3	4.6 E3	(100)
Control	E4	4.2 E4	(100)
	E5	3.7 E5	(100)
	E6	4.0 E6	(100)
MEM +	E4	4.4 E4	100
EDTA	E5	5.1 E5	100
	E6	5.6 E6	100
L1 + EDTA	E4	3.8 E4	90
	E5	3.3 E5	89
	E6	4.2 E6	100
L2 + EDTA	E4	4.7 E4	100
	E5	5.7 E5	100
	E6	6.6 E6	100
L3 + EDTA	E4	3.5 E4	83
	E5	4.6 E5	100
	E6	4.4 E6	100

^aAbbreviations: MEM = Eagle's Minimum Essential Medium;

L1 = Leachate 1; L2 = Leachate 2; L3 = Leachate 3.

^bVirus stock dilutions as orders of magnitude (e.g., E4 is expected to give about 5×10^4 PFU/ml).

^cIn experiment 1, leachates were supplemented with EDTA before viruses were added.

^dIn experiment 2, leachates were seeded with viruses and after 45 minutes EDTA was added.

VIRUS SURVIVAL EXPERIMENTS

Fecal Suspension and Disposable Diapers

Temperature

A summary of the results of experiments on virus survival in fecally soiled diapers at 5, 25 and 40°C under aerobic and anaerobic conditions can be seen in Appendix Table 5 as estimated times for 1 $\log_{10}$ through 4 $\log_{10}$ reductions of viruses over time, in Table 4 as estimated times for 4 $\log_{10}$ reduction, and in Table 5 as the slopes and y-intercepts of the regression lines for virus survival. Figures 1-6 show surviving viruses as $\log N_t/N_0$ versus time (days). N_0 is the initial number of viruses and N_t is the number of viruses at time=t. Additional data showing linear regression analyses of virus survival are shown in Figures 7-11. As shown by the results in Tables 4 and 5 for estimated times to achieve 4 $\log_{10}$ (99.99%) virus reduction, temperature was the most important factor affecting virus survival in the aerobic and aerobic fecal suspensions. Virus inactivation was greatest at 40°C and least at 5°C, regardless of sample, other experimental conditions or viruses. At 40°C, 4 $\log_{10}$ reduction times averaged 20 days in fecal suspensions. The shortest times for virus inactivation occurred in the anaerobic fecal suspensions at 40°C with estimated 4 $\log_{10}$ inactivation times of 7.5 days for poliovirus and 7.7 days for HAV. The longest times for

virus reductions were at 5°C in aerobic fecal suspensions, with estimated 4 log₁₀ reduction times of about 2500 days for HAV, 750 days for F-specific coliphages and 600 days for poliovirus. At 5°C and aerobic conditions, the time to achieve even 1 log₁₀ (90%) reduction of all three viruses exceeded 150 days. HAV was the most persistent virus under these conditions with an estimated time for 1 log₁₀ (90%) reduction in excess of 630 days.

Virus survival was considerably lower at 25°C than at 5°C, with mean 4 log₁₀ reductions being achieved after approximately 104 days for the aerobic fecal suspension. Viruses in the anaerobic fecal suspension reached mean 4 log₁₀ reductions at around 110 days. F-specific coliphages and poliovirus demonstrated similar survival times at 25°C in the aerobic fecal suspension with 22 days to attain a 1 log₁₀ reduction and 88 and 83 days to achieve 4 log₁₀ reductions, respectively. In comparison, HAV survived the longest at 25°C in aerobic fecal suspensions, with an estimated 33 days to reach a 1 log₁₀ reduction and 140 days to reach a 4 log₁₀ reduction. In anaerobic fecal suspensions at 25°C, there was more disparity among the results for all three viruses. F-specific coliphages survived the longest: 42 days and 167 days for 1 log₁₀ reductions and 4 log₁₀ reductions, respectively. Poliovirus was reduced by 1 log₁₀ in <1 day and by 4 log₁₀ in 78 days, while HAV was reduced by 1 log₁₀ in 12 days and by 4 log₁₀ in 84 days.

Table 4
VIRUS REDUCTIONS IN FECAL SUSPENSIONS
UNDER DIFFERENT CONDITIONS

Anaerobic or Aerobic	Temp (°C)	Virus Type	4 Log ₁₀ (99.99%) Reduction (days)*
Aerobic	5	F-specific	748 A*
	5	Polio	607 A
	5	HAV	2511 A
Aerobic	25	F-specific	83 A,B
	25	Polio	84 B
	25	HAV	139 A
Aerobic	40	F-specific	21 A
	40	Polio	20 A
	40	HAV	29 A
Anaerobic	25	F-specific	167 A
	25	Polio	78 A,B
	25	HAV	83 B
Anaerobic	40	F-specific	22 A
	40	Polio	20 A,B
	40	HAV	8 B

* Same upper case letter within each temperature indicates no significant difference between the slope of each best fit model for virus survival in sample. Different upper case letter within each temperature indicates a significant difference between slope of each best fit model for virus survival in sample.

TABLE 5

COMPARATIVE VIRUS SURVIVAL IN FECAL SUSPENSIONS
UNDER DIFFERENT CONDITIONS

Virus Type	Temp (°C)	AnO ₂ or O ₂	Slope*
F-specific	5	O ₂	-0.005 A
Polio	5	O ₂	-0.007 A
HAV	5	O ₂	-0.002 A
F-specific	25	O ₂	-0.046 A,B
Polio	25	O ₂	-0.049 B
HAV	25	O ₂	-0.028 A
F-specific	40	O ₂	-0.171 A
Polio	40	O ₂	-0.173 A
HAV	40	O ₂	-0.116 A
F-specific	25	AnO ₂	-0.024 A
Polio	25	AnO ₂	-0.035 A,B
HAV	25	AnO ₂	-0.044 B
F-specific	40	AnO ₂	-0.169 A
Polio	40	AnO ₂	-0.152 A,B
HAV	40	AnO ₂	-0.371 B

* Same upper case letter within each temperature indicates no significant difference between slope of each best fit model for virus survival in sample. Different uppercase letter within each temperature indicates a significant difference between slope of each best fit model for virus survival in sample.

Figure 1

SURVIVAL OF MALE-SPECIFIC COLIPHAGES
IN DIAPERS, AEROBIC CONDITIONS

Figure 2

SURVIVAL OF MALE-SPECIFIC COLIPHAGES
IN DIAPERS, ANAEROBIC CONDITIONS

Figure 3

SURVIVAL OF POLIOVIRUS 1
IN DIAPERS, AEROBIC CONDITIONS

Figure 4

SURVIVAL OF POLIOVIRUS 1
IN DIAPERS, ANAEROBIC CONDITIONS

Figure 5

SURVIVAL OF HEPATITIS A VIRUS
IN DIAPERS, AEROBIC CONDITIONS

Figure 6

SURVIVAL OF HEPATITIS A VIRUS
IN DIAPERS, ANAEROBIC CONDITIONS

Figure 7

FECAL SUSPENSION AT
5 DEGREES CELSIUS, AEROBIC CONDITIONS

Figure 8

FECAL SUSPENSION AT
25 DEGREES CELSIUS, AEROBIC CONDITIONS

Figure 9

Figure 10

FECAL SUSPENSION AT
25 DEGREES CELSIUS, ANAEROBIC CONDITIONS

Figure 11

Aerobic versus Anaerobic Conditions

Overall, HAV survived the longest of the test viruses in the aerobic fecal suspensions, with estimated 4 log₁₀ reductions of 2437, 139 and 29 days at 5, 25 and 40°C, respectively. Survival of poliovirus and F-specific coliphages in aerobic fecal suspensions was quite similar at all three temperatures. F-specific coliphages survived the longest in the anaerobic fecal suspensions with estimated 4 log₁₀ reductions of 167 and 22 days at 25 and 40°C, respectively.

In fecal suspensions, overall virus survival was generally similar at 25°C under both aerobic and anaerobic conditions, with mean 4 log₁₀ reduction times for all three viruses combined of 104 days for aerobic conditions and 110 days for anaerobic conditions. The range of 4 log₁₀ reduction times varied from 78 to 167 days. At 40°C, poliovirus and F-specific coliphages had similar times at aerobic and anaerobic conditions. However, 4 log₁₀ reduction times of HAV at 40°C were much longer under aerobic than anaerobic conditions.

The influence of aerobic versus anaerobic conditions for each virus and incubation temperature in fecal suspensions was determined statistically by comparison of slopes of regression lines. Results can be seen in Table 6 where the slopes of the regression lines are presented and Figures 12-17 where the inactivation data are plotted. At

40°C, there was essentially no difference between aerobic or anaerobic conditions in terms of slopes of regression lines for survival of all three test viruses. For poliovirus and F-specific coliphages, there were significant differences between the slopes of regression lines for virus survival at 25°C between aerobic and anaerobic conditions. However, for HAV at 25°C, there was no statistical difference between the slopes of regression lines for anaerobic and aerobic conditions at the 5% level of significance. However, visual examination of the plotted data indicate that HAV survival was longer under aerobic conditions than under anaerobic conditions, and the slopes of the regression lines differed by nearly two-fold (Table 6).

TABLE 6

COMPARATIVE VIRUS SURVIVAL IN AEROBIC
VERSUS ANAEROBIC FECAL SUSPENSIONS

Virus Type	Temp (°C)	AnO ₂ or O ₂	Slope*
HAV	25	O ₂	-0.028
HAV	25	AnO ₂	-0.044
HAV	40	O ₂	-0.116
HAV	40	AnO ₂	-0.371
Polio	25	O ₂	<u>-0.049</u>
Polio	25	AnO ₂	<u>-0.035</u>
Polio	40	O ₂	-0.173
Polio	40	AnO ₂	-0.152
F-specific	25	O ₂	<u>-0.046</u>
F-specific	25	AnO ₂	<u>-0.024</u>
F-specific	40	O ₂	-0.171
F-specific	40	AnO ₂	-0.169

* Underlined values indicate a significant difference between aerobic and anaerobic conditions ($p < 0.05$).

Figure 12

F-SPECIFIC COLIPHAGES AT
25 DEGREES CELSIUS

Figure 13

POLIOVIRUS 1 AT
25 DEGREES CELSIUS

Figure 14

HEPATITIS A VIRUS AT
25 DEGREES CELSIUS

Figure 15

F-SPECIFIC COLIPHAGES AT
40 DEGREES CELSIUS

Figure 16

POLIOVIRUS 1 AT
40 DEGREES CELSIUS

Figure 17

HEPATITIS A VIRUS AT
40 DEGREES CELSIUS

Landfill Leachates

Temperature

Results of the experiments on virus survival in solid waste landfill leachates at 5, 25 and 40°C under anaerobic conditions are given in Appendix Table 6 as estimated times for 1 $\log_{10}$ through 4 $\log_{10}$ reductions of viruses over time and summarized in Table 7 as 4 $\log_{10}$ reduction times. Figures 18-23 show $\log_{10}$ surviving viruses, N_t/N_0 , versus time (days). Additional data including statistical comparisons of regression lines for virus survival data are shown in Tables 8 and in Figures 24-29. As seen by the results in Tables 7 as estimated times to achieve 4 $\log_{10}$ virus reductions, temperature was the most important factor affecting virus survival in different samples. Virus inactivation was greatest at 40°C and least at 5°C, regardless of sample or virus. At 40°C, 4 $\log_{10}$ reduction times averaged 10 days in solid waste landfill leachates. The most rapid inactivation time for viruses in landfill leachates at 40°C was 2 days in leachate 1 (the "old" leachate) and 3 days in leachate 2 (the "young" leachate) for F-specific coliphages. At 40°C, viruses were inactivated at similar rates overall in leachates 1 and 2 with mean 4 $\log_{10}$ reduction times of 9.3 and 11.7 days, respectively. At the other extreme, the longest times for virus survival were at 5°C in landfill leachates, with estimated 4 $\log_{10}$ reduction times of >650 days for poliovirus

in leachate 2, >600 days for HAV leachate 1 and >400 days for F-specific coliphages in leachate 2. At 5°C, overall virus survival was generally similar in leachate 1 and leachate 2, with mean 4 log₁₀ reduction times of 439 versus 452 days. However, in all cases estimated times for 4 log₁₀ virus reductions in leachates at 5°C exceeded 250 days.

Virus survival at 25°C was intermediate between that at 40 and 5°C, with 4 log₁₀ reductions of all three test viruses combined of 67 days for landfill leachate 1 and between 50 and 293 days (mean 144 days) for leachate 2. Thus, at 25°C, viruses survived longer in leachate 2 (from the "younger" landfill) than in leachate 1 (from the "older" landfill). HAV exhibited the longest survival of the three viruses in both leachates at 25°C, with 4 log₁₀ reduction times of 89 and 283 days in leachate 1 and 2, respectively.

Table 7

VIRUS REDUCTIONS IN LEACHATE SAMPLES
AT DIFFERENT TEMPERATURES

Leachate	Temp (°C)	Virus Type	4 Log ₁₀ (99.99%) Reduction (days)*
No. 1	5	F-specific	308 A*
	5	Polio	367 A
	5	HAV	643 A
No. 1	25	F-specific	57 A
	25	Polio	46 A
	25	HAV	88 A
No. 1	40	F-specific	2 A
	40	Polio	19 A
	40	HAV	7 B
No. 2	5	F-specific	422 A
	5	Polio	681 A
	5	HAV	254 B
No. 2	25	F-specific	50 A
	25	Polio	100 A
	25	HAV	293 B
No. 2	40	F-specific	3 A
	40	Polio	23 A
	40	HAV	9 A

* Same upper case letter within each temperature indicates no significant difference between slope of each best fit model for virus survival in sample. Different upper case letter within each temperature indicates a significant difference between slope of each best fit model for virus survival in sample.

TABLE 8

COMPARATIVE VIRUS SURVIVAL IN LEACHATE SAMPLES
AT DIFFERENT TEMPERATURES

Virus Type	Temp (°C)	Leachate	Slope*
F-specific	5	No. 1	-0.013 A
Polio	5	No. 1	-0.011 A
HAV	5	No. 1	-0.006 A
F-specific	25	No. 1	-0.057 A
Polio	25	No. 1	-0.081 A
HAV	25	No. 1	-0.042 A
F-specific	40	No. 1	-1.367 A
Polio	40	No. 1	-0.133 A
HAV	40	No. 1	-0.565 B
F-specific	5	No. 2	-0.009 A
Polio	5	No. 2	-0.006 A
HAV	5	No. 2	-0.015 B
F-specific	25	No. 2	-0.064 A
Polio	25	No. 2	-0.038 A
HAV	25	No. 2	-0.012 B
F-specific	40	No. 2	-1.278 A
Polio	40	No. 2	-0.143 A
HAV	40	No. 2	-0.396 A

* Same upper case letter within each temperature indicates no significant difference between slope of each best fit model for virus survival in sample. Different uppercase letter within each temperature indicates a significant difference between slope of each best fit model for virus survival in sample.

Figure 18

SURVIVAL OF MALE-SPECIFIC COLIPHAGES
IN LEACHATE No. 1, ANAEROBIC CONDITIONS

Figure 19

SURVIVAL OF MALE-SPECIFIC COLIPHAGES
IN LEACHATE No. 2, ANAEROBIC CONDITIONS

Figure 20

SURVIVAL OF POLIOVIRUS 1
IN LEACHATE No. 1, ANAEROBIC CONDITIONS

Figure 21

SURVIVAL OF POLIOVIRUS 1
IN LEACHATE No. 2, ANAEROBIC CONDITIONS

Figure 22

SURVIVAL OF HEPATITIS A VIRUS
IN LEACHATE No. 1, ANAEROBIC CONDITIONS

Figure 23

SURVIVAL OF HEPATITIS A VIRUS
IN LEACHATE No. 2, ANAEROBIC CONDITIONS

Figure 24

Figure 25

LEACHATE No. 1 AT
25 DEGREES CELSIUS, ANAEROBIC CONDITIONS

Figure 26

Figure 27

Figure 28

Figure 29

Comparison of Virus Survival within each Leachate

The survival of individual viruses was not consistent among leachate samples and temperatures. HAV survived longer than the other two viruses at 25°C in both leachate 1 and 2, although the difference of survival was statistically significant ($p < 0.05$) only in leachate 2. HAV also appeared to survive longer than the other viruses in leachate 1 at 5°C, but there was no significant difference in the slopes of the regression lines. However, HAV survival was significantly shorter than other viruses only in leachate 2 at 5°C. Poliovirus survival appeared to be longer than the other viruses at 40°C in both leachate 1 and 2, with 2.6 to 9.5-fold longer survival than HAV or F-specific coliphages for a 4 log₁₀ reduction. However, survival was not significantly greater by regression analysis ($p > 0.05$). F-specific coliphages survived the least well in 4 of the 6 conditions tested, but this survival difference was generally not statistically significant. The results suggest that no appreciable differences exist among the survival of enteric viruses in landfill leachates.

Comparisons of virus survivals between the two leachates at the same temperatures suggest no major differences. Estimated mean 99.99% inactivation times in leachates 1 and 2, respectively, were 439 versus 452 days at 5°C, 83 versus 148 days at 25°C, and 9.3 versus 11.7 days at 40°C. However, at 25°C, HAV and poliovirus survived longer

in leachate 2 than in leachate 1. Physical-chemical analysis of the leachates (Table 2 in the Material and Methods section) show considerable differences in quality for many parameters tested. However, the lack of a consistent difference in virus inactivation rates between the two leachates precludes any interpretation of virus inactivation in terms of chemical or physical quality. Only incubation temperature had an effect on virus survival.

Fecal Suspension versus Landfill Leachate

Comparisons of virus survival in fecal suspensions versus sanitary landfill leachates indicate that viruses survive longer in the former than in the latter. Linear regression plots showing the comparative virus survivals in fecal suspensions and landfill leachates can be seen in Figures 30 through 38. By visual examination, most viruses appear to survive better in fecal suspensions than in leachates under most conditions tested. Mean survival of all three viruses at 5°C was estimated at a 4 log₁₀ reduction time of 1290 days in fecal suspensions and 446 days in leachates. At 25°C, viruses survived least well overall in leachate 1, with an estimated mean 4 log₁₀ reduction time of 64 days. Virus survival at 25°C was longest in leachate 2, with an estimated mean 4 log₁₀ reduction time of 148 days. In fecal suspensions at 25°C estimated average 4 log₁₀ virus reduction was about 106 days. At 40°C, viruses survived

longer in fecal suspensions than in landfill leachates. The estimated mean 4 $\log_{10}$ virus reduction time in fecal suspensions was 20 days but only 10.5 days in leachate samples.

Figure 30

F-SPECIFIC COLIPHAGES AT
5 DEGREES CELSIUS

Figure 31

F-SPECIFIC COLIPHAGES AT
25 DEGREES CELSIUS

Figure 32

F-SPECIFIC COLIPHAGES AT
40 DEGREES CELSIUS

Figure 33

POLIOVIRUS 1 AT
5 DEGREES CELSIUS

Figure 34

POLIOVIRUS 1 AT
25 DEGREES CELSIUS

Figure 35
POLIOVIRUS 1 AT
40 DEGREES CELSIUS

Figure 36

HEPATITIS A VIRUS AT
5 DEGREES CELSIUS

Figure 37

HEPATITIS A VIRUS AT
25 DEGREES CELSIUS

Figure 38

HEPATITIS A VIRUS AT
40 DEGREES CELSIUS

DISCUSSION

Diaper-aged infants and children are infected by enteric viruses and foster the transmission of viruses to others with whom they come in direct or indirect contact. An important contact source includes fecally-soiled disposable diapers. Since relatively little is known about the survival of enteric viruses in fecally soiled disposable diapers in the solid waste stream and in solid waste landfills, it is important to investigate environmental virology aspects of the potential health risk that soiled disposable diapers pose to the public. This research project was intended to determine the fate and persistence of three experimental viruses, hepatitis A virus, poliovirus type 1 and male-specific (F+) coliphages, in soiled disposable diapers and landfill leachates under a number of specific environmental conditions.

Virus Adsorption to Diaper Material in the Presence of Feces or Leachates

In preliminary experiments, it was determined that viruses did not adsorb to disposable diapers over an adequate period of time for physical-chemical adsorption to occur and they were easily recovered from disposable diaper-fecal suspension mixtures. Diaper pulp, AGM and diaper

material-free controls in either phosphate buffered saline (PBS) or a 20% fecal suspension were used in the virus adsorption and recovery studies. 100% recoveries were observed for the F+ coliphages under all but two sample conditions AGM (67%) and PBS (92%) control samples. Poliovirus 1 recovery was 100% in all samples except the PBS control (92%) and hepatitis A virus recovery ranged from 44-100%. The lowest recoveries were in the PBS control samples with 44% recovery in both diaper pulp and control samples, and 62% recovery in the AGM sample. Virus survival was the greatest from the samples suspended in fecal material: 100% from diaper pulp, 86% from the control and 72% from AGM samples. These preliminary results indicate that viruses are readily eluted from fecally soiled disposable diapers and therefore may be leached out of soiled disposable diapers under conditions of water saturation. Diaper material appears to have no appreciable virus adsorption or selective retention capacity.

Virus Survival on Diaper Material in Feces or Leachates

In studies on virus survival in fecal suspensions and two different solid waste landfill leachates, viruses were suspended in either 20% fecal suspensions with disposable diaper disks or one of two different leachate samples. They were then incubated at 5°, 25° and 40°C. All of the leachate samples were kept in anaerobic conditions; half of the

disposable diapers-plus-fecal suspension samples were stored in anaerobic conditions and the other half were stored in aerobic conditions.

Fecal Suspensions in Disposable Diapers: Temperature Effects

The results from the survival experiments indicate that temperature was the most important factor in the inactivation of viruses. At 40°C, estimated 4 log₁₀ virus inactivation in the fecal suspensions under both aerobic and anaerobic conditions averaged approximately 20 days. HAV 4 log₁₀ reduction was estimated at 29 days in the aerobic condition at 40°C but only 8 days in anaerobic conditions. Poliovirus survived least well of the three test viruses in the aerobic conditions with an estimated 4 log₁₀ reduction time of 20 days. F-specific coliphages reduction was the least in anaerobic conditions, with 22 days for an estimated mean 4 log₁₀ reduction. In contrast to the relatively short times for 4 log₁₀ virus reduction at high temperature (40°C), regression models predicted that viruses survived much longer at 5°C with 4 log₁₀ reductions of >500 days. These results are substantiated by other results indicating that virus inactivation is temperature dependent and enteric viruses are more persistent at lower temperatures than at higher temperatures under a variety of environmental conditions (such as water, wastewater, sludges, groundwater, soil, municipal solid waste landfills and landfill

leachates) (Biziagos, et al., 1988, Hurst, et al., 1986, Yates, et al., 1985, McGeady, et al., 1979, Ward, et al., 1976, Sobsey, et al., 1975). Although these results are from laboratory conditions and are a basis for estimating virus survival in soiled disposable diapers, they do not adequately simulate realistic exposure conditions. Depending on the location, time of year and method of disposal, soiled disposable diapers may undergo a wide temperature range for various amounts of time before reaching solid waste landfills or some other final receiver. Due to the diversity of environmental conditions that soiled disposable diapers may experience, the kinetics and extent of environmental virus inactivation may differ considerably from the results that were obtained in this study, especially if diapers experience temperatures $>40^{\circ}\text{C}$.

Anaerobic versus Aerobic Conditions

The results of this study show that on average, no appreciable difference exists between virus survival under aerobic and anaerobic conditions. No anaerobic experiments were run at 5°C so results include only comparative outcomes from 25 and 40°C . HAV had the greatest survival at both temperatures, 25°C and 40°C , in aerobic conditions. Male-specific coliphages survived the longest in anaerobic conditions at the two test temperatures. Poliovirus and F-specific coliphages did not vary greatly in their survival in aerobic conditions. In anaerobic conditions at 25°C ,

poliovirus and HAV were similar with an estimated 78 and 83 days required to reach 4 $\log_{10}$ inactivation, respectively. However, HAV at 40°C in anaerobic conditions averaged much shorter survival compared to the other test viruses. The average time for 4 $\log_{10}$ reduction of all three test viruses at 25°C was similar, but survival was somewhat longer in anaerobic conditions than in aerobic conditions. However, in aerobic conditions average survival at 40°C was longer than in anaerobic conditions. These results at 40°C differ from other studies in which aerobic conditions were found to be more antagonistic than anaerobic conditions, leading to more rapid virus inactivation in the former. Hurst (1988) reported that there was an increased inactivation rate attributed to aerobic microorganisms and no comparable effect was observed for anaerobic conditions. Similar results were observed for non-sterile conditions at 1, 23 and 37°C. However, in sterile samples at 23°C, 90% virus inactivation in aerobic conditions required 31 days and in anaerobic conditions it was 30 days. Similarly, little variation was observed under the 37°C condition, where 3.0 and 1.7 days were required for 90% inactivation under sterile aerobic and anaerobic conditions, respectively.

At 25°C, F-specific coliphages survived twice as long in anaerobic fecal suspensions as in aerobic fecal suspensions and the slopes of regression lines were significantly different ($p < 0.05$). In contrast, poliovirus

survived significantly longer in aerobic conditions than anaerobic conditions. HAV survival also appeared to be better in aerobic conditions than anaerobic conditions. The slopes of the regression lines were not significantly different ($p = 0.11$). At 40°C no significant difference was observed in either the slope of regression lines for both F-specific coliphages and poliovirus. However, HAV exhibited a considerable difference in 4 $\log_{10}$ inactivation between aerobic and anaerobic conditions: 29 versus 8 days respectively.

The relatively better survival rates observed for poliovirus at 25°C and HAV at 40°C under aerobic than anaerobic conditions are not supported by the previous literature on virus survival in other media. Environmental microorganisms considered antagonistic to enteric viruses are thought to be much more active under aerobic than anaerobic conditions. They are considered likely to create an antagonistic environment against viruses more quickly than in anaerobic conditions. One explanation may be that under aerobic conditions, these viruses have a higher tendency to sorb to and associate with particulate matter. It should be noted that anaerobic sludge digestion is the most common form of treatment, and it has some capacity for inactivation of pathogens such as enteric viruses. Though these experiments do not model conventional sludge digestion, the rapid virus inactivation observed for HAV and

poliovirus may be a function of some phenomenon that is similar in the anaerobic digestion process. At the experimental conditions tested, some viruses were susceptible to this activity more than the other test viruses.

Differences Among Viruses for Survival in Fecal Suspensions

It is not possible to generalize about causative factors which influence virus survival in all fecal suspensions, aerobic or anaerobic, at all three temperatures. However, one consistent outcome is that HAV survived better than the other viruses at 5°, 25° and 40°C in aerobic conditions. These results are substantiated by previous findings that HAV is more persistent in the environment than most other enteric viruses, including poliovirus. F-specific coliphages, which are not enteric viruses causing human illness but rather indicators, are not as resistant to environmental factors as enteric viruses under most conditions. However, F-specific coliphages survived better than the other viruses at 25° and 40°C in anaerobic conditions. One explanation for F-specific coliphages having the best survival in anaerobic conditions may be that F-specific phages are commonly found in the lower gut where anaerobic conditions prevail. Therefore, they may be adapted to these conditions and hence less sensitive to inactivation in anaerobic fecal suspensions.

At 5°C, no statistically significant difference between virus survivability was observed. Since the inactivation rate is so low and the length of time for any of the viruses to be inactivated by 4 log₁₀ is >500 days, the slopes are not very different and are close to zero. At 25°C for the anaerobic conditions, there is a significant difference between the slopes of HAV and poliovirus ($p < 0.05$) and a noticeable and nearly significant difference between the slopes of HAV and F-specific phages ($p = 0.052$). It appears that a variety of factors influence the survival (or conversely, die-off) of viruses in fecal suspensions and that depending on temperature and test conditions, different viruses will be affected somewhat differently.

Solid Waste Landfill Leachates

Preliminary experiments were run to determine the efficiency of recovering viruses from the landfill leachates. Direct recovery yielded low titers indicating that one or several components of the leachates were enhancing adsorption to constituents in the liquid or otherwise preventing the viruses from producing plaques. In order to control for this masking effect by leachates, 0.1 M of EDTA was added to the samples to increase detection efficiency. When both leachate samples were tested for virus detection without the addition of EDTA, an average of less than 3.0% was detected from the "old" leachate sample

and only 1.0-53% of the viruses were detectable in the "young" leachate. When EDTA was added to both the "young" and "old" leachate samples, virus detection increased to between 89 and 100% of the control samples. The 0.1 M EDTA concentration was used previously by Sobsey *et al.*, (1974) for detecting viruses in solid waste landfill leachates by preventing the apparent loss of viruses in leachates (Sobsey, *et al.*, 1974). The ability of EDTA to prevent or reverse leachate masking of viruses that are still infectious suggests that such masking is easily reversible by chelating agents. Hence, it must be assumed that such viruses are still potentially infectious for humans because the human alimentary tract also contains chelating agents.

Temperature

Analogous to the results obtained in the disposable diaper-fecal suspension experiments, temperature was the dominant factor in the inactivation of viruses in solid waste landfill leachates, both "young" and "old". At 40°C, estimated 4 log₁₀ virus inactivation in both leachates averaged approximately 10.5 days. At 5°C, estimated 4 log₁₀ virus inactivation in both leachates averaged 446 days. At 25°C, average 4 log₁₀ virus reduction was estimated at 106 days. Studies by Sobsey *et al.*, (1975) corroborate the conclusion that virus survival in leachates is temperature dependent by reporting that maximum inactivation rates occurred at 37°C and minimum rates at 4°C. Although these

were "natural" leachates from municipal solid waste landfills, the survival experiments were performed in the laboratory under simulated conditions. The results of these types of model studies indicate the effects of temperature on virus survival in leachates, even though conditions and temperatures may vary widely in solid waste landfill environments.

Virus Survival Differences Between "Old" and "Young"
Leachates

The tested landfill leachates came from solid waste landfills that had been in operation for an extended period of time. In one, the source of leachate was landfilled refuse >15 years old in the other case the source of leachate was landfilled refuse <2 years old. The leachates that were collected from these landfills were designated leachate 1 and 2 for the "older" leachate and "younger" leachate, respectively. Results from the survival experiments indicate that the viruses generally survived better in the "young" leachate rather than the "older" leachate. At 40°C, all three test viruses had significantly faster 4 log₁₀ inactivation times in the "older" leachate than in the "young" leachate. At 25°C, poliovirus and HAV also exhibited faster 4 log₁₀ inactivation times in the "older" leachate. The inactivation rates for F-specific coliphages in the "old" leachate (leachate 1) and the "young" leachate (leachate 2) were not significantly

different, suggesting similar die-off rates. The only case where viruses did not have the same inactivation rates or were not inactivated more rapidly in the "older" leachate than in the "young" leachate was at 5°C. HAV survived significantly better in leachate 1 than in leachate 2. However, HAV inactivation rates were so slow at 5°C that the differences between the two leachates is of little consequence. Previous studies suggest that leachate has an "inactivation property" and when poliovirus was tested, greater inactivation was observed with increasing age of the landfill leachate (Engelbrecht, *et al.*, 1974). One reason for appreciable virus loss in older leachates may be due to chemical changes which occur in aging leachates leading to production of concentrated toxins which may exert antimicrobial properties. The 4 log₁₀ inactivation of poliovirus and F-specific coliphages were not statistically significant between leachate 1 and leachate 2 at 5°C and therefore, it cannot be concluded that viruses are inactivated quicker in "old" leachates at low temperatures. Although the two leachates differed in chemical composition, the lack of a consistent and significant difference in virus survival between the young and old leachate precluded any explanation of virucidal properties based on measured chemical quality.

Differences Among Viruses in Survival Times in Leachates

Survival in the "older" leachate at 5°C showed no statistical difference among viruses for estimated 4 log₁₀ inactivation rates. All viruses survived for considerable periods of time. This was also true for virus survival in older leachate at 25°C. However, at 40°C some differences in estimated 4 log₁₀ inactivation times were noted, with poliovirus surviving the longest. Perhaps some activity or component of the leachate that affected viruses differently was more active at 40°C.

In the "young" landfill leachate at 5°C, HAV had a significantly "shorter" 4 log₁₀ inactivation rate time of 254 days compared to poliovirus (681 days) and F-specific coliphages (422 days). However, all viruses were inactivated very slowly. Survival of HAV at 25°C in the "young" leachate was also different and considerably longer than the other two viruses. At 40°C, inactivation rates were not significantly different but poliovirus appeared to survive somewhat longer than the other viruses.

Differences Between Virus Survival in Disposable Diaper-Fecal Suspensions versus Landfill Leachates

In some cases there were differences in virus survival between fecal suspensions, either aerobic or anaerobic, and landfill leachates. At 5°C, F-specific coliphages had different inactivation rates between leachate 1 and the

fecal suspension (aerobic) and HAV had different inactivation rates between the fecal suspension (aerobic) and leachate 2. At 25°C, F-specific coliphage 4 log₁₀ inactivation times were not statistically significant between both leachate 1 and leachate 2 for the aerobic fecal suspensions. At the same temperature, poliovirus results did not show significant 4 log₁₀ differences between both leachate 1 and leachate 2 in the aerobic fecal suspension and for leachate 2 in the anaerobic fecal suspension. HAV had similar 4 log₁₀ inactivation rates in leachate 1 for both the aerobic and the anaerobic fecal suspension at 25°C. The remainder of the viruses and test conditions at 25°C had different 4 log₁₀ virus inactivation times. At 40°C, viruses had significant differences for 4 log₁₀ inactivation rates between some leachate and fecal samples. Significantly different 4 log₁₀ reductions were seen between leachate 1 and the the anaerobic fecal suspension for F-specific coliphages and between leachate 1 and the aerobic fecal suspension for the aerobic fecal suspension for poliovirus. Other significant differences seen for 4 log₁₀ reductions occurred between leachate 1 and both the aerobic and anaerobic fecal suspensions at 40°C. No further significant 4 log₁₀ differences at 40°C were seen for the remainder of the viruses between leachates and fecal suspensions. In general, viruses survived longer in the fecal suspensions (aerobic and anaerobic) than in solid waste landfill

leachates. This may be due to the fact that landfill leachates contain chemicals, such as metals and various organics which may exert toxic effects on viruses. Viruses in fecal suspensions have the ability to associate with fecal material and be protected to some extent. Rao and Melnick (1986) reported that 95% of polioviruses became associated with sewage sludge after 10 minutes of experimental contact. The protection afforded by sludge solids may perhaps be a property of fecal solids, too, causing viruses to survive longer in fecal suspension than in leachates. Due to the fact that viruses were easily removed from the diaper pulp material in the presence of leachates, there is the potential for leachates to act on the feces; desorb the viruses and inactivate them.

This study demonstrates that viruses have the ability to survive for varying lengths of time in fecally contaminated disposable diapers and in landfill leachates under a variety of different temperatures and conditions. These experiments were done with actual fecal and leachate samples but under laboratory conditions. Further research to evaluate the environmental conditions e.g., temperature, moisture content, etc. that viruses in fecally soiled disposable diapers experience before being placed in municipal solid waste landfills is necessary to better characterize the fate of viruses and their potential risks to human health from this possible source of exposure.

CONCLUSIONS

Temperature was a very important factor influencing the rate and extent of reduction of hepatitis A virus, poliovirus 1 and F-specific coliphages in fecally soiled diapers over time. At the highest test temperature of 40°C, viruses were inactivated at a much faster rate than they were inactivated at 5 and 25°C. The longest virus survival occurred at 5°C and intermediate virus survival occurred at 25°C in fecally soiled diapers.

Temperature also proved to be an important factor influencing the rate and extent of reduction of hepatitis A virus, poliovirus 1 and F-specific coliphages in diaper material and solid waste landfill leachates over time. As for diaper material and feces, viruses had the shortest survival times at 40°C and the longest survival times at 5°C in landfill leachates.

There were some differences in virus survival between anaerobic and aerobic conditions in fecally soiled diapers at 25°C. At 40°C, there was no significant difference in the survival of HAV, poliovirus or F-specific coliphages between anaerobic and aerobic fecal suspensions. However, the inactivation rate of HAV was slower in aerobic conditions rather than anaerobic conditions. At 25°C, HAV survival in

aerobic and anaerobic fecal suspensions was not significantly different, but the inactivation rate was considerably slower in aerobic than in anaerobic conditions. However, poliovirus and F-specific phages were significantly different between anaerobic and aerobic conditions.

In most cases, survival of all three viruses was quite similar, although HAV showed the greatest survival in 6 of the 11 cases of experimental conditions tested. Poliovirus survived the longest in 3 of the cases and F-specific phages survived the longest in 2 of the cases. F-specific phages survived the longest in the anaerobic fecal suspensions and HAV survived the longest in the aerobic fecal suspensions. Virus survival in leachates varied among virus types and test conditions with HAV having greater survival in half of the leachate conditions and poliovirus surviving the longest in the other half of the leachate conditions.

Virus survival between the "older" and "younger" landfill leachates was only slightly different. On average, viruses survived longer in the "younger" leachate than in the "older" leachate. The difference in survival times between the "young" and the "old" leachate was not very great at 5 and 40°C. However, viruses tended to survive twice as long in the "younger" leachate at 25°C than they survived in the "older" leachate at 25°C.

Viruses were inactivated more rapidly in the presence of solid waste landfill leachates than in fecal suspensions

inactivated viruses. Overall, viruses survived longer in fecal suspension than in solid waste landfill leachates at both 5 and 40°C. At 25°C, viruses had the greatest survival in the "younger" leachate, intermediate survival in the fecal suspensions and the poorest survival in the "older" leachate.

FUTURE STUDIES

1. Repeat similar experiments using a longer time frame to characterize the inactivation of viruses. Collect samples at several time points while extending the overall length of the experiment.
2. Use other enteric viruses that have been found to be important environmental contaminants for which the technology is now available for their detection.
3. Follow and model the transport of soiled disposable diapers from point of contamination (generation) to final destination and test for persistence of viruses along the route.
4. Test more rigorously for differences between aerobic and anaerobic conditions to determine the physical, chemical and/or biological factors that are responsible for the inactivation of certain viruses in anaerobic or aerobic conditions.

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APPENDIX

Table 1

Adsorption of F+ Coliphages, Hepatitis A Virus and Poliovirus Type 1 to Diaper Pulp Saturated with PBS

Exp. #	Sample	Plaque Phage	Forming HAV	Units per ml Polio 1	Percent Phage	Virus HAV	Adsorbed Polio 1
PBS; Phages							
1	PBS	1.0 E3	ND	ND	--	ND	ND
	PBS-F	no data	ND	ND	no data	ND	ND
	DP-F-a	1.0 E3	ND	ND	0	ND	ND
	DP-F-b	1.4 E3	ND	ND	0	ND	ND
PBS; HAV and Polio							
1	PBS	ND	3.5 E4	ND	ND	--	ND
	PBS-F	ND	1.3 E5	ND	ND	0	ND
	DP-F-a	ND	5.5 E4	ND	ND	0	ND
	DP-F-b	ND	1.2 E4	ND	ND	66	ND
	DP-F-c	ND	1.0 E4	ND	ND	71	ND
2	PBS	ND	ND	4.4 E4	ND	ND	--
	PBS-F	ND	ND	1.9 E4	ND	ND	57
	DP-F-a	ND	ND	1.7 E4	ND	ND	61
	DP-F-b	ND	ND	1.4 E4	ND	ND	69
	DP-F-c	ND	ND	3.1 E4	ND	ND	30
3	PBS	ND	8.8 E5	ND	ND	--	ND
	PBS-F	ND	5.2 E5	ND	ND	0	ND
	DP-F-a	ND	5.2 E5	ND	ND	0	ND
	DP-F-b	ND	3.7 E5	ND	ND	0	ND
	PBS	ND	1.0 E6	ND	ND	--	ND
	PBS-F	ND	1.5 E6	ND	ND	0	ND
	DP-F-a	ND	6.9 E5	ND	ND	31	ND
	DP-F-b	ND	7.9 E5	ND	ND	21	ND
	DP-F-c	ND	8.0 E5	ND	ND	20	ND

Abbreviations: PBS = phosphate buffered saline; FS = fecal suspension; ND = not done; F = sample filtered; DP = Diaper Pulp; a, b & c = replicate samples

Table 2

Adsorption of F+ Coliphages, Hepatitis A Virus and Poliovirus Type 1 to Diaper Pulp Saturated Fecal Suspension

Exp. #	Sample	Plaque Forming Phage	Units HAV	per ml Polio 1	Percent Virus Adsorbed Phage	HAV	Polio 1
Feces Suspension; Phages							
1	FS	6.0 E3	ND	ND	--	ND	ND
	FS-F	2.9 E4	ND	ND	0	ND	ND
	DP-F-a	3.0 E4	ND	ND	0	ND	ND
	DP-F-b	3.5 E4	ND	ND	0	ND	ND
2	FS	2.8 E3	ND	ND	--	ND	ND
	FS-F	3.5 E3	ND	ND	0	ND	ND
	DP-F-a	4.2 E3	ND	ND	0	ND	ND
	DP-F-b	3.1 E3	ND	ND	0	ND	ND
3	FS	5.7 E2	ND	ND	--	ND	ND
	FS-F	3.0 E2	ND	ND	47	ND	ND
	DP-F-a	1.3 E2	ND	ND	77	ND	ND
	DP-F-b	4.7 E3	ND	ND	18	ND	ND
Feces Suspension; HAV and Polio							
1	FS	ND	3.0 E6	1.2 E5	ND	--	--
	FS-F	ND	2.5 E6	9.0 E4	ND	18	25
	DP-F-a	ND	2.8 E5	1.4 E5	ND	7	0
	DP-F-b	ND	2.7 E5	6.2 E4	ND	11	48
	DP-F-c	ND	2.4 E5	1.2 E5	ND	23	0
2	FS	ND	4.5 E4	6.5 E4	ND	--	--
	FS-F	ND	7.5 E4	9.7 E4	ND	0	0
	DP-F-a	ND	7.5 E4	8.0 E4	ND	0	0
	DP-F-b	ND	6.8 E4	1.2 E5	ND	0	0
	DP-F-c	ND	7.5 E4	1.4 E5	ND	0	0
3	FS	ND	1.3 E5	2.9 E5	ND	--	--
	FS-F	ND	7.3 E4	2.6 E5	ND	43	10
	DP-F-a	ND	9.8 E4	3.3 E5	ND	25	0
	DP-F-b	ND	5.7 E4	2.8 E5	ND	56	3
	DP-F-c	ND	1.1 E5	2.7 E5	ND	15	7

Abbreviations: PBS = phosphate buffered saline; FS = fecal suspension; ND = not done; F = sample filtered; DP = Diaper Pulp; a, b & c = replicate samples.

Table 3

Adsorption of F+ Coliphages, Hepatitis A Virus and Poliovirus Type 1 to
Absorptive Gel Material Saturated with PBS or Fecal Suspension

Exp. #	Sample	Plaque Forming Units per ml			Percent Virus Adsorbed		
		Phage	HAV	Polio 1	Phage	HAV	Polio 1
PBS; Phages							
1	PBS	2.8 E3	ND	ND	--	ND	ND
	PBS-F	3.5 E3	ND	ND	0	ND	ND
	DP-F-a	4.2 E2	ND	ND	0	ND	ND
	DP-F-b	3.1 E2	ND	ND	0	ND	ND
PBS; HAV and Polio							
1	PBS	ND	8.8 E5	8.3 E4	ND	--	--
	PBS-F	ND	5.2 E5	3.2 E4	ND	41	61
	DP-F-a	ND	5.2 E5	3.4 E4	ND	41	58
	DP-F-b	ND	3.7 E5	2.2 E4	ND	58	73
Feces Suspension; Phages							
1	FS	5.7 E2	ND	ND	--	ND	ND
	FS-F	3.0 E2	ND	ND	47	ND	ND
	DP-F-a	7.3 E2	ND	ND	0	ND	ND
	DP-F-b	4.7 E2	ND	ND	18	ND	ND
Feces Suspension; HAV and Polio							
1	FS	ND	ND	2.1 E5	ND	ND	--
	FS-F	ND	ND	2.3 E5	ND	ND	0
	AGM-F-a	ND	ND	1.9 E5	ND	ND	10
	AGM-F-b	ND	ND	2.2 E5	ND	ND	0
2	FS	ND	2.8 E5	ND	ND	--	ND
	FS-F	ND	4.0 E5	ND	ND	0	ND
	AGM-F-a	ND	3.7 E5	ND	ND	0	ND
	AGM-F-b	ND	3.7 E5	ND	ND	0	ND

Abbreviations: AGM = absorptive gel material.

Table 4

Adsorption of F+ Coliphages, HAV and Poliovirus Type 1 to Diaper Pulp Saturated with PBS or Landfill Leachates

Exp. #	Sample	PFU per ml		% Virus Adsorbed	
		HAV	Polio 1	HAV	Polio 1
PBS					
1	PBS	9.9 E4	1.4 E6	--	--
	PBS-F	1.6 E5	9.6 E5	0	31
	DP-F-a	1.7 E5	1.6 E6	0	0
	DP-F-b	8.0 E4	1.1 E6	11	21
Leachate					
1	L1	1.6 E5	1.4 E6	--	--
	L1-F	7.3 E4	1.7 E6	54	0
	L1-DP-Fa	1.1 E5	3.4 E5	31	76
	L1-DP-Fb	1.0 E5	5.2 E5	38	63
1	L2	8.0 E4	1.8 E6	--	--
	L2-F	1.0 E5	1.2 E6	0	33
	L2-DP-Fa	9.0 E4	1.0 E6	0	44
	L2-DP-Fb	1.7 E5	6.0 E5	0	67
1	L3	1.6 E5	1.4 E6	--	--
	L3-F	6.3 E4	5.6 E5	61	60
	L3-DP-Fa	1.0 E5	1.5 E6	38	0
	L3-DP-Fb	1.5 E5	1.0 E6	6	29

Abbreviations: L = leachate; DP = diaper pulp; F = filtered.

Table 5

Estimated Times for 1, 2, 3 and 4 log₁₀ (90, 99, 99.9 and 99.99%)
Virus Reductions in Fecally Soiled Diapers

Anaerobic or Aerobic	Temp (°C)	Virus Type	Estimated Days for Indicated Reduction			
			1 Log ₁₀ (90%)	2 Log ₁₀ (99%)	3 Log ₁₀ (99.9%)	4 Log ₁₀ (99.99%)
Aerobic	5	F-specific	(1) 188	404	620	836
			(2) 147	318	490	661
Aerobic	5	Poliovirus	(1) 164	326	489	651
			(2) 143	283	423	563
Aerobic	5	HAV	(1) 556	1117	1677	2238
			(2) 749	1428	2106	2785
Aerobic	25	F-specific	(1) 18	40	61	83
			(2) 19	40	62	84
Aerobic	25	Poliovirus	(1) 23	43	63	84
			(2) 25	45	65	85
Aerobic	25	HAV	(1) 35	71	108	145
			(2) 32	66	100	134
Aerobic	40	F-specific	(1) 3.9	9.8	15.7	21.6
			(2) 3.8	9.6	15.4	21.2
Aerobic	40	Poliovirus	(1) 2.3	8.1	13.9	19.7
			(2) 2.2	8.1	13.9	19.6
Aerobic	40	HAV	(1) 3.1	11.7	20.3	28.9
			(2) 3.0	11.7	20.3	29.0
Anaerobic	25	F-specific	(1) 43	87	131	175
			(2) 40	80	120	160
Anaerobic	25	Poliovirus	(1) 0	21	49	76
			(2) 0	20	50	80
Anaerobic	25	HAV	(1) 15	38	62	85
			(2) 12	35	58	81
Anaerobic	40	F-specific	(1) 4.4	10.3	16.2	22.1
			(2) 3.7	9.7	15.6	21.6
Anaerobic	40	Poliovirus	(1) 0	4.6	11.0	17.3
			(2) 2.5	9.4	16.3	23.2
Anaerobic	40	HAV	(1) 0	2.4	5.0	7.7
			(2) 0	2.2	4.9	7.6

Table 6

Estimated Times for 1, 2, 3 and 4 log₁₀ (90, 99, 99.9 and 99.99%)
Virus Reductions in Landfill Leachates

Leachate	Temp (°C)	Virus Type	Estimated Days for Indicated Reduction			
			1 Log ₁₀ (90%)	2 Log ₁₀ (99%)	3 Log ₁₀ (99.9%)	4 Log ₁₀ (99.99%)
No. 1	5	F-specific	(1) 76	158	239	321
			(2) 71	146	221	296
No. 1	5	Poliovirus	(1) 89	185	280	376
			(2) 80	172	265	357
No. 1	5	HAV	(1) 132	273	415	557
			(2) 179	362	545	729
No. 1	25	F-specific	(1) 4	18	32	46
			(2) 0	22	45	68
No. 1	25	Poliovirus	(1) 9	22	34	46
			(2) 9	21	33	45
No. 1	25	HAV	(1) 17	42	66	91
			(2) 15	38	61	84
No. 1	40	F-specific	(1) 0	0.6	1.4	2.1
			(2) 0.4	1.1	1.7	2.4
No. 1	40	Poliovirus	(1) 0	4.2	11.7	19.3
			(2) 0	4.2	11.7	19.2
No. 1	40	HAV	(1) 1.9	3.7	5.5	7.3
			(2) 1.9	3.7	5.4	7.2
No. 2	5	F-specific	(1) 105	211	318	424
			(2) 101	207	313	420
No. 2	5	Poliovirus	(1) 167	353	539	724
			(2) 144	308	473	638
No. 2	5	HAV	(1) 54	118	183	247
			(2) 50	120	190	260
No. 2	25	F-specific	(1) 4	19	35	51
			(2) 3	19	35	50
No. 2	25	Poliovirus	(1) 22	47	73	98
			(2) 20	47	75	102
No. 2	25	HAV	(1) 50	131	212	293
			(2) 26	115	205	294
No. 2	40	F-specific	(1) 0.6	1.4	2.2	3.0
			(2) 0.8	1.5	2.3	3.1
No. 2	40	Poliovirus	(1) 2.1	9.1	16.1	23.2
			(2) 1.5	8.5	15.4	22.3
No. 2	40	HAV	(1) 1.4	3.9	6.5	9.0
			(2) 1.5	4.1	6.6	9.1

Table 7

Survival of F-specific Coliphages in Disposable Diapers
Incubated Under Aerobic Conditions

<u>5° celsius</u>	<u>PFU/ml</u>	<u>log</u>
0	4.930*10 ⁴	4.693
0	6.000*10 ⁴	4.778
1	3.630*10 ⁴	4.560
1	4.700*10 ⁴	4.672
3	4.400*10 ⁴	4.643
3	5.270*10 ⁴	4.722
10	2.170*10 ⁴	4.336
10	2.600*10 ⁴	4.415
30	2.700*10 ⁴	4.431
30	2.333*10 ⁴	4.368
60	1.127*10 ⁴	4.052
60	1.142*10 ⁴	4.058
90	2.050*10 ⁴	4.312
90	2.033*10 ⁴	4.308
<u>25° celsius</u>		
0	6.30*10 ⁴	4.799
0	5.67*10 ⁴	4.753
1	3.80*10 ⁴	4.580
1	3.30*10 ⁴	4.519
3	3.80*10 ⁴	4.580
3	2.63*10 ⁴	4.420
10	1.33*10 ⁴	4.124
10	1.83*10 ⁴	4.262
30	2.20*10 ³	3.342
30	1.63*10 ³	3.212
60	1.07*10 ³	1.029
60	2.70*10 ³	1.431
90	9.67*10 ⁰	0.985
90	5.67*10 ⁰	0.754
<u>40° celsius</u>		
0	5.20*10 ⁴	4.716
0	5.60*10 ⁴	4.748
1	1.83*10 ⁴	4.262
1	2.60*10 ⁴	4.415
3	1.00*10 ⁴	4.000
3	9.67*10 ³	3.985
10	8.33*10 ³	1.921
10	6.33*10 ³	1.801
30	<0.33*10 ⁰	<-0.481
30	<0.33*10 ⁰	<-0.481
60	<0.33*10 ⁰	<-0.481
60	<0.33*10 ⁰	<-0.481
90	<0.33*10 ⁰	<-0.481
90	<0.33*10 ⁰	<-0.481

Table 8

Survival of F-specific Coliphages in Disposable Diapers
Incubated Under Anaerobic Conditions

<u>25° celsius</u>	<u>PFU/ml</u>	<u>log</u>
0	$5.27 \cdot 10^4$	4.722
0	$4.90 \cdot 10^4$	4.690
1	$5.47 \cdot 10^4$	4.738
1	$3.80 \cdot 10^4$	4.580
3	$6.63 \cdot 10^4$	4.822
3	$3.47 \cdot 10^4$	4.540
10	$1.53 \cdot 10^4$	4.185
10	$2.23 \cdot 10^4$	4.348
30	$1.24 \cdot 10^4$	4.093
30	$1.70 \cdot 10^4$	4.230
60	$1.63 \cdot 10^3$	3.212
60	$2.73 \cdot 10^3$	3.436
90	$2.90 \cdot 10^2$	2.462
90	$2.97 \cdot 10^2$	2.473
<u>40° celsius</u>		
0	$4.73 \cdot 10^4$	4.675
0	$4.60 \cdot 10^4$	4.663
1	$2.00 \cdot 10^4$	4.301
1	$2.30 \cdot 10^4$	4.362
3	$6.00 \cdot 10^3$	3.778
3	$8.33 \cdot 10^3$	3.921
10	$6.67 \cdot 10^1$	1.824
10	$1.37 \cdot 10^2$	2.137
30	$<0.33 \cdot 10^0$	<-0.481
30	$<0.33 \cdot 10^0$	<-0.481
60	$<0.33 \cdot 10^0$	<-0.481
60	$<0.33 \cdot 10^0$	<-0.481
90	$<0.33 \cdot 10^0$	<-0.481
90	$<0.33 \cdot 10^0$	<-0.481

Table 9

Survival of Poliovirus in Disposable Diapers
Incubated Under Aerobic Conditions

<u>5° celsius</u>	<u>PFU/ml</u>	<u>log</u>
0	2.325×10^6	6.366
0	2.475×10^6	6.394
1	2.575×10^6	6.411
1	2.475×10^6	6.394
3	2.425×10^6	6.385
3	2.550×10^6	6.407
10	2.375×10^6	6.376
10	2.175×10^6	6.337
30	9.000×10^5	5.954
30	1.375×10^6	6.138
60	1.425×10^6	6.154
60	1.475×10^6	6.169
90	6.250×10^5	5.796
90	4.667×10^5	5.669
<u>25° celsius</u>		
0	2.350×10^6	6.371
0	2.550×10^6	6.407
1	2.225×10^6	6.317
1	2.450×10^6	6.389
3	2.525×10^6	6.402
3	2.375×10^6	6.376
10	2.200×10^6	6.342
10	2.175×10^6	6.337
30	1.205×10^5	5.081
30	7.955×10^5	5.901
60	8.500×10^2	2.929
60	1.675×10^3	3.224
90	2.500×10^2	2.398
90	1.508×10^2	2.176
<u>40° celsius</u>		
0	2.575×10^6	6.411
0	2.625×10^6	6.419
1	5.425×10^5	5.735
1	4.950×10^5	5.695
3	2.475×10^5	5.394
3	2.725×10^5	5.435
10	8.000×10^2	2.903
10	8.250×10^2	2.916
30	$<1.000 \times 10^1$	<1.000
30	$<1.000 \times 10^1$	<1.000
60	$<1.000 \times 10^1$	<1.000
60	$<1.000 \times 10^1$	<1.000
90	$<1.000 \times 10^1$	<1.000
90	$<1.000 \times 10^1$	<1.000

Table 10

Survival of Poliovirus in Disposable Diapers
Incubated Under Anaerobic Conditions

<u>25° celsius</u>	<u>PFU/ml</u>	<u>log</u>
0	1.85×10^6	6.267
0	1.725×10^6	6.237
1	5.50×10^4	4.740
1	6.00×10^4	4.778
3	5.50×10^4	4.740
3	3.75×10^4	4.574
10	1.275×10^4	4.106
10	9.50×10^3	3.978
30	5.796×10^3	3.763
30	4.364×10^3	3.640
60	5.00×10^2	2.699
60	5.00×10^2	2.699
90	1.75×10^2	2.243
90	1.00×10^2	2.000
<u>40° celsius</u>		
0	2.05×10^5	5.312
0	1.775×10^6	6.249
1	6.50×10^4	4.813
1	7.50×10^4	4.875
3	7.75×10^4	4.889
3	6.75×10^4	4.829
10	1.00×10^1	1.000
10	1.00×10^1	1.000
30	$<1.00 \times 10^1$	<1.000
30	$<1.00 \times 10^1$	<1.000
60	$<1.00 \times 10^1$	<1.000
60	$<1.00 \times 10^1$	<1.000
90	$<1.00 \times 10^1$	<1.000
90	$<1.00 \times 10^1$	<1.000

Table 11

Survival of Hepatitis A Virus in Disposable Diapers
Incubated Under Aerobic Conditions

<u>5° celsius</u>	<u>PFU/ml</u>	<u>log</u>
0	3.63×10^4	4.560
0	3.52×10^4	4.547
1	4.36×10^4	4.639
1	1.075×10^5	5.031
3	2.93×10^4	4.467
3	3.32×10^4	4.521
10	3.50×10^4	4.544
10	3.70×10^4	4.568
30	2.95×10^4	4.470
30	2.80×10^4	4.447
60	2.675×10^4	4.427
60	3.975×10^4	4.599
90	2.60×10^4	4.415
90	3.575×10^4	4.553
<u>25° celsius</u>		
0	4.295×10^4	4.633
0	4.659×10^4	4.668
1	4.43×10^4	4.646
1	4.73×10^4	4.675
3	3.36×10^4	4.526
3	4.23×10^4	4.626
10	3.017×10^4	4.480
10	3.217×10^4	4.507
30	1.70×10^3	3.230
30	1.20×10^3	3.079
60	1.00×10^3	3.000
60	8.75×10^2	2.942
90	1.75×10^2	2.243
90	1.25×10^2	2.097
<u>40° celsius</u>		
0	3.614×10^4	4.558
0	3.386×10^4	4.530
1	1.727×10^4	4.237
1	1.500×10^4	4.176
3	7.270×10^2	2.862
3	8.180×10^2	2.913
10	1.333×10^2	2.125
10	1.000×10^2	2.000
30	$<0.500 \times 10^1$	<0.699
30	$<0.500 \times 10^1$	<0.699
60	$<0.500 \times 10^1$	<0.699
60	$<0.500 \times 10^1$	<0.699
90	$<0.500 \times 10^1$	<0.699
90	$<0.500 \times 10^1$	<0.699

Table 12

Survival of Hepatitis A Virus in Disposable Diapers
Incubated Under Anaerobic Conditions

<u>25° celsius</u>	<u>PFU/ml</u>	<u>log</u>
0	1.15×10^5	5.061
0	1.00×10^5	5.000
1	2.55×10^4	4.407
1	3.63×10^4	4.560
3	2.45×10^4	4.389
3	3.18×10^4	4.502
10	2.60×10^4	4.415
10	2.75×10^4	4.439
30	4.545×10^2	2.658
30	1.068×10^3	3.029
60	5.00×10^1	1.699
60	2.50×10^1	1.398
90	1.00×10^1	1.000
90	2.00×10^1	1.301
<u>40° celsius</u>		
0	1.375×10^5	5.138
0	1.225×10^5	5.088
1	2.458×10^3	3.391
1	2.917×10^3	3.465
3	2.500×10^1	1.398
3	2.500×10^1	1.398
10	$<0.500 \times 10^1$	<0.699
10	$<0.500 \times 10^1$	<0.699
30	$<0.500 \times 10^1$	<0.699
30	$<0.500 \times 10^1$	<0.699
60	$<0.500 \times 10^1$	<0.699
60	$<0.500 \times 10^1$	<0.699
90	$<0.500 \times 10^1$	<0.699
90	$<0.500 \times 10^1$	<0.699

Table 13

Survival of F-specific Coliphages in Leachate No. 1
Incubated Under Anaerobic Conditions

5° celsius		
<u>DAY</u>	<u>PFU/ml</u>	<u>log</u>
0	$7.27 \cdot 10^3$	3.862
0	$8.07 \cdot 10^3$	3.907
1	$6.67 \cdot 10^3$	3.824
1	$7.73 \cdot 10^3$	3.888
3	$6.63 \cdot 10^3$	3.822
3	$6.83 \cdot 10^3$	3.834
10	$2.30 \cdot 10^3$	3.362
10	$2.60 \cdot 10^3$	3.415
30	$3.167 \cdot 10^3$	3.501
30	$3.967 \cdot 10^3$	3.565
60	$1.67 \cdot 10^3$	3.223
60	$1.73 \cdot 10^3$	3.238
90	$3.867 \cdot 10^2$	2.587
90	$3.30 \cdot 10^2$	2.519
<u>25° celsius</u>		
0	$5.80 \cdot 10^3$	3.763
0	$5.00 \cdot 10^3$	3.699
1	$4.50 \cdot 10^3$	3.653
1	$4.57 \cdot 10^3$	3.660
3	$1.30 \cdot 10^3$	3.114
3	$1.12 \cdot 10^3$	3.049
10	$6.67 \cdot 10^0$	0.824
10	$3.33 \cdot 10^0$	0.533
30	$0.667 \cdot 10^0$	-0.176
30	$0.667 \cdot 10^0$	-0.176
60	$<0.333 \cdot 10^0$	<-0.478
60	$0.666 \cdot 10^0$	-0.176
90	$<0.333 \cdot 10^0$	<-0.478
90	$<0.333 \cdot 10^0$	<-0.478
<u>40° celsius</u>		
0	$1.37 \cdot 10^4$	4.137
0	$9.61 \cdot 10^3$	3.983
1	$3.33 \cdot 10^1$	1.522
1	$<0.333 \cdot 10^0$	<-0.478
3	$<0.333 \cdot 10^0$	<-0.478
3	$<0.333 \cdot 10^0$	<-0.478
10	$<0.333 \cdot 10^0$	<-0.478
10	$<0.333 \cdot 10^0$	<-0.478
30	$<0.333 \cdot 10^0$	<-0.478
30	$<0.333 \cdot 10^0$	<-0.478
60	$<0.333 \cdot 10^0$	<-0.478
60	$<0.333 \cdot 10^0$	<-0.478
90	$<0.333 \cdot 10^0$	<-0.478
90	$<0.333 \cdot 10^0$	<-0.478

Table 14

Survival of F-specific Coliphages in Leachate No. 2
Incubated Under Anaerobic Conditions

5° celsius		
<u>DAY</u>	<u>PFU/ml</u>	<u>log</u>
0	$2.27 \cdot 10^3$	3.356
0	$2.47 \cdot 10^3$	3.393
1	$2.17 \cdot 10^3$	3.336
1	$2.03 \cdot 10^3$	3.307
3	$1.67 \cdot 10^3$	3.223
3	$2.23 \cdot 10^3$	3.348
10	$9.80 \cdot 10^2$	2.291
10	$1.15 \cdot 10^3$	3.061
30	$2.70 \cdot 10^3$	3.431
30	$1.30 \cdot 10^3$	3.114
60	$7.21 \cdot 10^2$	2.858
60	$8.57 \cdot 10^2$	2.933
90	$2.20 \cdot 10^2$	2.342
90	$2.43 \cdot 10^2$	2.386
<u>25° celsius</u>		
0	$2.73 \cdot 10^3$	3.436
0	$2.87 \cdot 10^3$	3.458
1	$1.57 \cdot 10^3$	3.196
1	$1.23 \cdot 10^3$	3.090
3	$8.11 \cdot 10^2$	2.909
3	$8.28 \cdot 10^2$	2.918
10	$3.33 \cdot 10^0$	0.522
10	$3.33 \cdot 10^0$	0.522
30	$0.667 \cdot 10^0$	-0.176
30	$0.667 \cdot 10^0$	-0.176
60	$<0.333 \cdot 10^0$	<-0.478
60	$<0.333 \cdot 10^0$	<-0.478
90	$<0.333 \cdot 10^0$	<-0.478
90	$<0.333 \cdot 10^0$	<-0.478
<u>40° celsius</u>		
0	$2.67 \cdot 10^3$	3.427
0	$2.60 \cdot 10^3$	3.415
1	$3.33 \cdot 10^1$	1.522
1	$1.33 \cdot 10^2$	2.124
3	$<0.333 \cdot 10^0$	<-0.478
3	$<0.333 \cdot 10^0$	<-0.478
10	$<0.333 \cdot 10^0$	<-0.478
10	$<0.333 \cdot 10^0$	<-0.478
30	$<0.333 \cdot 10^0$	<-0.478
30	$<0.333 \cdot 10^0$	<-0.478
60	$<0.333 \cdot 10^0$	<-0.478
60	$<0.333 \cdot 10^0$	<-0.478
90	$<0.333 \cdot 10^0$	<-0.478
90	$<0.333 \cdot 10^0$	<-0.478

Table 15

Survival of Poliovirus in Leachate No. 1
Incubated Under Anaerobic Conditions

5° celsius		
<u>DAY</u>	<u>PFU/ml</u>	<u>log</u>
0	8.250×10^6	6.916
0	9.750×10^6	6.989
1	9.000×10^6	6.954
1	9.000×10^6	6.954
3	4.762×10^6	6.678
3	4.670×10^6	6.669
10	8.500×10^6	6.929
10	7.750×10^6	6.889
30	1.450×10^6	6.161
30	1.425×10^6	6.154
60	1.750×10^6	6.243
60	2.125×10^6	6.327
90	9.750×10^5	5.989
90	8.250×10^5	5.916
<u>25° celsius</u>		
0	9.250×10^6	6.966
0	1.000×10^7	7.000
1	4.000×10^6	6.602
1	4.119×10^6	6.615
3	2.000×10^6	6.301
3	2.825×10^6	6.451
10	9.000×10^5	5.954
10	7.750×10^5	5.889
30	1.000×10^4	4.000
30	7.500×10^3	3.875
60	$<1.000 \times 10^2$	<2.000
60	$<1.000 \times 10^2$	<2.000
90	$<1.000 \times 10^2$	<2.000
90	$<1.000 \times 10^2$	<2.000
<u>40° celsius</u>		
0	7.500×10^6	6.875
0	7.750×10^6	6.889
1	3.932×10^5	5.595
1	5.136×10^5	5.711
3	1.425×10^4	4.154
3	1.125×10^4	4.051
10	5.000×10^2	2.699
10	5.000×10^2	2.699
30	$<1.000 \times 10^2$	<2.000
30	$<1.000 \times 10^2$	<2.000
60	$<1.000 \times 10^2$	<2.000
60	$<1.000 \times 10^2$	<2.000
90	$<1.000 \times 10^2$	<2.000
90	$<1.000 \times 10^2$	<2.000

Table 16

Survival of Poliovirus in Leachate No. 2
Incubated Under Anaerobic Conditions

5° celsius		
<u>DAY</u>	<u>PFU/ml</u>	<u>log</u>
0	5.250×10^6	6.720
0	6.500×10^6	6.813
1	4.024×10^6	6.605
1	6.167×10^6	6.790
3	3.050×10^6	6.484
3	4.125×10^6	6.615
10	7.750×10^6	6.889
10	6.000×10^6	6.778
30	1.225×10^6	6.088
30	1.025×10^6	6.011
60	1.800×10^6	6.255
60	2.200×10^6	6.342
90	1.800×10^6	6.255
90	1.875×10^6	6.273
<u>25° celsius</u>		
0	4.500×10^6	6.653
0	5.500×10^6	6.740
1	3.595×10^6	6.556
1	3.643×10^6	6.561
3	2.550×10^6	6.407
3	1.650×10^6	6.217
10	1.725×10^6	6.237
10	1.600×10^6	6.204
30	6.250×10^4	4.796
30	9.250×10^4	4.966
60	1.575×10^4	4.197
60	1.575×10^4	4.197
90	1.250×10^3	3.097
90	2.250×10^3	3.352
<u>40° celsius</u>		
0	4.250×10^6	6.628
0	4.750×10^6	6.677
1	1.075×10^6	6.031
1	1.325×10^6	6.122
3	1.425×10^5	5.154
3	1.250×10^5	5.097
10	3.500×10^3	3.544
10	1.750×10^3	3.243
30	$<1.000 \times 10^2$	<2.000
30	$<1.000 \times 10^2$	<2.000
60	$<1.000 \times 10^2$	<2.000
60	$<1.000 \times 10^2$	<2.000
90	$<1.000 \times 10^2$	<2.000
90	$<1.000 \times 10^2$	<2.000

Table 17

Survival of Hepatitis A Virus in Leachate No. 1
Incubated Under Anaerobic Conditions

<u>5° celsius</u>		
<u>DAY</u>	<u>PFU/ml</u>	<u>log</u>
0	1.975×10^6	6.296
0	1.750×10^6	6.243
1	2.400×10^6	6.380
1	2.200×10^6	6.342
3	1.650×10^6	6.217
3	1.725×10^6	6.237
10	8.750×10^5	5.942
10	9.500×10^5	5.978
30	7.750×10^5	5.889
30	8.500×10^5	5.929
60	7.000×10^5	5.845
60	1.275×10^6	6.106
90	4.159×10^5	5.619
90	4.450×10^5	5.648
<u>25° celsius</u>		
0	2.125×10^6	6.327
0	1.875×10^6	6.273
1	1.925×10^6	6.284
1	1.850×10^6	6.267
3	1.375×10^6	6.138
3	8.250×10^5	5.916
10	1.750×10^5	5.243
10	NO DATA	
30	1.525×10^4	4.183
30	1.400×10^4	4.146
60	1.500×10^3	3.176
60	2.000×10^3	2.301
90	7.500×10^2	2.875
90	7.500×10^2	2.875
<u>40° celsius</u>		
0	1.800×10^6	6.255
0	2.000×10^6	6.301
1	6.500×10^5	5.813
1	8.000×10^5	5.903
3	5.500×10^4	4.740
3	5.250×10^4	4.720
10	$<0.500 \times 10^1$	<0.699
10	$<0.500 \times 10^1$	<0.699
30	$<0.500 \times 10^1$	<0.699
30	$<0.500 \times 10^1$	<0.699
60	$<0.500 \times 10^1$	<0.699
60	$<0.500 \times 10^1$	<0.699
90	$<0.500 \times 10^1$	<0.699
90	$<0.500 \times 10^1$	<0.699

Table 18

Survival of Hepatitis A Virus in Leachate No. 2
Incubated Under Anaerobic Conditions

5° celsius		
<u>DAY</u>	<u>PFU/ml</u>	<u>log</u>
0	5.750×10^4	4.760
0	6.500×10^4	4.813
1	3.500×10^4	4.544
1	1.750×10^4	4.243
3	9.250×10^4	4.966
3	9.500×10^4	4.978
10	9.100×10^3	3.959
10	1.130×10^4	4.053
30	1.075×10^4	4.031
30	8.000×10^3	3.903
60	3.500×10^3	3.544
60	2.400×10^3	3.380
90	2.250×10^3	3.352
90	3.250×10^3	3.512
<u>25° celsius</u>		
0	5.500×10^4	4.740
0	9.500×10^4	4.978
1	1.500×10^4	4.176
1	1.000×10^4	4.000
3	1.250×10^4	4.097
3	1.250×10^4	4.097
10	1.575×10^4	4.197
10	5.000×10^3	3.699
30	1.600×10^4	4.204
30	1.350×10^4	4.130
60	2.000×10^3	3.301
60	2.800×10^3	3.447
90	2.500×10^3	3.398
90	2.250×10^3	3.352
<u>40° celsius</u>		
0	9.000×10^4	4.954
0	7.500×10^4	4.875
1	1.000×10^4	4.000
1	1.250×10^4	4.097
3	7.500×10^3	2.875
3	5.000×10^3	2.699
10	$<0.500 \times 10^1$	<0.699
10	$<0.500 \times 10^1$	<0.699
30	$<0.500 \times 10^1$	<0.699
30	$<0.500 \times 10^1$	<0.699
60	$<0.500 \times 10^1$	<0.699
60	$<0.500 \times 10^1$	<0.699
90	$<0.500 \times 10^1$	<0.699
90	$<0.500 \times 10^1$	<0.699