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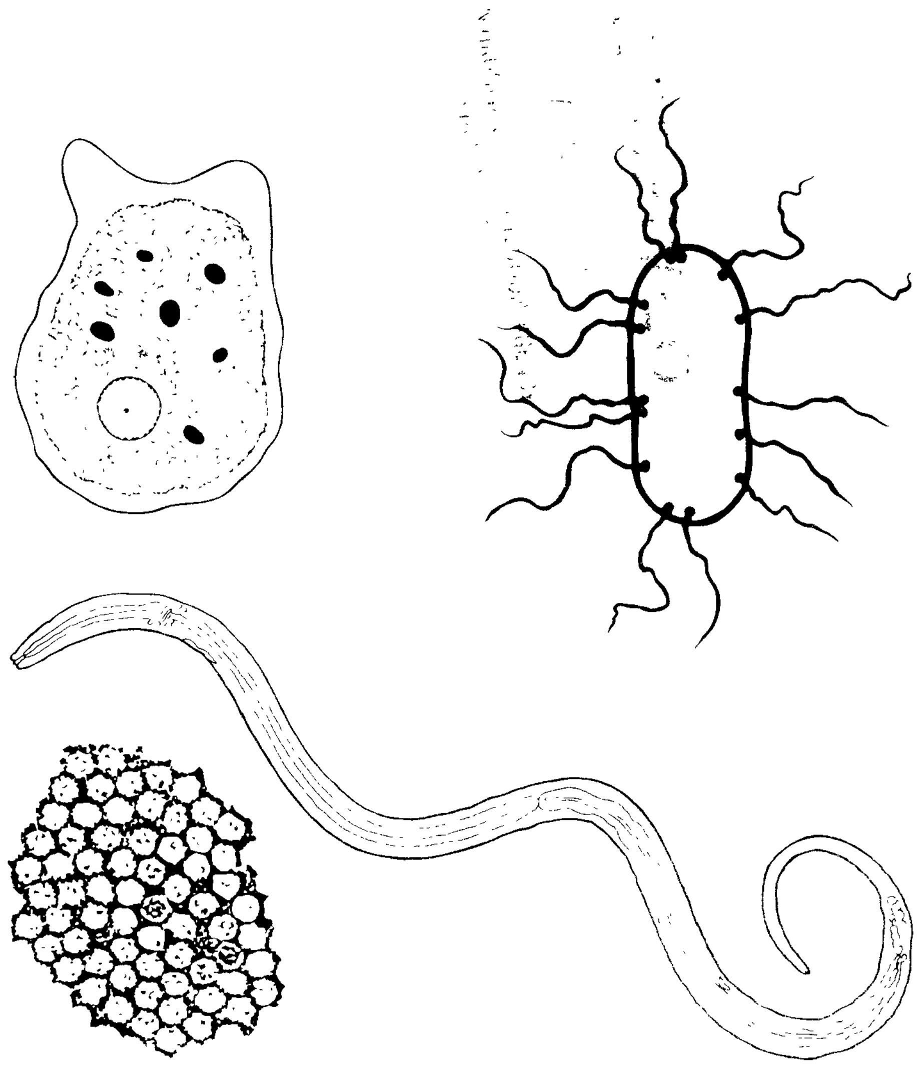
Health Effects of Land Treatment: Microbiological



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HEALTH EFFECTS OF LAND TREATMENT: MICROBIOLOGICAL

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and governmental concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay among its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report provides a general appraisal of the impact of microbiological contaminants in wastewater when applied to land. It is assumed that only a minimum of pre-application treatment is given so that the land itself serves as part of the treatment system. With a better understanding of such factors as microbiological densities, die-off rates, and minimum infective dose, more informed decisions may be made on proper management practices necessary to protect public health in the community.

James B. Lucas
Acting Director
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ABSTRACT

The potential health effects arising from the land treatment of wastewater are examined, and an appraisal of these effects made. The agents, or pollutants, of concern from a health effects viewpoint are divided into the categories of pathogens and toxic substances. Only the former are considered in this volume, the latter to be discussed in a subsequent volume. The pathogens include bacteria, viruses, protozoa, and helminths. These agents form the basis of the main sections of this report.

For each agent of concern the types and levels commonly found in municipal wastewater and the efficiency of preapplication treatment (usually stabilization pond) are briefly reviewed. A discussion of the levels, behavior, and survival of the agent in the medium or route of potential human exposure, i.e., aerosols, surface soil and plants, subsurface soil and groundwater, and animals, follows as appropriate. Infective dose, risk of infection, and epidemiology are then briefly reviewed. Finally, conclusions and research needs are presented.

This report covers a period from October 1979 to April 1981 and work was completed as of April 1981.

CONTENTS

TABLES

ACKNOWLEDGMENT

The editorial and scientific contributions of Herbert R. Pahren and Elmer W. Akin are gratefully acknowledged.

SECTION 1
INTRODUCTION

For centuries Western man has been conscious of the potential value of the application of human wastes to the land. Thus, von Liebig, in his 1863 work, *The Natural Laws of Husbandry* (Jewell and Seabrook 1979) wrote:

> Even the most ignorant peasant is quite aware that the rain falling upon his dung-heap washes away a great many silver dollars, and that it would be much more profitable to him to have on his fields what now poisons the air of his house and the streets of the village; but he looks on unconcerned and leaves matters to take their course, because they have always gone on in the same way.

In the context of present-day conventional wastewater treatment we might add "poisons the rivers and streams" as well. More recently, the Committee on Water Quality Criteria of the National Academy of Sciences-National Academy of Engineering (1972) stated that:

> An expanding population requires new sources of water for irrigation of crops and development of disposal systems for municipal and other wastewaters that will not result in the contamination of streams, lakes, and oceans. Irrigation of crops with wastewater will probably be widely practiced because it meets both needs simultaneously.

Still more recently the U.S. General Accounting Office (1978) concluded that greater use of land application as an alternative wastewater treatment technique is needed, because it provides the benefits of (1) the elimination of point discharge to surface waters, (2) higher levels of treatment than generally provided by conventional secondary treatment, and (3) recharge of groundwaters. The GAO felt that land application techniques have not been widely used because (1) restrictive State pretreatment requirements have caused these techniques to compare unfavorably with conventional treatment alternatives, (2) limited technical and health effects information is available, and (3) suitable land may not be available.

The legislative mandate for the greater use of land application treatment techniques is found in the Clean Water Act of 1977 (PL 95-217), Title II (Grants for Construction of Treatment Works), Section 201, which states that the:

> Administrator shall encourage waste treatment management which results in the construction of revenue producing facilities providing for (1) the recycling of potential sewage pollutants through the production of agriculture, silviculture, or aquaculture products, or any combination thereof . . .

Moreover, the Act requires that a construction grant not be made unless:

> the grant applicant has satisfactorily demonstrated to the Administrator that innovative and alternative wastewater treatment processes and techniques which provide for the reclaiming and reuse of water, otherwise eliminate the discharge of pollutants, and utilize recycling techniques, land treatment, new or improved methods of waste treatment management for municipal and industrial waste (discharged into municipal systems) and the confined disposal of pollutants, so that pollutants will not migrate to cause water or other environmental pollu-

tion, have been fully studied and evaluated by the applicant taking into the account section 201(d) of this Act and taking into account and allowing to the extent practicable the more efficient use of energy and resources.

One of the most important of these innovative and alternative wastewater treatment processes and techniques is land treatment.

There are three types of land treatment systems in general use: slow rate (or "irrigation"), rapid infiltration (or "infiltration-percolation"), and overland flow. Slow rate is the most commonly used land treatment system. Wastewater, usually pretreated by some process, is applied by sprinklers, surface flooding, or ridge-and-furrow irrigation, at a rate of 2-20 feet (0.6-6 m) per year. Soils are usually medium to fine textured with moderate permeability, and percolated water is either collected by drainage tile or reaches the groundwater. Surface vegetation has included lawns and golf courses for highly treated wastewater, pastures, and forests, but is most commonly crops, usually for animal consumption. Climatic constraints often require some winter storage of wastewater (Reed 1979) Recycling benefits include moderate groundwater recharge and the utilization of wastewater nutrients in crop production.

In rapid infiltration wastewater is flooded, usually intermittently, into shallow basins at a rate of 20-600 feet (6-183 m) per year. Soils are usually coarse textured with high permeability, and percolated water moves to groundwater, recovery wells, or underdrains Surface vegetation is usually absent, and climatic constraints not of concern (Reed 1979). Groundwater recharge is a recycling benefit.

Overland flow is the least commonly used land treatment system. Wastewater is applied to the top of gently sloping (2-4 percent) fields at a rate of 10-70 feet (3-21 m) per year, and moves by sheet flow down the slope to collection ditches at the base. Soils are usually fine textured with very low permeability. Surface vegetation consists of water-tolerant grasses, e.g., reed canary grass. Climatic constraints may require some winter storage of wastewater (Reed 1979). Recycling benefits may include the utilization of wastewater nutrients in harvested grass for animal forage.

Many of the examples of land application systems in the U.S. utilize wastewater treated by conventional means up to tertiary level (secondary in the case of overland flow). The objectives of these systems are usually to produce clean irrigation water (e.g., for golf course application) or highly treated water for groundwater recharge. From a wastewater treatment point of view, land application in these systems is a form of tertiary treatment or effluent "polishing," rather than true land treatment. In land *treatment* systems, the subject of this report, raw wastewater is given a minimum preapplication treatment, or "pretreatment," e.g., by a stabilization pond, before being applied to the land, and the land itself is the site of the major portion of the wastewater treatment.

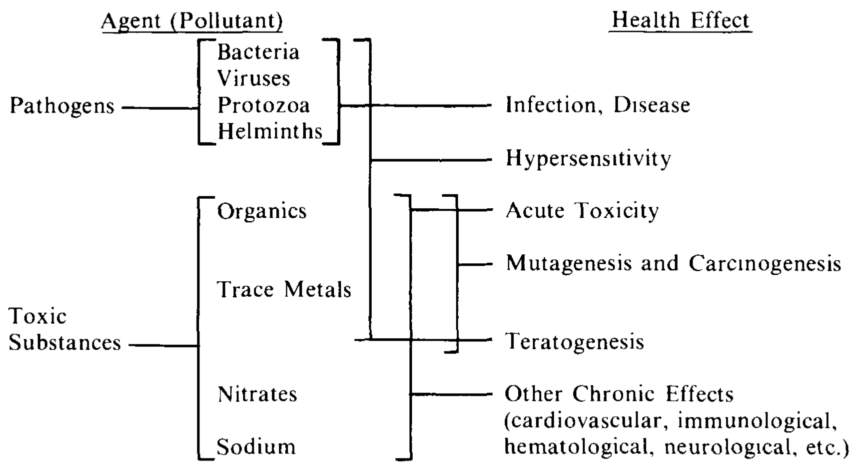
With the application to land of large volumes of minimally pretreated wastewater, it is evident that considerable potential for adverse health effects exists. These potentials have been briefly summarized by Lance and Gerba (1978) in Table. 1. They identified the greatest health risks as arising from aerosols in slow rate, groundwater pollution in rapid infiltration, and surface water pollution in overland flow.

TABLE 1. POTENTIAL LAND TREATMENT HEALTH EFFECTS*

Type of land treatment system	Food contamination	Groundwater pollution	Surface Water pollution	Aerosols
Slow rate	+	+	+	++
Rapid infiltration	-	++	-	-
Overland flow	-	-	++	+

-Little or no potential problem
+Moderate potential
++Considerable potential
*Source Lance and Gerba 1978

It is the purpose of this report to examine the potential health effects of land treatment, and to provide an appraisal of these effects. The agents, or pollutants, of concern from a health effects viewpoint can be divided into the two broad categories of pathogens and toxic substances. The pathogens include bacteria (e.g., *Salmonella* and *Shigella*), viruses (i.e., enteroviruses, hepatitis virus, adenoviruses, rotaviruses, and Norwalk-like agents), protozoa (e.g., *Entamoeba* and *Giardia*), and helminths (or worms, e.g., *Ascaris*, *Trichuris*, and *Toxocara*). The protozoa and helminths are often grouped together under the term, "parasites," although in reality all the pathogens are parasites. The toxic substances include organics, trace elements (or heavy metals, e.g., cadmium and lead), nitrates, and sodium. Nitrates and sodium are usually not viewed as "toxic" substances, but are here so considered because of their potential hematological and long-term cardiovascular effects when present in water supplies at high levels. These agents form the basis of the main sections of this report. The major health effects of these agents are listed below:



For each agent of concern the types and levels commonly found in municipal wastewater and the efficiency of preapplication treatment (usually stabilization pond) are briefly reviewed. A discussion of the levels, behavior, and survival of the agent in the medium or route of potential human exposure, i.e., aerosols, surface soil and plants, subsurface soil and groundwater, and animals, follows as appropriate. For the pathogens, infective dose, risk of infection, and epidemiology are then briefly reviewed. Finally, conclusions and research needs are presented.

Surface water pollution from land treatment site runoff is not considered since proper system design should prevent direct runoff to surface waters (Sorber and Guter 1975, Reed 1979). Surface discharge of overland flow effluent may have similar consequences to those of conventional treatment, but little is known in this area since examples are so few.

The present volume is devoted to the pathogens. A subsequent volume will cover the toxic substances.

SECTION 2
CONCLUSIONS

The types and levels in wastewater of most pathogens are fairly well understood. However, viruses are less well understood. The occurrence of virus in an environmental setting should probably be based on viral tests rather than bacterial indicators since failures in this indicator system have been reported.

Although untreated wastewater should never be used for irrigation, the level of preapplication treatment required for the protection of public health may be as little as properly-designed sedimentation at land treatment sites with limited public access, where crops are protected by appropriate crop choice and waiting periods, and groundwater is protected by appropriate hydrological studies and selection of application rate. Where protection of groundwater cannot be assured, wastewater stabilization ponds should be considered for virus removal. Because of potential contamination of crops and infection of animals, slow-rate irrigation and overland-flow systems should have complete removal of helminth eggs. These relatively simple pretreatment requirements would be appropriate for many land treatment systems in the United States, e.g., for many slow-rate sites where crops for animal feed are grown

This appraisal assumes only a minimum level of preapplication treatment, i.e., properly-designed sedimentation. In situations with greater public access (e.g., water disposal on golf courses), shorter waiting periods before grazing or harvest of crops (e.g., agriculture in arid areas), or threat of groundwater contamination (e.g., shallow water table), more extensive preapplication treatment may be required. This treatment may consist of wastewater stabilization ponds, conventional treatment unit processes, or even disinfection. The exact degree of pretreatment required for these situations is site-specific, and recommendations should be determined separately for each system.

Because of the potential exposure to aerosolized viruses at land treatment sites, it would be prudent to limit public access to 100–200 m from a spray source. At this distance bacteria are also unlikely to pose a significant risk. Human exposure to pathogenic protozoa or helminth eggs through aerosols is extremely unlikely.

Suppression of aerosol formation by the use of downward-directed, low-pressure nozzles, ridge-and-furrow irrigation, or drip irrigation is recommended where these application techniques are feasible.

The survival times of pathogens on soil and plants are summarized as follows. Since pathogens survive for a much longer time on soil than plants, the recommended waiting periods before harvest are based upon probable contamination with soil.

	Soil		Plants	
Pathogen	**Absolute maximum**	**Common maximum**	**Absolute maximum**	**Common maximum**
Bacteria	1 year	2 months	6 months	1 month
Viruses	6 months	3 months	2 months	1 month
Protozoa	10 days	2 days	5 days	2 days
Helminths	7 years	2 years	5 months	1 month

Aerial crops with little chance for contact with soil should not be harvested for human consumption for at least one month after the last wastewater application; subsurface and low-growing crops for human consumption should not be grown at a land treatment site for at least six months after last application. These waiting periods need not apply to the growth of crops for animal feed, however.

Properly designed slow-rate land treatment systems pose little threat of bacterial or viral contamination of groundwater.

Considerable threat of bacterial contamination exists, however, at rapid-infiltration sites where the water table is shallow, particularly if the soil is porous. Likewise, considerable potential for viral contamination of groundwater exists at rapid-infiltration sites, and appropriate preapplication treatment or management techniques should be instituted, e.g., intermittent application of wastewater. Until then, groundwater drawn for use as potable water supplies should be disinfected.

Human exposure to pathogenic protozoa or helminths through groundwater is extremely unlikely.

There appears to be little danger of bacterial, viral, or protozoal disease to animals grazing at land treatment sites if grazing does not resume until four weeks after application. Removal of helminth eggs during preapplication treatment should eliminate the potential of disease from those long-lived parasites.

Because of the possibility of picking up an infection, it would be wise for humans to maintain a minimum amount of contact with an active land treatment site.

Epidemiological studies to date suggest little effect of land treatment on disease incidence. However, well planned and implemented prospective studies have not been completed.

SECTION 3
RECOMMENDATIONS

This appraisal brought out a number of areas where additional research is needed to fill gaps in knowledge or substantiate information which has an insufficient data base. Research areas recommended are:

1. Develop better methods to recover and detect viruses, since only a small fraction of the total viruses in wastewater and other environmental samples may actually be detected.
2. Survival of viruses and protozoan cysts in storage ponds and waste stabilization ponds.
3. Determination of the effect of drying of the soil between wastewater applications on the survival of surface-soil viruses.
4. The factors controlling the migration of viruses in soils and the survival of viruses and bacteria in groundwater at rapid-infiltration sites.
5. The role of animals in transmitting human diseases at land application sites to animals or man off site.
6. The comparison of the respiratory infective dose of enteric viruses with the oral infective dose.
7. Well-planned and funded prospective acute disease epidemiological studies at land treatment sites involving a large number of exposed people should be completed.

SECTION 4
BACTERIA

TYPES AND LEVELS IN WASTEWATER

The pathogenic bacteria of major concern in wastewater are listed in Table 2. All have symptomless infections and human carrier states, and many have important nonhuman reservoirs as well. The pathogenic bacteria of minor concern are listed in Table 3; this list is perforce somewhat arbitrary since almost any bacterium can become an opportunistic pathogen under appropriate circumstances, e.g., in the immunologically compromised or in the debilitated. Recent reviews of pathogens in wastewater include those by Benarde (1973), Burge and Marsh (1978), Elliott and Ellis (1977), Kristensen and Bonde (1977), and Menzies (1977).

TABLE 2. PATHOGENIC BACTERIA OF MAJOR CONCERN IN WASTEWATER

Name	Nonhuman reservoir
Campylobacter jejuni	Cattle, dogs, cats, poultry
Escherichia coli (pathogenic strains)	—
Leptospira spp.	Domestic and wild mammals, rats
Salmonella paratyphi (A, B, C)*	—
Salmonella typhi	—
Salmonella spp.	Domestic and wild mammals, birds, turtles
Shigella sonnei, S. flexneri, S. boydii, S. dysenteriae	—
Vibrio cholerae	—
Yersinia enterocolitica, Y. pseudotuberculosis	Wild and domestic birds and mammals

*Correct nomenclature *Salmonella paratyphi* A, *S schottmuelleri*, *S hirschfeldii*, respectively

Campylobacter jejuni (formerly *C. fetus* subsp. *jejuni*) is a recently-recognized cause of acute gastroenteritis with diarrhea. It is now thought to be as prevalent as the commonly recognized enteric bacteria *Salmonella* and *Shigella*, having been isolated from the stools of 4-8% of patients with diarrhea (MMWR 1979).

Pathogenic strains of the common intestinal bacterium *Escherichia coli* are of three types—enterotoxigenic, enteropathogenic, and enteroinvasive (WHO Scientific Working Group 1980). All produce acute diarrhea, but by different mechanisms. Fatality rates may range up to 40% in newborns. Outbreaks occasionally occur in nurseries and institutions, and the disease is common among travelers to developing countries.

Leptospira spp. are bacteria excreted in the urine of domestic and wild animals, and enter municipal wastewater primarily from the urine of infected rats inhabiting sewers. Leptospirosis is a group of diseases caused by the bacteria, and may manifest itself through fever, headache, chills, severe malaise, vomiting, muscular aches, and conjunctivitis, and occasionally meningitis, jaundice, renal insufficiency, hemolytic anemia, and skin and mucous membrane hemorrhage. Fatality is low, but increases

TABLE 3. PATHOGENIC BACTERIA OF MINOR CONCERN IN WASTEWATER

Aeromonas spp
Bacillus cereus
Brucella spp.
Citrobacter spp.
Clostridium perfringens
Coxiella burnetii
Enterobacter spp.
Erysipelothrix rhusiopathiae
Francisella tularensis
Klebsiella spp.
Legionella pneumophila
Listeria monocytogenes
Mycobacterium tuberculosis
M. spp
Proteus spp.
Pseudomonas aeruginosa
Serratia spp.
Staphylococcus aureus
Streptococcus spp.

with age, and may reach 20% or more in patients with jaundice and kidney damage (Benenson 1975). In the U.S., 498 cases were reported in 1974-78 (Martone and Kaufmann 1980). Direct transmission from humans is rare, with most infection resulting from contact with the urine of infected animals, e.g., by swimmers, outdoor workers, sewer workers, and those in contact with animals.

Salmonella paratyphi (A, B, C) causes paratyphoid fever, a generalized enteric infection, often acute, with fever, spleen enlargement, diarrhea, and lymphoid tissue involvement. Fatality rate is low, and many mild attacks exhibit only fever or transient diarrhea. Paratyphoid fever is infrequent in the U.S. (Benenson 1975).

Salmonella typhi causes typhoid fever, a systemic disease with a fatality rate of 10% untreated or 2-3% treated by antibiotics (Benenson 1975). It occurs sporadically in the U.S., where 647 cases were reported in 1979 (MMWR 1980a), but is more common in the developing countries.

Salmonella spp., including over 1000 serotypes, cause salmonellosis, an acute gastroenteritis characterized by abdominal pain, diarrhea, nausea, vomiting, and fever. Death is uncommon except in the very young, very old, or debilitated (Benenson 1975). In 1979, 30,476 cases were reported to the Center for Disease Control (CDC) (MMWR 1980a).

Shigella sonnei, S. flexneri, S. boydii, and *S. dysenteriae* cause shigellosis, or bacillary dysentery, an acute enteritis primarily involving the colon, producing diarrhea, fever, vomiting, cramps, and tenesmus. There is negligible mortality associated with shigellosis (Butler *et al.* 1977). In 1979, 15,265 cases were reported to CDC (MMWR 1980b).

Vibrio cholerae causes cholera, an acute enteritis characterized by sudden onset, profuse watery stools, vomiting, and rapid dehydration, acidosis, and circulatory collapse. Fatality rates are about 50% untreated, but less than 1% treated (Benenson 1975). Cholera is rare in the U.S., there being no reported cases between 1911 and 1972, although one case occurred in 1973 in Texas and 11 in 1978 in Louisiana (Blake *et al.* 1980).

Yersinia enterocolitica and *Y. pseudotuberculosis* cause yersiniosis, an acute gastroenteritis and/or mesenteric lymphadenitis, with diarrhea, abdominal pain, and numerous other symptoms Death is uncommon. Yersiniosis occurs only sporadically in the U.S., and its transmitted from either infected animals or humans.

At this point it might be useful to clarify a few points of bacterial terminology. The term, "enteric bacteria," includes all those bacteria whose natural habitat is the intestinal tract of humans and animals, including members of several families, particularly Enterobacteriaceae and Pseudomonadaceae (e.g., *Pseudomonas*). They are all aerobic, gram-negative, nonsporeforming rods (Jawetz *et al.* 1978). The family Enterobacteriaceae includes the following tribes and genera (Holt 1977).

Escherichieae
- *Escherichia*
- *Edwardsiella*
- *Citrobacter*
- *Salmonella* (including *Arizona*)

Klebsielleae
- *Klebsiella*
- *Enterobacter*
- *Hafnia*
- *Serratia*

Proteeae
- *Proteus*

Yersinieae
- *Yersinia*

Erwinieae
- *Erwinia*

The terms, "total coliform" and "fecal coliform," are operationally-defined entities used for indicator purposes. Their taxonomic composition is variable, but all are members of the Enterobacteriaceae. A recent study of fecally-contaminated drinking water (Lamka *et al.* 1980) found the following composition:

Total Coliform Species	
Citrobacter freundii	46%
Klebsiella pneumoniae	18%
Escherichia coli	14%
Enterobacter agglomerans	12%
E. cloacae	4%
E. hafniae	3%
Serratia liquifaciens	1%
Fecal Coliform Species	
Escherichia coli	73%
Serratia liquifaciens	18%
Citrobacter freundii	9%

Most bacteria of concern in wastewater get there from human feces, although a few, such as *Leptospira*, enter through urine. The contribution from wash water, or "grey water," is probably relatively insignificant, except as it may contain opportunistic pathogens. Human feces contains 25–33% by weight of bacteria, most of these dead. Although the exact viable bacteria composition of feces is dependent on such factors as the age and nutritional habits of the individual, some gross estimates appear in the literature. Two such estimates are summarized in Table 4. The bacteria listed are normal fecal flora, and are only occasionally associated with disease as opportunistic pathogens.

In the case of those persons infected with one of the pathogenic bacteria of major concern, the fecal content of that bacterium may be quite high. Estimates are presented in Table 5 (Feachem *et al.* 1978).

Since the bacteria of feces are predominantly anaerobes while the environment of wastewater is often aerobic, and thus toxic to the anaerobes, the bacterial composition of wastewater is drastically different from that of feces. The composition also

TABLE 4. VIABLE BACTERIA IN HUMAN FECES (number/g wet weight)

	Carnow *et al* 1979	Feachem *et al.* 1978
Anaerobes		
Bacteroides	10^9–10^{10}	10^8–10^{10}
Bifidobacterium	10^9–10^{10}	10^9–10^{10}
Lactobacillus	10^3–10^5	10^6–10^8
Clostridium	10^3–10^5	10^5–10^6
Fusobacterium	10^3–10^5	-
Eubacterium	-	10^8–10^{10}
Veillonella	$<10^3$	-
Aerobes		
Enterobacteria*	10^6	10^7–10^9
Enterococci (fecal *Streptococcus*)	10^5	10^5–10^8
Staphylococcus	$<10^3$	-
Bacillus, Proteus, Pseudomonas, Spirochetes	$<<10^3$	-

*Enterobacteria are primarily *Escherichia coli*, with some *Klebsiella* and *Enterobacter* (Carnow *et al* 1979)

TABLE 5. PATHOGENIC BACTERIA IN FECES OF INFECTED PERSONS

Name	Number/g wet weight
Campylobacter jejuni	?
Escherichia coli (enteropathogenic strains)	10^8
Salmonella paratyphi (A, B, C)	10^6
Salmonella typhi	10^6
Salmonella spp.	10^6
Shigella sonnei, S. flexneri, S. boydii, S. dysenteriae	10^6
Vibrio cholerae	10^6
Yersinia enterocolitica, Y. pseudotuberculosis	10^5

varies with geographic region and season of the year, higher densities being found in summer. According to Carnow *et al.* (1979) the most prominent bacteria of human origin in raw municipal wastewater are *Proteus*, Enterobacteria (10^5/ml), fecal *Streptococcus* (10^3–10^4/ml), and *Clostridium* (10^2–10^3/ml). Less prominent bacteria include *Salmonella* and *Mycobacterium tuberculosis*. The total bacterial content of raw wastewater, as recovered on standard media at 20°C (Carnow *et al.* 1979), is about 10^6–10^7 organisms/ml. The presence and levels in wastewater of any of the pathogens listed in Tables 1 and 2 depend, of course, on the levels of infection in the contributing population.

PREAPPLICATION TREATMENT

Although any level of bacterial inactivation could theoretically be accomplished by disinfection with chlorine, such a practice on raw wastewater would be very costly (because of the high BOD, and thus high chlorine consumption), could produce carcinogenic chloromethanes, and could cause damage to the soil biota. Thus, simpler methods of preapplication treatment should be considered, if indeed they are necessary for the protection of public health.

An important point to keep in mind when discussing the degree of pathogen removal or survival during various wastewater treatment unit processes is the health significance of the number of organisms remaining. For example, if a wastewater contains 10^5 pathogenic bacteria per liter, a superficially impressive 99% removal, or

1% survival, will produce an effluent with 10^3 pathogenic bacteria per liter. This level may still be of great public health concern, depending on how the effluent is used. As Feachem *et al.* (1978) summarize the issue:

> When considering treatment technologies in terms of their ability to remove pathogens, it is necessary not to dwell on trivial differences, as between 92.3% removal and 97.8% removal, but to look at orders of magnitude. Conventional treatment works remove between 1 and 2 log units of enteric bacteria and should be contrasted with technologies, like waste stabilization ponds, which remove 5 or more log units. When considering technologies, like stabilization ponds or thermophilic digesters, with very high removal performance it is also misleading to talk in terms of percentage removal. Use of this convention disguises, for instance, the important difference between 99.99% removal and 99.999% removal.

The minimum preapplication treatment system likely to be used in land treatment is sedimentation, or conventional primary treatment. Typical degrees of bacterial removals have been summarized by Crites and Uiga (1979) and Sproul (1978), and are presented in Table 6.

TABLE 6. BACTERIAL REMOVAL DURING WASTEWATER SEDIMENTATION

Total coliforms	10%
Fecal coliforms	35%, 27-96%
Escherichia coli	15%
Mycobacterium tuberculosis	50%
Salmonella spp.	15%
Shigella spp.	15%

As a result of the need for winter storage of wastewater in most land treatment systems, and the possible need for low-cost further pathogen removal, wastewater stabilization ponds are likely to be the most common preapplication treatment system. Wastewater stabilization ponds, or "lagoons," are large shallow ponds in which organic wastes are decomposed by the action of microorganisms, especially bacteria. There are three types of ponds in common use, often used in a series (Feachem *et al.* 1978):

1. Anaerobic pretreatment ponds, 2–4 m deep, 1–5 day retention time.
2. Facultative ponds, with oxygen supplied by algae, 1–1.5 m deep, 10–40 day retention time.
3. Maturation ponds, 1–1.5 m deep, 5–10 day retention time.

Feachem *et al.* (1978) have surveyed a large body of literature on bacterial survival in ponds, and concluded that:

1. In single anaerobic ponds *E. coli* removals of 46–85% after 3.5–5 days at various temperatures have been reported.
2. In single facultative and aerobic ponds *E. coli* removals of 80 to over 99% after 10–37 days at various temperatures have been reported.
3. In single facultative and aerobic ponds fecal streptococci removals are similar to or greater than *E. coli.*
4. Removals of 99.99% or greater have been reported for series of 3 or more ponds.
5. One or two ponds will remove 90–99% of *Salmonella* or other pathogenic bacteria.
6. Complete elimination of pathogenic bacteria can be achieved with 30–40 day retention times, particularly at high temperatures (over 25°C).
7. A series of 5–7 ponds, each with a 5 day retention time, can produce an effluent with less than 100 fecal coliforms and fecal streptococci per 100 ml.

Aerated lagoons, i.e., ponds with mechanical aerators, have been reported (Crites and Uiga 1979) to provide removal rates of 60 to 99.99% for total coliforms and 99% for fecal coliforms, total bacteria, *Salmonella typhi*, and *Pseudomonas aeruginosa*.

Thus, wastewater stabilization ponds can be designed to achieve practically any degree of bacterial pathogen removal deemed necessary for the protection of public health, including complete wastewater treatment. Such a high degree of preapplication treatment, of course, should not be necessary for most land treatment systems.

AEROSOLS

Where wastewater is applied to the land by spray equipment of some sort, e.g., impact sprinklers, fan sprinklers, rain guns, and fixed-aperture rocker-arm sprayers, aerosols that travel beyond the wetted zone of application will be produced (Shaub *et al.* 1978a). These are suspensions of solid or liquid particles up to about 50 μm in diameter, formed, for example, by the rapid evaporation of small droplets to form droplet nuclei. Their content of microorganisms depends upon the concentration in the wastewater and the aerosolization efficiency of the spray process, a function of nozzle size, pressure, angle of spray trajectory, angle of spray entry to the wind, impact devices, etc. (Schaub *et al.* 1978a).

Although aerosols represent a means by which pathogens may be deposited upon fomites such as clothing and tools, the major health concern with aerosols is the possibility of direct human infection through the respiratory route, i.e., by inhalation. The exact location where aerosol particles are actually deposited upon inhalation is a function of their size. Those above about 2 μm in diameter are deposited primarily in the upper respiratory tract (including the nose for larger particles), from which they are carried by cilia into the oropharynx. They then may be swallowed, and enter the gastrointestinal tract. The smaller airways and alveoli do not possess cilia, so that pathogens deposited there would have to be combatted by local mechanisms. Although the pattern of deposition is variable, the greatest alveolar deposition appears to occur in the 1–2 μm range, decreasing to a minimum at about 0.25 μm, and increasing (due to Brownian motion) below 0.25 μm (Sorber and Guter 1975).

When aerosols are generated, bacteria are subject to an immediate "aerosol shock," or "impact factor," which may reduce their level by one log within seconds (Schaub *et al.* 1978a). There is some evidence that this might be caused by rapid pressure changes (Biederbeck 1979). Their survival is subsequently determined primarily by relative humidity and solar radiation (Carnow *et al.* 1979, Teltsch and Katzenelson 1978). At low relative humidities rapid desiccation occurs, resulting in rapid die-off (Sorber and Guter 1975), although concentration of protective materials within the droplet may occur (Schaub *et al.* 1978a). Solar radiation, particularly the ultraviolet portion, is destructive to bacteria, and increases the rate of desiccation. Teltsch and Katzenelson (1978) have found bacterial survival at night up to ten times that during daytime in Israel. High temperature is another factor decreasing bacterial survival While biological aerosol decay is occurring, the rate of physical aerosol decay, or deposition, simultaneously affects the distance of dissemination of the bacteria This is influenced by wind speed, air turbulence, and local topography, e.g., a windbreak of trees.

Any of the bacteria listed earlier as present in feces, urine, or wastewater could appear in aerosols emanating from land treatment sites. In aerosols generated by activated sludge aeration tanks, Kenline and Scarpino (1972) found *Klebsiella, Enterobacter, Escherichia, Citrobacter, Shigella, Arizona, Hafnia,* and *Serratia*, but no *Salmonella* (other than *Arizona*) or *Proteus*. Carnow *et al.* (1979), after reviewing the literature on wastewater treatment plant aerosols, concluded that recovered bacteria include *Klebsiella, Enterobacter, Proteus, Staphylococcus, Streptococcus, Mycobacterium*, and other nonpathogens. The dominance of *Klebsiella*, a respiratory pathogen, in the aerosol literature may be in error since Johnson *et al.* (1980)

have recently shown that further bacteriological confirmation steps of "*Klebsiella*" isolates reveal them to be nonpathogenic bacteria, true *Klebsiella* dying off rapidly during the aerosolization process.

Because of the low density of aerosol bacteria normally emanating from land treatment sites, high-volume samplers, e.g., 1 m^3/min electrostatic precipitators, are often necessary for aerosol analysis. Likewise, because of the normally low density of pathogenic bacteria compared with nonpathogens, most measurements of aerosol bacteria have utilized traditional indicator bacteria, e.g., standard plate count, total coliforms, and fecal coliforms. The measurements of Johnson *et al.* (1980) have shown little correlation between densities of these indicator bacteria and densities of the pathogens which they are intended to indicate. This results in "extreme underestimation of pathogen levels," since the pathogens which they studied, i.e., *Pseudomonas, Streptococcus,* and *Clostridium perfringens,* survived the aerosolization process much better than did the indicator bacteria. They suggest that fecal streptococci might be a more appropriate indicator organism because of its similar hardiness upon impact and viability to those of pathogens. Similarly, Teltsch *et al.* (1980) measured densities of coliforms, *Salmonella,* and the enteroviruses in aerosols and wastewater at an Israeli land treatment site, and from ". . . the ratios of salmonellae to coliforms and enteroviruses to coliforms in the air, as compared to these ratios in the wastewater, it was concluded that the suitability of coliforms as an indication of airborne contamination caused by spray irrigation is questionable."

The results of some of the most important studies of aerosol bacteria production at land treatment spray sites are summarized in Table 7. Although local environmental conditions, e.g., wind speed, vary among and within these studies, the results give a general idea of aerosol bacteria levels to be expected at land treatment sites.

The results suggest that the aerosol bacteria are usually detected at a maximum distance less than 400 m from the spray site. Experiments in Israel (Katzenelson *et al.* 1977) found that *Escherichia coli* could be detected in aerosols 10 m from the sprinkler only when its concentration in the wastewater reached 10^4/ml or more. The Pleasanton, California data (Johnson *et al.* 1978) suggest that a threshold value of 10^3/ml might be more reasonable for wastewater bacteria. There is some evidence (Reploh and Handloser 1957) that the type of sprinkler and spray diameter has little effect on the distance of aerosol bacteria transport. It is generally felt, however, that downward-directed, low-pressure sprinklers (usually on center-pivot spray rigs) produce much less aerosol than the upward-directed, high-pressure types used to obtain the data in Table 7. The Ft. Huachuca, Arizona results indicate a much greater transport distance during night than day, likewise the 400 m measurement in Germany (Bringmann and Trolldenier 1960) occurred at night. The high nighttime transport of aerosol bacteria is probably due to high humidity and absence of solar radiation. Most of the aerosols represented by the data in Table 7 are probably respirable, since Bausum *et al.* (1978) found that, at 30 m in Deer Creek, 75% of the particles fell in the range of 1–5 μm, with a median of 2.6 μm.

The human exposure to aerosol bacteria at land treatment sites can be roughly estimated from the data at Kibbutz Tzora, Israel, where raw wastewater was sprayed, thus yielding higher bacterial levels than those found at Deer Creek, Ft. Huachuca, or Pleasanton, where treated wastewater was sprayed. Thus, an adult male, engaged in light work, breathing at a rate of 1.2 m^3/hr, and exposed to 34 coliforms/m^3 (the Kibbutz Tzora average) at 100 m downwind from a sprinkler, would inhale approximately 41 coliforms per hour. Since the ratio of aerosolized *Salmonella* to coliforms is 1:10^5 (Grunnet and Tramsen 1974) the rate of inhalation of *Salmonella* would be about 10^5-fold less, an extremely low rate of bacterial exposure. More recent data from Kibbutz Tzora allows a more accurate estimate of human exposure (Teltsch *et al.* 1980). During a period of time in 1977–78, when the wastewater total coliforms were 2.4×10^6 to 1.4×10^7/100 ml and *Salmonella* was 0–60/100 ml, the density of aerosol *Salmonella* at 40 m, the maximum distance found, was 0–0.054/m^3, with a mean of 0.014/m^3. This would result in an inhalation

TABLE 7. AEROSOL BACTERIA AT LAND TREATMENT SITES

Wastewater type	Location (reference)	Distance (m)	Bacteria	Density (/m³)
Raw or primary	Germany (Reploh & Handloser 1957)	90–160	Coliform	Detected at maximum distance
	Germany (Bringmann & Trolldenier 1960)	63–400	Coliform	Detected at maximum distance (night)
	California (Sepp 1971)	32	Coliform	Detected at maximum distance
	Kibbutz Tzora, Israel (Katzenelson & Teltch 1976)	10	Coliform	11–496
			Fecal coliform	35–86
		20	Coliform	0–480
		60	"	0–501
			Salmonella	Detected at maximum distance
		70	Coliform	30–102
		100	"	0–88
		150	"	4–32
		200	"	0–25
		250	"	0–17
		300	"	0–21
		350	"	0–7
		400	"	0–4
Ponded, chlorinated	Deer Creek, Ohio (Bausum *et al* 1978)	Upwind	Std. plate count	111(23–403)
		21–30	"	485(46–1582)*
		41–50	"	417(0–1429)*
		200	"	37(<0–223)*

Continued

TABLE 7. Continued

Wastewater type	Location (reference)	Distance (m)	Bacteria	Density (/m³)
Secondary, nonchlorinated	Ft. Huachuca, Arizona (Schaub *et al.* 1978a)	Upwind	Std plate count	28(12-170)
			Coliform	2.4(0-58)
		45-49	Std. plate count	430-1400(day) 560-6300(night)
			Klebsiella	1-23
		120-152 m	Std plate count	86-130(day) 170-410(night)
	Pleasanton, California (Johnson *et al* 1978, 1980)	Upwind	Std plate count	300-805
		30-50	"	450-1560
			Total coliform	2 4-2.5
			Fecal coliform	0 4
			Fecal streptococci	0.3-1.7
			Pseudomonas	34
			Klebsiella	<5
			Clostridium perfringens	0 9
			Mycobacterium	0 8
		100-200	Std plate count	330-880
			Total coliform	0 6-1 2
			Fecal coliform	<0.3
			Fecal streptococci	0.3-1 9
			Pseudomonas	43
			Klebsiella	<5
			Clostridium perfringens	1 1
			Mycobacterium	0 8

*Corrected for upwind background value

rate of 0.017/hr at 40 m, higher than the previous estimate, but still an extremely low rate of bacterial exposure (cf. the infective dose discussion below).

SURFACE SOIL AND PLANTS

The surface soil and plants of an active land treatment site are constantly heavily laden with enteric bacteria; these are the specific locations where the actual treatment of the wastewater and inactivation of the bacteria occur. (In some situations bacteria may be deposited on plants in the environs of a land treatment site, due to aerosol drift.) The survival time of bacteria in surface soil and on plants is only of concern when decisions must be made on how long a period of time must be allowed after last application before permitting access to people or animals, or harvesting crops.

The factors affecting bacterial survival in soil (Gerba *et al.* 1975, USEPA 1977) are:

1. Moisture content. Moist soils and periods of high rainfall increase survival time. This has been demonstrated for *Escherichia coli*, *Salmonella typhi*, and *Mycobacterium avium*.
2. Moisture-holding capacity. Survival time is shorter in sandy soils than those with greater water-holding capacity.
3. Temperature. Survival time is longer at lower temperatures, e.g., in winter.
4. pH. Survival times are shorter in acid soils (pH 3–5) than in neutral or alkaline soils. Soil pH is thought to have its effect through control of the availability of nutrients or inhibitory agents. The high level of fungi in acid soils may play a role.
5. Sunlight. Survival time is shorter at the surface, probably due to desiccation and high temperatures, as well as ultraviolet radiation.
6. Organic matter. Organic matter increases survival time, in part due to its moisture-holding capacity. Regrowth of some bacteria, e.g., *Salmonella*, may occur in the presence of sufficient organic matter. In highly organic soils anaerobic conditions may increase the survival of *Escherichia coli* (Tate 1978).
7. Soil microorganisms. The competition, antagonism, and predation encountered with the endemic soil microorganisms decrease survival time. Protozoa are thought to be important predators of coliform bacteria (Tate 1978). Enteric bacteria applied to sterilized soil survive longer than those applied to unsterilized soil.

In view of the large number of environmental factors affecting bacterial survival in soil, it is understandable that the values found in the literature vary widely. Two useful summaries of this literature are those of Bryan (1977) and Feachem *et al.* (1978). The ranges given in Table 8 are extracted from these summaries, as well as other literature. "Survival" as used in this table, and throughout this report, denotes days of detection. It should be noted that inactivation is a rate process and therefore detection depends upon the initial level of organisms, sensitivity of detection methodology, and other factors. If kept frozen, most of these bacteria would survive longer than indicated in Table 8, but this would not be a realistic soil situation.

TABLE 8. SURVIVAL TIMES OF BACTERIA IN SOIL

Coliform	4–77 days
Fecal coliform	8–55 days
Fecal streptococci	8–>70 days
Leptospira	<15 days
Mycobacterium	10 days–15 months
Salmonella paratyphi	>259 days
Salmonella typhi	1–120 days
Salmonella spp.	11–>280 days
Streptococcus faecalis	26–77 days

The survival of bacteria on plants, particularly crops, is especially important since these may be eaten raw by animals or humans, may contaminate hands of workers touching them, or may contaminate equipment contacting them. Such ingestion or contact would probably not result in an infective dose of a bacterial pathogen, but if contaminated crops are brought into the kitchen in an unprocessed state they could result in the regrowth of pathogenic bacteria, e.g., *Salmonella*, in a food material affording suitable moisture, nutrients, and temperature (Bryan 1977). It should be kept in mind that many bacteria on plants, as well as soil, are not contaminants from human beings. For example, *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., and *Pseudomonas aeruginosa* are believed to be part of the natural flora of vegetables (Remington and Schimpff 1981).

Pathogens do not penetrate into vegetables or fruits unless their skin is broken (Bryan 1977, Rudolfs *et al.* 1951a), and many of the same factors affect bacterial survival on plants as those in soil, particularly sunlight and desiccation. The survival times of bacteria on subsurface crops, e.g., potatoes and beets, would be similar to those in soil. Useful summaries of the literature on the survival times of bacteria on aerial crops are those of Bryan (1977), Sepp (1971), and Feachem *et al.* (1978). The ranges given in Table 9 are extracted from these summaries, as well as other literature.

TABLE 9. SURVIVAL TIMES OF BACTERIA ON CROPS

Bacterium	Crop	Survival
Coliform	Tomatoes	>1 month
	Fodder	6-34 days
	Leaf vegetables	35 days
Escherichia coli	Vegetables	<3 weeks
	Grass	<8 days
Mycobacterium	Grass	10-14 days
	Lettuce	>35 days
	Radishes	>13 days
Salmonella typhi	Vegetables (leaves and stems)	10-31 days
	Radishes	24-53 days
	Lettuce	18-21 days
Salmonella spp.	Leaf vegetables	7-40 days
	Beet leaves	3 weeks
	Tomatoes	3-7 days
	Cabbage	5 days
	Gooseberries	5 days
	Clover	12 days
	Grass	>6 weeks
	Orchard crops	>2 days
Shigella spp.	Tomatoes	2-5 days
	Apples	8 days
	Leaf vegetables	2-7 days
	Fodder	<2 days
	Orchard crops	6 days
Vibrio cholerae	Vegetables	5-7 days
	Dates	<1-3 days

On the basis of New Jersey field experiments with tomatoes irrigated with municipal wastewater, Rudolfs *et al.* (1951a) concluded that: (1) cracks and split stem ends provide protected harboring places for enteric bacteria to survive for long periods, and such portions should be cut away before consumption, (2) on normal tomatoes, without cracks, after direct application of wastewater to the surface of the fruit the residual coliform concentration decreases to or below that of uncontaminated controls by the end of 35 days or less, (3) survival of *Salmonella* and *Shigella* on tomato surfaces in the field did not exceed 7 days, even when applied with fecal organic material, and (4) if wastewater application is stopped about one month before the harvest, the chances for the transmission of enteric bacterial diseases will decrease to almost nil.

On the basis of field experiments with lettuce and radish irrigated with municipal wastewater, Larkin *et al.* (1978a) concluded that leafy vegetables cannot be considered safe from *Salmonella* contamination until the soil can be shown to be free of *Salmonella*. They also noted that, because of regrowth in soil and on leaf crops, total coliforms and fecal streptococci bore no relationship to *Salmonella* levels, and are unacceptable indicators of fecal contamination; they recommended using fecal coliforms or *Salmonella* itself.

Thus, the consumption of subsurface and low-growing food crops, e.g., leafy vegetables and strawberries, harvested from an irrigated site within about six months of last application, is likely to increase the risk of disease transmission, because of contamination with soil and bacterial survival in cracks, leaf folds, leaf axils, etc. Possible approaches to avoid this problem are (1) use of the subsurface or covered drip irrigation method for aerial crops (Sadovski *et al.* 1978a, 1978b), (2) growth of crops the harvested portion of which does not contact the soil, e.g., grains and orchard crops, or (3) growth of crops used for animal feed only, e.g., corn (maize), soybeans, or alfalfa. The last alternative is probably the most common and most economic. In the situation where the harvested portion does not contact the soil nor is within splash distance, stopping wastewater application a month prior to harvest would be prudent.

MOVEMENT IN SOIL AND GROUNDWATER

Over 60 million people in the United States are served by public water supplies using groundwater, and about 54 percent of the rural population and 2 percent of the urban population obtain their water from individual wells (Duboise *et al.* 1979). Thus, it is imperative that land treatment systems do not result in the transmission of disease through groundwater. This is not to imply that groundwater in the U.S. is now pristine. Almost half of the waterborne disease outbreaks in the U.S. between 1971 and 1977 were caused by contaminated groundwater (Craun 1979), and a recent examination of individual groundwater supplies in a rural neighborhood of Oregon (Lamka *et al.* 1980) showed more than one-third to be fecally contaminated. But, thus far, no disease outbreaks have been attributed to wastewater land treatment systems (Gerba and Lance 1980)

It is generally felt that the removal of bacteria at land treatment sites occurs primarily by filtration, or straining, with most bacteria retained within about 50 cm of the soil surface. Under optimum conditions 92–97% of coliforms have been observed to be trapped in the first centimeter of soil (Gerba *et al.* 1975). Coarse sandy or gravelly soils or fissured subsurface geology would, of course, allow the bacteria to penetrate to great depths. Adsorption of bacteria also plays a secondary role, being increased by the presence of clay-sized particles, high cation concentration, and low pH. This adsorption is reversible, and the bacteria can be released and moved down the soil profile by distilled water or any water with low conductivity, e.g., rainfall (Sagik *et al.* 1978).

Once retained, the bacteria are inactivated by sunlight, oxidation, desiccation, and predation and antagonism by the soil microbial community. Intermittent application and drying periods result in more rapid die-off of enteric bacteria (USEPA 1977). However, too long a drying period has been found to result in deeper penetration of bacteria in rapid-infiltration systems upon resumption of flooding (Bouwer *et al.* 1974). This phenomenon is probably due to the decomposition of filtering organic matter on the soil surface and the decrease in soil microbial activity during extended drying. With the resumption of flooding, the filtering organic matter starts to accumulate on the surface and the soil microbial activity increases, causing greater bacterial removal.

Summaries of data on the soil penetration of bacteria at some of the most important land treatment sites are presented in Table 10 for slow-rate systems, and Table 11 for rapid-infiltration systems. Overland-flow systems should result in negligible penetration of soil by bacteria.

It is evident from Table 10 that most slow-rate sites pose little threat to the groundwater, but that the combination of high bacterial densities and shallow water table, resulting in seeping, such as that at the San Angelo site, should be avoided. The data of Table 11 suggest that bacteria at rapid-infiltration sites may penetrate about 10 m vertically and variable distances laterally. These distances are, of course, highly site-specific, and the vertical distance may be more than 10 m, but is usually much less.

To prevent the entry of enteric bacteria into groundwater, it would thus be advisable (unless an underdrain system is installed) not to site land treatment systems where the water table is shallow, particularly if the soil is sandy or gravelly, large cracks or root tunnels are present, or a thin soil mantle overlies rock with solution channels or fissures. This is especially true for rapid-infiltration systems.

Once in the groundwater the bacteria may travel long distances underground in situations where coarse soils or solution channels are present, but normally the filtering action of the matrix should restrict horizontal travel to only a few hundred feet (Sorber and Guter 1975). The actual distance travelled also depends upon the rate of movement of the groundwater and the survival time of the bacteria. The rate of movement of groundwater is highly site-specific, but often is extremely slow. The survival time of bacteria in groundwater would be expected to be longer than that in surface soil because of the moisture, low temperature, nearly neutral pH, absence of sunlight, and usual absence of antagonistic and predatory microorganisms. Groundwater survival times found in both field and laboratory measurements have been summarized by Gerba *et al.* (1975):

Coliforms	17 hours (for 50% reduction)
Escherichia coli	63 days–4.5 months
Salmonella	44 days
Shigella	24 days
Vibrio cholerae	7.2 hours

Concern has recently been expressed over the presence of endotoxin in municipal wastewater, and the possibility of it entering groundwater at land treatment sites (Goyal *et al.* 1980). Endotoxin is a lipopolysaccharide which is a natural component of cell walls of gram-negative bacteria, including enterobacteria, and, upon entry into the bloodstream, may cause acute nonspecific inflammation, fever, nausea, and shock. Goyal *et al.* (1980) showed that 90–99% of endotoxin is removed after travel of wastewater through 100–250 cm of loamy sand, but that the endotoxin can be desorbed and moved by rainfall, and can be detected in the groundwater beneath several land treatment sites. Since the concentration of endotoxin in the normal gut would be expected to be high due to endemic bacteria, the significance of endotoxin in groundwater is questionable. Chills, fever, and hypotension may occur if endotoxin-contaminated water is used for hemodialysis without pretreatment, but it would be impractical for water authorities to maintain endotoxin-free water since blue-green algae (closely related taxonomically to gram-negative bacteria), common in the flora of lakes and rivers, also produce endotoxin (Hindman *et al.* 1975).

TABLE 10. SOIL PENETRATION OF BACTERIA AT SLOW-RATE LAND TREATMENT SITES

Location (soil and substrate)	Type of applied effluent	Application rate	Bacterium	Concentration in applied effluent	Bacteria recovered: Depth	Bacteria recovered: Concentration	Ref.
Taber, Alberta (Loamy sand)	Aerobic lagoon	4 5 cm/wk	FC*	230–1700/ml	≥69 cm	None detected	1
Swift Current, Saskatchewan (Clay loam)	Aerobic lagoon		FC	8400/100 ml	>30 cm	None detected	2
San Angelo, Texas (Sandy clay)	Primary	2–3 times crop requirement	TC* FC *Salmonella*	10^2–10^5/ml 10–10^4/ml Present	Seepage creeks	10–10^2/ml 10–10^2/ml Present	3
Dickenson, North Dakota (Sandy alluvium)	Series lagoon	140 cm/yr	FC	9100/100 ml	2 5–75 ft (20 wells)	None detected	4
Roswell, New Mexico (Silty clay loams)	Chlorinated secondary	80 cm/yr	TC & FC		18–105 ft (groundwater)	No increase over control	5

*FC - fecal coliform, TC - total coliform

1 - Bell and Bole 1978
2 - Biederbeck and Bole 1979
3 - Weaver *et al* 1978
4 - Benham-Blair *et al* 1979
5 - Koerner and Haws 1979a

TABLE 11. SOIL PENETRATION OF BACTERIA AT RAPID-INFILTRATION LAND TREATMENT SITES

Location (soil and substrate)	Type of applied effluent	Application rate	Bacterium	Concentration in applied effluent (per 100 ml)	Bacteria recovered: Depth (meters)	Bacteria recovered: Concentration (per 100 ml)	Ref.
Lodi, California	Undisinfected		Coliforms		1.2-2.1	<1	1
(Sandy loam)					3.9	Detected in one case	
Santee, San Diego, California (Coarse gravel & sand)	Oxidation pond		Fecal streptococci	4500	61 L**	20	1
					122 L	48	
					450 L	6.8	
Flushing Meadows, Phoenix, Arizona (Sand & gravel)	Secondary	330 ft (99 m)/yr					
		2 wk/3 wk dry	TC*	10^6	9	200	1
		2 day/3 day dry	TC	10^6	9	5	
			FC*	10^5-10^6	60 L	ND***	2
Hollister, California (Gravelly sand over clay & silt)	Primary	15.4 m/yr, intermittent	TC	27.6×10^6	7-10	0.23-1.1×10^6	3
					21-24	<2-1,570	
					48	9	
			FC	12.4×10^6	7-10	156-186×10^3	
					21-24	0-11	
					48	<1	
Vineland, New Jersey (Sand)	Primary		FC		6-9	0-300	4
					>9	0	
Fort Devens, Massachusetts (Sand & gravel)	Primary	27.1 m/yr, intermittent	TC	6.8×10^6	18.3	3500	5
				32×10^6	60-100 L	<200	6
			FC		60-100 L	ND	

*TC - total coliform, FC - fecal coliform, ** L - Lateral, *** ND - Not detected

1 - Gerba *et al* 1975
2 - Bouwer and Rice 1978
3 - Pound *et al* 1978
4 - Koerner and Haws 1979b
5 - Satterwhite *et al* 1976b
6 - Satterwhite *et al* 1976a

ANIMALS

The major bacterial concerns with respect to animals grazing at land treatment sites are *Salmonella* infections and bovine tuberculosis *(Mycobacterium bovis* and *M. tuberculosis);* both can be passed on to man.

That the transmission of salmonellosis to cattle grazing at land treatment sites is at least possible was demonstrated by Taylor and Burrows (1971), who showed that calves grazing pastures, to which 10^6 *Salmonella dublin* organisms/ml of slurry had been applied, became infected. No infection occurred when the rate was decreased to 10^3/ml, suggesting that *Salmonella* may only be of concern when high concentrations are present. At the San Angelo, Texas, slow-rate soil treatment site, although *Salmonella* was isolated from the soil and the seepage creeks, the proportion of cattle grazing the pastures that were shedding *Salmonella* in their manure was not unusually high (Weaver *et al.* 1978). Feachem *et al.* (1978) concluded that there is no clear evidence that cattle grazed at land treatment sites are more at risk from salmonellosis than other cattle, probably because the required infectious doses are high and *Salmonella* infections are transmitted among cattle in many other ways. On the basis of *Salmonella* measurements in wastewater and sludge in England, Jones *et al.* (1980) concluded that a four-week waiting period would prevent salmonellosis in grazing animals.

Several investigations on tuberculosis infection of cattle grazing on wastewater-irrigated land have been performed in Germany, with the conclusion that if application is stopped 14 days before pasturing, there is no danger that grazing cattle will contract bovine tuberculosis (Sepp 1971).

Other possible bacterial concerns with respect to animals grazing at land treatment sites are *Leptospira* (causing leptospirosis), *Brucella* (causing brucellosis), and *Bacillus anthracis* (causing anthrax). Wastewater, however, probably contains insignificant numbers of these pathogens, and plays a negligible role in the transmission of these diseases (Feachem *et al.* 1978).

INFECTIVE DOSE, RISK OF INFECTION, EPIDEMIOLOGY

Upon being deposited on or in a human body a pathogen may be destroyed by purely physical factors, e.g., desiccation or decomposition. Before it can cause an infection, and eventually disease, it must then overcome the body's natural defenses. In the first interaction with the host, whether in the lungs, in the gastrointestinal tract, or other site, the pathogen encounters nonspecific immunologic responses, i.e., inflammation and phagocytosis. Phagocytosis is carried out primarily by neutrophils or polymorphonuclear leukocytes in the blood, and by mononuclear phagocytes, i.e., the monocytes in the blood and macrophages in the tissues (e.g., alveolar macrophages in the lungs). Later interactions with the host result in specific immunologic responses, i.e., humoral immunity via the B-lymphocytes, and cell-mediated immunity via the T-lymphocytes (Bellanti 1978).

With these barriers to overcome it is understandable that an infection resulting from inoculation by a few bacterial cells is a most unlikely occurrence; usually large numbers are necessary. Some representative oral infection dose data for enteric bacteria, based upon numerous studies using nonuniform techniques, are presented in Table 12 (adapted from Bryan 1977).

Although the terms, "infective dose," "minimal infectious dose," etc., are used in the literature, it is obvious from Table 12 that these are misnomers, and that we are really dealing with dose-response relationships, where the dose is the number of cells to which the human is exposed, and the response is lack of infection, infection without illness, and infection with illness (in an increasing proportion of the test subjects). The response is affected by many factors, making it highly variable. Some of the most important factors are briefly discussed below.

TABLE 12. INFECTIVE DOSE TO MAN OF ENTERIC BACTERIA

Bacterium	No Infection or no illness	Infections without illness	Percent of volunteers developing illness 1-25	26-50	51-75	76-100
Clostridium perfringens				10^8	10^9	10^9
Escherichia coli						
0124:K72:H-		10^{10}	10^8			
0148 H28			10^8			10^{10}
0111:B4					10^6–10^9	
Several strains	10^4	10^4–10^6	10^6	10^8	10^8–10^{10}	10^{10}
Salmonella typhi						
Ty2W			10^8			
Zermat vi					10^4	
Most strains	10^3		10^5	10^5–10^8		10^8–10^9
S. newport			10^5	10^6		
S. bareilly			10^5	10^6		
S. anatum	10^4–10^6		10^5–10^8	10^6		
S. meleagridis	10^4–10^6		10^6	10^7	10^7–10^8	
S. derby	10^5–10^6			10^7		
S. pullorum	10^4–10^9			10^9		10^9–10^{10}
Shigella dysenteriae			10–10^2	10^2–10^4	10^3	10^4
S. flexneri			10^2–10^4		10^3–10^9	10^6–10^8
Streptococcus faecalis var. *liquefaciens*	10^8		10^9	10^{10}		
Vibrio cholerae						
$NaHCO_3$-buffered	10	10^3		10^3–10^8	10^4–10^6	
Unbuffered	10^4–10^{10}			10^8–10^{11}		

1. The site of exposure determines what types of defense mechanisms are available, e.g., alveolar macrophages and leukocytes in the lungs, and acidity and digestive enzymes in the stomach. The effect of acidity is clearly shown by the cholera (*Vibrio cholerae*) data in Table 12, where buffering reduces the infective dose by about a thousandfold. Direct inoculation into the bloodstream results in the fewest barriers being presented to the pathogen; Hellman *et al.* (1976) found 10 tuleremia organisms injected to be comparable to 10^8 by mouth.
2. Previous exposure to a given pathogen often produces varying degrees of immunity to that pathogen, through the induction of specific immune responses. A study in Bangladesh showed that repeated ingestion of small inocula (10^3–10^4 organisms) of *Vibrio cholerae* produced subclinical or mild diarrhea infection followed by specific antibody production. For this reason the peak incidence of endemic cholera occurs in the one to four-year old age group, and decreases with age thereafter as immunity developes (Levine 1980).
3. Other host factors, such as age and general health, also affect the disease response. Infants, elderly persons (Gardner 1980), malnourished people, those with concomitant illness, and people taking antiinflammatory, cytotoxic, and immunosuppressant drugs would be more susceptible to pathogens. An example

of human variability (possibly genetic) is the following response of men orally challenged with several different doses of *Salmonella typhi* (Hornick *et al.* 1970):

Number of *S. typhi*	Percent developing typhoid fever
10^3	0
10^5	28
10^7	50
10^9	95

Twenty-eight percent of the men came down with typhoid fever after 10^5 organisms, while five percent were still resistant to 10^9 organisms, four orders of magnitude as many.

4. The number of organisms that must be swallowed for intestinal colonization (subclinical infection), and consequent risk of clinical disease, to be established is affected by treatment with antibiotics (Remington and Schimpff 1981). Due to its normal content of anaerobic bacteria and their products, the gut can resist colonization when an oral dose of about 10^6 organisms is given. Once resistance is reduced by systemic or oral antibiotics, the dose required to induce colonization is only ten to 100 organisms.
5. The timing of the exposure to pathogens, e.g., as a single exposure or an exposure over a long period of time, would be expected to affect the response.
6. Finally, as illustrated by *Escherichia coli* and *Salmonella typhi* in Table 12, the virulence, or pathogenicity, of bacteria varies among strains. Thus, three different strains of *Shigella flexneri* have been found to have infective doses of 10^{10} or higher, 10^5–10^8, and 180 organisms (NRC 1977).

The risk of infection is probably greatest for *Salmonella* spp. and *Shigella* spp., because they are the most common bacterial pathogens in municipal wastewater. The infective dose for *Salmonella* is high (10^5–10^8 organisms) but this dose might be reached on a contaminated foodstuff under conditions that allow multiplication. On the other hand the infective dose for *Shigella* is low—as few as 10 to 100 organisms. "Because of this miniscule inoculum it is rather simple for shigellae to spread by contact without interposition of a vehicle such as food, water or milk to amplify the infectious dose" (Keusch 1979). Consequently, it would be prudent for humans to maintain a minimum amount of contact with an active land treatment site, and to rely on "time" to reduce the bacterial survival, as discussed earlier, when growing crops for human consumption.

A number of epidemiological reports have attested to the fact that transmission of enteric disease can occur when *raw* wastewater is used in the cultivation of crops to be eaten raw (Geldreich and Bordner 1971, Hoadley and Goyal 1976, and Sepp 1971). Salmonellosis has been traced to the consumption of wastewater-irrigated celery, watercress, watermelon, lettuce, cabbage, endive, salad vegetables, and fruits; shigellosis to wastewater-irrigated pastureland; and cholera to wastewater-irrigated vegetables in Israel. These data support the view that untreated wastewater should never be used for irrigation.

Perhaps the largest epidemiological study of the health effects of land treatment was a retrospective study of 77 kibbutzim (agricultural cooperative settlements) in Israel practicing slow-rate land treatment with nondisinfected oxidation pond effluent, and 130 control kibbutzim (Katzenelson *et al.* 1976). The incidence of typhoid fever, salmonellosis, shigellosis, and infectious hepatitis was 2–4 times higher in the land-treatment kibbutzim than the controls. The study, however, did not rule out a number of pathways of infection other than aerosols, e.g., direct contact via clothing or bodies of sewage irrigation workers, and there were problems with the data reporting methods. Consequently, it is generally felt that no conclusive findings may be based on the report, and the study is currently being repeated, correcting for the deficits of the original study (Shuval and Fattal 1980). Preliminary results suggest little effect of land treatment on disease incidence.

Other epidemiological reports on the health effects of land treatment have been more superficial. Examination of the workers on sewer farms in Berlin and Memmingen in Germany has not shown them to have a higher rate of infectious diseases or worm infestation than the rest of the population (Sepp 1971). At land treatment sites near Paris, grain for cattle, beef cattle, and vegetables (e.g., beans, onions, and celeriac) are raised (Dean 1978). The vegetables are checked for *Salmonella*, with none having been found, and no disease has been traced to the farms. During a cholera outbreak, no cholera bacteria were found on the vegetables. At Werribee Farm in Melbourne, Australia, there has never been a reported epidemic or outbreak of disease among employees or residents, although no precautions other than normal hygiene practices have been taken, and the general health of employees and residents is no different from that of the community in general (Croxford 1978).

Although these retrospective studies are reassuring, a better measure of the health effects of land treatment will come from well-planned prospective epidemiological studies. Two such studies are currently underway—at Lubbock, Texas, and in Israel. The results of these two projects may well modify the conclusions and recommendations of this report in the future

CONCLUSIONS AND RESEARCH NEEDS

The level of preapplication treatment required is highly site-specific. It may be as little as simple sedimentation at sites with limited public access where crops are protected by appropriate choice of crops and waiting periods, and groundwater is protected by appropriate hydrological studies and application rate selection. In any case, wastewater stabilization ponds or other pretreatment systems can be designed to achieve practically any degree of bacterial pathogen removal deemed necessary for the protection of public health.

The human exposure to aerosol bacteria at land treatment sites does not appear to pose a high public health risk. Limiting public access to about 50 m should prevent any problem from developing.

Aerial crops with little chance for contact with soil should not be harvested for human consumption for at least one month after the last wastewater application; subsurface and low-growing crops for human consumption should not be grown at a land treatment site for at least six months after last application. Growth of crops for animal feed, however, is probably a safe practice.

Properly designed slow-rate land treatment systems pose little threat of bacterial contamination of groundwater. Considerable threat exists, however, at rapid-infiltration sites where the water table is shallow, particularly if the soil is porous. The survival of bacteria in groundwater, once they get there, is poorly understood, and is an important research need

There appears to be little danger to animals grazing at land treatment sites, if grazing does not resume until four weeks after last application

Although the infective doses of bacteria appear to be fairly high, it would be wise for humans to maintain a minimum amount of contact with an active land treatment site.

SECTION 5

VIRUSES

Transmission of viruses by feces is the second most frequent means of spread of common viral infections, the first being the respiratory route. Transmission by urine has not been established as being of epidemiological or clinical importance, although some viruses, e.g., cytomegalovirus and measles, are excreted through this route. The gastrointestinal tract is an important portal of entry of viruses into the body, again second to the respiratory tract (Evans 1976). There has been some recent concern that land treatment may increase the population size of the tree-hole breeding mosquito, *Aedes triseriatus*, a vector of California encephalitis virus (Zaim *et al.* 1979), but this would likely only be a problem in wooded and waste areas, and is not further considered here.

TYPES AND LEVELS IN WASTEWATER

The human enteric viruses that may be present in wastewater are listed in Table 13 (Melnick *et al.* 1978, Holmes 1979). These are referred to as the enteric viruses and new members are constantly being identified. Since no viruses are normal inhabitants of the gastrointestinal tract and none of these have a major reservoir other than man (with the likely exception of rotaviruses), all may be regarded as pathogens, although most can produce asymptomatic infections.

TABLE 13. HUMAN WASTEWATER VIRUSES

Enteroviruses
Poliovirus
Coxsackievirus A
Coxsackievirus B
Echovirus
New Enterovirus
Hepatitis A Virus
Rotavirus ("Duovirus," "Reovirus-like Agent")
Norwalk-Like Agents (Norwalk, Hawaii, Montgomery County, etc)
Adenovirus
Reovirus
Papovavirus
Astrovirus
Calicivirus
Coronavirus-Like Particles

Upon entry into the alimentary tract, if not inactivated by the hydrochloric acid, bile acids, salts, and enzymes, enteroviruses, hepatitis A virus, rotavirus, adenovirus, and reovirus may multiply within the gut. The multiplication and shedding of adenovirus and reovirus here has not been shown to be of major epidemiological importance in their transmission (Evans 1976). The rotavirus often produces diarrhea in children, but the local multiplication of enteroviruses and (possibly) hepatitis A virus in cells lining the area rarely produces local symptoms, i.e., diarrhea, vomiting, and abdominal pain. Most enterovirus infections, even with the more virulent types, cause few or no clinical symptoms. Occasionally, after continued multiplication in the lymphoid tissue of the pharynx and gut, viremia may occur, i.e., virus enters the blood stream, leading to further virus proliferation in the cells of the reticuloendothelial system, and finally to involvement of the major target organs—the central nervous system, myocardium, and skin for the enteroviruses, and the liver for hepatitis A virus (Melnick *et al.* 1979, Evans 1976).

Polioviruses cause poliomyelitis, an acute disease which may consist simply of fever, or progress to aseptic meningitis or flaccid paralysis (slight muscle weakness to to complete paralysis caused by destruction of motor neurons in the spinal cord). Polio is rare in the United States, but may be fairly common in unimmunized populations in the rest of the world. No reliable evidence of spread by wastewater exists (Benenson 1975).

Coxsackieviruses may cause aseptic meningitis, herpangina, epidemic myalgia, myocarditis, pericarditis, pneumonia, rashes, common colds, congenital heart anomalies, fever, hepatitis, and infantile diarrhea.

Echoviruses may cause aseptic meningitis, paralysis, encephalitis, fever, rashes, common colds, epidemic myalgia, pericarditis, myocarditis, and diarrhea.

The new enteroviruses may cause pneumonia, bronchiolitis, acute hemorrhagic conjunctivitis, aseptic meningitis, encephalitis, and hand-foot-and-mouth disease. The prevalence of the diseases caused by the coxsackieviruses, echoviruses, and new enteroviruses is poorly known, but 7,075 cases were reported to the Center for Disease Control (CDC) in the years 1971–75 (Morens *et al.* 1979). These enteroviruses are practically ubiquitous in the world, and may spread rapidly in silent (asymptomatic) or overt epidemics, especially in late summer and early fall in temperate regions. Because of their antigenic inexperience, children are the major target of enterovirus infections, and serve as the main vehicle for their spread. Most of these infections are asymptomatic, and natural immunity is acquired with increasing age. The poorer the sanitary conditions, the more rapidly immunity develops, so that 90% of children living under poor hygienic circumstances may be immune to the prevailing enteroviruses (of the approximately 70 types known) by the age of 5. As sanitary conditions improve, the proportion of nonimmunized in the population increases, and infection becomes more common in older age groups, where symptomatic disease is more likely and is more serious (Melnick *et al.* 1979, Benenson 1975). Thus, decreasing the human exposure to the common enteric viruses through the water and food route has its disadvantages, as well as advantages.

Hepatitis A virus causes infectious hepatitis, which may range from an inapparent infection (especially in children) to fulminating hepatitis with jaundice. Recovery with no sequelae is normal. Approximately 40,000–50,000 cases are reported annually in the U.S. About half the U.S. population has antibodies to hepatitis A virus, and the epidemiological pattern is similar to that of enteroviruses, with childhood infection common and asymptomatic (Duboise *et al.* 1979).

Rotavirus causes acute gastroenteritis with severe diarrhea, sometimes resulting in dehydration and death in infants. It may be the most important cause of acute gastroenteritis in infants and young children, especially during winter (Konno *et al.* 1978), but also may strike older children and adults (Holmes 1979).

Norwalk-like agents include the Norwalk, Hawaii, Montgomery County, Ditchling, W, and cockle viruses, and cause epidemic gastroenteritis with diarrhea, vomiting, abdominal pain, headache, and myalgia or malaise. The illness is generally mild and self-limited (Kapikian *et al.* 1979). These agents have been associated with sporadic outbreaks in schoolchildren and adults (Holmes 1979).

Adenoviruses are primarily causes of respiratory and eye infection, transmitted by the respiratory route, but several strains are now believed to be important causes of sporadic gastroenteritis in young children (Richmond *et al.* 1979, Kapikian *et al.* 1979).

Reoviruses have been isolated from the feces of patients with numerous diseases, but no clear etiological relationship has yet been established. It may be that reovirus infection in humans is common, but associated with either mild or no clinical manifestations (Rosen 1979).

Papovaviruses have been found in urine, and may be associated with progressive multifocal leukoencephalopathy (PML), but are poorly understood (Warren 1979).

Astroviruses, caliciviruses, and coronavirus-like particles may be associated with human gastroenteritis, producing diarrhea, but are also poorly understood (Holmes 1979, Kapikian *et al.* 1979).

Viruses are not normal inhabitants of the gastrointestinal tract nor regular components of human feces, while certain types of bacteria are. Because of this difference, the concept of using bacteria, e.g., coliforms and fecal streptococci, as indicators of potential viral contamination in the environment has been a very attractive one. Unfortunately the response of viruses to wastewater treatment and their behavior in the environment are very different from those of bacteria (Berg *et al.* 1978); for example, viruses are less easily removed during passage through soil than are bacteria (Sobsey *et al.* 1980). Thus, Goyal *et al.* (1979) provided data to indicate that current bacteriological standards for determining the safety of shellfish-growing waters do not reflect the occurrence of enteroviruses. Likewise, Marzouk *et al.* (1979) isolated enteroviruses from 20% of Israeli groundwater samples, including 12 samples which contained no detectable fecal bacteria. They found no significant correlation between the presence of virus in groundwater and levels of bacterial indicators, i.e., total bacteria, fecal coliforms, and fecal streptococci. An expansion of the study to include potable, surface, and swimming pool waters resulted in the same conclusion (Marzouk *et al.* 1980). It appears, therefore, that estimates of virus presence or levels in the environment will have to be made on the basis of measurements of viral indicators, e.g., vaccine poliovirus or bacteriophage, or of the viral pathogens themselves, e.g., coxsackievirus or echovirus, rather than of indicator bacteria.

The concentration of viruses in the feces of an uninfected person is normally zero. The concentration in the feces of an infected person has not been widely studied. However, from the available data it has been estimated to be about 10^6 per gram (Feachem *et al.* 1978), but may be as high as 10^{10} per gram in the case of rotavirus (Bitton 1980).

Estimates of the concentration of viruses in wastewater in the United States vary widely, but it is thought to be lower than that in many developing countries. Numbers tend to be higher in later summer and early fall than other times of the year because of the increase in enteric viral infections at this time, except for vaccine polioviruses, whose concentration tend to remain constant. The concentrations reported in the literature may be as little as one-tenth to one-hundredth of the actual concentrations because of the limitations of virus recovery procedures and the use of inefficient cell-culture detection methods (Akin *et al.* 1978, Keswick and Gerba 1980). (The use of several cell lines usually detects more viral types than a single cell line does, and many viruses cannot yet be detected by cell-culture methods, e.g., hepatitis A virus and Norwalk-like agents.) Some representative levels of enteric viruses in raw U.S. wastewaters are summarized in Table 14. It is evident that reported concentrations are highly variable; Akin and Hoff (1978) have concluded that ". . . from the reports that are available from field studies and with reasonable allowances for the known variables, it would seem extremely unlikely that the total concentration would ever exceed 10,000 virus units per liter of raw sewage and would most often contain less than 1,000 virus units/liter."

PREAPPLICATION TREATMENT

Chlorine, although effective as a bactericide, is a very inefficient inactivator of wastewater viruses. Levels as high as 8 mg/l have little effect in secondary effluent

TABLE 14. LEVELS OF ENTERIC VIRUSES IN U.S. WASTEWATERS

Description	Viral units/liter	Reference
St. Petersburg	10->183	Wellings *et al.* 1978
Various souces	100-400	Akin and Hoff 1978
Chicago	Up to 440	Fannin *et al.* 1977
Honolulu	0-820	Ruiter and Fujioka 1978
Cincinnati	0-1450	Akin and Hoff 1978
Urban	Up to 6000	Vaughn 1977
Calculated U S. average	7000	Clarke *et al.* 1961

(Berg 1973) because of the difficulty in maintaining the more viricidal form, free chlorine, under acidic conditions. Although very high doses of chlorine will destroy viruses in wastewater, cost, production of carcinogens, and toxicity make this impractical.

Processes for virus inactivation in wastewater have been briefly reviewed by Melnick *et al.* (1978). Sedimentation, or conventional primary treatment, results in low rates of removal, most of which is associated with the settling of solids in which the viruses are embedded or on which they are adsorbed (Lance and Gerba 1978). Removal rates of up to 90 percent have been reported (Melnick *et al.* 1978), with 10 percent or less being more common (Sproul 1978, Crites and Uiga 1979).

The survival of viruses in wastewater stabilization ponds is poorly known (Feachem *et al.* 1978). Some representative survival data is summarized in Table 15 (Feachem *et al.* 1978, Kott *et al.* 1978). The data suggest that long retention times, of the order of 50 days, particularly in combination with ponds in series, might accomplish quite significant virus removals.

TABLE 15. ENTERIC VIRUS SURVIVAL IN WASTEWATER STABILIZATION PONDS

Description	Retention time (days)	Removal rate
Model ponds	38	0
Pond fed by activated sludge effluent	30	20% of samples positive
Pond	20	0-96%
3 ponds in series	7 (total)	>90%
Secondary effluent pond in Israel, summer (water temp. 18-20°C)	11	96%
	35	100%
Secondary effluent pond in Israel, winter (water temp down to 8°C)	8	51%
	15	81%
	22	96%
	29	91%
	34	96%
	40	97%
	47	97%
	73	100%

AEROSOLS

Aerosols have been of concern as a potential route of transmission of disease caused by enteric viruses because, as with bacteria, once they are inhaled they may be carried from the respiratory tract by cilia into the oropharynx, and then swallowed into the gastrointestinal tract. Some enteroviruses may also multiply in the respiratory tract itself (Evans 1976). Another reason for concern is the theoretically possible transmission of respiratory viruses through wastewater aerosols. On the basis of actual viral sampling of wastewater, however, Johnson *et al.* (1980) concluded that the likelihood of finding respiratory viruses in treated wastewater is very small.

The initial aerosol shock during the process of aerosolization may result in a half log loss of virus level (Sorber 1976). The subsequent die-off, estimated to be about one log every 40 seconds (Sorber 1976), is determined primarily by solar radiation, temperature, and relative humidity (Lance and Gerba 1978). The effect of relative humidity appears to depend upon the lipid content of viruses, lipid-containing viruses surviving better at low humidities, and those without lipid (e.g., most of the enteric viruses) surviving better at high humidities (Carnow *et al.* 1979). Sorber (1976) has estimated that, under the least desirable meteorological conditions studied, less than 200 meters would be required to provide a reduction of three logs in aerosolized virus concentrations.

Very few measurements of aerosol viruses from the spraying of wastewater have been reported in the literature. The spraying in Israel of 3–5 day detention time oxidation pond effluent, having a coliform density of about 10^6/100 ml, resulted in the detection of poliovirus, coxsackievirus, and echovirus up to 100 m downwind (Shuval 1978). To obtain quantitative measurements of aerosol virus concentrations in air may require heroic efforts. Johnson *et al.* (1980) operated ten high-volume samplers (1 m^3/min electrostatic precipitators) for three-hour sample periods, at 50 m downwind from the source, to measure the aerosol enteroviruses produced by the spraying of unchlorinated aerated-pond effluent in Pleasanton, California. Likewise, Teltsch *et al.* (1980) used a large-volume scrubber-cyclone sampler to extract 27±11 m^3 of air downwind from an irrigation line spraying raw wastewater at Kibbutz Tzora, Israel. The results of these two studies are summarized in Table 16.

TABLE 16. AEROSOL ENTEROVIRUSES AT LAND TREATMENT SITES

Site	Distance (m)	Wastewater total coliforms (/l)	Wastewater Enteroviruses (mean) (PFU/l)	Aerosol Enteroviruses (mean) (/m³)
Pleasanton, California (Pond effluent)	50	6.4×10^5–1.9×10^6	45–330 (188)	0.011–0.017 (0.014)
Kibbutz Tzora, Israel (Raw wastewater)	36–42	3.1×10^7–1.5×10^9	0–650 (125)	0–0.082 (0.015)
	50	1.0×10^8	650	0.14
	70	1.0×10^7–1.7×10^8	170–13,000 (6585)	0–0.026 (0.013)
	100	2.4×10^7–3.0×10^8	0–82,000 (16,466)	0–0.10 (0.038)

The results obtained from these two studies are highly variable, but it appears reasonable to make use of the Pleasanton aerosol virus density, i.e., 0.014/m^3, to make human exposure estimates, since (1) the Pleasanton wastewater virus level is similar to that in U.S. wastewaters in general (cf. Table 14), (2) the high Israeli wastewater virus levels are not typical of those found in the U.S., and, in any case, a wastewater stabilization pond would decrease these levels, and (3) the 0.14/m^3 value found at 50 m in Israel is based on only one sample, and does not appear to be representative of the other values.

From these data it can be calculated that an adult male, engaged in light work, breathing at a rate of 1.2 m^3/hour and exposed to 0.014 PFU/m^3 at 50 m downwind from a sprayer, would inhale approximately 0.13 PFU of enterovirus during an 8-hour work day. This is probably an insignificant level of exposure. However, since the recovery of enteric viruses from environmental samples is not perfectly efficient, isolation of viruses increases as more cell culture types are used, and some enteric viruses cannot yet be isolated on cell cultures, the actual exposure to enteric viruses may be as much as ten to a hundred times the reported level (Teltsch *et al.* 1980). Thus, it might be prudent to recommend a 100 m or 200 m minimum exposure distance of the general public to a land treatment spray souce.

SURFACE SOIL AND PLANTS

As is the case with bacteria, the surface soil and plants of an active land treatment site are constantly receiving enteric viruses. The survival time of viruses is primarily of concern when decisions must be made on how long a period of time must be allowed after last application before permitting access to people or animals, or harvesting crops. Another concern is that the longer viruses survive at the surface the greater opportunity they have for being desorbed and moving into the soil toward the groundwater. This is not a problem with overland flow systems, which, although 68 to 85% of the enteric viruses are deposited at the surface, little virus penetrates into the soil profile (Schaub *et al.* 1978b, 1980).

The factors affecting virus survival in soil are solar radiation, moisture, temperature, pH, and adsorption to soil particles. The soil microorganisms appear to have a less important effect on virus degradation. Although it is often believed that adsorption to inorganic surfaces prolongs the survival of viruses, there is some evidence that adsorption may result in their physical disruption (Murray and Laband 1979). Desiccation and higher temperatures decrease survival time (Sagik *et al.* 1978). On the basis of studies with coxsackievirus, echovirus, poliovirus, rotavirus, and bacteriophages, Hurst *et al.* (1980b) have concluded that temperature and adsorption to soil appear to be the most important factors affecting virus survival. The soil is a complex medium, however, with fluctuations in soil moisture, temperatures, ionic strength, pH, dissolved gas concentrations, nutrient concentrations, etc. These may be caused by meteorological changes, by the action of other soil organisms, or by the activities of metazoans including humans (Duboise *et al.* 1979), and understanding of the behavior of viruses in soil will be slow developing.

It is believed that most virus inactivation occurs in the top few centimeters of soil where drying and radiation forces are maximal. The persistence of virus particles that survive surface forces and enter the soil matrix is not well studied. However, Wellings *et al.* (1978) has reported data that indicates virus may penetrate up to 58 feet of sandy soil.

Much of the recent literature on survival times of enteric viruses in soil is summarized in Table 17. Approximately one hundred days appears to be the maximum survival time of enteric viruses in soil, unless subject to very low temperatures, which prolong survival beyond this time. Exposure to sunlight, high temperatures, and drying greatly reduce survival times. Thus, Yeager and O'Brien (1979) could recover no infectivity of poliovirus and coxsackievirus from dried soil regardless of temperature, soil type, or type of liquid amendment. They suggested that the main effect of temperature on virus survival in the field may be its influence on evaporation rates. They also suggested that enterovirus contamination of soil, and possible migration to underlying groundwater, might be reduced or eliminated by allowing the soil to dry between wastewater applications.

TABLE 17. SURVIVAL TIMES OF ENTERIC VIRUSES IN SOIL

Virus	Soil	Moisture and temperature	Survival (days)	Reference
Enterovirus	Sandy or loamy podzol	10–20% 3–10°C	70–170	Bagdasaryan 1964
		10–20% 18–23°C	25–110	
		Air dry, 18–23°C	15–25	
Poliovirus	Sand	Moist Dry	91 <77	Lefler and Kott 1974
Poliovirus	Loamy fine sand	Moist, 4°C	84 (<90% reduction)	Duboise *et al.* 1976
		Moist, 20°C	84 (99.999% reduction)	
Coxsackievirus	Clay	300 mm rain-fall, -12-26°C	<161	Damgaard-Larsen *et al* 1977
Poliovirus	—	-14-27°C 15-33°C	89-96 <11	Tierney *et al.* 1977
Poliovirus	Sugarcane field	Open, direct sunlight	7-9	Lau *et al.* 1975
		Mature sugarcane	≤60	
Poliovirus and coxsackievirus	Sandy loam	Saturated, 37°C	12	Yeager and O'Brien 1979
		Saturated, 4°C	≥180	
		Dried, 37°C and 4°C	<3-<30	

The phenomenon of virus inactivation by evaporative dewatering has been documented by Ward and Ashley (1977), who observed a decrease in poliovirus titer of greater than three orders of magnitude when the solids content of sludge was increaed from 65% to 83%. This loss of infectivity was due to irreversible inactivation of poliovirus because viral particles were found to have released their RNA molecules which were extensively degraded Both Ward and Ashley's (1977) and Yeager and O'Brien's (1979) studies made use of radiolabeled viruses to correct for virus recovery efficiency (affected by irreversible sludge and soil binding).

The absorption of enteric viruses by plants is a theoretical possibility. Murphy and Syverton (1958) found enterovirus to be absorbed by tomato plant roots grown in hydroponic culture under some conditions, and in some cases to be translocated to the aerial parts. However, the rapid adsorption of virus by soil particles under natural conditions may make them unavailable for plant absorption, thereby indicating that plants or plant fruits would be unlikely reservoirs or carriers of viral pathogens. The intact surfaces of vegetables are probably impenetrable for enteroviruses (Bagdasaryan 1964).

On the surface of aerial crops virus survival would be expected to be shorter than in soil because of the exposure to deleterious environmental effects, especially sunlight, high temperature, drying, and washing off by rainfall (USEPA 1977). Some of the literature on survival times is summarized in Table 18 (Feachem *et al.* 1978). The data are similar to those for bacteria (cf. Table 9), and likewise appear to support a one-month waiting period after last wastewater application before harvest.

TABLE 18. SURVIVAL TIMES OF ENTERIC VIRUSES ON CROPS

Virus	Crop	Conditions	Survival (days)	Reference
Enterovirus	Tomatoes	3-8°C	10 (90% reduction)	Bagdasaryan 1964
		18-21°C	10 (99% reduction)	
Poliovirus	Radishes	5-10°C	20 (99% reduction), >60	Bagdasaryan 1964
Poliovirus	Tomatoes	Indoors, 22-25°C	<12	Kott and Fishelson 1974
		Indoors, 37°C	<5	
		Outdoors	<1	
	Parsley	15-31°C	<2	
Poliovirus	Lettuce and radishes	Sprayed, summer-fall	6 (99% reduction) 36 (100% reduction)	Larkin *et al.* 1976
Poliovirus	Lettuce and radishes	Flooded, summer	23	Tierney *et al.* 1977
Enterovirus	Cabbage	—	4	Grigor'Eva *et al.* 1965
	Peppers	—	12	
	Tomatoes	—	18	

Because of the possible contamination of subsurface and low-growing crops with soil, in which viruses have a longer survival time, about one hundred days would probably be a safe waiting period. As with bacteria, this period could be shortened by (1) the use of subsurface or covered drip irrigation (Sadovski *et al.* 1978a, 1978b), (2) the growth of crops the harvested portion of which does not contact the soil, or (3) the growth of crops used for animal feed only. At a site where (2) and (3) were practiced, the Roswell, New Mexico, slow-rate land treatment site where secondary effluent has been applied by ridge-and-furrow irrigation for 33 years, no enteroviruses were found on or in the leaf and grain portions of corn (Koerner and Haws 1979a).

MOVEMENT IN SOIL AND GROUNDWATER

While viruses near the soil surface are rapidly inactivated due to the combined effects of sunlight, drying, and the antagonism of aerobic soil microorganisms, those that penetrate the aerobic zone can be expected to survive over a more prolonged period of time. The longer they survive, the greater the chance that an event will occur to promote their penetration into groundwater (Gerba and Lance 1980).

In contrast with bacteria, filtration plays a minor role in the removal of viruses in soils, virus removal being almost totally dependent on adsorption. Since adsorption is a surface phenomenon, soils with a high surface area, i.e., those with a high clay content, would be expected to have high virus removal capabilities. Although the physical-chemical reasons for virus adsorption to soil surfaces are poorly understood, it appears that adsorption is increased by high cation exchange capacity, high exchangeable aluminum, low pH (below 5), and increased cation concentration (Gerba and Lance 1980). As the flowrate, or application rate, increases, virus removal declines (Lance and Gerba 1980, Vaughn *et al.* 1981). Although it is commonly believed that soluble organics compete with viruses for adsorption sites on the soil particles, resulting in decreased virus adsorption or even elution of already adsorbed viruses, Gerba and Lance (1978) found that adsorption of poliovirus from primary effluent was similar to that from secondary effluent, and that the adsorbed virus from the two sources had similar desorption properties. These results suggest that adsorption of poliovirus and movement through the soil are not affected by the higher organic content of primary effluent.

The degree of adsorption of viruses to soil is highly variable. Thus, Goyal and Gerba (1979) found virus adsorption to differ greatly among virus types, virus strains (within a type), and soils. Differences in adsorption among different strains of the same virus type may be due to differences in the configuration of proteins in the outer capsid of the virus, which affects the net charge on the virus. This affects the electrostatic potential between virus and soil, which, in turn, affects the degree of interaction between the two particles. They concluded that ". . . no one enterovirus or coliphage can be used as the sole model for determining the adsorptive behavior of viruses to soils and that no single soil can be used as the model for determining viral adsorptive capacity of all soil types."

Much of the research in the past on virus behavior in soils has been done with vaccine strains of poliovirus, because of their availability and safety, but poliovirus adsorb better to soils than most other viruses (Gerba *et al.* 1980). Thus, the existing literature may underestimate the mobility of viruses in soil.

With respect to variability among soils, the generalization can probably be made that clayey soils are good virus adsorbers and sandy and organic soils poor virus adsorbers. Sobsey *et al.* (1980) found $\geqslant$95% virus removal from intermittently-applied wastewater in unsaturated 10-cm-deep columns of sandy and organic soils. However, considerable quantities of the retained viruses were washed out by simulated rainfall. Under the same conditions clayey soils resulted in $\geqslant$99.995% virus removal, but none were washed out by simulated rainfall. The reason for the poor adsorption of sandy soils is probably the low level of available surface area. The reason for the poor adsorption of organic soils, in spite of their high surface area, has been suggested to be the complexation of virus by naturally-occuring low molecular weight (<50,000) humic substances (Bixby and O'Brien 1979, Scheuerman *et al.* 1979).

Upon the application of wastewater to land most of the viruses are adsorbed in the top few centimeters of the soil profile. In packed column studies with Flushing Meadows, Arizona, soil, 90–99% of applied polioviruses was retained in the top 5–10 cm at infiltration rates of 15–55 cm/day. Removal appeared to be independent of the concentration of the virus and was the same in both primary and secondary effluent, but was less at flow rates exceeding 1.2 m/day. Tests with echovirus 1 and echovirus 29 showed somewhat different adsorption patterns from that of poliovirus, but all three viruses has approximately a 99% removal in the top 40 cm of soil (Gerba and Lance 1980). Hurst and Gerba (1979), using the same soil in the field, found enterovirus concentrations above 2.5 cm depth to be ten times those at 2.5–25 cm, confirming the laboratory column studies. Application of poliovirus-seeded sewage effluent (1 cm/hr) to *in situ* soil cores in Long Island resulted in 77% of the viruses adsorbed by the first 5 cm of soil, 11% by 5–10 cm, 8% by 10–25 cm, and the remainder (4%) by 25-50 cm (Landry *et al.* 1980).

After being adsorbed to the soil, viruses may remain infective and, under certain conditions, may be desorbed and migrate down the soil profile. Thus, at a land treatment site in Florida, viruses were not detected in 3 m and 6 m wells until periods of heavy rainfall occurred (Wellings *et al.* 1975). Subsequent laboratory studies have shown that poliovirus, previously adsorbed in the top 5 cm of soil, can be desorbed and eluted to a depth of 160 cm (Lance *et al.* 1976). The degree of desorption and migration is inversely related to the specific conductance of the percolated water (Duboise *et al.* 1976). Viruses desorbed near the surface will usually readsorb further down the soil profile (Landry *et al.* 1980), but might gradually migrate downward in a chromatographic effect in response to cycles of rainfall. Lance *et al.* (1976) have found that drying for one day between viral application and flooding with deionized water prevented desorption (or enhanced inactivation). The importance of drying is emphasized by the fact that poliovirus may retain its ability to migrate through the soil for 84 days if the soil is kept moist (Duboise *et al.* 1976). As is the case with soil adsorption of viruses, the degree of desorption of enteroviruses varies with type and strain (Landry *et al.* 1979).

As a result of the migration of viruses down the soil profile, they have been detected in the groundwater beneath several rapid-infiltration land treatment sites. These events are summarized in Table 19 (modified from Gerba and Lance 1980). The two Florida land treatment sites showed viruses only after periods of heavy rainfall. Vaughn *et al.* (1978) noted that none of the enteroviruses isolated at the two New York sites were polioviruses, supporting the observation that poliovirus is an especially strong soil adsorber. No viruses were found in the groundwater at a third New York land treatment site, where the infiltration basins were 24 m above the water table. The Hawaiian groundwater contamination was attributed to underlying soil fissures and fractures, which channeled the percolating waters at the land treatment site (Hori *et al.* 1970). Most of these land treatment sites were underlain by very coarse soils, i.e., sandy and/or gravelly, which would be expected to have a high rate of water percolation. Since coarse-textured soils and a high rate of effluent application are characteristic of rapid-infiltration sites, it would appear that rapid-infiltration land treatment sites have a high potential for the contamination of groundwater with enteric viruses.

In contrast to the above, in the Flushing Meadows, Arizona, rapid-infiltration land treatment site, although viruses were present in the secondary effluent used to flood the basins, no viruses were detected in wells 6 m deep and 3 m away horizontally (Gilbert *et al.* 1976). At this site, basins in loamy sand underlaid at about 1 m with coarse sand and gravel are intermittently flooded at an average application rate of about 90 m/year. The excellent virus removal and prevention of groundwater

TABLE 19. GROUNDWATER PENETRATION OF VIRUSES AT RAPID-INFILTRATION LAND TREATMENT SITES

Location	Depth (m)	Horizontal distance (m)	Reference
St. Petersburg, Florida	6	—	Wellings *et al.* 1975
Cypress Dome, Florida	3	7	Wellings *et al.* 1974
Fort Devens, Massachusetts	18 3	183	Schaub and Sorber 1977
Vineland, New Jersey	16.8	250	Koerner and Haws 1979b
East Meadow, New York	11 3	3	Vaughn *et al.* 1978
Holbrook, New York	6 1	45 7	Vaughn *et al.* 1978
Oahu, Hawaii	Groundwater	—	Hori *et al.* 1970

contamination has been attributed to the low rainfall in the region, the fine loamy sand, and the practice of intermittent flooding of the soil (Gerba and Lance 1980).

It might be advisable, therefore, to site rapid-infiltration systems only on fine sandy soils, avoiding coarse gravelly or organic ones, and to apply the effluent intermittently, allowing the soil to dry between applications. The drying should help to inactivate both viruses and bacteria, and has recently been recommended by Hurst *et al.* (1980a) to prevent virus contamination of groundwater. If, for climatic reasons, drying between applications is impossible, as would be the case for much of eastern U.S., many viruses would survive to the next application, at which time they could be desorbed and migrate to the groundwater. To obviate this problem it would be prudent to utilize wastewater stabilization ponds or some other form of pretreatment which would decrease virus levels in the effluent. Serious consideration also should be given to performing standard soil column tests (e.g., those described by Lance and Gerba 1980) to assess virus retention at potential rapid-infiltration sites (Bitton 1980).

As is the case with bacteria, slow-rate land treatment sites probably pose little threat of viral contamination of groundwater. This is because of the finer-textured soils, the low rate of application, and the usual drying between applications. Thus, test wells at a Roswell, New Mexico, site had no viruses after 33 years of application of secondary effluent (Koerner and Haws 1979a).

Once enteric viruses get into groundwater, they can survive for long periods of time, 2 to 188 days having been reported in the literature (Akin *et al.* 1971), and probably migrate for long distances (Keswick and Gerba 1980). Low temperatures prolong survival, but the factors affecting survival in groundwater are poorly understood. It might be possible, for example, that entry of viruses into the groundwater would be tolerable if sufficient underground detention time could be provided before movement of the groundwater to wells or streams (Lance and Gerba 1978). Until these factors are well-understood, it would be prudent to assume that groundwater underlying coarse sandy or gravelly soils, and in the vicinity of rapid-infiltration land treatment sites, or septic tank-leaching field systems, is contaminated with viruses. Groundwater drawn from such sources for use as potable water supplies should be disinfected; this advice is consistent with that of the World Health Organization Scientific Group on Human Viruses in Water, Wastewater and Soil (WHO 1979).

The conclusion to this section on viruses in soil and groundwater at land treatment sites can be taken from the recent review by Gerba and Lance (1980):

> Although the presence of viruses in groundwater has been demonstrated, it would appear that with proper site selection and management the presence of viruses could be minimized or eliminated. The key is to define the processes involved in the survival and transport of pathogens in groundwater. With proper design, land treatment could be used as an

effective method for reducing the number of pathogens in wastewater. With the proper soil type, viruses and bacteria can be reduced to levels as effective as chlorination as currently practiced, after the travel of wastewater through only a few centimeters of soil.

ANIMALS

Human polioviruses, coxsackieviruses, echoviruses, and reoviruses have been recovered from, or found to produce infection in, at least six species of animals—dogs, cats, swine, cattle, horses, and goats (Metcalf 1976). Dogs and cats were found to be involved in a majority of instances, probably because of their intimate association with man in the household. The present state of information on virus transmission in animals and man does not appear to allow an evaluation of the effect of land treatment on animal infections or the role of animals as reservoirs of human disease (Metcalf 1976).

Polley (1979) noted that, under experimental conditions, rotaviruses of human origin have infected pigs, calves, and lambs, but concluded that in Canada their transmission to livestock via effluent irrigation was a slight and unproven risk.

INFECTIVE DOSE, RISK OF INFECTION, EPIDEMIOLOGY

In contrast with bacteria, where large numbers of cells are usually necessary to produce an infection, a few virus particles are currently thought to be able to produce an infection under favorable conditions. The most important studies on the oral infective dose of enteric viruses in humans are summarized in Table 20 (modified from National Research Council 1977). The results are highly variable, and may reflect differences in experimental conditions as well as states of the hosts. The recent data do suggest, however, that the infective dose of enteroviruses to man is low, possibly of the order of 10 virus particles or less. The same factors discussed earlier, that affect bacteria also affect the virus dose-response relationship.

Since a potential route of exposure to viruses at land treatment sites is aerosols, it is of great importance to compare the infective dose through the respiratory route with that through the ingestion route. Couch *et al.* (1965) and Gerone *et al.* (1966) reported the human inhalation infective dose of coxsackievirus A21 to be ≤ 18 TCD_{50}, which is comparable with the oral infective dose of the enteroviruses.

Theoretically, a single virus particle is capable of establishing infection both in a cell in culture and in a mammalian host (Westwood and Sattar 1976). If this were to be the case, extreme care should be taken to avoid human exposure to enteric viruses through aerosols or crops grown on land treatment sites. On the other hand, the concept that a single virus particle often constitutes an infective dose in the real world has been argued against (Lennette 1976) on the basis of the oral poliovaccine studies, nonimmunologic barriers, human immunologic responses, and probabilistic factors.

Viruses do not regrow on foods or other environmental media, as bacteria sometimes do. Therefore, the risk of infection is completely dependent upon being exposed to an infective dose (which may be very low) in the material applied. In any event, as is the case with bacteria, it would seem prudent for humans to maintain a minimum amount of contact with an active land treatment site, and to rely on the viral survival data discussed earlier for limiting the hazard from crops grown for human consumption on wastewater-amended soils.

Fecally-polluted vegetable-garden irrigation water in Brazil has been found to contain polioviruses and coxsackieviruses, and has been associated with earlier epidemics (Christovao *et al.* 1967a, 1967b), but current epidemiological techniques are probably not sufficiently sensitive to detect the low levels of viral disease transmission that might occur from a modern land treatment site (Melnick 1978, WHO 1979).

TABLE 20. ORAL INFECTIVE DOSE TO MAN OF ENTERIC VIRUSES

Virus	Subjects	Dose*	Percent infected	Reference
Vaccine poliovirus	Infants	0.2 PFU**	0	Koprowski 1956
		2 PFU	67	
		20 PFU	100	
		$10^{5.5}$	50	Gelfand *et al.* 1960
		$10^{7.5}$	100	
		$10^{6.6}$	60	Krugman *et al* 1961
		$10^{7.6}$	75	
		5.5×10^{6} PFU	89	Holgiun *et al.* 1962
		$10^{3.5}$	29	Lepow *et al.* 1962
		$10^{4.5}$	46	
		$10^{5.5}$	57	
		$10^{3.5}$	68	Warren *et al.* 1964
		$10^{5.5}$	79	
	Premature infants	1	30	Katz and Plotkin 1967
		2.5	33	
		10	67	
	Infants	7-52†	1	Miner *et al.* 1981
		24-63	10	
		55-93	50	
Echovirus 12	Young adults	10 PFU	18	Schiff *et al.* (personal communication) 1980
		100 PFU	67	

*Tissue Culture Dose 50% (TCD_{50}) unless indicated
**Plaque-Forming Unit
†95% Confidence Limits

CONCLUSIONS AND RESEARCH NEEDS

Since only one-tenth to one-hundredth of the total viruses in wastewater and other environmental samples may actually be detected, the development of methods to recover and detect viruses continues to be a research need.

As in the case with bacteria, the level of preapplication treatment required is highly site-specific, but may be minimal where crops and groundwater are protected. Where protection of groundwater cannot be assured, wastewater stabilization ponds should be considered, but the survival of viruses in these ponds is an important research need.

Because of the potential exposure to aerosol viruses at land treatment sites, it would be prudent to limit public access to 100–200 m from a spray source.

Aerial crops with little chance for contact with soil should not be harvested for human consumption for at least one month after the last wastewater application; for subsurface and low-growing crops about one hundred days would probably be a safe waiting period. An important research need is the effect of drying of the soil between wastewater applications on the survival of surface-soil viruses.

As with bacteria, properly designed slow-rate land treatment systems pose little threat of viral contamination of groundwater. Considerable threat exists, however, at rapid-infiltration sites, and appropriate management or preapplication treatment techniques should be instituted; until then, groundwater drawn for use as potable water supplies should be disinfected. The factors controlling the migration of viruses in soils and the survival of viruses in groundwater are poorly understood, and are significant research needs.

The role of animals at land treatment sites in transmitting human viral disease is poorly known, and is a research need.

Since the infective doses of viruses are low, it would be wise for humans to maintain a minimum amount of contact with an active land treatment site. The comparison of the respiratory infective dose of enteric viruses with the oral infective dose is a significant research need.

SECTION 6
PROTOZOA

The protozoa and helminths (or worms) are often grouped together under the term, "parasites," although in reality all the pathogens are biologically parasites. Because of the large size of protozoan cysts and helminth eggs, compared with bacteria and viruses, it is extremely unlikely that they will find their way into either aerosols or groundwater at land treatment sites, and, thus, these routes of exposure are not further considered in this report. Little attention has been given to the presence of parasites in wastewater, and their potential for contaminating food crops in the United States, probably because of the popular impression that the prevalence of parasite infection in the U.S. is minimal (Larkin *et al.* 1978b). However, because of the increasing recognition of parasite infections in the U.S., the return of military personnel and travelers from abroad, the level of recent immigration and food imports from countries with a high parasitic disease prevalence, and the existence of resistant stages of the organisms, a consideration of parasites is warranted.

TYPES AND LEVELS IN WASTEWATER

The most common protozoa which may be found in wastewater are listed in Table 21. Of these, only three species are of major significance for transmission of disease to humans through wastewater: *Entamoeba histolytica, Giardia lamblia,* and *Balantidium coli. Toxoplasma gondii* also causes significant human disease, but the wastewater route is probably not of importance. *Eimeria* spp. are often identified in human fecal samples, but are considered to be spurious parasites, entering the gastrointestinal tract from ingested fish.

TABLE 21. TYPES OF PROTOZOA IN WASTEWATER

Name	Protozoan class	Nonhuman reservoir
Human Pathogens		
Entamoeba histolytica	Ameba	Domestic and wild mammals
Giardia lamblia	Flagellate	Beavers, dogs, sheep
Balantidium coli	Ciliate	Pigs, other mammals
Toxoplasma gondii	Sporozoan (Coccidia)	Cats
Dientamoeba fragilis	Ameba	
Isospora belli	Sporozoan (Coccidia)	
I. hominis	Sporozoan (Coccidia)	
Human Commensals		
Endolimax nana	Ameba	
Entamoeba coli	"	
Iodamoeba butschlii	"	
Animal pathogens		
Eimeria spp.	Sporozoan (Coccidia)	Fish, birds, mammals
Entamoeba spp.	Ameba	Rodents, etc.
Giardia spp.	Flagellate	Dogs, cats, wild mammals
Isospora spp.	Sporozoan (Coccidia)	Dogs, cats

Entamoeba histolytica causes amebiasis, or amebic dysentery, an acute enteritis, whose symptoms may range from mild abdominal discomfort with diarrhea to fulminating dysentery with fever, chills, and bloody and mucoid diarrhea. Most infections are asymptomatic, but in severe cases dissemination may occur, producing liver, lung, or brain abscesses, and death may result. Amebiasis is rare in the U.S. (Krogstad *et al.* 1978), and is transmitted by cysts contaminating water or food.

Giardia lamblia causes giardiasis, an often asymptomatic infection of the small intestine, which may be associated with chronic diarrhea, malabsorption of fats, steatorrhea, abdominal cramps, bloating, fatigue, and weight loss. The carrier rate in different areas of the U.S. may range between 1.5 and 20% (Benenson 1975), and it is transmitted by cysts contaminating water or food, and by person-to-person contact (Osterholm *et al.* 1981).

Balantidium coli causes balantidiasis, a disease of the colon, characterized by diarrhea or dysentery. Infections are often asymptomatic, and the incidence of disease in man is very low (Benenson 1975). Balantidiasis is transmitted by cysts contaminating water, particularly from swine.

Toxoplasma gondii causes toxoplasmosis, a systemic disease which rarely gives rise to clinical illness, but which can damage the fetus if infection, and subsequent congenital transmission, occurs during pregnancy. Approximately 50% of the population of the U.S. is thought to be infected (Krick and Remington 1978), but the infection is probably transmitted by oocysts in cat feces or the consumption of cyst-contaminated, inadequately-cooked meat of infected animals (Teutsch *et al.* 1979), rather than through wastewater.

The active stage of protozoans in the intestinal tract of infected individuals is the trophozoite. The trophozoites, after a period of reproduction, may round up to form precysts, which secrete tough membranes to become environmentally-resistant cysts, in which form they are excreted in the feces (Brown 1969). The number of cysts excreted by a carrier of *Entamoeba histolytica* has been estimated to be 1.5×10^7 per day (Chang and Kabler 1956), and by an adult infected with *Giardia lamblia* at 2.1–7.1×10^8 per day (Jakubowski and Ericksen 1979). The concentration of *Entamoeba histolytica* cysts in the feces of infected individuals has been estimated to be 1.5×10^5/g (Feachem *et al.* 1978). The concentration of *Giardia lamblia* cysts in the feces has been estimated to be 10^5/g in infected individuals (Feachem *et al.* 1978), up to 2.2×10^6/g in infected children, and up to 9.6×10^7/g in asymptomatic adult carriers (Akin *et al.* 1978).

The types and levels of protozoan cysts actually present in wastewater depend on the levels of disease in the contributing human population, and the degree of animal contribution to the system. Some estimates are present in Table 22.

TABLE 22. LEVELS OF PROTOZOA IN WASTEWATER

Species	Wastewater type	Concentration (cysts/l)	Reference
Entamoeba histolytica	Untreated	4.0	Foster and Engelbrecht 1973
	Municipal effluent	2 2	Kott and Kott 1967
	During epidemic (50% carrier rate)	5000	Chang and Kabler 1956
Giardia lamblia	Raw sewage (1-25% prevalence)	9.6×10^3–2.4×10^5	Jakubowski and Ericksen 1979
	Raw sewage	Up to 8×10^4	Weaver *et al.* 1978

PREAPPLICATION TREATMENT

Entamoeba histolytica and *Giardia* are very chlorine resistant, *Entamoeba* being one of the most chlorine-resistant pathogens known (Hoff 1979). Sedimentation, or conventional primary treatment, appears to result in poor removals of protozoan cysts from wastewater, as indicated by data on *Entamoeba histolytica*. Thus, Cram (1943) reported lack of removal, Foster and Engelbrecht (1973) 15% removal, Sproul (1978) 0 to incomplete removal, and Crites and Uiga (1979) 10–50% removal. These authors reported very poor secondary treatment removals as well.

Wastewater stabilization ponds may accomplish much better removals of protozoan cysts. Thus, 100% reduction of protozoan cysts from the effluent was accomplished by a series of 3 ponds, with a 7-day retention time, in India (Arceivala *et al.* 1970), and of *Giardia* cysts by a storage lagoon in Texas (Weaver *et al.* 1978). However, it is likely that this treatment resulted in significant concentration of the cysts in sludge rather than complete inactivation. *Entamoeba* cysts have been found to survive several months in water at 0° C, 3 days at 30° C, 30 minutes at 45° C, and 5 minutes at 50°C (Freeman 1979). *Giardia* cysts can survive up to about 77 days in water at 8° C, 5–24 days at 21° C, and 4 days or less at 37° C (Bingham *et al.* 1979).

SOIL AND PLANTS

Protozoan cysts are highly sensitive to drying. Rudolfs *et al.* (1951b) have reported survival times for *Entamoeba histolytica* of 18–24 hours in dry soil and 42–72 hours in moist soil. Somewhat longer times, i.e., 8–10 days, have been reported by Beaver and Deschamps (1949) in damp loam and sand at 28–34° C.

Because of their exposure to the air, protozoan cysts deposited on plant surfaces would also be expected to die off rapidly. The fact that cysts can survive long enough to get into the human food supply under poor management conditions is confirmed by the recent isolation of high levels of *Entamoeba histolytica, E. coli, Endolimax nana,* and *Giardia lamblia* on the wastewater-irrigated fruits and vegetables in Mexico City's marketplaces (Tay *et al.* 1980). Rudolfs *et al.* (1951b) found contaminated tomatoes and lettuce to be free from viable *Entamoeba* cysts within 3 days, and the survival rate to be unaffected by the presence of organic matter in the form of fecal suspensions. They concluded that field-grown crops "... consumed raw and subject to contamination with cysts of *E. histolytica* are considered safe in the temperate zone one week after contamination has stopped and after two weeks in wetter tropical regions."

Therefore, if the recommendations, based on bacteria, for harvesting human food crops are followed, it is extremely unlikely than any public health risk will ensue.

ANIMALS

Although it would be theoretically possible for protozoan diseases to be transmitted through animals at a land treatment site, little relevent information on the subject appears to exist. However, in view of the survival times discussed above, the four week waiting period before the resumption of grazing, recommended on the basis of bacteria, should prevent any problem from developing.

INFECTIVE DOSE, RISK OF INFECTION, EPIDEMIOLOGY

Human infections with *Giardia lamblia* and the nonpathogenic *Entamoeba coli* have been produced with ten cysts administered in a gelatin capsule (Rendtorff 1954a, 1954b). Infections have been produced with single cysts of *Entamoeba coli*, and there is no biological reason why single cysts of *Giardia* would not also be infectious (Rendtorff 1979). This is probably true for *E. histolytica* as well (Beaver *et al.* 1956). The pathogenicity of protozoa is highly variable among strains, and human responses likewise are variable. Thus, many infections are asymptomatic.

Because of the low infective doses of protozoan cysts, it would be prudent for humans to maintain a minimum amount of contact with an active land treatment site. However, if the recommended waiting periods for crop harvest are followed, the risk of infection should be minimal, because of the cysts' sensitivity to drying.

A few epidemiological reports have linked the transmission of amebiasis to vegetables irrigated with raw wastewater or fertilized with night soil (Bryan 1977, Geldreich and Bordner 1971).

CONCLUSIONS AND RESEARCH NEEDS

The required level of preapplication treatment is site-specific, and wastewater stabilization ponds can probably accomplish a significant degree of removal of protozoan cysts. The effectiveness of ponds in the treatment for protozoa is a significant research need, however.

Human exposure to pathogenic protozoa through aerosols or groundwater is extremely unlikely, and, if crops are not harvested nor animals allowed to graze until two weeks after the last wastewater application, exposure through these routes will be minimized.

Because of the low infective doses of protozoan cysts, it would be wise for humans to maintain a minimum amount of contact with an active land treatment site.

SECTION 7
HELMINTHS
TYPES AND LEVELS IN WASTEWATER

The pathogenic helminths whose eggs are of major concern in wastewater are listed in Table 23. They are taxonomically divided into the nematodes, or roundworms, and cestodes, or tapeworms. The trematodes, or flukes, are not included since they require aquatic conditions and intermediate hosts, usually snails, to complete their life cycles, and thus are unlikely to be of concern at land treatment sites. Some common helminths, pathogenic to domestic or wild animals, but not to humans, are listed in Table 24 (after Reimers *et al.* 1980), since their eggs are likely to be identified in wastewater. Several of the human pathogens listed in Table 23, e.g., *Toxocara* spp., are actually animal parasites, rather than human parasites, infesting man only incidentally, and not completing their life cycle in man.

TABLE 23. PATHOGENIC HELMINTHS OF MAJOR CONCERN IN WASTEWATER

Pathogen	Common name	Disease	Nonhuman reservoir
NEMATODES (Roundworms)			
Enterobius vermicularis	Pinworm	Enterobiasis	
Ascaris lumbricoides	Roundworm	Ascariasis	
A. suum	Swine roundworm	Ascariasis	Pig*
Trichuris trichiura	Whipworm	Trichuriasis	
Necator americanus	Hookworm	Necatoriasis	
Ancylostoma duodenale	Hookworm	Ancylostomiasis	
A. braziliense	Cat hookworm	Cutaneous larva migrans	Cat, dog*
A. caninum	Dog hookworm	Cutaneous larva migrans	Dog*
Strongyloides stercoralis	Threadworm	Strongyloidiasis	Dog
Toxocara canis	Dog roundworm	Visceral larva migrans	Dog*
T. cati	Cat roundworm	Visceral larva migrans	Cat*
CESTODES (Tapeworms)			
*Taenia saginata***	Beef tapeworm	Taeniasis	
T solium	Pork tapeworm	Taeniasis, Cysticercosis	
Hymenolepis nana	Dwarf tapeworm	Taeniasis	Rat, mouse
Echinococcus granulosus	Dog tapeworm	Unilocular hydatid disease	Dog*
E. multilocularis		Alveolar hydatid disease	Dog, fox, cat*

*Definitive host, man only incidentally infested
**Eggs not infective for man

TABLE 24. ANIMAL-PATHOGENIC HELMINTHS IN WASTEWATER

Pathogen	Definitive host
Trichuris suis	Pig
T. vulpis	Dog
*Toxascaris leonina**	Dog, cat
Ascaridia galli	Poultry
Heterakis gallinae	Poultry
Trichosomoides crassicauda	Rat
Anatrichosoma buccalis	Opossum
Cruzia americana	Opossum
Capillaria hepatica	Rat
C. gastrica	Rat
C. spp.	Poultry, wild birds, wild mammals
Hymenolepis diminuta	Rat
H. spp.	Birds
Taenia pisiformis	Cat
Hydatigera taeniaeformis	Dog
Macracanthorhynchus hirudinaceous	Pig

**Toxascaris leonina* may produce visceral larva migrans in experimental animals, but its role in human disease is undefined (Quinn *et al* 1980)

Enterobius vermicularis, the pinworm, causes itching and discomfort in the perianal area, particularly at night when the female lays her eggs on the skin. A 1972 estimate of the prevalence of pinworm infections in the U.S. was 42 million (Warren 1974). Although it is by far the most common helminth infection, the eggs are not usually found in feces, are spread by direct transfer, and live only for a few days.

Ascaris lumbricoides, the large roundworm, produces numerous eggs, which require 1–3 weeks for embryonation. After the embryonated eggs are ingested, they hatch in the intestine, enter the intestinal wall, migrate through the circulatory system to the lungs, enter the alveoli, and migrate up to the pharynx. During their passage through the lungs they may produce ascaris pneumonitis, or Loeffler's syndrome, consisting of coughing, chest pain, shortness of breath, fever, and eosinophilia, which can be especially severe in children. The larval worms are then swallowed, to complete their maturation in the small intestine, where small numbers of worms usually produce no symptoms. Large numbers of worms may cause digestive and nutritional disturbances, abdominal pain, vomiting, restlessness, and disturbed sleep, or, occasionally, intestinal obstruction. Death due to migration of adult worms into the liver, gallbladder, peritoneal cavity, or appendix occurs infrequently. The prevalence of ascariasis in the U.S. was estimated to be about 4 million in 1972 (Warren 1974).

Ascaris suum, the swine roundworm, may produce Loeffler's syndrome, but probably does not complete its life cycle in man (Phillis *et al.* 1972).

Trichuris trichiura, the human whipworm, lives in the large intestine with the anterior portion of its body threaded superficially through the mucosa. Eggs are passed in the feces, and develop to the infective stage after about four weeks in the soil (Reimers *et al.* 1980), and direct infections of the cecum and proximal colon result from the ingestion of infective eggs. Light infections are often asymptomatic, but heavy infections may cause intermittent abdominal pain, bloody stools, diarrhea, anemia, loss of weight, or rectal prolapse in very heavy infections. Human infections with *T. suis*, the swine whipworm, and *T. vulpis*, the dog whipworm, have been reported, but are uncommon (Reimers *et al.* 1980). The prevalence of trichuriasis in the U.S. was estimated to be about 2.2 million in 1972 (Warren 1974). Reimers *et al.* (1980) have found *Ascaris*, *Trichuris*, and *Toxocara* to be the most frequently recovered helminth eggs in municipal wastewater sludge in southeastern United States.

Necator americanus and *Ancylostoma duodenale*, the human hookworms, live in the small intestine attached to the intestinal wall. Eggs are passed in the feces, and develop to the infective stage in 7–10 days in warm, moist soil. Larvae penetrate bare skin, usually of the foot (although *Ancylostoma* may also be acquired by the oral route), pass through the lymphatics and and blood stream to the lungs, enter the alveoli, migrate up the pharynx, are swallowed, and reach the small intestine. During lung migration, a pneumonitis, similar to that produced by *Ascaris*, may occur (Benenson 1975). Light infections usually result in few clinical effects, but heavy infections may result in iron-deficiency anemia (because of the secreted anti-coagulant causing bleeding at the site of attachment) and debility, especially in children and pregnant women. The prevalence of hookworm in the U.S. (usually due to *Necator*) was estimated to be about 700,000 in 1972 (Warren 1974).

Ancylostoma braziliense and *A. caninum*, the cat and dog hookworm, do not live in the human intestinal tract. Larvae from eggs in cat and dog feces penetrate bare skin, particularly feet and legs on beaches, and burrow aimlessly intracutaneously, producing "cutaneous larva migrans" or "creeping eruption." After several weeks or months the larva dies without completing its life cycle.

Strongyloides stercoralis, the threadworm, lives in the mucosa of the upper small intestine. Eggs hatch within the intestine, and the reinfection may occur, but usually noninfective larvae pass out in the feces. The larva in the soil may develop into an infective stage or a free-living adult, which can produce infective larvae. The infective larvae penetrate the skin, usually of the foot, and complete their life cycle similarly to hookworms. Intestinal symptoms include abdominal pain, nausea, weight loss, vomiting, diarrhea, weakness, and constipation. Massive infection and autoinfection may lead to wasting and death in patients receiving immunosuppressive medication (Benenson 1975). The prevalence of strongyloidiasis in the U.S. was estimated to be about 400,000 in 1972 (Warren 1974). Dog feces is another source of threadworm larvae.

Toxocara canis and *T. cati*, the dog and cat roundworms, do not live in the human intestinal tract. When eggs from animal feces are ingested by man, particularly children, the larvae hatch in the intestine and enter the intestinal wall, similarly to *Ascaris*. However, since *Toxocara* cannot complete its life cycle, the larvae do not migrate to the pharynx, but, instead, wander aimlessly through the tissues, producing "visceral larva migrans," until they die in several months to a year. The disease may cause fever, appetite loss, cough, asthmatic episodes, abdominal discomfort, muscle aches, or neurological symptoms, and may be particularly serious if the liver, lungs, eyes (often resulting in blindness), brain, heart, or kidneys become involved (Fiennes 1978). The infection rate of *T. canis* is more than 50% in puppies and about 20% in older dogs in the U.S. (Gunby 1979), and *Toxocara* is one of the most common helminth eggs in wastewater sludge (Reimers *et al.* 1980).

Taenia saginata and *T. solium*, the beef and pork tapeworms, live in the intestinal tract, where they may cause nervousness, insomnia, anorexia, loss of weight, abdominal pain, and digestive disturbances, or be asymptomatic. The infection arises from eating incompletely cooked meat (of the intermediate host) containing the larval stage of the tapeworm, the cysticercus, however, rather than from a wastewater-contaminated material. Man serves as the definitive host, harboring the self-fertile adult. The eggs (contained in proglottids) are passed in the feces, ingested by cattle and pigs (the intermediate hosts), hatch, and the larvae migrate into tissues, where they develop to the cysticercus stage. The hazard, then, is principally to livestock grazing on land-treatment sites. The major direct hazard to man is the possibility of him acting as the intermediate host. While *Taenia saginata* eggs are not infective for man, those of *T. solium* are infective for man, in which they can produce cysticerci. Cysticercosis can present serious symptoms when the larvae localize in the ear, eye, central nervous system, or heart. Taeniasis with *Taenia solium* is rare in the U.S., and with *T. saginata* is only occasionally found. However, human infections with these tapeworms are fairly common in some other areas of the world.

Hymenolepis nana, the dwarf tapeworm, lives in the human intestinal tract, where it may be asymptomatic or produce the same symptoms as *Taenia*. Infective eggs are released, and internal autoinfection may occur, or, more usually, eggs may be passed in the feces. No intermediate host is required, and, upon ingestion, eggs develop into adults in the intestinal tract. The prevalence of infection in southern U.S. is 0.3 to 2.9 percent, mostly among children under 15.

Echinococcus granulosus and *E. multilocularis*, two dog tapeworms, do not live in the human intestinal tract. Dogs and other carnivores are their definitive hosts. Eggs in animal feces are usually ingested by an herbivore, in which they hatch into larval forms, which migrate into tissues, where they develop into hydatid cysts. When the herbivore is eaten by a carnivore the cysts develop into adult tapeworms in the carnivore's intestinal tract. If man ingests an egg, he can play the role of the herbivore, just as in cysticercosis. A hydatid cyst can develop in the liver, lungs, or other organs, where serious symptoms can be produced as the cyst grows in size or ruptures. The disease is rare in the U.S., but has been reported from the western states, Alaska, and Canada, particularly where dogs are used to herd grazing animals, and where dogs are fed animal offal.

Since no helminths are normal inhabitants of the human gastrointestinal tract, i.e., commensals, there are no normal levels of helminth eggs in feces. Levels suggested by Feachem *et al.* (1978) for eggs in the feces of infected humans (eggs/g) are:

Enterobius	0
Ascaris	10,000
Trichuris	1,000
Necator and *Ancylostoma*	800
Strongyloides	10
Taenia	10,000
Hymenolepis	?

Obviously, these values will depend on intensity of infection.

The presence and levels in wastewater of any of these helminth eggs, or of those from animal feces *(Ancylostoma, Toxocara,* and *Echinococcus)*, depend on the levels of disease in the contributing population, and the degree of animal contribution to the system. Foster and Engelbrecht (1973) suggested a value of 66 helminth ova/l in untreated wastewater, and Larkin *et al.* (1978b) cited values of 15–27 *Ascaris* eggs/l and 6.2 helminth eggs/l in primary effluent.

PREAPPLICATION TREATMENT

Since helminth eggs are denser than water, ordinary sedimentation, or conventional primary treatment, is a fairly efficient method of removal. German sanitary engineers have found one to two hours of sedimentation detention time to be sufficient to remove most helminth eggs (Sepp 1971). Newton *et al.* (1949) showed 98% removal of *Taenia saginata* eggs by 2-hour sedimentation in the laboratory, but lower removal under field conditions.

Conventional secondary treatment, i.e., activated sludge or trickling filter, results in very poor helminth egg removal rates (Sproul 1978).

Wastewater stabilization ponds accomplish excellent degrees of helminth egg removal, as indicated in Table 25 (after Feachem *et al.* 1978). Feachem *et al.* (1978) have concluded that complete removal of helminth eggs occurs in all cases of well-designed multicelled stabilization ponds with an overall retention time of more than 20 days. Stabilization ponds with variable retention times at a land treatment site in San Angelo, Texas, resulted in complete removal of helminths (Weaver *et al.* 1978).

It should be kept in mind that the sludge or pond sediment resulting from these processes will have high densities of viable helminth eggs, and will require proper treatment before utilization.

TABLE 25. HELMINTH EGG SURVIVAL IN WASTEWATER STABILIZATION PONDS

Organism	Retention time or pond description	Removal rate	Reference
Helminths	7 days, 3 ponds	100%	Arceivala *et al.* 1970
Helminths	4 ponds	100%	Koltypin 1969
Enterobius	38 days	100%	Hodgson 1964
Enterobius	6 days, 3 ponds	100 %	Lakshminarayana and Abdulappa 1972
Ascaris	6 days, 3 ponds	100%	Lakshminarayana and Abdulappa 1972
Trichuris	6 days, 3 ponds	100%	Lakshminarayana and Abdulappa 1972
Ancylostoma duodenale	38 days	100%	Hodgson 1964
Ancylostoma	6 days, 3 ponds	90%	Lakshminarayana and Abdulappa 1972
Hymenolepis	6 days, 3 ponds	100%	Lakshminarayana and Abdulappa 1972

SOIL AND PLANTS

Helminth eggs and larvae, in contrast to protozoan cysts, live for long periods of time when applied to the land, probably because soil is the transmission medium for which they have evolved, while protozoa have evolved toward water transmission. Thus, under favorable conditions of moisture, temperature, and sunlight, *Ascaris, Trichuris,* and *Toxocara* can remain viable and infective for several years (Little 1980). Hookworms can survive up to 6 months (Feachem *et al.* 1978), and *Taenia* a few days to seven months (Babayeva 1966); other helminths survive for shorter periods.

Because of desiccation and exposure to sunlight, helminth eggs deposited on plant surfaces die off more rapidly. Thus, Rudolfs *et al.* (1951c) found *Ascaris* eggs, the longest-lived helminth egg, sprayed on tomatoes and lettuce, to be completely degenerated after 27–35 days.

At rapid-infiltration land treatment sites there should be little risk to public health from helminths, as long as the site is dedicated to rapid infiltration. However, because of the growth of crops and presence of people at slow-rate and overland-flow sites, and the longevity of helminth eggs, it would be advisable to select a preapplication treatment method, e.g., stabilization ponds, which will completely remove helminth eggs at these land treatment sites.

ANIMALS

The most serious threat to cattle at land treatment sites is the beef tapeworm, *Taenia saginata* (Feachem *et al.* 1978). The increased incidence of cysticercosis in cattle results in economic losses (because of condemnation of carcasses), as well as increased incidence of disease in man. The application of wastewater sludge to pastures has resulted in outbreaks of cysticercosis in grazing cattle in England (Macpherson *et al.* 1978, 1979), but wastewater land treatment sites at San Angelo, Texas (Weaver *et al.* 1978), and Melbourne, Australia (Croxford 1978, McPherson 1978), have resulted in no increase of cysticercosis in grazing cattle.

Nevertheless, because of the longevity of helminth eggs in the soil, and the fact that cattle consume considerable quantities of soil as they graze, it would be prudent to select a pretreatment method which will completely remove helminth eggs at land treatment sites where cattle are allowed to graze. Arundel and Adolph (1980) have suggested that stabilization ponds remove *Taenia saginata* quite efficiently. They found no cysticercosis in cattle grazed on pasture irrigated with effluent from lagooning, compared with a 3.3% infection rate from trickling filter effluent, 9.0–12.5% from activated sludge effluent, and 30.0% from raw sewage.

INFECTIVE DOSE, RISK OF INFECTION, EPIDEMIOLOGY

Single eggs of helminths are infectious to man, although, since the symptoms of helminth infections are dose-related, many light infections are asymptomatic. However, *Ascaris* infection may sensitize individuals so that the passage of a single larval stage through the lungs may result in allergic symptoms, i.e., asthma and urticaria (Muller 1953).

Because of the low infective doses of helminth eggs, and their longevity, it would be prudent for humans to maintain a minimum amount of contact with an active or inactive land treatment site, unless the wastewater has been pretreated to remove helminths.

A few epidemiological reports have linked the transmission of *Ascaris* and hookworm to the use of night soil on gardens and small farms in Europe and the Orient (Geldreich and Bordner 1971).

CONCLUSIONS AND RESEARCH NEEDS

The required level of preapplication treatment for rapid-infiltration systems is site-specific, but slow-rate and overland-flow systems should have complete removal of helminth eggs. This would probably be most easily done by wastewater stabilization ponds.

Human exposure to helminths through aerosols or groundwater is extremely unlikely, and, if wastewater is properly pretreated, exposure through crops and animals will be minimized.

It would be wise for humans to maintain a minimum amount of contact with an active or inactive land treatment site, unless the wastewater has been pretreated to remove helminths.

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