Chapter 13

MICROBIOLOGICAL HEALTH EFFECTS ASSOCIATED WITH THE USE OF MUNICIPAL WASTEWATER FOR IRRIGATION

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I. INTRODUCTION

For centuries 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,

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> 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.

Slow rate land application, or irrigation, 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 between 0.6 and 6 m/yr. 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 pastures, forests, lawns and golf courses (for highly treated wastewater), but crops (usually for animal consumption) are most commonly grown. Climatic constraints may require some winter storage of wastewater (Reed, 1979). Recycling benefits include moderate groundwater recharge and the utilization of wastewater nutrients in crop production.

Many of the examples of land application systems in the United States utilize wastewater treated by conventional means up to tertiary level. 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 paper, it is assumed that raw wastewater is given only 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. It is assumed that raw sewage will not be used for irrigation.

With the application to land of large volumes of minimally pretreated wastewater, it is evident that considerable potential for adverse health effects exists. Problems could occur from food crop contamination, pathogen-laden aerosols, groundwater pollution, or surface water pollution.

It is the purpose of this paper to examine the potential health effects of land treatment, and to provide an appraisal of these effects. The microbial agents of concern from a health effects viewpoint include bacteria such as Salmonella and Shigella; viruses such as enteroviruses, hepatitis virus, adenoviruses, rotaviruses, and Norwalk-like agents; protozoa such as Entamoeba and Giardia; and helminths or worms such as Ascaris, Trichuris, and Toxocara. The protozoa and helminths are often grouped together under the term, "parasites," although in reality all the pathogens are parasites.

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. There then follows 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, as appropriate. For the pathogens, infective dose, risk of infection, and epidemiology are then briefly reviewed. Finally, conclusions are presented.

II. BACTERIA

A. Types and Levels in Wastewater

The pathogenic bacteria of major concern in wastewater are listed in Table I. 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 II; 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.

Campylobacter jejuni (formerly C. fetus ssp. 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 between 4 and 8% of patients with diarrhea (MMWR, 1979).

TABLE I. 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)[a]	--
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

[a]Correct nomenclature: Salmonella paratyphi A, S. schottmuelleri, S. hirschfeldii, respectively.

TABLE II. 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 aereus
Streptococcus spp.

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. Outbreaks occasionally occur in nurseries and institutions, and the enterotoxigenic strain accounts for about 60 to 70% of diarrheal episodes 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 with age, and may reach 20% or more in patients with jaundice and kidney damage (Benenson, 1975). 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 United States (Benenson, 1975).

Salmonella typhi causes typhoid fever, systemic disease with a fatality rate of 10% untreated or 2 to 3% when treated by antibiotics (Benenson, 1975). It occurs sporadically in the United States, 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% when treated (Benenson, 1975). Cholera is rare in the United States, 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 United States, and is 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, nonspore-forming rods (Jawetz et al., 1978). The family Enterobacteriaceae includes the following tribes and genera (Holt, 1977):

(1) Escherichieae
  Escherichia
  Edwardsiella
  Citrobacter
  Salmonella (including Arizona)

(2) Klebsielleae
  Klebsiella
  Enterobacter
  Hafnia
  Serratia

(3) Proteeae
  Proteus

(4) Yersinieae
  Yersinia

(5) 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 fruendii	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 between 25 and 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 III. 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 IV.

Since the bacteria of feces are predominantly anaerobes while the environment of wastewater is aerobic, and thus toxic to the anaerobes, the bacterial composition of wastewater is drastically different from that of feces. The composition also 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). 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 to 10^7 organisms/ml. The presence and levels in wastewater of any of the pathogens listed in Tables I and II depend, of course, on the levels of infection in the contributing population.

TABLE III. Viable Bacteria in Human Feces

Bacterium	Carnow et al., 1979	Feachem et al., 1978
ANAEROBES	- - - - number/gm (wet weight) - - - -	
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[a]	10^6	10^7-10^9
Enterococci (fecal Streptococcus)	10^5	10^5-10^8
Staphylococcus	$<10^3$	-
Bacillus, Proteus, Pseudomonas, Spirochetes	$<<10^3$	-

[a] Enterobacteria are primarily Escherichia coli with some Klebsiella and Enterobacter (Carnow et al., 1979).

TABLE IV. Pathogenic Bacteria in Feces of Infected Persons[a]

Bacterium	Number/gm 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

[a] From Feachem et al. (1978)

B. 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 halomethanes, 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/liter, a superficially impressive 99% removal, or 1% survival, will produce an effluent with 10^3 pathogenic bacteria/liter. This level may still be of great public health concern, depending on how the effluent is used.

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 V.

TABLE V. Bacterial Removal During Wastewater Sedimentation

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

As a result of the need for 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 to 4 m deep, 1 to 5 day retention time), (2) facultative ponds, with oxygen supplied by algae (1 to 1.5 m deep, 10 to 40 day retention time), and (3) maturation ponds (1 to 1.5 m deep, 5 to 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 to 85% after 3.5 to 5 days at various temperatures have been reported.

(2) In single facultative and aerobic ponds E. coli removals of 80 to >99% after 10 to 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 between 90 to 99% of Salmonella or other pathogenic bacteria.

(6) Complete elimination of pathogenic bacteria can be achieved with 30 to 40 day retention times, particularly at high temperatures (>25°C).

(7) A series of 5 to 7 ponds, each with a 5 day retention time, can produce an effluent with less than 100 fecal coliforms and fecal streptococci/100 ml.

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

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.

C. 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 (Schaub et al., 1978a). These are suspensions of solid or liquid particles up to about 50 um 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 um 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 combated by local mechanisms. Although the pattern of deposition is variable, the greatest alveoli deposition appears to occur in the 1 to 2 um range, decreasing to a minimum at about 0.25 um, and increasing below 0.25 um (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 10 fold 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.

TABLE VI. Aerosol Bacteria at Land Treatment Sites

Wastewater Type	Location (reference)	Distance	Bacterium	Density ($/m^3$)
Raw or primary	Germany (Reploh and Handloser, 1957)	90-160m	Coliform	Detected at maximum distance
	Germany (Bringmann and Trolldenier, 1960)	63-400m	Coliform	Detected at maximum distance (night)
	California (Sepp, 1971)	32m	Coliform	Detected at maximum distance
	Kibbutz Tzora, Israel (Katzenelson and Teltch, 1976)	10m	Coliform	11-496
			Fecal coliform	35-86
		20m	Coliform	0-480
		60m	Coliform	0-501
			Salmonella	Detected at maximum distance
		70m	Coliform	30-102
		100m	Coliform	0-88
		150m	Coliform	4-32
		200m	Coliform	0-25
		250m	Coliform	0-17
		300m	Coliform	0-21
		350m	Coliform	0-7
		400m	Coliform	0-4

TABLE VI. Aerosol Bacteria at Land Treatment Sites (Continued)

Wastewater Type	Location (reference)	Distance	Bacterium	Density (/m^3)
Ponded, chlorinated	Deer Creek, Ohio (Bausum et al., 1978)	Upwind	Std. plate count	111 (23-403)
		21-30m	Std. plate count	485 (46-1582)[a]
		41-50m	Std. plate count	417 (0-1429)[a]
		200m	Std. plate count	37 (<0-223)[a]
Secondary, nonchlorinated	Ft. Huachuca, Arizona (Schaub et al., 1978a)	Upwind	Std. plate count	28 (12-170)
			Coliform	2.4 (0-58)
		45-49m	Std. plate count	430-1400 (day) 560-6300 (night)
			Klebsiella	1-23
		120-152m	Std. plate count	86-130 (day) 170-410 (night)
	Pleasanton, California (Johnson et al., 1978, 1980)	Upwind	Std. plate count	300-805
		30-50m	Std. plate count	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

TABLE VI. Aerosol Bacteria at Land Treatment Sites (Continued)

Wastewater Type	Location (reference)	Distance	Bacterium	Density (/m^3)
			Mycobacterium	0.8
		100-200m	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

[a] Corrected for upwind background value.

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 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 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 VI. 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 VI. 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 VI 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 to 5 um, with a median of 2.6 um.

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/hr. 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 to 60/100 ml, the density of aerosol Salmonella at 40 m, the maximum distance found, was 0 to 0.054/m^3, with a mean of 0.014/m^3. This would result in an inhalation 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).

D. 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 dessication 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 decreases 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 VII are extracted from these summaries, as well as other literature. "Survival" as used in this table, and throughout this report, denotes days of detention. 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 VII, but this would not be a realistic soil situation.

TABLE VII. Survival Times of Bacteria in Soil

Bacterium	Survival time (days)
Coliform	4 - 77
Fecal coliform	8 - 55
Fecal streptococci	8 - >70
Leptospira	<15
Mycobacterium	10 - >450
Salmonella paratyphi	>259
Salmonella typhi	1 - 120
Salmonella spp.	11 - >280
Streptococcus faecalis	26 - 77

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).

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 dessication. 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 VIII are extracted from these summaries, as well as other literature.

TABLE VIII. Survival Times of Bacteria on Crops

Bacterium	Crop	Survival time (days)
Coliform	Tomatoes	>30
	Fodder	6 - 34
	Leaf vegetables	35
Escherichia coli	Vegetables	<21
	Grass	<8
Mycobacterium	Grass	10 - 14
	Lettuce	>35
	Radishes	>13
Salmonella typhi	Vegetables (leaves and stems)	10 - 31
	Radishes	24 - 53
	Lettuce	18 - 21
Salmonella spp.	Leaf vegetables	7 - 40
	Beet leaves	21
	Tomatoes	3 - 7
	Cabbage	5
	Gooseberries	5
	Clover	12
	Grass	>42
	Orchard crops	>2
Shigella spp.	Tomato	2 - 5
	Apple	8
	Leaf vegetables	2 - 7
	Fodder	<2
	Orchard crops	6
Vibrio cholerae	Vegetables	5 - 7
	Dates	<1 - 3

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 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 irrigation 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.

E. Infective Dose, Risk of Infection, and 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 an unlikely occurrence with most pathogens; usually large numbers are necessary. Some representative oral infective dose data for enteric bacteria, based upon numerous studies using nonuniform techniques, are presented in Table IX (adapted from Bryan, 1977).

Although the terms, "infective dose," "minimal infectious dose," etc., are used in the literature, it is obvious from Table IX 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, infections 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.

(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 IX, where buffering reduces the infective dose by about a thousand fold. 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 diarrheal 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 developed (Levine, 1980).

TABLE IX. Infective Dose (number of bacterial cells) 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		

TABLE IX. Infective Dose (number of bacterial cells) to Man of Enteric Bacteria (Continued)

Bacterium	No Infection or no illness	Infections without illness	Percent of volunteers developing illness			
			1-25	26-50	51-75	76-100
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			10^1-10^2	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	10^1	10^3		10^3-10^8	10^4-10^6	
Unbuffered	10^4-10^{10}			10^8-10^{11}		

(3) Other host factors, such as age and general health, also affect the disease response. Infants, elderly persons, malnourished people, those with concomitant illness, and people taking antiinflammatory, cytotoxic, and immunosuppressant drugs would be more susceptable 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 5% were still resistant to 10^9 organisms, four orders of magnitude as many.

(4) The timing of the exposure to pathogens, e.g., as a single exposure or over a long period of time, would be expected to affect the response.

(5) Finally, as illustrated by Escherichia coli and Salmonella typhi in Table IX, 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 to 10^8, and 180 organisms (NRC, 1977).

The risk of infection is probably greatest for Salmonella spp. and Shigella spp., bacause they are the most common bacterial pathogens in municipal wastewater. The infective dose for Salmonella is high, between 10^5 and 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.

II. 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).

A. Types and Levels in Wastewater

The human enteric viruses that may be present in wastewater are listed in Table X (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.

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 epidemilogical 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 enteroviral infections, even with the more virulent types, cause few or no clinical symptoms. Occasionally, after continued multipli-

TABLE X. Human Viruses in Wastewater

Enteroviruses

- Poliovirus
- Coxsackievirus A
- Coxsackievirus B
- Echovirus
- New Enteroviruses

Hepatitis A Virus
Rotavirus ("Duovirus," "Reovirus-like Agent")
Norwalk-Like Agents (Norwalk, Hawaii, Montgomery County, etc.)
Adenovirus
Reovirus
Papovavirus
Astrovirus
Calicivirus
Coronavirus-Like Particles

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cation 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 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 7075 cases were reported to 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 low level 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 to 50,000 cases are reported annually in the United States, and about half the population has antibodies to hepatitis A virus. 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).

Adenovirses 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 viruses 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. Thus, Goyal et al. (1979) provided data to indicate that current bacteriological standards for determining the safety of shellfish and 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. 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 bacterial indicators.

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 (Feachem et al., 1978) to be about 10^6/gm.

Reports of 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 late 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 tends 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). (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 viruses.) Some representative levels of enteric viruses in raw wastewaters of the United States are summarized in Table XI. 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/liter of raw sewage and would most often contain less than 1000 virus units/liter."

TABLE XI. Levels of Enteric Viruses in United States Wastewaters

Description	Viral units/liter	Reference
St. Petersburg	10->183	Wellings et al., 1978
Various sources	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

B. Preapplication Treatment

Chlorine, although effective as a bacteriacide, is a very inefficient inactivator of wastewater viruses. Levels as high as 8 mg/liter have little effect in secondary effluent (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% have been reported (Melnick et al., 1978), with 10% 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 XII (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.

C. 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 respirable 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 sec (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 lipids (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

TABLE XII. 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%

200 m 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 to 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 3 hr sampling periods, at 50 m downwind from the source, to measure the aerosol enteroviruses produced by the spraying of unchlorinated secondary 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 XIII.

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 United States wastewaters in general (cf. Table XI), (2) the high Israeli wastewater virus levels are not typical of those found in the United States, 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 concluded that an adult male, engaged in light work, breathing at a rate of 1.2 m^3/hr, 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 hr work day. This is probably an insignificant level of exposure. However, the recovery of enteric viruses from environmental samples is not perfectly efficient. Since 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 source.

D. 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, and 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.

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

TABLE XIII. Aerosol Enteroviruses at Land Treatment Sites

Site	Distance (m)	Wastewater Total Coliforms (/liter)	Wastewater Enteroviruses (mean) (PFU/liter)	Aerosol Enteroviruses (mean) ($/m^3$)
Pleasanton California (Secondary Effluent)	50	$6.4-19 \times 10^5$	45-330 (188)	0.011-0.017 (0.014)
Kibbutz Tzora, Israel (Raw Wastewater)	36-42	$3.1-150 \times 10^7$	0-650 (125)	0-0.082 (0.015)
	50	1.0×10^8	650	0.14
	70	$1.0-17 \times 10^7$	170-13,000 (6585)	0-0.026 (0.013)
	100	$2.4-30 \times 10^7$	0-82,000 (16,466)	0-0.10 (0.038)

result in their physical disruption (Murray and Laband, 1979). Dessication and higher temperatures decrease survival time (Sagik et al., 1978). The soil is a complex medium, however, with fluctuations in soil moisture, temperatures, pH, ionic strength, dissolved gas concentrations, nutrient concentrations, etc., which 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 minimal. The persistance 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 XIV. Although dose dependent, approximately 100 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, and suggested that the main effect of temperature on virus survival in the field may be its influence on evaporation rates. They suggest 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.

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 increased 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 XV (Feachem et al., 1978). The data are similar to those for bacteria (cf. Table VIII), and likewise appear to support a one month waiting period after last wastewater application before harvest.

Because of the possible contamination of subsurface and low-growing crops with soil, in which viruses have a longer survival time, about 100 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 yr, no enteroviruses were found on or in the leaf and grain portions of corn (Koerner and Haws, 1979).

E. Infective Dose, Risk of Infection, and 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 XVI (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 does suggest, however, that the infective dose of enteroviruses to man is low, possibly on the order of 10 virus particles or less. The same factors, discussed earlier, that affect bacteria affect the virus dose-response relationship.

TABLE XIV. 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	91	Lefler and Kott, 1974
		Dry	<77	
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 rainfall, -12-26°C	<161	Damgaard-Larsen et al., 1977

TABLE XIV. Survival Times of Enteric Viruses in Soil (Continued)

Virus	Soil	Moisture and Temperature	Survival (days)	Reference
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	

TABLE XV. 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	

TABLE XV. Survival Times of Enteric Viruses on Crops
(Continued)

Virus	Crop	Conditions	Survival (days)	Reference
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	

TABLE XVI. Oral Infective Dose to Man of Enteric Viruses

Virus	Subjects	Dose[a]	Percent Infected	Reference
Vaccine poliovirus	Infants	0.2 PFU[b]	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	

TABLE XVI. Oral Infective Dose to Man of Enteric Viruses (Continued)

Virus	Subjects	Dose[a]	Percent Infected	Reference
	Premature infants	1	30	Katz and Plotkin, 1967
		2.5	33	
		10	67	
	Infants	7-52[c]	1	Minor et al., 1981
		24-63	10	
		55-93	50	
Echovirus 12	Young adults	10 PFU	18	Schiff et al., 1980 (personal communication)
		100 PFU	67	

[a] Tissue culture dose 50% (TCD_{50}) unless indicated.
[b] Plaque-forming unit.
[c] 95% Confidence limits.

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 human beings 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 level of viral disease transmission that might occur from a modern land treatment site (Melnick, 1978; WHO 1979).

III. 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 irrigation sites. 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 parasitic infection in the United States is minimal (Larkin et al., 1978b). However, because of the increasing recognition of parasitic infections in the United States, 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 existance of resistant stages of the organisms, a consideration of parasites is warranted.

A. Types and Levels in Wastewater

The most common protozoa which may be found in wastewater are listed in Table XVII. 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.

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 or 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 United States (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 United States 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.

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.

TABLE XVII. 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	Ameba	
Iodamoeba butschlii	Ameba	
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

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 United States 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/day (Chang and Kabler, 1956), and by an adult infected with Giardia lamblia at $2.1\text{-}7.1\times10^8$/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/gm (Feachem et al., 1978). The concentration of Giardia lamblia cysts in the feces has been estimated to be 10^5/gm in infected individuals (Feachem et al., 1978), up to 2.2×10^6/gm in infected children, and up to 9.6×10^7/gm 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 presented in Table XVIII.

B. 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) lack of to incomplete removal, and Crites and Uiga (1979) 10 to 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),

TABLE XVIII. Levels of Protozoa in Wastewater

Species	Wastewater Type	Concentration (cysts/liter)	Reference
E. 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
G. lamblia	Raw sewage (1 to 25% prevalence)	$9.6x10^3$-$2.4x10^5$	Jakubowski and Ericksen, 1979
	Raw sewage	Up to $8x10^4$	Weaver et al., 1978

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 min at 45°C, and 5 min at 50°C (Freeman, 1979). Giardia cysts can survive up to about 77 days in water at 8°C, 5 to 24 days at 21°C, and 4 days or less at 37°C (Bingham et al., 1979).

C. Soil and Plants

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

Because of their exposure to the air, protozoan cysts deposited on plant surfaces would also be expected to die off rapidly. Thus, 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.

D. Infective Dose, Risk of Infection, and 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

TABLE XIX. 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[a]
Trichuris trichiura	Whipworm	Trichuriasis	
Necator americanus	Hookworm	Necatoriasis	
Ancylostoma duodenale	Hookworm	Ancylostomiasis	
A. braziliense	Cat hookworm	Cutaneous larva migrans	Cat, dog[a]
A. caninum	Dog hookworm	Cutaneous larva migrans	Dog[a]
Strongyloides stercoralis	Threadworm	Strongyloidiasis	Dog
Toxocara canis	Dog roundworm	Visceral larva migrans	Dog[a]
T. cati	Cat roundworm	Visceral larva migrans	Cat[a]

TABLE XIX. Pathogenic Helminths of Major Concern in Wastewater (Continued)

Pathogen	Common Name	Disease	Nonhuman Reservoir
CESTODES (Tapeworms)			
Taenia saginata[b]	Beef tapeworm	Taeniasis	
T. solium	Pork tapeworm	Taeniasis, Cysticercosis	
Hymenolepis nana	Dwarf tapeworm	Taeniasis	Rat, mouse
Echinococcus granulosus	Dog tapeworm	Unilocular hydatid disease	Dog[a]
E. multilocularis		Alveolar hydatid disease	Dog, fox, cat[a]

[a] Definitive host; man only incidentally infested.
[b] Eggs not infective for man.

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).

IV. HELMINTHS

A. Types and Levels in Wastewater

The pathogenic helminths whose eggs are of major concern in wastewater are listed in Table XIX. 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 XX (after Reimers et al., 1980), since their eggs are likely to be identified in wastewater. Several of the human pathogens listed in Table XIX, e.g., Toxocara spp., are actually animal parasites, rather than human parasites, infesting man only incidentally, and not completing their life cycle in man.

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 United States 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 for only a few days.

Ascaris lumbricoides, the large roundworm, produces numerous eggs, which require 1 to 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

TABLE XX. Animal-Pathogenic Helminths in Wastewater

Pathogen	Definitive Host
Trichuris suis	Pig
T. vulpis	Dog
Toxascaris leonina[a]	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

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

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 United States 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 (Phills 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 4 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 United States 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 to 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 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 anticoagulant causing bleeding at the site of attachment) and debility, especially children and pregnant women. The prevalence of hookworm in the United States (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 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 United States 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 United States (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 United States, 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 United States is 0.3 to 2.9% 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 United States, 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/gm) are: Enterobius (0), Ascaris (10,000), Trichuris (1000), Necator and Ancylostoma (800), Strongyloides (10), Taenia (10,000), and 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/liter in untreated wastewater, and Larkin et al. (1978b) cited values of 15 to 27 Ascaris eggs/liter and 6.2 helminth eggs/liter in primary effluent.

B. 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 1 to 2 hr 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 hr sedimentation in the laboratory, but lower removals 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 XXI (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 viable 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.

C. 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 20 weeks, and Taenia up to 2 yr (Feachem et al., 1978); 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 to 35 days.

Because of the growth of crops and presence of people at irrigation 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.

TABLE XXI. 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

D. Infective Dose, Risk of Infection, and 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).

V. CONCLUSIONS

A. Types and Levels in Wastewater

The types and levels in wastewater of most pathogens are fairly well understood. Recent reviews of the subject include those by Benarde (1973), Burge and Marsh (1978), Elliott and Ellis (1977), Kristensen and Bonde (1977), and Menzies (1977). Viruses, however, are less well understood. 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. 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.

B. Preapplication Treatment

In 1978, the U.S. General Accounting Office (1978) found that concern over adverse health effects has been a major obstacle to the development of land treatment of municipal wastewater, resulting in the establishment of state pretreatment requirements which may be overly stringent. These requirements often make land treatment expensive compared to conventional treatment and surface-water discharge.

This review suggests that 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. Because of potential contamination of crops and infection of animals, slow-rate irrigation systems should have complete removal of helminth eggs. These relatively simple pretreatment requirements would be appropriate for many land treatment systems.

The recommendations made in the paragraphs below assume 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) or shorter waiting periods before grazing or harvest of crops (e.g., agriculture in arid areas), more extensive preapplication treatment will 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 (Lance and Gerba, 1978).

C. Aerosols

Because of the potential exposure to aerosolized viruses at land treatment sites, it would be prudent to limit public access to between 100 and 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.

D. Surface Soil and Plants

The survival times of pathogens on soil and plants are summarized in Table XXII (after Feachem et al., 1978). Since pathogens survive for a much longer time on soil than plants, the recommended waiting periods before harvest are based upon probable soil contamination.

TABLE XXII. Survival Times of Pathogens on Soil and Plants

	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.

E. Infective Dose, Risk of Infection, and Epidemiology

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. Untreated wastewater should never be used for irrigation. The comparison of the respiratory infective dose of enteric viruses with the oral infective dose is a significant research need.

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 to 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 then 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, one at Lubbock, Texas, and another in Israel. The results of these two projects may well modify the conclusions and recommendations of this paper in the future.

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