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Health Effects of Land Application of Municipal Sludge

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by

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FOREWORD

The many benefits of our modern, developing industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides, and non ionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This report provides information on the health effects of land application of municipal sludge. The results of this study suggest that the land application of sludge can be a safe practice, provided that the proper precautions are taken.

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ABSTRACT

The potential health effects arising from the land application of municipal sludge are examined, and an appraisal of these effects made. The agents, or pollutants, of concern from a health effects viewpoint are divided into the categories of pathogens and toxic substances. The pathogens include bacteria, viruses, protozoa, and helminths; the toxic substances include organics, trace elements, and nitrates.

For each agent of concern the types and levels commonly found in municipal wastewater and sludge are briefly reviewed. A discussion of the levels, behavior, and survival of the agent in the medium or route of potential human exposure, i.e., aerosols, surface soil and plants, subsurface soil and groundwater, and animals, follows as appropriate. Infective dose, risk of infection, and epidemiology are then briefly reviewed. Finally, some general conclusions are presented.

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INTRODUCTION

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

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

In spite of von Liebig's pessimism, farmers in many areas of the world have been applying sewage sludge to agricultural land for centuries. The practice has continued for millennia in the Far East. Sewage sludge (or "municipal sludge") has characteristics that make it valuable as a fertilizer and a soil conditioner it contains fair amounts of nitrogen, phosphorus,and micronutrients, and it increases soil friability, tilth, pore space, and water-holding capacity.

In the United States a mandate for the greater use of land application of both municipal wastewater and sludge has been provided by the Clean Water Act of 1977 (PL 95-217), Title II (Grants for Construction of Treatment Works), Section 201, which states that the:

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

The land application of wastewater (or "land treatment") has been discussed in previous reports (Kowal 1982, 1985); the land application of sludge is the subject of the present report.

Land application of sludge consists of the low-rate application (compared with a purely disposal operation) to agricultural, forest, or reclaimed land of municipal wastewater sludge which has been "stabilized" in some way, e.g., anaerobic digestion or composting. That land application of sludge is an important and probably growing practice in the U.S. is indicated by the results of a recent survey of 1008 publicly owned treatment works, accounting for over 2 million dry metric tons per day of sludge (Peirce and Bailey 1982). The survey found 17% of the total sludge to be utilized in large scale food-chain landspreading, 12% in large scale nonfood-chain landspreading, and 21% in distribution and marketing systems (much of which probably ends up in gardens and lawns).

With the application to land of large volumes of wastewater and sludge, it is evident that considerable potential for adverse health effects exists. The major health concerns with land treatment of wastewater and land application of sludge are somewhat different. Thus, the potential exposure of humans through the routes of aerosols and groundwater is frequently emphasized with wastewater, and through the food chain with sludge. Nevertheless, the agents, or pollutants, of concern from a health effects viewpoint are almost the same in wastewater and sludge. These agents can be divided into the two broad categories of pathogens and toxic substances. The pathogens include bacteria (e.g., *Salmonella* and *Shigella*), viruses (i.e., enteroviruses, hepatitis virus, adenoviruses, rotaviruses, and Norwalk-like agents), protozoa (e.g., *Entamoeba* and *Giardia*), and the helminths (or worms, e.g., *Ascaris*, *Trichuris*, and *Toxocara*). The protozoa and helminths are often grouped together under the term, "parasites," although in reality all the pathogens are parasites. The toxic substances[1] include organics, trace elements (or heavy metals, e.g., cadmium and lead), and nitrates. Nitrates are usually not viewed as "toxic" substances, but are here so considered because of their potential hematological effects when present in water supplies at high levels. These agents form the basis of the main sections of this report. The major health effects of these agents are listed in Figure 1.

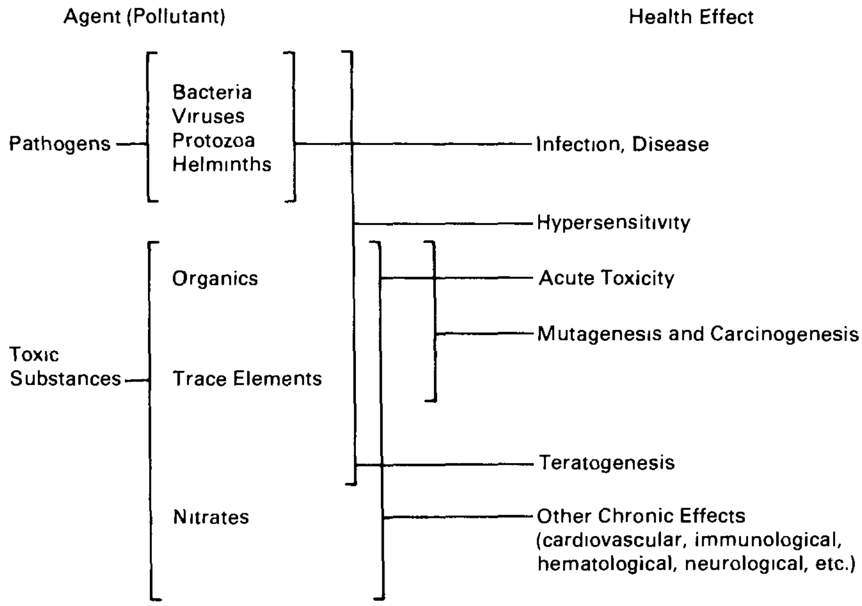

Figure 1. Health effects of pathogens and toxic substances.

For each agent of concern the types and levels commonly found in municipal sludge are briefly reviewed. A discussion of the levels, behavior, and survival of the agent in the medium or route of potential human exposure, i.e., aerosols, surface soil and plants, subsurface soil and groundwater, and animals, follows as appropriate. (Runoff to surface water is not considered, since it is assumed that this will be prevented in a well-managed sludge land application operation.) For the pathogens, infective dose, risk of infection, and epidemiology are then briefly reviewed.

[1]The term "toxin" is often incorrectly used as a synonym. A toxin is a poisonous, often proteinaceous, product of the metabolism of a living organism, e.g., snake, wasp, or pathogenic bacterium. Correct synonyms for "toxic substance" include "toxicant" and "toxic" when used as a noun.

SECTION 2

GENERAL CONCLUSIONS

Types and Levels of Agents in Wastewater and Sludge

The types and levels in wastewater and sludge of most pathogens are fairly well understood, with the exception of viruses. Since only a fraction of the total viruses in wastewater and other environmental samples may actually be detected, the development of methods to recover and detect viruses needs to be continued. The occurrence of viruses in an environmental setting should probably be based on viral tests rather than bacterial indicators since failures in this indicator system have been reported.

The tremendous number of organic chemicals possibly present in sludge, together with their myriad health effects and poorly understood behavior in the environment, represent a potential for public health risk when the sludge is applied to agricultural land. Among the trace elements, probably only cadmium, under ordinary circumstances, is likely to be of health concern to humans as a result of the land application of sludge, with the exposure being through food plants or organ meats. Minimizing of health risks can probably be accomplished by the monitoring of sludge composition, and the regulation of maximum concentrations and cumulative application of toxic substances in land-applied sludge. The complexity of the organics composition of sludges, however, might require the development and use of biological assays to screen for toxicity (Babish *et al.* 1982).

Aerosols

Because of the potential exposure to aerosolized bacteria, and possibly viruses, at land application sites, it would be prudent to limit public access to a sludge spray source, such as an active spray gun or tank truck. Human exposure to pathogenic protozoa or helminth eggs through aerosols is unlikely.

Surface Soil and Plants

The survival times of pathogens on soil and plants are summarized in Table 1 (after Feachem *et al.* 1978). Since pathogens survive for a much longer time on soil than on plants, recommended waiting periods before harvest are based upon probable contamination with soil. However, what is a safe waiting period before crop harvest for human consumption is really an unsettled issue.

Table 1. 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 probably not be harvested for human consumption for at least one month after the last sludge application; subsurface and low-growing crops for human consumption would probably require a six-month waiting period after last application. These waiting periods need not apply to the growth of crops for animal feed, however.

The levels of toxic organics likely to be present in soils at land application sites will probably result in very low levels in above-ground portions of plants, but levels in roots, tubers, and bulbs may present a health hazard.

The potential increase in cadmium levels in human food due to land application of sludge is still an unsettled question. Present levels of total dietary intake of cadmium for most people appear to be fairly safe. However, in view of human variability in sensitivity and the variability in food supply, these levels probably should not be allowed to rise greatly.

Movement in Soil and Groundwater

Properly designed sludge application sites may pose little threat of bacterial or viral contamination of groundwater. Human exposure to pathogenic protozoa or helminths through groundwater is unlikely. Groundwater is unlikely to represent a significant organic or trace element threat.

There is a possibility that land application of sludge may raise the nitrate concentration of groundwater above the drinking water standard of 10 mg/l as N. This can be prevented, however, by proper siting and management practice, e.g., matching loading rate to crop uptake.

Animals

The literature to date suggests little danger of bacterial, viral, or protozoan disease to animals grazing at land application sites if grazing does not resume until four weeks after last application (Yeager 1980), but the need for complete inactivation of helminths in sludge before land application is still unsettled. The feeding of land-application-site-grown plants to animals is unlikely to pose a health problem, but grazing animals may accumulate significant levels of toxic organics. The issue of accumulation of organics from the soil by plants and animals (particularly into milk), and into the human food supply, is poorly understood.

Infective Dose, Risk of Infection, Epidemiology

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

Epidemiological studies to date suggest little effect of land application on disease incidence. However, many questions on the public health consequences of land application of wastewater and sludge remain (Larkin 1982).

SECTION 3
BACTERIA

Types and Levels in Wastewater and Sludge

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

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

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

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

Table 2. Pathogenic Bacteria of Major Concern

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

*Correct nomenclature: *Salmonella paratyphi* A, *S. schottmuelleri, S. hirschfeldi,* respectively.

Table 3. Pathogenic Bacteria of Minor Concern

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

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

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

Salmonella typhi causes typhoid fever, a systemic disease with a fatality rate of 10% untreated or 2-3% treated by antibiotics (Benenson 1975). It occurs sporadically in the U.S., where about 500 cases occur per year (Taylor *et al.* 1983), 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 1980, 30,004 cases were reported to the Centers for Disease Control (CDC) (CDC 1982).

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 1980, 14,168 cases were reported to CDC (MMWR 1981).

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

Yersinia enterocolitica and *Y. pseudotuberculosis* cause yersiniosis, an acute gastroenteritis and/or mesenteric lymphadenitis, with diarrhea, abdominal pain, and numerous other symptoms. Death is uncommon. Yersiniosis occurs only sporadically in the U.S., and 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 facultative 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 gram-negative, nonspore-forming rods (Jawetz *et al.* 1978). The family Enterobacteriaceae includes the following tribes and genera (Holt 1977):

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

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

Proteeae
- *Proteus*

Yersinieae
- *Yersinia*

Erwinieae
- *Erwinia*

Obligate anerobic bacteria constitute 95-99% of the gut flora, but these are usually not included in the term, "enteric bacteria" (Davis *et al.* 1980). The terms, "total coliform" and "fecal coliform," are operationally defined entities used for indicator purposes. Their taxonomic composition is variable, but all are members of the Enterobacteriaceae. A recent study of fecally contaminated drinking water (Lamka *et al.* 1980) found the following composition:

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

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

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

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

Table 4. Viable Bacteria in Human Feces (number/g wet weight)

	Carnow *et al.* 1979	Feachem *et al.* 1978	Tomkins *et al.* 1981
Anaerobes			
Bacteroides	10^9-10^{10}	10^8-10^{10}	10^{10}-10^{11}
Bifidobacterium	10^9-10^{10}	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	10^4-10^7
Fusobacterium	10^3-10^5	--	--
Eubacterium	--	10^8-10^{10}	--
Veillonella	$<10^3$	--	--
Aerobes or Facultative Bacteria			
Enterobacteria*	10^6	10^7-10^9	10^5 -10^9
Enterococci (fecal *Streptococcus)*	10^5	10^5-10^8	10^4 -10^{10}
Staphylococcus	$<10^3$	--	--
Bacillus, Proteus, Pseudomonas, Spirochetes	$<<10^3$	--	--

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

Table 5. Pathogenic Bacteria in Feces of Infected Persons

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

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/ml). Less prominent bacteria include *Salmonella* and *Mycobacterium tuberculosis*. The total bacterial content of raw wastewater, as recovered on standard media at 20°C (Carnow *et al.* 1979), is about 10^6-10^7 organisms/ml. The presence and levels in wastewater of any of the pathogens listed in Tables 2 and 3 depend, of course, on the levels of infection in the contributing population.

The density of bacteria in municipal sludges is highly variable. Pedersen (1981) has surveyed most of the available literature on density levels of microbes in sludge for the period 1940-1980. Table 6 summarizes the results for bacteria in raw sludge. Sludge treatment provides variable reduction of these levels; Pedersen (1981) concluded that anaerobic digestion results in a 1-2 log reduction, aerobic digestion less than one log, and lime stabilization more than 2 log. It must be realized that there may be great site-specific variation about these values.

Table 6. Density of Bacteria in Municipal Sludge (geometric means, number/g dry wt) (Pedersen 1981)

	Raw Primary Sludge	Raw Secondary Sludge	Raw Mixed Sludge
Total Coliforms	1.2×10^8	7.0×10^8	1.1×10^9
Fecal Coliforms	2.0×10^7	8.3×10^6	1.9×10^5
Fecal Streptococci	8.9×10^5	1.7×10^6	3.7×10^6
Salmonella	4.1×10^2	8.8×10^2	$2 9 \times 10^2$

Aerosols

Where liquid sludge is applied to the land by spray equipment of some sort, aerosols that travel beyond the zone of application will be produced. These are suspensions of solid or liquid particles up to about 50 μm in diameter, formed, for example, by the rapid evaporation of small droplets. Their content of microorganisms depends upon the concentration in the sludge 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* 1978).

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 the size, shape, and density of the particles; respiratory anatomy; breathing pattern; dead space; disease state; etc. (Brain and Valberg 1979). Those above about 2 μm in aerodynamic diameter are deposited primarily in the upper respiratory tract (including the nose for larger particles), from which they are carried by cilia into the oropharynx. They then may be swallowed, and enter the gastrointestinal tract. The smaller airways and alveoli do not possess cilia, so that pathogens deposited there would have to be combatted by local mechanisms. About 40% of 1 pm particles are removed (about half in the pulmonary region—respiratory bronchiole, alveolar ducts, and alveoli) by the respiratory system at resting breathing rates, increasing to nearly 100% for 10 μm particles. Deposition increases (greater than 70%) for particles smaller than 0.1 μm, primarily in the pulmonary region (Brain and Valberg 1979).

When aerosols are generated, bacteria are subject to an immediate "aerosol shock," or "impact factor," which may reduce their level tenfold within seconds (Schaub *et al.* 1978). 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.* 1978). Solar radiation, particularly the ultraviolet portion, is destructive to bacteria, and also increases the rate of desiccation. Teltsch and Katzenelson (1978) have found bacterial survival at night up to ten times that during daytime in Israel. High temperature is another factor decreasing bacterial survival. While biological aerosol decay is occurring, the rate of physical aerosol decay, or deposition, simultaneously affects the distance of dissemination of the bacteria. This is influenced by wind speed, air turbulence, and local topography, e.g., a windbreak of trees.

Any of the bacteria listed earlier as present in feces, urine, or wastewater could appear in aerosols emanating from land application sites. Harding *et al.* (1981) have studied the production of microbial aerosols by the land application of liquid municipal sludge at two sites using tank-truck application and two sites using high-volume spray guns. Very low bacterial aerosol levels were found at the tank-truck sites, but elevated levels of fecal coliforms, fecal streptococci, and mycobacteria were found at the spray sites. Levels were significantly less, however, than those observed at wastewater spray application sites, and it was concluded that the spray application of sludge does not represent a serious threat to health for individuals more than 100 m downwind (Sorber *et al.* 1984).

Surface Soil and Plants

The surface soil, and occasionally plants, of a sludge application site may be initially heavily laden with enteric bacteria, depending on the level of prior treatment. 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 1981) 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 in those with greater water-holding capacity.
3. Temperature. Survival time is longer at lower temperatures, e.g., in winter.
4. pH. Survival times are shorter in acid soils (pH 3-5) than in neutral or alkaline soils. Soil pH is thought to have its effect through control of the availability of nutrients or inhibitory agents. The high level of fungi in acid soils may play a role.
5. Sunlight. Survival time is shorter at the surface, probably due to desiccation and high temperatures, as well as ultraviolet radiation.
6. Organic matter. Organic matter increases survival time, in part due to its moisture-holding capacity. Regrowth of some bacteria, e.g., *Salmonella*, may occur in the presence of sufficient organic matter. In highly organic soils anaerobic conditions may increase the survival of *Escherichia coli* (Tate 1978).
7. Soil microorganisms. The competition, antagonism, and predation encountered with the endemic soil microorganisms 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 7 are extracted from these summaries, as well as other literature. "Survival" as used in this table, and throughout this report, denotes days of detection. It should be noted that inactivation is a rate process and therefore detection depends upon the initial level of organisms, sensitivity of detection

Table 7. Survival Times of Bacteria in Soil

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

methodology, and other factors. If kept frozen, most of these bacteria would survive longer than indicated in Table 7, but this would not be a realistic soil situation.

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

Pathogens do not penetrate into vegetables or fruits unless their skin is broken (Bryan 1977, Rudolfs *et al.* 1951a), and many of the same factors affect bacterial survival on plants as those in soil, particularly sunlight and desiccation. The survival times of bacteria on subsurface crops, e.g., potatoes and beets, would be similar to those in soil. Useful summaries of the literature on the survival times of bacteria on aerial crops are those of Bryan (1977), Sepp (1971), and Feachem *et al.* (1978). The ranges given in Table 8 are extracted from these summaries, as well as other literature.

Table 8. Survival Times of Bacteria on Crops

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

On the basis of New Jersey field experiments with tomatoes irrigated with municipal wastewater, Rudolfs *et al.* (1951a) concluded that: (1) cracks and split stem ends provide protected harboring places for enteric bacteria to survive for long periods, and such portions should be cut away before consumption, (2) on normal tomatoes, without cracks, after direct application of wastewater to the surface of the fruit the residual coliform concentration decreases to or below that of uncontaminated controls by the end of 35 days or less, (3) survival of *Salmonella* and *Shigella* on tomato surfaces in the field did not exceed 7 days, even when applied with fecal organic material, and (4) if wastewater application is stopped about one month before 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 application 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) growth of crops the harvested portion of which does not contact the soil, e.g., grains and orchard crops, or (2) 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 application a month prior to harvest would be prudent, although in a typical sludge-application operation harvesting would normally occur much longer than one month after the last (often only) application.

Movement in Soil and Groundwater

Approximately 117 million people in the United States obtain their drinking water from groundwater, supplied by 48,000 community public water systems and approximately 12 million individual wells; the concentration is highest in rural areas (USEPA 1984). Thus, it is imperative that land application of sludge does not result in the transmission of disease through groundwater. This is not to imply that groundwater in the U.S. is now pristine. Almost half of the waterborne disease outbreaks in the U.S. between 1971 and 1977 were caused by contaminated groundwater (Craun 1979), and a recent examination of individual groundwater supplies in a rural neighborhood of Oregon (Lamka *et al.* 1980) showed more than one-third to be fecally contaminated.

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

Land application of sludge may pose little bacterial threat to ground water. Liu (1982) in Canada has found that after 4 years of heavy sludge application sewage bacteria were incapable of moving through the soil columns tested, and over 90% of the surviving sludge bacteria were still detained in the top 20 cm layer of soil. He concluded that there was little possibility of bacterial contamination of groundwater by the practice of sludge farmland application, provided that the water table was not too high and the soil was well drained. Similar results have been found in leachate experiments in South Africa (Nell *et al.* 1981).

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

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

Animals

The disease hazards to farm animals from land application of sludge have been reviewed by Argent *et al.* (1977) and Carrington (1978). The major bacterial concerns with respect to animals grazing at land application sites are *Salmonella* infections and bovine tuberculosis (*Mycobacterium bovis* and *M. tuberculosis*); both can be passed on to man.

That the transmission of salmonellosis to cattle grazing at land application sites is at least possible was demonstrated by Taylor and Burrows (1971), who showed that calves grazing in pastures, to which 10^6 *Salmonella dublin* organisms/ml of slurry had been applied, became infected. No infection occurred when the rate was decreased to 10^3/ml, suggesting that *Salmonella* may only be of concern when high concentrations are present. Feachem *et al.* (1978) concluded that there is no clear evidence that cattle grazed at land wastewater treatment sites are more at risk from salmonellosis than other cattle, probably because the required infectious doses are high and *Salmonella* infections are transmitted among cattle in many other ways. On the basis of *Salmonella* measurements in wastewater and sludge in England, Jones *et al.* (1980) concluded that a four-week waiting period would prevent salmonellosis in grazing animals.

Argent *et al.* (1981) applied raw sludge (11 *Salmonella*/100 ml) to a field at the rate of 44.8 m^3/ha, and confined 10 lambs to the field for 2 months. None of the lambs became infected, as measured by feces, rumen, and tissue samples, and clinical symptoms. Ayanwale *et al.* (1980) raised goats on corn silage grown on sludge-amended land, and found no *Salmonella* infections in spite of the presence of *Salmonella* in the sludge, supporting the position that the potential public health hazard resulting from the use of sludge as fertilizer when properly treated has so far proven not to be a threat. Nevertheless the significance of *Salmonella* in land-applied sludge is an issue yet to be settled. Evidence in Switzerland from studies of carrier rates and serotypes in cattle grazed on sludge-treated pastures has indicated a positive association and a cycle of infection from man to sludge to animals to man.

Experience in the Netherlands is similar, but there is no evidence of such a link in the United Kingdom, despite the compulsory reporting of incidents there (WHO 1981).

Animal feed raised on sludge-amended land appears to be even less of a risk. Thus, after feeding for 36 months on corn silage grown on land fertilized with *Salmonella*-containing sewage sludge, a goat herd was free of clinical and subclinical *Salmonella* infection (Ayanwale and Kaneene 1982).

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

Other possible bacterial concerns with respect to animals grazing at land application sites are *Leptospira* (causing leptospirosis), *Brucella* (causing brucellosis), and *Bacillus anthracis* (causing anthrax). Sludge, however, probably contains insignificant numbers of these pathogens, and plays a negligible role in the transmission of these diseases (Feachem *et al.* 1978). Jones *et al.* (1981) examined sludges in England for *Leptospira, Mycobacterium, Escherichia, Brucella*, and *Bacillus anthracis*, and concluded that the application of sludge to agricultural land should present no greater hazard than the spreading of animal manure if sensible grazing restrictions are observed.

Infective Dose, Risk of Infection, Epidemiology

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

With these barriers to overcome it is understandable that an infection resulting from inoculation by a few bacterial cells is a most unlikely occurrence; usually large numbers are necessary. Some representative oral infective dose data for enteric bacteria, based upon numerous studies using nonuniform techniques, are presented in Table 9 (adapted from Bryan 1977).

Although the terms, "infective dose," "minimal infectious dose," etc., are used in the literature, it is obvious from Table 9 that these are misnomers, and that we are really dealing with dose-response relationships, where the dose is the number of cells to which the human is exposed, and the response is lack of infection, infection without illness, and infection with illness (in an increasing proportion of the test subjects). The response is affected by many factors, making it highly variable. Some of the most important factors are briefly discussed below.

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 9, where buffering reduces the infective dose by about a thousandfold. Direct inoculation into the bloodstream results in the fewest barriers being presented to the pathogen; Hellman *et al.* (1976) found 10 tuleremia organisms injected to be comparable to 10^8 by mouth.
2. Previous exposure to a given pathogen often produces varying degrees of immunity to that pathogen, through the induction of specific immune

Table 9. **Infective Dose to Man of Enteric Bacteria**

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

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 develops (Levine 1980).

3. Other host factors, such as age and general health, also affect the disease response. Infants, elderly persons (Gardner 1980), malnourished people, those with concomitant illness, and people taking anti-inflammatory, cytotoxic, and immunosuppressant drugs would be more susceptible to pathogens. An example of human variability (possibly genetic) is the following response of men orally challenged with several different doses of *Salmonella* typhi (Hornick *et al.* 1970):

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

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

4. The number of organisms that must be swallowed for intestinal colonization (subclinical infection), and consequent risk of clinical disease, is affected by treatment with antibiotics (Remington and Schimpff 1981). Due to its normal content of anaerobic bacteria and their products, the gut can resist colonization when an oral dose of about 10^6 organisms is given. Once competition is reduced by systemic or oral antibiotics, the dose required to induce colonization is only 10 to 100 organisms.
5. The timing of the exposure to pathogens, e.g., as a single exposure or an exposure over a long period of time, would be expected to affect the response.
6. Finally, as illustrated by *Escherichia coli* and *Salmonella typhi* in Table 9, the virulence, or pathogenicity, of bacteria varies among strains. Thus, three different strains of *Shigella flexneri* have been found to have infective doses of 10^{10} or higher, 10^5-10^6 and 180 organisms (NRC 1977).

The risk of infection is probably greatest for *Salmonella* spp. and *Shigella* spp., because they are the most common bacterial pathogens in municipal wastewater. The infective dose for *Salmonella* is high (10^5-10^8 organisms) but this dose might be reached on a contaminated foodstuff under conditions that allow multiplication. A recent review of experimentally induced salmonellosis and salmonellosis outbreaks, however, has resulted in the conclusion that the infective dose for *Salmonella* may well be below 10^3 organisms (Blaser and Newman 1982). 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 application site, and to rely on the passage of 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.

A multiyear prospective epidemiological study of the health effects of the application of sewage sludge to agricultural land in Ohio has recently been completed (Brown 1985). Digested municipal sludge was applied to family-operated farms at the rate of 2-10 dry metric tons per hectare per year. Health of humans and livestock on 47 sludge-receiving farms and 46 control farms was evaluated by questionnaires, blood samples, fecal samples, and tuberculin testing. No significant differences between sludged and control farms were found in symptoms of respiratory, digestive, or other disease, or exposure to *Salmonella*, *Shigella*, or *Campylobacter* in humans, nor in animal health. No tuberculin conversions occurred on the sludged farms.

SECTION 4

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).

Types and Levels in Wastewater and Sludge

The human enteric viruses that may be present in wastewater and sludge are listed in Table 10 (Melnick *et al.* 1978, Holmes 1979). These are referred to as the enteric viruses and new members are constantly being identified. Since no viruses are normal inhabitants of the gastrointestinal tract and none of these have a major reservoir other than man (with the likely exception of rotaviruses), all may be regarded as pathogens, although most can produce asymptomatic infections.

Table 10. Human Wastewater Viruses

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

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 have not been shown to be of major epidemiological importance in their transmission (Evans 1976). The rotavirus often produces diarrhea in children, but the local multiplication of enteroviruses and (possibly) hepatitis A virus in cells lining the area rarely produces local symptoms, i.e., diarrhea, vomiting, and abdominal pain. Most enteroviral infections, even with the more virulent types, cause few or no clinical symptoms. Occasionally, after continued multiplication in the lymphoid tissue of the pharynx and gut, viremia may occur, i.e., virus enters the bloodstream, 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 7,075 cases were reported to the Centers for Disease Control (CDC) in the years 1971-75 (Morens *et al.* 1979). These enteroviruses are practically ubiquitous in the world, and may spread rapidly in silent (asymptomatic) or overt epidemics, especially in late summer and early fall in temperate regions. Because of their antigenic inexperience (i.e., lack of previous exposure), 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 unimmunized in the population increases, and infection becomes more common in older age groups, where symptomatic disease is more likely and is more serious (Melnick *et al.* 1979, Benenson 1975). Thus, decreasing the human exposure to the common enteric viruses through the water and food route has its disadvantages, as well as advantages.

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

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

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

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

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

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

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

Viruses are not normal inhabitants of the gastrointestinal tract nor regular components of human feces, while certain types of bacteria are. Because of this difference, the concept of using bacteria, e.g., coliforms and fecal streptococci, as indicators of potential viral contamination in the environment has been a very attractive one. Unfortunately the response of viruses to wastewater treatment and their behavior in the environment are very different from those of bacteria (Berg *et al.* 1978); for example, viruses are less easily removed by treatment processes and during passage through soil than are bacteria (Sobsey *et al.* 1980). Thus, Goyal *et al.* (1979) provided data to indicate that current bacteriological standards for determining the safety of shellfish 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. An expansion of the study to include potable, surface, and swimming pool waters resulted in the same conclusion (Marzouk *et al.* 1980). It appears, therefore, that estimates of virus presence or levels in the environment will have to be made on the basis of measurements of viral indicators, e.g., vaccine poliovirus or bacteriophage, or of the viral pathogens themselves, e.g., coxsackievirus or echovirus, rather than of indicator bacteria.

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

Estimates of the concentration of viruses in wastewater in the United States vary widely, but it is thought to be lower than that in many developing countries. Numbers tend to be higher in 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, Keswick and Gerba 1980). (The use of several cell lines usually detects more viral types than a single cell line does, and many viruses cannot readily be detected by cell culture methods, e.g., hepatitis A virus and Norwalk-like agents.) Some representative levels of enteric viruses in raw U.S. wastewaters are summarized in Table 11. 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

Table 11. Levels of Enteric Viruses in U.S. 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	192-1040	Sorber 1983

allowances for the known variables, it would seem extremely unlikely that the total concentration would ever exceed 10,000 virus units per liter of raw sewage and would most often contain less than 1,000 virus units/liter."

Reported concentrations of enteric viruses in sludge have been summarized by Gerba (1983). Ranges, in virus units/gram, found in the U.S. were: 2-215 for raw, 0.04-17 for anaerobically digested, and 0-260 for aerobically digested sludges.

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).

The initial aerosol shock during the process of aerosolization may result in a half log loss of virus level (Sorber 1976). The subsequent dieoff, estimated to be about one log every 40 seconds (Sorber 1976), is determined primarily by solar radiation, temperature, and relative humidity (Lance and Gerba 1978). The effect of relative humidity appears to depend upon the lipid content of viruses, with 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).

The concentration of viruses in aerosols at liquid sludge spray-application sites has been examined by Harding *et al.* (1981; Sorber *et al.* 1984). On a special virus run, 1470 m^3 of air was sampled and no human enteric viruses were detected from the pooled sample. This converts to a concentration of less than 0.0016 PFU/m^3 of air at a distance of 40 m downwind from the spray gun, and probably results from low viral concentration in sludge (0.7 PFU/g) and viral adsorption into poorly aerosolized solid matter. This suggests that aerosolization of viruses in liquid sludge may not present a significant health risk.

Surface Soil and Plants

The survival time of viruses at a sludge application site is primarily of concern when decisions must be made on how long a period of time must be allowed after last application before permitting access to people or animals, or harvesting crops. Another concern is that the longer viruses survive at the surface the greater opportunity they have for being desorbed and moving in the soil toward the groundwater.

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

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

Much of the recent literature on survival times of enteric viruses in soil is summarized in Table 12. Approximately one hundred days appear to be the maximum survival time of enteric viruses in soil, unless subject to very low temperatures, which prolong survival beyond this time. Exposure to sunlight, high temperatures, and drying greatly reduce survival times. Thus, Yeager and O'Brien (1979) could recover no infectivity of poliovirus and coxsackievirus from dried soil regardless of temperature, soil type, or type of liquid amendment. They suggested that the main effect of temperature on virus survival in the field may be its influence on evaporation rates, which causes dessication and inactivation of virus without high temperature.

The phenomenon of virus inactivation by evaporative dewatering has heen documented by Ward and Ashley (1977), who observed a decrease in poliovirus titer of greater that 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. Recent studies with high concentrations of bacteriophage in hydroponically grown corn and bean plants have shown little viral uptake in uncut roots, more in cut roots, and viral transport to all plant parts examined, but with survival times of limited duration. The authors concluded that the possible public health significance associated with viral uptake through the root systems of plants was minimal (Ward and Mahler 1982). Moreover, the rapid adsorption of virus by soil particles under natural conditions may make them unavailable for plant absorption, thereby suggesting 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 1981). Some of the literature on survival times is summarized in Table 13 (Feachem *et al.* 1978). The data are similar to those for bacteria (cf. Table 8), and likewise appear to support a minimum one-month waiting period after last 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 one hundred days might be required as a minimum safe waiting period. As with bacteria, this period could be shortened by (1) the growth of crops the harvested portion of which does not contact the soil, or (2) the growth of crops used for animal feed only.

Movement in Soil and Groundwater

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

In contrast with bacteria, filtration plays a minor role in the removal of viruses in soils, virus removal being almost totally dependent on adsorption. Since adsorption is a surface phenomenon, soils with a high surface area, i.e., those with a high clay content, would be expected to have high virus removal capabilities. Although the

Table 12. **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	
Polovirus	Sand	Moist Dry	91 <77	Lefler and Kott 1974
Poliovirus	Loamy fine sand	Moist, 4°C	84 (<90% reduction)	Duboise *et al.* 1976
		Moist, 20°C	84 (99.999% reduction)	
Coxsackievirus	Clay	300 mm rainfall, -12-26°C	<161	Damgaard-Larsen *et al.* 1977
Poliovirus	--	-14-27°C 15-33°C	89-96 <11	Tierney *et al.* 1977
Poliovirus	Sugarcane field	Open, direct sunlight	7-9	Lau *et al.* 1975
		Mature crop, moist, shaded	≤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 13. Survival Times of Enteric Viruses on Crops

Virus	Crop	Conditions	Survival (days)	Reference
Enterovirus	Tomatoes	3-8°C	10 (90% reduction)	Bagdasaryan 1964
		18-2°C	10 (99% reduction)	
Poliovirus	Radishes	5-10°C	20 (99% reduction), >60	Bagdasaryan 1964
Poliovirus	Tomatoes	Indoors, 22-25°C	<12	Kott and Fishelson 1974
		Indoors, 37°C	<5	
		Outdoors	<1	
	Parsley	15-31°C	<2	
Poliovirus	Lettuce and radishes	Sprayed, summer-fall	6 (99% reduction), 36 (100% reduction)	Larken *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	

physical-chemical reasons for virus adsorpticn to soil surfaces are poorly understood, it appears that adsorption is increased by high cation exchange capacity, high exchangeable aluminum, low pH (below 5), and increased cation concentration (Gerba and Lance 1980). For a review of virus adsorption see Gerba (1984).

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

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

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

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

There appears to be little reliable information on viruses getting into groundwater beneath sludge application sites, although one would expect the threat to be low because of virus binding to sludge solids. Studies with sludge-amended soil indicate that viruses are not easily eluted by rainfall and are efficiently retained by sludge-soil mixtures (Damgaard-Larsen *et al.* 1977, Farrah *et al.* 1981), even on sandy soils (Bitton *et al.* 1984).

Once enteric viruses get into groundwater, they can survive for long periods of time, 2 to 188 days having been reported in the literature (Akin *et al.* 1971), and probably migrate for long distances (Keswick and Gerba 1980). For example, Vaughn *et al.* (1983) have recovered human enteroviruses at 18 m depth and 67 m down gradient from a septic tank leach field in a shallow sandy aquifer. Low temperatures prolong survival, but the factors affecting survival in groundwater are poorly understood. It might be possible, for example, that entry of viruses into the groundwater would be tolerable if sufficient underground detention time could be provided before movement of the groundwater to wells or streams (Lance and Gerba 1978). For a review of virus in soil and groundwater see Vaughn and Landry (1983).

Animals

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

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

Infective Dose, Risk of Infection, Epidemiology

In contrast with bacteria, where large numbers of cells are usually necessary to produce an infection, a few virus particles are currently thought to be able to produce an infection under favorable conditions. The most important studies on the oral infective dose of enteric viruses in humans are summarized in Table 14 (modified from National Research Council 1977). The results are highly variable, and may reflect differences in experimental conditions as well as states of the hosts. The recent data do suggest, however, that the infective dose of enteroviruses to man is low, possibly of the order of 10 virus particles or less. The same factors discussed earlier, that affect bacteria, also affect the virus dose-response relationship.

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

Table 14. Oral Infective Dose to Man of Enteric Viruses

Virus	Subjects	Dose*	Percent Infected	Reference
Vaccine poliovirus	Infants	0.2 PFU**	0	Koprowski 1956
		2 PFU	67	
		20 PFU	100	
		$10^{5.5}$	50	Gelfand *et al.* 1960
		$10^{7.5}$	100	
		$10^{6.6}$	60	Krugman *et al.* 1961
		$10^{7.6}$	75	
		5.5×10^{6} PFU	89	Holguin *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
		$10^{3.5}$	79	
	Premature infants	1	30	Katz and Plotkin 1967
		2.5	33	
		10	67	
	Infants	7-52†	1	Minor *et al.* 1981
		24-63	10	
		55-93	50	
Echovirus 12	Young Adults	17 PFU	1	Schiff *et al.* 1984
		919 PFU	50	

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

be the case in the real world, 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 on the basis of oral poliovaccine studies, nonimmunologic barriers, human immunologic responses, and probabilistic factors (Lennette 1976).

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

Fecally polluted vegetable-garden irrigation water in Brazil has been found to contain polioviruses and coxsackieviruses, and has been associated with epidemics among the consumers of the garden products (Christovao *et al.* 1967a, 1967b). However, at the Muskegon, Michigan, land treatment spray irrigation site, where no products for human consumption are grown and where much higher exposure to aerosols would be expected than at sludge application sites, there was no increase in clinical illness among the site workers and there was no evidence of an increased risk of infection, for either viruses or bacteria (Linnemann *et al.* 1984). With a minor exception, they did not have increased prevalence of infection by hepatitis A, poliovirus (1, 2, 3), coxsackievirus (B2, B5), or echovirus (7, 11), as measured by serology. The exception was a high antibody titer to coxsackievirus B5 in the spray nozzle cleaners, a group with presumably high exposure to wastewater.

In the previously mentioned epidemiological study of sludge application to agricultural land in Ohio (Brown 1985), no significant difference in frequency of viral infections, as evidenced by serological examinations, was found between sludge and control groups.

In spite of these negative epidemiological results, however, some virologists feel that current epidemiological techniques are probably not sufficiently sensitive to detect the low levels cf viral disease transmission that might occur from a modern land application site (Melnick 1978, WHO 1979).

SECTION 5

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 unlikely that they will find their way into either aerosols or groundwater at land application sites, and, thus, these routes of exposure are not further considered in this report. Little attention has been given to the presence of parasites in wastewater, and their potential for contaminating food crops in the United States, probably because of the popular impression that the prevalence of parasitic infection in the U.S. is minimal (Larkin *et al.* 1978b). However, because of the increasing recognition of parasitic infections in the U.S., the return of military personnel and travelers from abroad, the level of recent immigration and food imports from countries with a high parasitic disease prevalence, and the existence of resistant stages of the organisms, a consideration of parasites is warranted.

Types and Levels in Wastewater and Sludge

The most common protozoa which may be found in wastewater and sludge are listed in Table 15. Of these, only three species are of major significance for

Table 15. 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

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 amehic 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 U.S. (Krogstad *et al.* 1978), and is transmitted by cysts contaminating water or food.

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

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

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

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

The types and levels of protozoan cysts actually present in wastewater depend on the levels of disease in the contributing human population, and the degree of animal contribution to the system. Some estimates are presented in Table 16. The sparse literature (Pedersen 1981) suggests that protozoan cysts will be absent, or at least nonviable, from anaerobically digested sludge.

Soil and Plants

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

Because of their exposure to the air, protozoan cysts deposited on plant surfaces would also be expected to die off rapidly. The fact that cysts can survive long enough to get into the human food supply under poor management conditions is confirmed

Table 16. Levels of Protozoa in Wastewater

Species	Wastewater	Concentration (cysts/l)	Reference
Entamoeha histolytica	Untreated	4.0	Foster and Engelbrecht 1973
	Municipal effluent	2.2	Kott and Kott 1967
	During epidemic (50% carrier rate)	5000	Chang and Kabler 1956
Giardia lamblia	Raw sewage (1-25% prevalence)	9.6×10^3- 2.4×10^5	Jakubowski and Ericksen 1979
	Raw sewage	Up to 8×10^4	Weaver *et al.* 1978

by the recent isolation of high levels of *Entamoeba histolytica, E. coli, Endolimax nana,* and *Giardia lamblia* on wastewater irrigated fruits and vegetables in Mexico City's marketplaces (Tay *et al.* 1980). Rudolfs *et al.* (1951b) found contaminated tomatoes and lettuce to be free from viable *Entamoeha* 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 unlikely that any public health risk will ensue.

Animals

Although it would be theoretically possible for protozoan diseases to be transmitted through animals at a land application site, little relevant information on the subject appears to exist. However, in view of the survival times discussed above, the four-week waiting period before the resumption of grazing, recommended on the basis of bacteria, would probably limit the risk of human illness.

Infective Dose, Risk of Infection, Epidemiology

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

Because of the low infective doses of protozoan cysts, it would be prudent for humans to maintain a minimum amount of contact with an active land application site. However, a waiting period for crop harvest after application would significantly reduce the risk of infection 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).

SECTION 6
HELMINTHS

Types and Levels in Wastewater and Sludge

The pathogenic helminths whose eggs are of major concern in wastewater and sludge are listed in Table 17. 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 sludge application sites. Some common helminths, pathogenic to domestic or wild animals, but not to humans, are listed in Table 18 (after Reimers *et al.* 1981), since their eggs are likely to be identified in wastewater and sludge. Several of the human pathogens listed in Table 17, 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 U.S. was 42 million (Warren 1974). Although it is by far the most common helminth infection, the eggs are not usually found in feces, are spread by direct transfer, and live for only a few days.

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

Ascaris suum, the swine roundworm, may produce Loeffler's syndrome, but probably does not complete its life cycle in man (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 four weeks in the soil (Reimers *et al.* 1981), 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.* 1981). The prevalence of trichuriasis in the U.S. was estimated to be about 2.2 million in 1972 (Warren 1974). Reimers *et al.* (1981, 1984) have found *Ascaris, Trichuris*, and *Toxocara* to be the most frequently recovered helminth eggs in municipal wastewater sludge in both southeastern and northern United States.

Table 17. Pathogenic Helminths of Major Concern

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

*Definitive host; man only incidentally infested.
**Eggs not infective for man.

Necator americanus and *Ancylostoma duodenale*, the human hookworms, live in the small intestine attached to the intestinal wall. Eggs are passed in the feces, and develop to the infective stage in 7-10 days in warm, moist soil. Larvae penetrate bare skin, usually of the foot (although *Ancylostoma* may also be acquired by the oral route), pass through the lymphatics and bloodstream to the lungs, enter the alveoli,

Table 18. Animal-Pathogenic Helminths

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

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

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 U.S. (usually due to Necator) was estimated to be about 700,000 in 1972 (Warrren 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 U.S was estimated to be about 400,000 in 1972 (Warren 1974). Dog feces is another source of threadworm larvae.

Toxocara canis and *T. cati*, the dog and cat roundworms, do not live in the human intestinal tract. When eggs from animal feces are ingested by man, particularly children, the larvae hatch in the intestine and enter the intestinal wall, similarly to

Ascaris. However, since *Toxocara* cannot complete its life cycle, the larvae do not migrate to the pharynx, but, instead, wander aimlessly through the tissues, producing "visceral larva migrans," until they die in several months to a year. The disease may cause fever, appetite loss, cough, asthmatic episodes, abdominal discomfort, muscle aches, or neurological symptoms, and may be particularly serious if the liver, lungs, eyes (often resulting in blindness), brain, heart, or kidneys become involved (Fiennes 1978). The infection rate of *T. canis* is more than 50% in puppies and about 20% in older dogs in the U.S. (Gunby 1979), and *Toxocara* is one of the most common helminth eggs in wastewater sludge (Reimers *et al.* 1981, 1984).

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 application sites. The major direct hazard to man is the possibility of him acting as the intermediate host. While *Taenia saginata* eggs are not infective for man, those of *T. solium* are infective for man, in which they can produce cysticerci. Cysticercosis can present serious symptoms when the larvae localize in the ear, eye, central nervous system, or heart. Taeniasis with *Taenia solium* is rare in the U.S., and with T. saginata is only occasionally found. However, human infections with these tapeworms are fairly common in some other areas of the world.

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

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

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

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

Obviously, these values will depend on the intensity of infection.

The presence and levels in wastewater of any of these helminth eggs, or of those from animal feces (*Ancylostoma, Toxocara*, and *Echinococcus*), depend on the levels of disease in the contributing population, and the degree of animal contribution to the system. Foster and Engelbrecht (1973) suggested a value of 66 helminth ova/l in untreated wastewater, and Larkin *et al.* (1978b) cited values of 15-27 *Ascaris* eggs/l and 6.2 helminth eggs/l in primary effluent. Since helminth eggs are denser than water, most will settle to the bottom during a sedimentation unit process, and primary effluent should have fairly low densities of eggs. As a consequence, sludge may have high densities of viable helminth eggs.

Reliable published figures for the density of helminth eggs in municipal sludge (mostly digested) are reproduced in Table 19. The data for the northern states have not been analyzed to date, but the densities are lower than those of the southern states (Reimers *et al.* 1984). The values for Chicago sludge probably reflect a lower rate of human infection and a higher contribution from pets than the southern states.

As with protozoa, the large size of helminth eggs makes it unlikely that they will find their way into either aerosols or groundwater at land application sites.

Table 19. Helminth Egg Density in Treated Municipal Sludge

	Southern States[1]		Chicago[2]	
Helminth	Mean Ova/ kg dry wt.	(Viability)	Mean Ova/ kg dry wt.	(Viability)
Ascaris spp.	9600	(69%)	2030	(64%)
Trichuris spp.	3300	(48-64%)	360	(20%)
Toxocara spp.	700	(52%)	1730	(53%)
Toxascaris leonina	--	--	480	(63%)

[1]Reimers *et al.* 1981.
[2]Arther *et al.* 1981.

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 in which they have evolved, while protozoa have evolved through water transmission. Thus, under favorable conditions of moisture, temperature, and sunlight, *Ascaris, Trichuris*, and *Toxocara* can remain viable and infective for several years (Little 1980). Hookworms can survive up to 6 months (Feachem *et al.* 1978), and *Taenia* a few days to seven months (Babayeva 1966); other helminths survive for shorter periods.

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

Because of the growth of crops and the presence of people at sludge application sites, and the longevity of helminth eggs, it might be considered advisable to select a sludge treatment method which will inactivate helminth eggs before use at these sites. From a less conservative point of view, Fitzgerald (1979) reviewed the potential impact on public health of parasites in soil/sludge systems, and concluded that the proper utilization of wastewater sludge did not pose any great threat to the health of society through actual transmission of pathogens that might be present in sludge.

Animals

The most serious threat to cattle at land application sites is the beef tapeworm, *Taenia saginata* (Feachem *et al.* 1978, WHO 1981). The increased incidence of cysticercosis in cattle results in economic losses (because of condemnation of carcasses), as well as increased incidence of disease in man. The application of wastewater sludge to pastures has resulted in outbreaks of cysticercosis in grazing cattle in England (Macpherson *et al.* 1978, 1979), but wastewater land treatment sites at San Angelo, Texas (Weaver et al. 1978), and Melbourne, Australia (Croxford 1978, McPherson 1978), have resulted in no increase of cysticercosis in grazing cattle. Arundel and Adolph (1980) have found no cysticercosis in cattle grazed on pasture irrigated with effluent from lagooning, compared with a 3.3% infection rate from trickling filter effluent, 9.0-12.5% from activated sludge effluent, and 30.0% from raw sewage.

Because of the longevity of helminth eggs in the soil, and the fact that cattle consume considerable quantities of soil as they graze, it might be prudent to select a sludge treatment method which will completely remove or inactivate helminth eggs at land application sites where cattle are allowed to graze, such as high-quality composting or heat treatment.

Infective Dose, Risk of Infection, Epidemiology

Single eggs of helminths are infectious to man, although, since the symptoms of helminth infections are dose-related, many light infections are asymptomatic. However, *Ascaris* infection may sensitize individuals so that the passage of a single larval stage through the lungs may result in allergic symptoms, i.e., asthma and urticaria (Mueller 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 application site, unless the sludge has been pretreated to remove or inactivate 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).

SECTION 7
ORGANICS

The potential health effects of toxic organic compounds are myriad. Systems affected range from the dermatological to the nervous to the subcellular, and effects produced range from rash to motor dysfunction to cancer. The degree of toxicity of organic compounds varies widely from essentially harmless (e.g., most carbohydrates) to moderately toxic (e.g.,most alcohols) to extremely toxic (e.g., aflatoxins).

A glance at the current edition of The Merck Index will reveal that the number of organic compounds described thus far is almost unfathomable.Nearly any of these may appear in wastewater, depending upon its sources.Thus, the discussion below must be perforce rather general, and the presence of any particular toxic organic in high concentration in sludge may require a site-specific evaluation of potential health effects.

Types and Levels in Wastewater and Sludge

Most common organics in domestic wastewater derive from feces, urine, paper products, food wastes, detergents, and skin excretions and contaminants (from bathing). In medium-strength sewage (700 ppm total solids content), organics make up about 75% of the suspended solids and about 40% of the filterable solids (colloidal and dissolved), consisting primarily of proteins (40-60%), carbohydrates (25-50%), and fats and oils (10%) (Metcalf and Eddy 1972). After secondary treatment, the more refractory and high-molecular-weight organics predominate, e.g., fulvic acid, humic acid, and hymathomelanic acid (Chang and Page 1978). In general, however, the chemical nature of domestic wastewater remains poorly characterized.

Although most of the organics found in municipal sludge of domestic origin are probably harmless in a land application context, it has recently been found that fecal material commonly contains mutagens. It is widely believed that mutagens form a large class of potential carcinogens (Weisburger and Williams 1980). Thus, there is evidence that one of the causes of colorectal cancer is the presence of carcinogens or co-carcinogens produced by the bacterial degradation in the gut of bile acids or cholesterol (Thornton 1981). The mutagenicity of feces can be increased by anaerobic incubation and by the presence of bile and bile acids (Van Tassel *et al.* 1982), and is lower in vegetarians than non-vegetarians (Kuhnlein *et al.* 1981). High levels of chromosome-breaking mutagenic activity have also been found in the feces of animals—dog, otter, gull, cow, horse, sheep, chicken, and goose (Stich *et al.* 1980). The chemical nature of the fecal mutagens is unknown. In the case of the latter animal mutagens, evidence suggests that at least part of the mutagenic action is due to hydrogen peroxide and the ensuing radicals which can be formed during oxidation of many organic compounds (Stich *et al.* 1980).

Ten domestic and industrial secondary effluents in Illinois were recently examined for mutagenicity by Johnston *et al.* (1982), with the results that all ten effluents assayed showed significant mutagenicity. Mutagenic activity per unit volume of effluent varied over a 4,500-fold range, and toxicity varied over a 120-fold range. Selective extraction of whole effluents appeared to unmask mutagenic activity, probably by separating mutagens and substances that interfere with the mutagen assay. In several effluents there was evidence of several mutagenic compounds present, and it appeared that the mutagens were predominantly nonpolar, neutral compounds. There was no obvious influence of disinfection by chlorination on the effluent mutagenicity, in spite of the fact that one would expect many mutagens to be formed by the action of chlorine on humic substances and other organics found in wastewater.

Whether natural mutagens are of any significance in sludge, however, is doubtful, since a recent small-scale survey has shown only municipal sludge with industrial input to have mutagenic activity. Mutagenic activity could not be demonstrated in purely domestic sludge (Hopke *et al.* 1982, 1984). However, since industrial input is characteristic of most American cities,it is reasonable to assume that most sludges will possess mutagenic activity. Thus Babish *et al.* (1983) found mutagenicity in 33 of 34 sludges from different American cities demonstrated by at least 1 of 5 tester strains of *Salmonella* in the Ames test.

The major contributors of toxic organics to municipal wastewaters are usually assumed to be industrial discharges. However, household wastewater discharge may represent an important contributor since many consumer products in daily use contain toxic substances. A recent study (Hathaway 1980) identified consumer products containing toxic compounds on EPA's list of 129 "priority" pollutants, which may eventually end up in wastewater. (It should be recognized that this list of "priority" pollutants is, to some extent, arbitrary. Although the list has been used by EPA and others for many purposes, there exist numerous other toxic organic compounds which are of public health concern.) The most frequently used products are cleaning agents and cosmetics, containing solvents and heavy metals as main ingredients. Next are deodorizers and disinfectants, containing naphthalene, phenol, and chlorophenols. Discarded into wastewater infrequently, but in large volumes, are pesticides, laundry products, paint products, polishes, and preservatives.The organic priority pollutants most frequently used and discharged into domestic wastewater were predicted to be the following:

benzene
phenol
2,4,6-trichlorophenol
2-chlorophenol
1,2-dichlorobenzene
1,4-dichlorobenzene
1,1,1-trichloroethane
naphthalene
toluene
diethylphthalate
dimethylphthalate
trichloroethylene
aldrin
dieldrin

Because of the difficulty of analysis of complex mixtures, it has only recently been possible to measure the actual levels of organics in wastewater using advanced methods of extraction, gas and other chromatography, mass spectrometry, and computer analysis. The U.S. Environmental Protection Agency has sponsored two extensive surveys of the types and levels of priority pollutants in municipal wastewaters, which, of course, result from both domestic and industrial discharges. The first (DeWalle *et al.* 1981), supported by the Municipal Environmental Research Laboratory in Cincinnati, covered 25 cities located throughout the United States, and the second (Burns and Roe 1982), supported by the Effluent Guidelines Division in Washington, D.C., covered 40 cities.

In the 25-city survey (DeWalle *et al.* 1981) most of the 24-hour composite samples of raw wastewaters contained less than 1 mg/l of priority organics,and the numbers of compounds detected clustered between 20 and 50. In the 40-city survey (Burns and

Roe 1982) six days of 24-hour sampling was completed. Comparison with other available data sets has shown the 40-city survey to be generally representative of municipal sludges (Fricke *et al.* 1985). The priority organics detected in at least 50% of the samples analyzed in either survey are listed, together with their concentrations, in Table 20. Comparison of the results of the two surveys with the list of organic priority pollutants most likely to be discharged into domestic wastewater, reveals considerable overlap, and gives one some confidence that these two studies have

Table 20. Most Frequently Detected Priority Organics in Raw Municipal Wastewater

	DeWalle *et al.* 1981		Burns and Roe 1982	
Compound	Detection Frequency (%)	Concentration Range (µg/l)	Detection Frequency (%)	Concentration Range (µg/l)
Phenol	94	0.90-2,440.00	79	1- 1,400
1,1,1-Trichloroethane	94	0.40- 97.50	85	1- 30,000
Trichloroethylene	94	0.90-1,553.00	90	1- 1,800
Tetrachloroethylene	94	1.50- 385.10	95	1- 5,700
Ethylbenzene	94	0.20- 304.40	80	1- 730
Trichloromethane (Chloroform)	94	0.25- 73.10	91	1- 430
Diethylphthalate	91	1.34- 290.00	53	1- 42
Di-n-butylphthalate	91	0.26- 123.00	64	1- 140
Toluene	90	0.70- 795.00	96	2- 1,300
Dichloromethane	90	0.50- 666.10	92	1- 49,000
Bis(2-ethylhexyl)phthalate	89	0.06- 117.00	92	2- 670
Naphthalene	86	1.25- 291.00	49	1- 150
1,4-Dichlorobenzene	83	1.70- 119.00	17	2- 200
Phenanthrene	83	0.20- 49.50	20	1- 93
Benzene	79	0.26- 243.00	61	1- 1,560
Heptachlor	77	0.30- 37.00	5	0.08-0.50
Butylbenzylphthalate	77	1.10- 237.00	57	2- 560
BHC-G (Lindane)	71	0.05- 11.20	26	0.02-3.9
1,2-Dichlorobenzene	69	0.78- 703.00	23	1- 440
Dimethylphthalate	66	0.09- 114.00	11	1- 110
BHC-D	63	0.01- 5.10	3	0.10-1.4
Dieldrin	63	0.02- 4.40	1	0.03-0.04
1,3-Dichlorobenzene	60	0.08- 548.00	7	2- 270
BHC-A	60	0.01- 2.90	8	0.02-4.4
DDT	60	0.10- 24.00	<1	1.2
Di-n-octylphthalate	57	0.31- 51.50	7	2- 210
1,1-Dichloroethane	55	0.20- 3.60	31	1- 24
1,2-Dichloroethane	55	0.20-3,950.00	15	1- 76,000
DDD	54	0.05- 10.00	1	0.31-0.77
Anthracene	51	0.04- 36.80	18	1- 93
Aldrin	51	0.02- 2.00	1	0.03- 5
Endosulfan-B	51	0.20- 8.80	--	--
1,2-Trans-dichloroethylene	20	0.20- 45.30	62	1- 200

yielded a reasonable characterization of the priority organics in municipal wastewater, at least of those identifiable by modern methods.

The priority organics in raw municipal sludge detected in at least 10% of the samples analyzed in the 40-city survey (Burns and Roe 1982) are listed, together with their concentrations, in Table 21. Note that, of the 30 compounds listed, 21 also appear in Table 20. The broad range of concentrations detected among the samples suggests that sludge applied to land should be regularly monitored for toxic organics. This measure is emphasized by the occasional discharge of toxic substances into municipal wastewater systems with resulting medical effects in treatment plant workers, such as the recent hexachlorocyclopentadiene episode in Louisville, Kentucky (Kominsky *et al.* 1980).

Table 21. Most Frequently Detected Priority Organics in Raw Municipal Sludge (Burns and Roe 1982)

Compound	Detection Frequency (%)	Concentration Range (μg/l)
Bis(2-ethylhexyl)phthalate	95	2- 47,000
Toluene	94	1- 427,300
Dichloromethane	73	1- 10,500
Ethylbenzene	63	1- 4,200
Benzene	61	1- 953
1,2-Trans-dichloroethylene	60	1- 96,000
Trichloroethylene	54	1- 32,700
Pyrene	53	1- 1,700
Phenanthrene	53	1- 10,100
Phenol	50	5- 17,000
Anthracene	48	1- 10,100
Di-n-butylphthalate	45	1- 6,900
Fluoranthene	44	1- 9,930
Butylbenzylphthalate	43	2- 45,000
Tetrachloroethylene	40	1- 2,800
1,1-Dichloroethane	34	1- 2,885
Naphthalene	34	1- 5,200
Chrysene	31	1- 1,500
1,2-Benzanthracene	27	1- 1,500
Trichloromethane (Chloroform)	24	1- 366
1,1,1-Trichloroethane	19	1- 10,910
1,4-Dichlorobenzene	17	2- 12,000
1,2-Dichlorobenzene	16	3- 1,319
1,1,2,2-Tetrachloroethane	15	1- 3,040
Pentachlorophenol	14	10- 10,500
Chlorobenzene	13	1- 687
1,2,4-Trichlorobenzene	13	2- 8,300
3,4-Benzofluoranthene	11	1- 2,400
Di-n-octylphthalate	10	4- 1,024
1,2-Dichloroethane	10	1- 10,010

Soil and Groundwater

Organic compounds in sludge may be volatilized, immobilized by adsorption, or transported through the soil column, possibly to reach the groundwater. At normal application rates and management techniques, however, leaching or soil migration of organics from a municipal sludge land application site is probably insignificant (Overcash 1983). Adsorbed organics may be subsequently chemically or photochemically degraded, microbially decomposed, or desorbed. A considerable body of research has been performed on the behavior of pesticides in soil. This research has shown that the affinity of soil components for pesticides, and presumably for organics in general, decreases in the following order (Chang and Page 1978):

Organic Matter
Vermiculite
Montmorillonite
Illite
Chlorite
Kaolinite

Iron and aluminum oxides also adsorb organics. Adsorption of organic pesticides tends to increase with the concentration of functional groups such as amine, amide, carboxyl, and phenol. Both laboratory and field experiments suggest that, because of adsorption by soil particles, most pesticide residues remain in surface soils during land treatment (Chang and Page 1978).

It has recently been shown that for polynuclear aromatic hydrocarbons adsorption increases with increasing organic carbon content of the soils and increasing effective chain length of the molecule (Means *et al.* 1980). The behavior of polychlorinated biphenyls (PCBs) in soil has been comprehensively reviewed by Griffin and Chian (1980), who concluded that PCBs are strongly adsorbed by soil, and that the nature of the surface, the soil organic matter content, and the chlorine content and/or hydrophobicity of the individual PCB isomers are factors affecting adsorption. Adsorption increases with increasing organic matter content of the soil, with increasing chlorine content, and with increasing hydrophobicity. One study of PCB percolation through soil columns showed that less than 0.05% of one isomer was leached in the worst case. Fairbanks and O'Connor (1984) have recently shown that PCBs remain tightly adsorbed to sludge-amended soil, with minimal transport by soil water.

Once organics are immobilized by adsorption on the surfaces of soil particles, microbial decomposition, or biodegradation, is probably the major mechanism of their breakdown. Although there are several abiotic mechanisms for chemical change, nonenzymatic reactions rarely result in appreciable changes in chemical structure, and it is biodegradation that brings about major alterations and mineralization of organics (Alexander 1981). The chief agents of this metabolism are the indigenous heterotrophic bacteria and fungi.

It is, of course, possible that high levels of toxic organics in sludge could have a severe inhibitory effect on the soil microflora. However, such levels are much greater than one would expect to find with the land application of municipal sludge (Overcash 1983).

The potential of microbial decomposition for removal of organics is demonstrated by the experience at two rapid infiltration and one overland flow wastewater land treatment sites. At Flushing Meadows in Arizona secondary effluent has resulted in no accumulation of organic carbon in the soil after ten years of operation and 754 m of total infiltration (Bouwer and Rice 1978). Secondly, the Lake George Village Sewage Treatment Plant in New York has been applying unchlorinated secondary effluent to natural delta sand beds by rapid infiltration since 1939 (Aulenbach and Clesceri 1978). After about forty years of daily infiltration rates of 0.08 to 0.30 m/day, there were no indications that the soil's capacity to treat the applied effluent was approaching exhaustion. The greatest removal of constituents occurred in the top 10 m of the sand beds. At a prototype overland flowland treatment system, at the U.S. Army Cold Regions Research and Engineering Laboratory in New Hampshire, greater than 94% removal of each of 13 trace organics by volatilization and adsorption was observed (Jenkins *et al.* 1983), with removal efficiencies decreasing as application rates increased and temperature decreased. With the possible exception of PCB, biodegradation resulted in the absence of contaminant buildup in the surface soil.

Although complete mineralization and detoxication of organic compounds is common, many compounds are acted on biologically in soils without the microorganisms being able to use them as their sources of nutrient or energy. The microorganisms are probably utilizing another substrate while performing the transformations known as "cometabolism" (Alexander 1981). Cometabolism may lead to detoxication, the formation of new toxic substances, or the synthesis of persistent products. There is evidence that cometabolism may be particularly common for toxic organics in very low concentrations in the environment (Rubin *et al.* 1982, Subba-Rao *et al.* 1982).

The metabolism of few chemicals has been studied in microbial cultures,and even fewer in natural ecosystems. Why certain intermediate compounds in a metabolic sequence accumulate outside or inside the active organism is not known, and it is extremely difficult to predict the chemical fate of toxic organics in the environment. The prediction of biodegradability from chemical structure, although theoretically possible, has thus far proven problematic. Alexander (1981) has described several common types of reactions that may occur, and these are listed in Table 22. It used to be thought that every organic compound could be completely decomposed by microorganisms. Thus a recent evaluation (Kobayashi and Rittmann 1982) indicated that the use of properly selected populations of microorganisms, and the maintenance of appropriate controlled environmental conditions, could be an important means of improving biological treatment of organic wastes, and that members of almost every class of synthetic compound can be degraded by some microorganism. However, field evidence has resulted in the conclusion that some synthetic organics are decomposed slowly, if at all, and may persist for long periods in the environment. Alexander (1981) has summarized the possible reasons for this, concluding primarily that various synthetic compounds, e.g., polymers and halogenated aromatics, are too far from the mainstream of catabolic pathways to be substrates for any microbial species.

Table 22. Common Types of Chemical Transformations in the Environment

Dehalogenation	Nitro metabolism
Deamination	Oxime metabolism
Decarboxylation	Nitrile/amide metabolism
Methyl oxidation	Cleavage reactions (many types)
Hydroxylation and ketone formation	Nondegradative reactions:
β oxidation	Methylation
Epoxide formation	Ether formation
Nitrogen oxidation	N-Acylation
Sulfur oxidation	Nitration
=S to =O	N-Nitrosation
Sulfoxide reduction	Dimerization
Reduction of triple bond	Nitrogen heterocycle formation
Reduction of double bond	Oligomer and polymer formation
Hydration of double bond	

A general idea of the relative degree of biodegradation of toxic organics to be expected in soil may be gained from Tabak *et al.*'s (1981)studies on organic priority pollutants. They collected data on the biodegradability and rate of microbial acclimation of 96 compounds (5 and 10 mg/l)in a static culture flask screening procedure, using domestic wastewater inoculum and synthetic medium. Microbial acclimation, or adaptation, was measured by making three weekly subcultures; percentage biodegradation was measured after seven days incubation in the dark at 25°C. Their overall results are summarized in Table 23. Significant biodegradation was found for phenolic compounds, phthalate esters, naphthalenes, and nitrogenous organics; variable results were found for monocyclic aromatics, polycyclic aromatics, polychlorinated biphenyls, halogenated ethers, and halogenated aliphatics; and no significant biodegradation was found for organochlorine pesticides.

Extrapolation of the above results to the behavior of toxic organics in the soil must be done with two provisos: (1) biodegradation in soil may be somewhat different from that in the aquatic medium used for the tests, and(2) the lower concentration of the organics at the land application site may not elicit microbial activity or enzyme induction. Nevertheless, a comparison of the results with the compounds to be expected in wastewater, as listed in Table 20, is instructive. Among the top ten compounds in the table, nine have significant degradation, and one has slow to moderate degradation with significant volatilization. Among the next ten compounds, eight have significant degradation, two of which are followed by toxicity. Only two compounds are not significantly degraded, the pesticides heptachlor and lindane.

Other than for pesticides, the literature on the microbial decomposition of toxic organics in soil is sparse. The degradation of petroleum hydrocarbons, a mixture of aliphatic, aromatic, and asphaltic compounds, has been reviewed by Atlas (1980, 1981). Factors which appear to be important in encouraging high decomposition rate of petroleum hydrocarbons are high temperature, low concentrations, high soil fertility, and an aerobic environment. There is little evidence for significant downward leaching of oil. Experiments with the high-rate application of high petroleum hydrocarbon sludge to land have shown a 77% degradation rate near the surface after one year, most of the degraded compounds being n-alkanes (Lin 1980), and it was concluded that sludge land disposal would not result in petroleum hydrocarbon buildup in the soil. In studies of organic substances in wastewaters used for irrigation, Dodolina *et al.* (1976) found acetaldehyde, crotonaldehyde, benzaldehyde, cyclohexanone, cyclohexanol, and dichloroethane to disappear from soil within ten days. The biodegradation in soil of polychlorinated biphenyls was reviewed by Griffin and Chian (1980) who concluded that they are degradable, but that resistance increases as isomers have higher chlorination. Polybrominated biphenyls, on the other hand, have shown little biodegradation after one year in soil (Jacobs *et al.* 1978).

It has recently been found that it might be possible for carcinogenic and teratogenic nitrosamines to be formed from secondary and tertiary amines at wastewater land treatment sites. Thus, Thomas and Alexander (1981) have shown that dimethylamine and trimethylamine can be formed in municipal wastewater from naturally-occurring precursors. Dimethylamine may then go on to be microbially nitrosated, forming N-nitrosodimethylamine, a process which can occur under conditions resembling land treatment of wastewater(Green *et al.* 1981). Whether this can actually occur under field conditions, resulting in a threat to groundwater, is unknown. The issue may be moot, however, since Mumma *et al.* (1984) have found nitrosamines already present in 14 of 15 municipal sludges analyzed. In any case, Dressel (1976) has demonstrated that nitrosamines are rapidly degraded in soil. Similarly, mutagens, as detected by the Ames test, in municipal sludge applied to soil at the rate of 112 dry t sludge/ha and mixed, could no longer be detected after 4 weeks (Angle and Baudler 1984).

Table 23. Biodegradability of Priority Organic Compounds (after Tabak *et al.* 1981)*

Test Compound	Performance Summary	Test Compound	Performance Summary
Phenols			
Phenol	D	p-Chloro-m-cresol	D
2-chloro phenol	D	2-Nitro phenol	D
2,4-Dichloro phenol	D	4-Nitro phenol	D
2,4,6-Trichloro phenol	D	2,4-Dinitro phenol	D
Pentachloro phenol	A	4,6-Dinitro-o-cresol	N
2,4-Dimethyl phenol	D		
Phthalate Esters			
Dimethyl phthalate	D	Bis-(2-ethyl hexyl) phthalate	A
Diethyl phthalate	D	Di-n-octyl phthalate	A
Di-n-butyl phthalate	D	Butyl benzyl phthalate	D
Naphthalenes			
Naphthalene	D		
2-Chloro naphthalene	D		
Acenaphthene	D		
Acenaphthylene	D		
Monocyclic Aromatics			
Benzene	D	Hexachlorobenzene	N
Chlorobenzene	D-A	Nitrobenzene	D
1,2-Dichlorobenzene	T	Ethylbenzene	D-N
1,3-Dichlorobenzene	T	Toluene	D
1,4-Dichlorobenzene	T	2,4-Dinitrotoluene	T
1,2,4-Trichlorobenzene	T	2,6-Dinitrotoluene	T
Polycyclic Aromatics (PAHs)			
Anthracene	A	1,2-Benzanthracene	N
Phenanthrene	D	Pyrene	D-N
Fluorene	A	Chrysene	A-N
Fluoranthene	A-N		

Table 23. (Continued)

Test Compound	Performance Summary	Test Compound	Performance Summary
Polychlorinated Biphenyls (PCBs)			
Aroclor-1016	N	Aroclor-1248	N
Aroclor-1221	D	Aroclor 1254	N
Aroclor-1232	D	Aroclor-1260	N
Aroclor-1242	N		
Halogenated Ethers			
Bis-(2-chloroethyl) ether	D	4-Bromodiphenyl ether	N
2-Chloroethyl vinyl ether	D	Bis-(2-chloroethoxy)methane	N
4-Chlorodiphenyl ether	N	Bis-(2-chloroisopropyl) ether	D
Nitrogenous Organics			
Nitrosamines		Acrylonitrile	D
N-Nitroso-di-N-propylamine	N	Acrolein	D
N-Nitrosodiphenylamine	D-A		
Substituted benzenes			
Isophorone	D		
1,2-Diphenylhydrazine	T		
Halogenated Aliphatics			
Chloroethanes		Chloroethylenes	
1,1-Dichloroethane	A	1,1-Dichloroethylene	A
1,2-Dichloroethane	B	1,2-Dichloroethylene-cis	B
1,1,1-Trichloroethane	B	1,2-Dichloroethylene-trans	B
1,1,2-Trichloroethane	C	Trichloroethylene	A
1,1,2,2-Tetrachloroethane	N	Tetrachloroethylene	A
Hexachloroethane	D	Chloropropanes	
Halomethanes		1,2-Dichloropropane	A
Methylene chloride	D	Chloropropylenes	
Bromochloromethane	D	1,3-Dichloropropylene	A
Carbon tetrachloride	D	Chlorobutadienes	

Table 23. (Continued)

Test Compound	Performance Summary	Test Compound	Performance Summary
Chloroform	A	Hexachloro-1,3-butadiene	D
Dichlorobromomethane	A	Chloropentadienes	
Bromoform	A	Hexachlorocyclopentadiene	D
Chlorodibromomethane	N		
Trichlorofluoromethane	N		
Organochlorine Pesticides			
Aldrin	N	Heptachlor	N
Dieldrin	N	Heptachlor epoxide	N
Chlordane	N	Hexachlorocyclohexane	
DDT p,p′	N	αBHC-alpha	N
DDE p,p′	N	Hexachlorocyclohexane	
DDD p,p′	N	βBHC-beta	N
Endosulfan-alpha	N	Hexachlorocyclohexane	
Endosulfan-beta	N	δBHC-delta	N
Endosulfan sulfate	N	Hexachlorocyclohexane	
Endrin	N	γBHC-gamma (lindane)	N

**D—significant degradation with rapid adaptation; A—significant degradation with gradual adaptation; T—significant degradation with gradual adaptation followed by a deadaptive process (toxicity); B—slow to moderate biodegradative activity, concomitant with significant rate of volatilization; C—very slow biodegradative activity, with long adaptation period needed; N—not significantly degraded under the conditions of test method.*

In view of multitudinous variety of organic compounds in existence, it is difficult to generalize about their biodegradation in soil. It appears, however, that most organics do become microbially decomposed in the soil, at least to some extent. This is especially true of naturally-occurring compounds, or those resembling them, because of the eons of evolution that have developed microbial enzyme systems to do the job. The more structurally complex the molecule is, e.g., condensed rings or dense branching, and more halogenated it is, the more difficult is biodegradation. Overcash (1983) has concluded that very few organic compounds can be said to be non-degradable in soil systems, in particular two classes: synthetic polymers manufactured for stability, and very insoluble large molecules, e.g., 5-10 chlorinated biphenyls.

Although few organics are likely to reach the groundwater at a sludge application site, those that do may be subject to some of the same removal processes that affect them at the surface, particularly adsorption and microbial decomposition, although certainly at much lower rates. These two processes, which largely govern the movement and fate of organics in the subsurface environment, have been reviewed by McCarty *et al.* (1980, 1981).The degree of adsorption of an organic compound in groundwater is to a great extent dependent upon its hydrophobicity, especially when the aquifer organic content is above about 0.1%. Thus, only compounds with octanol/water partition coefficients less than 10^3 are likely to readily move through the subsurface environment. Of course, these are the very compounds most likely to reach the groundwater, the more hydrophobic compounds having been adsorbed to the soil above. Likewise, it is probable that most microbial decomposition would have occurred before the organics reach the groundwater, although there is evidence that diverse microbial populations of sulfate reducers, methanogens, and heterotrophs exist and are metabolically active in aquifers, and that biodegradation of some organic pollutants occurs in groundwater (Gerba and McNabb 1981). Nevertheless, it is difficult to avoid the conclusion that once toxic organics get into the groundwater they may remain there for a long time.

Plants

At the low concentrations found in the soil at municipal sludge land application sites, very few organic compounds are likely to be toxic to plants (Overcash 1983). In a review of data on over 130,000 chemicals, Kenaga (1981) found only 0.17% of the chemicals killed seeds or seedlings at concentrations of 0.1-0.99 ppm. Crop plants, however, although not injured themselves, may accumulate organics that may be toxic to the animals to which they are fed or to humans who use them as food, either directly or through animal products. The issue is complicated by the fact that significant levels of toxic organics, e.g., polycyclic aromatic hydrocarbons (Borneff *et al.* 1968), may be synthesized by the plants themselves. Moreover, plant composition of biologically active compounds, e.g., natural mutagens, may be affected by growth on sludge-amended soil (Miller *et al.* 1983).

Among the organics, the pesticides appear to be the most notorious accumulators in crop plants. Thus, heptachlor, dieldrin, and chlordane are absorbed at low levels from the soil (Braude *et al.* 1978). Most herbicides, of course, are readily taken up and translocated within plants, but there is no reason to think that herbicides would present more of a problem at land application sites than they do at ordinary agricultural sites.

In contrast with pesticides, most organic compounds are only poorly absorbed and translocated by plants, with much of the "absorption" probably accounted for by root adsorption, often through vapor transport. Vapor transport from the soil may even result in shoot adsorption (Chaney 1984). Soil organic matter adsorbs lipophilic compounds, decreasing their availability to plants. Thus, sludge itself helps to retain toxic organics in the soil, and keeps them accessible to biodegradation.

Numerous studies of organics uptake by plants have shown that many organics can indeed be absorbed, but usually only at high soil levels and with little translocation to the upper parts of the plants.

Trace levels of polychlorinatd biphenyls (PCBs) from municipal sludge applied to an old field has resulted in no detectable PCBs in plant samples (Davis *et al.* 1981). Higher levels (50-100 ppm dry soil) resulted in 3-50% of the soil concentration in carrots (Iwata *et al.*, 1974), with concentration increasing with lesser-chlorinated biphenyls. Since 97% of the PCB was found in the carrot peel, very little translocation occurred in the plant tissue. The lower-chlorinated PCBs are much more volatile and biodegradable, and thus are less likely to be common in sludge; the higher-chlorinated PCBs are less absorbed by plants (Fries and Marrow 1981). As a consequence, PCB exposure through plants is probably minimal. For example, Lee *et al.* (1980) were unable to detect PCBs in carrots grown in land to which 0.93 ppm PCBs domestic sludge was applied at 224 t/ha, and Naylor and Mondy (1984) have obtained similar results with potatoes. On the basis of greenhouse and field studies of polybrominated biphenyls (PBBs), it has been concluded that little, if any, PBB will be translocated from contaminated soil to plant tops, and although some root crops from highly contaminated soil might contain traces of PBB, much of this PBB could probably be removed by peeling (Chou *et al.* 1978).

Irrigation of vegetables in test plots with contaminated wastewaters has shown no accumulation of polycyclic aromatic hydrocarbons, especially benzo(a)pyrene (Il'nitskii *et al.* 1974). 4-Chloroaniline and 3,4-dichloroaniline can be absorbed by .omato plants, oats, barley, and wheat, but 90-95% remains in the roots (Fuchsbichler *et al.* 1978); in carrots, however, the chloroanilines are translocated to the upper parts of the plants in significant quantities. In a study of aldehydes and other organics at agricultural land treatment sites Dodelina *et al.* (1976) found no uptake of acetaldehyde, crotonaldehyde, and benzaldehyde in the aboveground portions of potatoes and corn. Cyclohexanone and cyclohexanol could be found in corn plants four days after irrigation, but not later. Dichloroethane was taken up by beets and cereals, but was metabolized and absent within about two weeks after irrigation.

At the operating land treatment site in Muskegon, corn crop samples for 1980 did not contain detectable levels of any of the chemicals tested, and it was concluded that plant uptake of irrigated organic chemicals does not occur to any measurable extent (Demirjian *et al.* 1981). Thus, it is probably reasonable to assume that the health risk from toxic organics in plants is slight, provided that high levels in sludge are prevented by monitoring.

Animals

The low levels of toxic organics to be expected in the above ground portions of plants growing at land application sites probably pose little hazard to animals feeding upon them. Under certain site-specific conditions, however, high concentrations of particular organics in the sludge may cause problems. For example, PCBs in cabbage grown on sludge-amended soil have probably caused degenerative changes in liver and thyroid of sheep (Kienholz 1980, Haschek 1979).

Hansen *et al.* (1976) studied young swine fed for 56 days on corn grown on sludge-fertilized land. It was essentially a negative study: electroencephalograms, electrocardiograms, clinical chemistry, and histopathology were all normal. However, they observed elevated levels of hepatic microsomal mixed function oxidase (MFO) activity in the swine fed sludge-fertilized corn. Associated with this were non-statistically significant increased liver weights. Other liver enzymes (alkaline phosphatase and lactate dehydrogenase) were normal. This increased MFO activity may be caused by toxic organics, metals, or be of no significance, but the authors concluded that further study should be performed before such grain can be recommended as the major dietary component for animals over long periods.

Similar results were found by Telford *et al.* (1982), who examined sheep fed silage corn grown on soil amended with municipal sludge at a high rate (224 t/ha). The sheep had significantly higher hepatic microsomal p-nitroanisole-O-demethylase activity than controls, but no mutagenic responses for animal feed or feces, and no histopathological effects. In contrast, the same research group (Lisk *et al.* 1982) found no hepatic microsomal MFO response in swine fed corn grown on high-rate sludge-amended soil. Liver: body weight ratios, corn, feces, and urine mutagenicity, and histopathology were also unremarkable, suggesting absence or low levels of organic toxicants in the corn. Sludge-amended-soil-grown cabbage, beets, green beans, and squash have been fed to rats for 12 weeks, resulting in no effects on weight gain, alphafetoprotein (a marker for hepatic preneoplastic transformation), liver weight, hepatic MFO (aminopyrene-N-demethylase and p-nitroanisole-O-demethylase), or liver cell ultrastructure (Boyd *et al.* 1982). Mutagenicity, however, was found in the sludge-grown beans and in the urine of rats fed sludge-grown beets. Sludge-grown cabbage has also been shown to have mutagenic activity (Miller *et al.* 1983).

Forages grown on soils containing PCBs have PCB residues of about one-tenth or lower that of the soil during the first crop year (Chaney 1984). Delaying grazing for 30 days after surface sludge application and supplying alternative feeds during periods of low forage availability reduce sludge ingestion so that 10 ppm PCBs could be allowed in sludge surface-applied at 10 t ha^{-1} yr^{-1}. Subsurface injection could further reduce exposure.

A more serious route of exposure by animals to toxic organics is the soil itself. Most grazing animals ingest a certain amount of soil together with their food plants. Thus, dairy cows may ingest 100-500 kg of soil per year, with an average of about 200-300 kg/yr; expressed in other terms, dairy cows may consume soil up to 14% of dry matter intake when available forage is low and no supplemental feed is used (Kienholz 1980, Fries 1980). Lipophilic organics present in the soil may concentrate in animal fat. For example, feeding experiments with PCBs indicate that the steady-state milk fat concentrations are about five times the diet concentrations, which could result in milk fat levels of 0.7 ppm for each 1 ppm of PCBs in surface soil (Fries 1980). Body fat levels would be expected to be similar. Based upon FDA tolerances of 1.5 mg PCB/kg milk fat, Fries (1982) has concluded that PCBs should not exceed 2.0 mg/kg dry sludge if dairy cows are allowed to graze sludge-amended pastures. In a study of the pasture application of wastewater sludge with a high textile industry component, deHaan (1977) found 1.2 ppm of dieldrin (almost 19 times the acceptable level in The Netherlands) in the milk of grazing cows.

Studies at New Mexico State University (Smith 1982), involving direct feeding of sludges to rats, sheep, and cattle, indicate no hazard from toxicants, based on uptake, MFO activity, and histopathology.

Turning to humans, Baker *et al.* (1980) described the metabolic consequences of exposure to high levels of PCBs from contaminated wastewater sludge used as a soil amendment in Bloomington, Indiana. No skin or systemic symptoms were noted, and of the hematologic, hepatic, and renal functions measured, only serum triglyceride levels increased, suggesting altered lipid metabolism. Serum PCB levels were normal. Naylor and Loehr (1982) have recently performed a detailed toxicological analysis of the potential human health risks of the consumption of sludge-contaminated soils and crops associated with organic priority pollutants. They concluded that land application of sludge is not likely to result in the ingestion of amounts of organic priority pollutants exceeding the acceptable daily intake.

The long-term effects of chronic ingestion of these same organics, many of which are animal carcinogens, have been examined by Connor (1984). In his analysis Connor calculated the lifetime risk of cancer from the predicted doses of known carcinogens. The predicted doses were based upon the sludge pollutant concentrations and application rates summarized by Naylor and Loehr (1982), but included ingestion resulting from uptake of organics by plants and animals as well as direct consumption. Connor concluded that toxic organics are not likely to present a significant health risk, with the possible exception of polycyclic aromatic hydrocarbons (PAHs), and that it would be prudent to develop management techniques to decrease PAH concentrations in sludge.

SECTION 8

TRACE ELEMENTS

Types and Levels in Wastewater and Sludge

The trace elements (including the "heavy metals") in wastewater of public health concern, i.e., those for which primary drinking water standards (USEPA 1977) exist (but excluding silver since its effect is largely cosmetic), are:

	Primary Drinking Water Standard (mg/l)
Arsenic (As)	0.05
Barium (Ba)	1.0
Cadmium (Cd)	0.010
Chromium (Cr)	0.05
Lead (Pb)	0.05
Mercury (Hg)	0.002
Selenium (Se)	0.01

Of these, cadmium, lead, and mercury are usually regarded as of most concern, and barium of minor concern. Chromium and selenium are essential elements in man; arsenic and cadmium have been shown to be essential to experimental animals and, thus, may be essential to man as well (National Research Council 1980). Secondary drinking water standards (USEPA 1979), i.e., those related to aesthetic quality, also exist for copper, iron, manganese, and zinc. These latter elements, as well as all other trace elements, are toxic if ingested or inhaled at high levels for long periods (Underwood 1977), but this fact does not warrant considering them in the land application context, where low levels are expected.

Arsenic is popularly known as an acute poison, but chronic human exposure to low doses, as might be expected for all trace elements as a result of land application, may cause weakness, prostration, muscular aching, skin and mucosal changes, peripheral neuropathy, and linear pigmentations in the fingernails. Chronic arsenic intoxication may result in headache, drowsiness, confusion, and convulsions (Underwood 1977). Epidemiological evidence has implicated arsenic as a human carcinogen, but there is little evidence that arsenic compounds are carcinogenic in experimental animals (Sunderman 1977). Even with high concentrations in soil, however, plants rarely take up enough of the element to constitute a risk to human health (Underwood 1977, Council for Agricultural Science and Technology 1976).

Barium has a low degree of toxicity by the oral route. Because of its effect of intensely stimulating smooth, striated, and cardiac muscle in acute exposures, however, it may have cardiovascular effects in low doses, but this has not thus far been demonstrated (Brenniman *et al.* 1979).

Cadmium is widely regarded as the trace element of most concern from a human health effects viewpoint in the land application of sludge. Cadmium has a very long biological half-life in humans, with its concentration in the liver and kidneys continually increasing to the sixth decade of life (Kowal *et al.* 1979). The critical health effect of chronic environmental exposure via ingestion is proximal renal tubular damage due to accumulation of cadmium in the kidney. The initial consequence of this damage is the loss of low molecular weight serum proteins in the urine, followed by loss of other proteins, glucose, amino acids, and phosphate, i.e., the Fanconi syndrome. This kidney damage is often irreversible and constitutes a significant adverse health effect. There is evidence that the absorption and/or toxicity of cadmium are antagonized by zinc, selenium, iron, and calcium (Sandstead 1977). The carcinogenicity of cadmium is controversial; the epidemiological evidence is tenuous, and the experimental evidence is conflicting (Ryan *et al.* 1982). The human health effects of cadmium have been recently reviewed by Hallenbeck (1984) and Bernard and Lauwerys (1984).

Chromium is much more toxic in its hexavalent form than its trivalent form, its predominant state in wastewater and soil. Chronic oral exposure in experimental animals has been associated with growth depression, and liver and kidney damage (Underwood 1977). Hexavalent chromium causes respiratory cancer upon chronic exposure to chromate dust (Sunderman 1977). Most crops absorb relatively little chromium from the soil (Council for Agricultural Science and Technology 1976).

Lead chronic toxicity is characterized by neurological defects, renal tubular dysfunction, and anemia. Damage to the central nervous system is common, especially in children, who have low lead tolerance, resulting in physical brain damage, behavioral problems, intellectual impairment, and hyperactivity. At soil pH above 5.5 and high labile phosphorus content, common conditions at a land treatment site, little movement of lead from the soil into plant tops and seed would be expected (Council for Agricultural Science and Technology 1976, Stewart 1979).

Mercury in low levels can result in neurological symptoms such as tremors, vertigo, irritability, and depression, as well as salivation, stomatitis, and diarrhea. Mercury can enter plants through the roots, and appears to be readily translocated throughout the plant (Council for Agricultural Science and Technology 1976), although there is some contrary evidence (Stewart 1979).

Selenium exposure in its chronic form is associated with dental caries, jaundice, skin irruptions, chronic arthritis, deformed finger and toenails, and subcutaneous edema. It has also been found to have an inhibitory effect against several types of cancer (Fishbein 1977). Selenium is readily taken up by plants and passed onto animals, and has caused toxicity in livestock in high-selenium soils (Council for Agricultural Science and Technology 1976, Underwood 1977).

The concentrations of trace elements (after Chaney 1984) in typical dry digested municipal sludges and in typical agricultural soils are presented in Table 24. Also included in the table are the limits for the maximum cumulative application of trace elements in sludge to agricultural land, which have been recommended by various governmental agencies for the protection of public health and the prevention of phytotoxicity. [For a concise discussion of phytotoxicity from land application of sludge, see Logan and Chaney (1983).]

Soil and Plants

The availability of trace elements for uptake by plants (and thus entry into the human food chain) and transport to groundwater is controlled by chelation to organic matter, adsorption, and precipitation. Adsorption occurs on organic matter, hydrous oxides of iron and manganese, clays, and other soil minerals. Precipitation reactions include the formation of poorly soluble oxides, hydroxides, carbonates, phosphates, sulfides, etc., for the cations (the metals), and formation of anions for arsenic and selenium. Mercury, of course, may leave the soil through volatilization. As a result of these processes only small amounts of the trace elements remain free in the soil solution, from which they are available for absorption by plant roots. These processes are strongly affected by soil pH, cation levels decreasing and anion levels increasing in the soil solution with increasing pH (Chaney 1984). Repeated annual cropland sludge application does not appear to affect the form of many trace elements in the soil, e.g., cadmium and lead remain predominantly in the carbonate form, and chromium in the sulfide residue form (Chang *et al.* 1984a).

Table 24. Concentrations of Trace Elements (After Chaney 1984) in Typical Dry Digested Municipal Sludges and Agricultural Soils, and Maximum Cumulative Application Limits

				Agricultural Soil		Cumulative Limits	
Element	Sludge Minimum (ppm)	Sludge Maximum (ppm)	Sludge Median (ppm)	*(ppm)*	*(kg/ha)*[1]	USA[2] (kg/ha)	UK[3] (kg/ha)
Arsenic	1.1	230	10	6[4]	12	--	10
Barium	150[4]	4,000[4]	1,500[4]	500[4]	1,000	--	--
Cadmium	1	3,410	10	0.1	0.2	5/10/20[5]	5
Chromium	10	99,000	500	25	50	--	1,000
Lead	13	26,000	500	25	50	800	1,000
Mercury	0.6	56	6	0.03[4]	0.06	--	2
Selenium	1.7	17.2	5	0.2[4]	0.4	--	5

[1]Assuming tillage depth of 15 cm (thus soil volume of 1500 m^3/ha), and soil bulk density of 1330 kg/m^3 (Page 1974).
[2]USEPA, USFDA, and USDA 1981.
[3]National Water Council 1977.
[4]Page 1974.
[5]For soils with cation exchange capacities of <5,5-15, and >15 meq/100g, respectively, and soil pH ≥ 6.5. If soil pH <6.5, first figure holds.

The uptake of trace elements by plants has been reviewed by Logan and Chaney (1983). Important factors affecting uptake rate include: trace element properties, soil properties, the immediate environment (especially pH) of the roots, plant crop species, and plant crop cultivar (variety or strain). As an example of species effects, leafy vegetables, especially Swiss chard, are much better cadmium accumulators than most other plants. Cultivars of maize (corn) and wheat have been shown to have very different rates of cadmium accumulation.

The problem of cadmium uptake from sludge by crop plants, and the significance of its buildup in the soil, has been studied and argued about for many years. The current state of knowledge appears to allow the following generalizations to be made: (1) Low-cadmium sludges result in low plant uptake, and high-cadmium sludges result in high uptake. (2) While high cadmium land application rates using high-cadmium sludges result in high uptake, the same high cadmium application rates using low-cadmium sludges result in low uptake. (3) In soil to which sludge is amended annually, cadmium adsorption increases, and thus plant uptake (with reference to total soil cadmium) decreases with time (Chaney 1984, Logan and Chaney 1983). Uptake decreases very rapidly after sludge applications are terminated (Hinesly *et al.* 1984). Another potentially dangerous toxic trace element in municipal sludge, lead, has been shown to have low availability when applied to land, and no appreciable migration to the reproductive and reserve organs of vegetables (Berthet *et al.* 1984, Naylor and Mondy 1984).

After a trace element enters the root cells, translocation to shoots, and thus into above-ground human-food plant organs (leaves, fruits, seeds), depends upon the properties of the specific element and plant. Involved in the process are membrane surfaces, organic chelators, and cells specialized for pumping materials into the xylem, through which it reaches the shoot. Chromium, lead, and mercury are strongly held in the root cells, so that very little is translocated to the shoots of crop plants. On the other hand, cadmium and selenium are weakly chelated, and thus easily translocated (Logan and Chaney 1983).

These generalizations are supported by recent analyses of corn silage grown on sludge-amended soils (Bray *et al.* 1985). Silage was produced for three years on land amended by municipal sludge each year at high rates (15-90 metric tons/hectare). The silage contained elevated levels of cadmium and zinc, but not of any of the other 12 elements tested, including arsenic, chromium, lead, mercury, and selenium.

Cadmium, therefore, under ordinary circumstances is the only trace element likely to be of human health concern as a result of the application of municipal sludge to agricultural land. This is because of the potentially high concentrations in sludge and high sludge concentrations compared with normal soil concentrations (Table 24), cadmium's relative ease of absorption into and translocation through plants, its low level of phytotoxicity, and cadmium's human toxic effects.

Groundwater

At sludge application sites, trace elements are probably immobilized near the soil surface, especially at high pH. In sludge-applied soils, Chang *et al.* (1984b) have found over 90% of the deposited trace elements (e.g., cadmium, chromium, and lead) to accumulate in the 0-15 cm soil depth, with little movement occurring below 30 cm.

Leachate from sludge-applied land in South Africa regularly had cadmium concentrations below the drinking water standard of 10 $\mu g/l$ (Nell *et al.* 1981). Dowdy and Volk (1983) feel that the potential for groundwater contamination by sludge-borne trace elements is extremely limited. Trace element movement will be most likely with large applications to a sandy, acid, low organic-matter soil that receives high precipitation or irrigation, but even under these conditions the extent of movement will be limited.

Animals

Just as in the case of organics, animals can be exposed to trace elements through sludge residuals adhering to plants, sludge on the soil surface or mixed into the soil, or trace elements absorbed and translocated by plants. All three routes would operate on grazing land, but only the third when animals are fed sludge-amended-soil-grown feed.

Studies of the accumulation of trace elements in cattle grazed on sludge-amended pastures have revealed raised levels in liver and kidney, but not in muscle tissue (Bertrand *et al.* 1981a, Baxter *et al.* 1983). Sheep grazing on sludge-amended pasture have been found to have non-statistically-significant higher tissue levels of cadmium, but no toxic effects (Hogue *et al.* 1984). Bertrand *et al.* (1981b) observed no increases when cattle were fed sludge amended-soil-grown forage sorghum, nor did increases occur in mice or guinea pigs fed lettuce and Swiss chard grown on sludge-amended soil (Chaney 1984). Other studies, however, have shown significant increases in kidney and liver, but not muscle, cadmium in animals fed sludge-fertilized crops, e.g., swine and corn (Hansen and Hinesly 1979, Lisk *et al.* 1982), pheasants and corn (Hinesly *et al.* 1984), goats and corn silage (Bray *et al.* 1985), rats and beets (Boyd *et al.* 1982), and guinea pigs and cabbage (Babish *et al.* 1979); the latter two studies used extremely high cadmium and sludge application rates.

Trace element levels and disease conditions of cattle grazing on land reclaimed by Chicago sludge have been observed by Fitzgerald (1978) for four years; it was concluded that little risk to man or animals is associated with land application of anaerobically digested wastewater sludge. Experience at Werribee Farm in Melbourne, Australia, where cattle are grazed on wastewater-irrigated pastures, has shown higher organ levels of cadmium and chromium than in Farm cattle grazed on non-irrigated pastures, but comparable to non-Farm cattle (Croxford 1978). Organ levels of lead, however, did not increase, in spite of increases in both soil and pasture plants.

Since trace elements accumulate in very small quantities in animal muscle tissue, there is probably little concern about non-visceral meats in the marketplace. Liver and kidneys of animals do, however, accumulate high levels of cadmium, just as they do in man, so that these meats may be of concern to those people consuming large quantities of them.

Cadmium

It seems reasonable to conclude that cadmium is the only trace element likely to be of health concern to humans as a result of land application of sludge, with the exposure being through food plants or organ meats. Groundwater is unlikely to represent an exposure threat. Although the risk from sludge application is real, it should be kept in mind that, on a regional scale, agricultural land usually receives more cadmium from wind deposition and phosphate fertilizers than from municipal sludge (Davis 1984).

The significance of this concern with cadmium getting into the human food chain depends upon the cadmium levels presently existing in human food, the total dietary intake of cadmium, and the potential increase in cadmium levels in human food due to land application. [Drinking water and ambient air contribute relatively little to total daily cadmium intake (Pahren *et al.* 1979).]

The cadmium levels presently existing in human food can be estimated, at least for the United States, by data from the U.S. Food and Drug Administration's Compliance Program ("market-basket survey"). These levels, together with the calculated normal dietary intake and vegetarian dietary intake of cadmium, are summarized in Table 25. It should be noted that root and leafy vegetables have the highest concentrations of cadmium. More accurate estimates of cadmium (and other trace element) concentrations in crops grown in the United States, together with

Table 25. Cadmium Concentration in Foods and Calculated Dietary Intake (from Ryan *et al.* 1982)

		Normal Diet[b]		Vegetarian Diet[c]	
Food Classes	ppb Cd[a]	g/day	μg Cd/day	g/day	μg Cd/day
Dairy products	5.7	549	3.1	584	3.3
Meat, fish, poultry	15.3	204	3.1	--	--
Grain & cereal products	23.2	331	7.7	203	4.7
Potatoes	48.0	138	6.6	43	2.1
Leafy vegetables	40.5	42	1.7	252	10.2
Legume vegetables	6.2	51	0.3	166	1.0
Root vegetables	32.3	25	0.8	--	--
Garden fruits	14.7	69	1.0	--	--
Fruits	3.0	173	0.5	284	0.8
Oily fats, shortenings	15.3	56	0.9	107	1.6
Sugars & adjuncts	10.0	65	0.7	110	1.1
Beverages	3.0	534	1.6	600	1.8
Total Intake		2,237	28.0	2,349	26.6

[a]From FDA Compliance Program Evaluation 1974 Total Diet Studies.
[b]Adjusted on a caloric basis from the FDA 1974 Total Diet Studies to represent the normal diet which compares with the adult lacto-ovo-vegetarian diet.
[c]Loma Linda lacto-ovo-vegetarian diet. Based on response of 183 southern Californians in a food frequency questionnaire by the Department of Biostatistics and Epidemiology, Loma Linda University School of Health, 1978. Leafy vegetables class includes root vegetable and garden fruit classes from normal diet.

concentrations in the soils in which they are growing, will be available from a survey jointly supported by the USEPA, USFDA, and USDA. In this survey, 6,000 crop samples and 18,000 soil samples are being analyzed over a four-year period, and the results should be available in the near future. Initial results suggest that the USFDA levels are too high (Wolnik *et al.* 1983).

The present total dietary intake of cadmium was estimated in Table 25 to be about 28 μg/day. Other estimates based on the market-basket method have resulted in higher values: 30.9-36.9 μg/day in 15- to 20-year-old U.S. males, by the USFDA (1980), and 52 μg/day in Canadians (Kirkpatrick and Coffin 1977).

A more direct, and potentially more accurate, method of estimating dietary intake of cadmium is by measuring the cadmium content of human feces. This method is feasible because the absorption of cadmium from the gut is low (rarely more than 10%, and usually 4-6%) and the excretion of cadmium into the gut is also very low. It is more accurate because cadmium is generally about ten times more concentrated in feces than food, and because feces reflect actual food intake rather than predicted. A recent study, using existing fecal cadmium data collected in Chicago and Dallas, and estimating daily feces production, resulted in a final estimate of the average daily intake of cadmium in food for U.S. inhabitants of 13-16 μg/day (Kowal *et al.* 1979). (Since the ingestion rate of the teenage male is often used in discussions of cadmium intake, values of 24 μg/day, 19 μg/day, and 18 μg/day for 10- to 19-year-old males from Chicago 1974, Chicago 1976, and Dallas, respectively, were estimated.) A more recent study in California, using both measured cadmium concentration and measured feces production, has resulted in a value of 23.7 μg/day for 40- to 60-year olds (Willard 1984).

These estimates of the average daily intake of cadmium in food can be compared with other estimates by the fecal analysis method, where the daily feces production of each individual was measured. In Sweden, rates of 16 μg/ day in nonsmokers and 19 μg/ day in smokers (former and present) have been reported (see Kowal *et al.* 1979 for references). Nine μg/day fecal cadmium has been measured in Sweden, compared with a value of 10 μg/ day measured by the total diet collection method. In Germany, 31 μg/day has been measured, compared with 48 μg/day measured by the market-basket method. In Japan, where cadmium levels in food are higher because of industrial pollution, the fecal analysis method has resulted in several estimates ranging from 24 μg/day to 84 μg/day.

The issue of cadmium in tobacco is particularly significant since tobacco is a cadmium accumulator. For example, it has been recently found that growing tobacco on soils amended with municipal sludges at very high rates (224 t/ha) can result in a 30-fold increase in the cadmium content of cigarette smoke (Gutenmann *et al.* 1982). Since the absorption of cadmium from the lungs is much greater than from the gut, it is evident that tobacco should not be grown on sludge-amended land.

> "It has generally been concluded that ingestion of 200 to 350 mg Cd/day over a 50-year exposure period is a reasonable estimate for individuals (excluding smokers and occupationally exposed) within the population to reach the critical renal concentration (200 mg Cd/g wet weight in the renal cortex) associated with the initiation of proteinuria. This ingestion limit assumes background exposure levels of air and no exposure from smoking. If these exposures are increased, then the suggested ingestion limit must be correspondingly reduced. Smoking one pack of cigarettes/day will reduce the limit by about 25 μg/day. Again these exposures are assumed to occur over a 50-year exposure period and, in the case of cigarettes, since many smokers start as teenagers, this addition would be relevant for much (30 to 35 years) of the 50-year exposure period. Therefore, smokers must be considered as being at increased risk." (Ryan *et al.* 1982).

Thus, present levels of total dietary intake of cadmium for most people appear to be fairly safe. However, in view of human variability in sensitivity and the variability in food supply, these levels probably should not be allowed to rise greatly.

It is of interest to note that increased consumption by individuals of those leafy and root vegetable crops highest in cadmium, and of organ meats as well, would increase the dietary iron intake. Since iron-sufficient humans absorb only about 2.3% of dietary cadmium, compared to an average absorption of 4.6% in the generally iron-deficient American population (Flanagan *et al.* 1978, McLellan *et al.* 1978), the increased iron intake would tend to correct for the increased cadmium intake (Chaney 1980). The increased zinc and calcium intake would have similar effects.

The potential increase in cadmium levels in human food due to land application of sludge is still an unsettled question (see Ryan *et al.* 1982). It is clear, however, that increased cadmium in the soil results in increased cadmium in the plants grown in that soil, the degree of increase being a function of cadmium amendment, plant species and cultivar, soil pH, organic matter, and time since application, but especially sludge cadmium concentration, with low-cadmium sludges resulting in minimal cadmium uptakes (Logan and Chaney 1983). A detailed discussion of the food-chain impact of cadmium in sludge may be found in Hansen and Chaney (1984). Some are optimistic. Thus, Davis and Coker (1980) made an extensive review of cadmium in agriculture, particularly the potential transfer of cadmium from wastewater sludge into the human food chain. It was concluded that when sludge is applied to farmland in accordance with current practice, the hazard attributable to possible effects of the cadmium in the sludge on crops, animals, or the human food chain, is negligible.

This view is borne out by the results of the Seven Markets Garden study in England (Sherlock 1983). The dietary cadmium content of gardeners and their families, growing cash crops on land which had received massive applications of sewage sludge during previous decades, was measured. In spite of mean soil cadmium concentrations of 1.5-14.1 ppm, there was little difference in cadmium intake from the national average. Davis *et al.* (1983) have performed an analysis of the relationship between cadmium in sludge-treated soil and potential human dietary intake of cadmium. They concluded that a soil concentration of 6.0-12.0 ppm in calcareous soils (pH 7-8) is compatible with the WHO maximum acceptable dietary intake of cadmium of 70 μg/day for an average consumer taking all his crops from sludge-treated soil. This estimate is similar to the current cumulative cadmium limits of 5-20 kg/ha, since 6-12 ppm is equivalent to 10-22 kg/ha. Wheat and, to a lesser extent, potatoes were found to have a dominating influence on dietary cadmium; this is similar to the situation with the American diet (see Table 25). Naylor and Mondy (1984) have recently found potatoes grown in a well managed, sludge-treated soil at pH 4.9 to not have excessive cadmium uptake.

In a recent review, Davis (1984) has concluded that a cumulative cadmium limit of 5 kg/ha, equivalent to about 3.5 mg Cd/kg soil, results in adequate protection to the food chain where sludge is used on agricultural land. There would be no need for a limit to protect public health where the land is used to grow animal feed.

The degree of risk to man in general, of course, is dependent upon the amount of food supply affected and the diet selection of the individual.

SECTION 9

NITRATES

Nitrogenous wastes are important constituents of municipal wastewaters, consisting of (1) proteins and other nitrogenous organics from feces, food wastes, etc., (2) urea from urine, and (3) their breakdown products. Raw domestic wastewater has concentrations of about 8-35 mg/l organic nitrogen, 12-50 mg/l ammonium (plus ammonia), and, thus, 20-85 mg/l total nitrogen, all expressed as N (Metcalf and Eddy 1972). Nitrites and nitrates are normally present only in trace amounts in fresh wastewater. Municipal sludges contain <0.1-17.6 percent dry weight (median of 3.3) of total nitrogen, most of it organic (USEPA 1983).

Bacteria rapidly decompose most forms of organic nitrogen to ammonium (or ammonia) in wastewater or soil. Under aerobic conditions ammonium is oxidized by bacteria (*Nitrosomonas*) to nitrite, and the nitrite rapidly oxidized by bacteria (*Nitrobacter*) to nitrate; the two-step process is called "nitrification." Under anaerobic conditions, and in the presence of organic matter, bacteria can use nitrate as a source of oxygen, and convert nitrate to molecular nitrogen, which escapes to the atmosphere; this is called "denitrification." Both aquatic and terrestrial plants can use ammonium and nitrate as a nitrogen source, and this is usually the primary immediate economic benefit of sludge application to agricultural land, in addition to its function as a phosphorus source and soil conditioner.

Inorganic nitrogen is normally quite innocuous from a human health point of view, although high ammonia levels can present an aesthetic problem. The major health concern is that infants, less than about three months of age and consuming large quantities of high-nitrate drinking water through prepared formula, have a high risk of developing methemoglobinemia. The incompletely developed capacity to secrete gastric acid in the infant allows the gastric pH to rise sufficiently to encourage the growth of bacteria which reduce nitrate to nitrite in the upper gastrointestinal tract. The nitrite is absorbed into the bloodstream, and oxidizes the ferrous iron in hemoglobin to the ferric state, yielding methemoglobin, a form incapable of carrying oxygen. Fetal hemoglobin (Hb F), 50-89% of total hemoglobin at birth, is particularly susceptible to this transformation. Methemoglobin is normally present in the erythrocytes of adults, at a concentration of about 1% of total hemoglobin, being formed by numerous agents, but kept to a low level by the methemoglobin reductase enzyme system. This enzyme system is normally not completely developed in young infants. At a methemoglobin concentration of about 5-10% of total hemoglobin the body's oxygen deficit results in clinically-detectable cyanosis. As a result of epidemiological and clinical studies (Shuval and Gruener 1977, Craun *et al.* 1981, Fraser and Chilvers 1981) a primary drinking water standard of 10 mg/l of nitrate-nitrogen (i.e., nitrate expressed as N) has been established (USEPA 1977) to prevent this condition from developing.

Besides methemoglobinemia, there is also some concern about nitrates resulting in the formation of carcinogenic N-nitroso compounds in the gut, but this phenomenon probably involves higher concentrations than the 10 mg/l water standard (Fraser *et al.* 1980, Fraser and Chilvers 1981).

The relevance of land application, of course, centers on the possibility of highly soluble nitrates reaching groundwater which may be used as a potable water supply.

In the case of land application of sludge, there would probably be minimal threat if the sludge were applied at nitrogen rates not exceeding fertilizer nitrogen recommendations for the crop grown, but higher rates or application outside of seasons of nitrogen uptake might result in a hazard. Data from a liquid sludge application study (Duncomb *et al.* 1982) suggest that the ratio of nitrogen application to crop removal should not exceed approximately 2 to prevent nitrate buildup below the rooting zone of crops.

It should be kept in mind that land application sites are not the only source of nitrate in groundwater. Many groundwaters are naturally high in nitrates, e.g., that in the vicinity of San Angelo, Texas (Hossner *et al.* 1978), and in urban areas on-site absorption fields and lawn fertilizers have been shown to be sources of nitrates in groundwater (Porter 1980).

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