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VTEC & AGRICULTURE

REPORT BY KAARIN GOODBURN AND THE ECFF VTEC WG

14/10/98

VTEC AND AGRICULTURE

REPORT OF THE ECFF VTEC WORKING GROUP

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F. ECFF VTEC Working Group Membership

List of Tables and Illustrations

Abbreviations

ACMSF	Advisory Committee on the Microbiological Safety of Food (UK)
ADAS	UK consultancy, formerly the Agriculture Development Advisory Service
CAMR	Centre for Applied Microbiology Research (UK)
CIWEM	Chartered institute for Water and Environment Management (UK)
DETR	Department of Transport and the Regions (UK)
DH	Department of Health (UK)
DoE	Department of the Environment (UK) – now DETR
EA	Environment Agency (EA)
EPA	Environment Protection Agency (US)
MAFF	Ministry of Agriculture, Fisheries and Food (UK)
NRA	National Rivers Authority (UK) – now part of EA
NRC	National Research Centre (USA)
PHLS	Public Health Laboratory Service
RCP	UK Royal Commission on Environmental Pollution
WRc	Water Research Centre

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A. Introduction

This initial report has been developed in conjunction with the ECFF VTEC Working Group to provide a compilation of information on the occurrence and control of VTEC, and to make recommendations as to any changes required to be made to the ECFF Guidelines.

The report is not complete, more information being sought on a number of areas, particularly on the microbiological quality and use of organic materials other than sewage sludge. This draft should therefore be viewed as a working document that will be under continuous revision. Initial recommendations are made, however, with respect to the current requirements of the ECFF Guidelines and of areas for further work. These sections require detailed consideration by ECFF and its Members, and are expected to be developed further.

Although the Report focuses on VTEC, it should be borne in mind that there would appear to be potential for the transferability of VT (verocytotoxin) genes to other microorganisms. There is concern with respect to other pathogens, especially zoonotic, with a low infectious dose and causing severe disease. It is believed that, in many cases, the sources of VTEC and such organisms are similar.

The product focus is chilled ready to eat foods as these will not necessarily be subject to a treatment which would eliminate the risk of VTEC being present. ECFF will therefore be primarily concerned with fruit and vegetables, but raw protein may be an area of interest with products such as salami that is used, for example, on pizzas and in sandwiches. Concerns regarding other foodstuffs such as unpasteurised dairy products potentially resulting from the consideration of the report may need to be highlighted by ECFF to the appropriate sector associations.

Work will continue on information gathering through the Working Group, which will meet again in September 1998.

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B. Executive Summary

Is there an issue?

Verocytotoxigenic E coli (VTEC) is a group of toxigenic *E coli* in the Enterobacteriaeceae family, of which the main concern is currently *E coli* O157:H7. VTEC is an emerging group of pathogens which is causing an increasing number of major outbreaks of severe illness and deaths worldwide[1], and should therefore be treated as seriously as Salmonella. VTEC is of animal origin if present on produce. It is more persistent in the environment than Salmonella.

An overwhelming body of evidence indicates[1,2,3] that VTEC will continue to be an issue in the future, impacting on the food business and on regulation globally. *E. coli* O157:H7 is believed to be the most important VTEC in terms of human health due to the very low infectious dose and the severity of the disease it causes, but others are noted. The VTEC Working Group is concerned that changes in agricultural practices could exacerbate food safety issues, such as VTEC.

Organic materials in agricultural practice

Organic materials used in agricultural practice include treated and untreated human sewage sludge, and exempt animal wastes (see section 3.2).

There is evidence of survival of Salmonella in untreated human sewage sludge. This may be indicative of the behaviour of VTEC[4,5,6]. Cattle and other animals[7,8,9] have been shown to carry and/or shed *E. coli* O157, providing another potential source of contamination of the agricultural environment and food chain. In addition, under laboratory conditions, *E. coli* O157 has been shown to persist in soil cores and river water[10] for significant periods.

Minimising the risk

There is currently little guidance available on the control of VTEC in the field and little work has been carried out to determine which control measures are the most effective. Better understanding is required and effective controls need to be established at the point of entry of VTEC into the food chain, i.e. during primary production in the field, in order to break the cycle of infection. The industry needs to be involved earlier in the food chain, in agricultural practices relating to animal and human excretory waste, working in partnership with the farming sector in the EU and rest of the world.

The VTEC Working Group recognises the significance and importance of having a Best Agricultural Practice document at European level, for example through CIAA, and recommends that this be developed.

Further Reading

1. Ammon (1997)
2. Little *et al* (1997).
3. Johnson *et al* (1996)
4. Beuchat & Ryu (1997)
5. Grant *et al* (1996)
4 Watkins & Sleath (1981)
6. Armstrong *et al* (1996)
7. Porter *et al* (1997)
8. Faith et al (1996)
9. Maule (1997).

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C. Initial Recommendations

a) ECFF Guidelines

Text from the current Guidelines appears below in bold italics, followed by the initial recommendation of the VTEC Working Group.

Purchasing of Raw Materials

'Suppliers should be selected in order to obtain raw materials of required quality and safety. They should be audited as appropriate. The selection of suppliers should include an assessment of the Quality Management System.'

- Because there is not currently sufficient information on the relative risks of various agricultural practices, it is not possible to state what best agricultural practice would be. Manufacturers therefore need to consider carefully the particular nature of potential contamination of raw materials.

'Raw materials should be purchased to agreed specifications and from suppliers who comply with GMP and HACCP-based systems, if appropriate.'

- Because some coliforms are present naturally on produce as part of the normal flora, their use as indicators of VTEC may have no relevance to food safety. While microbiological criteria for VTEC may be employed, these should be used with extreme caution when considering the safety of a raw material. The Working Group is currently not aware of a satisfactory indicator for VTEC.
- If a supplier is using HACCP, this will facilitate auditing.
- If HACCP is not used by a supplier, the supplier should have at least identified and analysed the hazards using an approach that could be audited.
- Hazard analysis should include consideration of VTEC as a potential contaminant

'Raw materials should be checked against appropriate acceptance criteria as necessary.'

- The use of microbiological testing is currently of limited value, owing to the statistical uncertainty involved in sampling and since methods for many VTEC are still under development.
- It is appropriate to use monitoring and control systems based on non-microbiological criteria, which could include physical or visual inspection of crops, e.g. their cleanliness, physical quality, the nature and quality of the packaging.

Storage

'Raw materials should be stored in adequate, specifically designated areas and under hygienic conditions that prevent contamination by microorganisms, insects, rodents, foreign bodies and chemicals and to avoid adverse physical conditions.'

- The potential for cross contamination between raw materials and produce needs to be considered in the design and management of storage areas.
- Incorrect storage (e.g. at too high a temperature) can result in growth of VTEC, therefore storage conditions should minimise the potential for growth of contaminants on raw materials.

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- Damaged and/or diseased raw materials should not be taken into storage. Damaged produce is known to be able to support the growth of certain organisms, leading to tissue deterioration and potentially rapid growth of Enterobacteriaeceae, which includes VTEC.

Preparation

'The raw materials preparation area should be designed to hold and handle the range of raw material types to be prepared, in a hygienic manner.'

- The potential for contamination between materials needs to be considered in the design and management of storage areas.

- Storage conditions at this stage should also minimise the potential for growth of contaminants on raw materials.

'Raw materials should, when necessary, be decontaminated before use and chilled to control microbial growth.'

- The ECFF definition of 'decontamination' ('*destruction of microorganisms in a product by heat and/or chemicals or other means such that vegetative pathogens are absent or their numbers have been reduced by at least 6 logs*') means that this requirement cannot be met easily with produce without using heat. Where lesser reductions are achieved this should be taken into account in the overall risk assessment.

- Achieving the absence of coliforms is not always feasible, but the Group believes that treatments to reduce the level of VTEC are worthwhile and should be effected. It should be recognised that this is additional to Good Agricultural Practices and cannot replace them. Washing processes need to be designed effectively and adequately and the efficacy of any 'decontamination' process must be understood and not itself lead to any conditions that could aggravate the presence of any potential contaminants.

b) Areas Requiring Further Work

The items appearing below are an initial list of topics to be considered by the ECFF membership prior to their being put forward to relevant organisations for further work.

1. Farm/fields

a) Types of waste
   - Levels of contamination
   - Persistence and relevance of CAMR work to actual survival in the soil and water
   - Effective treatments

b) Application to land
   - Effect of time of year
   - Effect of means of application

c) Silage production
d) Vectors
e) Irrigation water

2. Raw Materials

a) Produce damage effects
b) Control of seed contamination
c) Controls applied in hydroponics
d) What is the extent of contamination – levels, seasonal effects

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e) Measurement techniques and indicator organisms

3, Factory

a) Raw material correct storage conditions on receipt: temperature, RH
b) Decontamination methods
c) Effective combinations of pH and acid in products

4. VTEC and their effects

a) Surveillance systems and case ID
b) Relative importance of types – *E. coli* O157:H7 is believed to be the most important VTEC in terms of human health owing to its prevalence, but others are noted. (See listing at http://www.sciencenet.com.au/frames/feature/vtec/brief03.html)

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D. CONSOLIDATED INFORMATION

1. DEFINITIONS

One of the problems that are becoming increasingly recognised is that the terminology used to describe diarrhoeagenic *E. coli* is both complex and by no means definitive. Since it was first recognised (some 50 years ago) that *E. coli* could cause diarrhoea, an array of virulence factors have been discovered and a number of categories of diarrhoeagenic *E. coli* have been proposed, generally based on the presence of non-overlapping virulence factors.

There are already a number of documented studies describing isolates which do not fit neatly into any of the recognised categories of diarrhoeagenic E. coli*. This should not be surprising considering that the virulence factors are encoded on 'pathogenicity islands', bacteriophages, transposons and transmissible plasmids. Some of these elements have also been found in other members of the* Enterobacteriaceae. *Therefore it should be anticipated that there will be other combinations of known and currently unknown virulence factors appearing in the group of organisms currently called* E. coli*, and other members of the* Enterobacteriaceae*. The ECFF VTEC Working Group acknowledges the limitations of the currently adapted scheme for classifying diarrhoeagenic* E. coli. Where terms are used in the text these are as appear in the source material.

Information sources*: 'Bacterial Pathogenesis: A Molecular Approach' (Salyers and Whitt, ASM Press, 1994); 'Management of Outbreaks of Foodborne Disease' (UK Department of Health, 1994); 'Growing Concerns and Recent Outbreaks Involving non-O157:H7 Serotypes of VTEC' (Johnson* et al *(1996); 'Backgrounder:* E. coli *O157:H7' (http://www.inform.umd.edu/EdRes/Topic/AgrEnv/ndd/health/)*

Coliforms Coliform bacteria are members of the family *Enterobacteriaceae*, but are not a taxonomic group in their own right. A number of definitions have been proposed, but they generally centre on the ability of the organism to ferment lactose with the production of gas, in the presence of bile salts or other surface-active agents. By and large, they are represented by four genera: *Citrobacter, Enterobacter, Escherichia* and *Klebsiella*.

EaggEC Enteroaggregative *E. coli* – cause persistent diarrhoea in children. These bacteria have been shown to produce a low molecular weight enterotoxin which may have a mode of action similar to other heat stable enterotoxins (Sts). EaggEC adhere to mucosa in patches, are not invasive, and produce two toxins (ST-like and haemolysin-like).

E. coli The genus *Escherichia* is a typical member of the *Enterobacteriaceae* that have their principal habitat in the bowel of humans and animals. It is closely related to a number of other genera in the family, most notably *Shigella*, to the extent that the two genera could be combined. Distinction is maintained because of the separate clinical and epidemiological importance of the various species.

***E. coli* O157** [See FDF Questions and Answers] This serogroup is one of 176 groups of *E. coli* that have been identified over the past 50 years, about 60 of which, including the O157 serogroup, are associated with intestinal diseases of animals and man. This serotype is the one most frequently associated with Human HC and HUS (see below) in the USA and several other countries (Johnson *et al*). Serogroup O157 is an enterohaemorrhagic *E. coli* (EHEC). Its virulence factors include Toxins, Adherence Factors and Haemolysins. *E coli* O157 is easily distinguished because of its inability to ferment sorbitol.

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EHEC Enterohaemorrhagic *E. coli* - see VTEC. EHEC strains are similar to EPEC strains except that they produce one or more vero cytotoxins (VTs, also called verotoxins) or Shiga-like toxins (SLTs or Stx), so-called because of their close structural, functional and sequence homology to the Shiga toxin of *Shigella dysenteriae*. Two principal VTs have been identified, VT1 and VT2 (or SLT-I, SLT-II or Stx1, Stx2) and variants of VT2 have been described in strains of animal and human origin.

EHEC are a subset of VTEC, that may be associated with bloody diarrhoea. Assigning the VTEC label to an isolate is based on an association with HC, production of VTs and possession of a large plasmid (EHEC plasmid) which codes for the EHEC haemolysin. There are a number of VTEC serotypes associated with HC or HUS which lack some of the pathogenic features typical of EHEC. Strains classified as EHEC possess the virulence characteristic of both EPEC and VTEC.

EIEC Enteroinvasive *E. coli* - less common cause of Travellers' Diarrhoea. Occasional outbreaks in industrialised countries. Causes dysenteric enteroinvasive *E. coli* enteritis which is indistinguishable symptomatically from the dysentry caused by *Shigella* spp, although no cases of HUS have been seen in people with EIEC dysentry. EIEC do not produce Shiga toxin. EIEC strains actively invade colonic cells and spread laterally to adjacent cells. A number of different serogroups are associated with EIEC, 3 of which predominate. Relatively low infectious dose, but can vary with gastric function and acidity of gastric contents. Sources: Human cases and excreters. Spread: Water, faecal-oral route. Has been associated with imported cheese imported into the UK.

Enterobacteriaeceae The nomenclature and classification of members of the family *Enterobacteriaeceae* was defined until recently by biochemical and antigenic analysis. Molecular techniques have better defined the relationships of all the organisms in the family. All are Gram negative and rod-shaped; do not form spores; are motile by peritrichous flagella or non-motile; grow on peptone or meat extract media; grow well on MacConkey agar; grow aerobically and anaerobically; ferment glucose; are catalase positive and oxidase negative; reduce nitrate to nitrite; and have a 39 to 59% guanine-plus-cytosine (G+C) content of DNA.

In the *Enterobacteriaceae*, the genera *Salmonella, Shigella, Escherichia*, and *Yersinia*, are the most significant for human disease, especially in association with enteric illness. The family also includes other genera including *Citrobacter, Enterobacter, Klebsiella, Morganella, Proteus, Providencia*, and *Serratia,* which are also known to be involved in human infection. There are also a number of less prominent genera.

EPEC Enteropathogenic *E. coli*. These strains are more invasive than ETEC or EaggEC strains and cause inflammatory response. These strains adhere to the intestinal mucosa and cause extensive rearrangement of host cell actin. The severe diarrhoea they cause is probably not due to an enterotoxin but to disruption in water absorption by mucosal cells.

ETEC Enterotoxigenic *E. coli* - a principal cause of Travellers' Diarrhoea, also a major cause of dehydrating diarrhoea in infants and children in less well developed countries. Not highly pathogenic, usually $>10^6$ bacteria required to cause illness. Sources: human cases and excreters. Spread: foodborne, waterborne, faecal-oral route, person to person. ETEC strains are similar to *Vibrio cholerae*. They adhere to the mucosa but do not invade and they

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produce 2 toxins (LT and ST). Genes for these toxins are carried on plasmids.

Exempt waste Waste which can be spread on agricultural land without a waste disposal licence (Framework Directive on Waste 75/442/EEC amended by 91/156/EEC): waste soil or compost; waste wood, bark or other plant matter, waste food, drink or materials used in or resulting from the preparation of food and drink; blood and gut contents from abattoirs; waste lime; paper waste sludge, waste paper and de-inked paper pulp, dredgings from any inland waters; textile waste; septic tank sludge; sludge from biological treatment plants; and waste hair and effluent treatment sludge from tanneries.

HC Haemorrhagic Colitis – the acute disease caused by *E.* coli O157:H7 and other VTEC. 0-15% of HC victims may develop HUS.

HUS Haemolytic Uraemic Syndrome. Disease characterised by renal failure and haemolytic anaemia. The disease can lead to permanent loss of kidney function. In the elderly, HUS, plus two other symptoms, fever and neurological symptoms, constitutes thrombotic thrombocytopenic purpura (TTP). This illness can have a mortality rate as high as 50% in the elderly.

Sludge Residual sludge from sewage plants treating domestic or urban waste waters and from other sewage plants treating waste waters of a composition similar to domestic and urban waste waters.

STEC Shiga toxin-producing *E. coli* . Synonym for VTEC (see below).

VTEC Verocytotoxin-producing *E. coli*. VTEC are responsible for a wide spectrum of diseases, including simple diarrhoea, HC and HUS. High infectivity: possibly as few as 10 cells required to cause illness. Source: Undercooked beef, contaminated/unpasteurised milk, raw vegetables, water, animals and animal excreta. Among food animals, ruminants have the highest rates of VTEC carriage. With the exception of a number of serogroups, many animal serogroups of VTEC differ from those isolated from humans. Surveys have shown that the cattle reservoir contains more than 100 serotypes of VTEC (Johnson *et al*). Spread: foodborne, waterborne, secondary cases by faecal-oral route from human cases.

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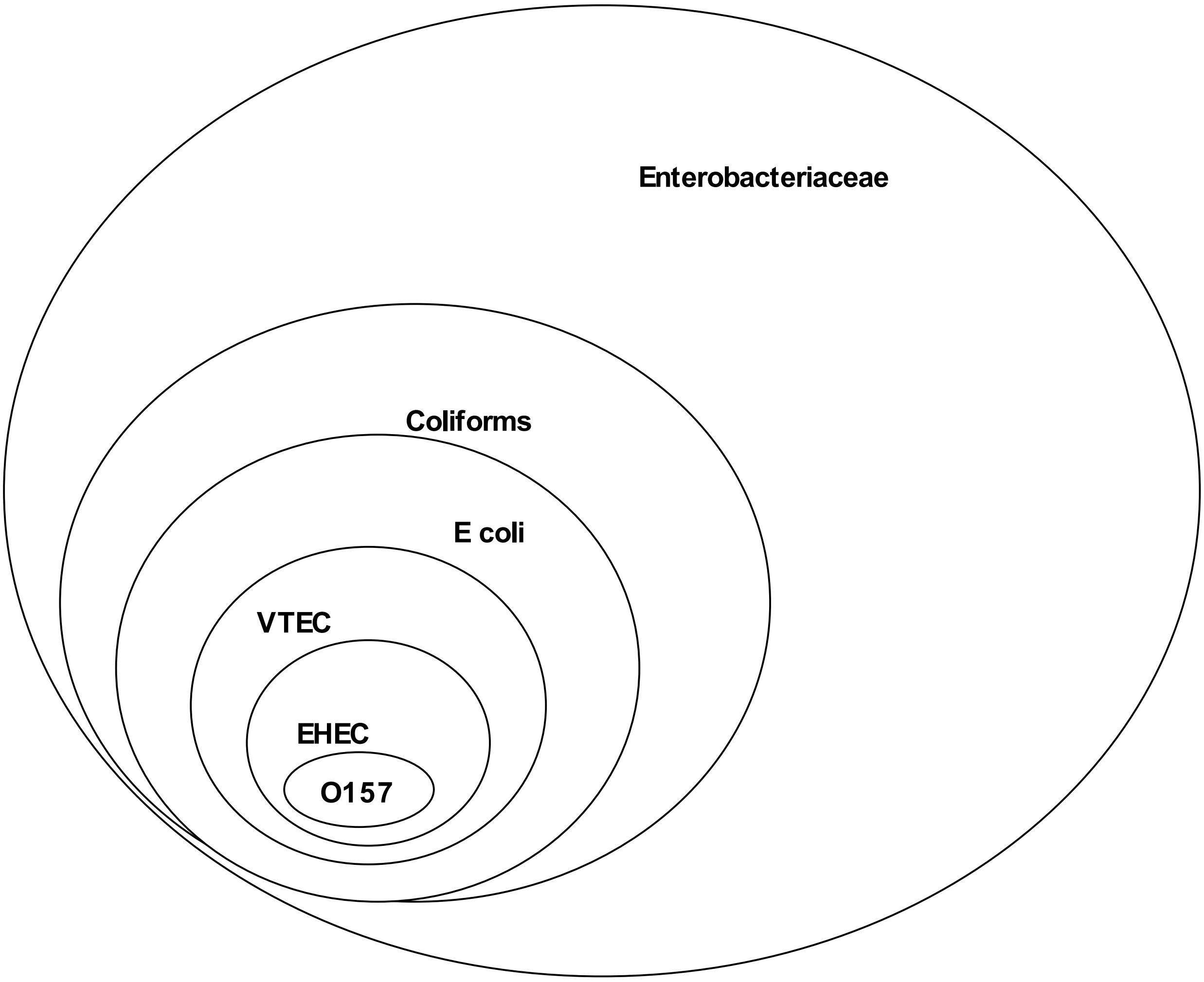
Relationship Between Enterobacteriaceae and VTEC



- There are some stains of *E. coli* O157:H7 that do not posses either of the genes required for vero cytotoxin production. It is thought that these isolates probably lost the ability because of their genetic instability – loss of phage that encode for the toxin production. Because virulence factors are encoded on mobile elements (transposons, plasmids, phages) or pathogenicity islands (shared amongst many genera), it is very likely that there will be a continuum of combinations of virulence factors arising in different isolates, making neat categorisation increasingly difficult.

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2. IS THERE AN ISSUE?

Variable information is available from European countries which is reflected in the following data.

2.1 Summary

VTEC is an emerging group of pathogens which is causing an increasing number of major outbreaks of serious disease and deaths worldwide[1], and should therefore be treated at least as seriously as Salmonella. VTEC is of animal origin if present on produce. It is persistent in the environment.

An overwhelming body of evidence indicates[1,2,3] that VTEC will continue to be an issue in the future, impacting on the food business and on regulation globally. *E. coli* O157:H7 is believed to be the most important VTEC in terms of human health due to the very low infectious dose and the severity of the disease it causes, but others are noted. The VTEC Working Group believes that VTEC is probably representative of problems caused by changes to agricultural practice. VTEC may require a reassessment of the significance of coliforms in foods.

Further Reading

1. Ammon (1997)
2. Little *et al* (1997)
3. Johnson et al (1996)

2.2 Core Information

Infectious disease agents associated with wastewater and sewage sludge include bacteria, viruses and parasites. According to the US National Research Council's 1996 report on the Use of Reclaimed Water and Sludge in Food Crop Production, it is reasonable to assume that any or all of these infectious agents might be present in the water and soil fractions of raw sewage.

Table 1
Examples of Pathogens Associated with Raw Domestic Sewage & Sewage Solids (NRC, 1996)

Pathogen Class	Examples	Disease
Bacteria	*Shigella sp*	Bacillary dysentery
	Salmonella sp	Salmonellosis (gastroenteritis)
	S typhi sp	Typhoid fever
	V cholerae	Cholera
	EHEC	A variety of gastroenteric diseases
	Yersinia sp	Yersiniosis (gastroenteritis)
	Campylobacter jejuni	Campylobacteriosis (gastroenteritis)
Viruses	*Hepatitis A virus*	Infectious hepatitis
	Norwalk virus	Acute gastroenteritis
	Rotavirus	Acute gastroenteritis
	Poliovirus	Poliomyelitis
	Coxsakie virus	'flu-like' symptoms
	Echovirus	'flu-like' symptoms
Protozoa	*Entamoeba histolytica*	Amebiaisis (amoebic dysentery)
	Giardia lamblia	Giardiasis (gastroenteritis)
	Cryptosporidium sp	Cryptosporidiosis (gastroenteritis)
	Balantium coli	Balantidiasis (gastroenteritis)
Helminths	*Ascaris sp*	Ascariasis (roundworm infection)
	Taenia sp	Taeniasis (tapeworm infection)
	Necator americanus	Ancylostomiasis (hookworm infection)
	Trichuris trichuria	Trichuriasis (whipworm infection)

2.2.1 Infectious Dose

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Tilden *et al* (1996) calculated that the estimated infectious dose in the US Garibaldi fermented meat incident was 2-5 organisms in one child and 7 organisms in a 24-year old male, based on the highest contamination level detected. The organisms were likely to be stressed.

However, the WRc (1998) contends that the concept of a minimum infectious dose has no justification since infectivity depends not only upon the invasiveness and pathogenicity of the pathogen but also on the immunity and general state of health of the individual.

2.2.2 Prevalence

2.2.2.1 Throughout Europe

Ammon (1997), reported that the main reservoir for EHEC is cattle and other ruminants, and many outbreaks have been associated with beef products and raw milk. A wide range of other food products have been implicated in outbreaks of EHEC infections, such as raw milk cheese, yoghurt, fermented sausage, apple juice, seed sprouts, and lettuce. Contaminated water and direct or indirect contact with animals are other routes of transmission.

7 countries have surveillance systems. EHEC infection is statutorily notifiable in 3 countries: Austria, Finland and Sweden. 5 countries – Belgium, Finland, Italy, Netherlands and the UK, have sentinel systems. England, Wales and Scotland have comprehensive national laboratory reporting schemes for Verocytotoxigenic *E. coli* (VTEC) O157. *Ad hoc* sources exist in 9 countries. Some countries have more than one data source to collect information about EHEC infections and 2 countries reported collecting no data on EHEC infections. Data sources in Germany vary from year to year.

10 countries reported EHEC infections in 1996. The reported incidence in 1996 varied between 0.1 cases per million inhabitants in Spain to 20.3/million in the UK

Table 2
Number of reported EHEC infections in Europe, 1996

Country	EHEC Infections	Millions of Inhabitants	Per million Inhabitants
Spain	4	39.6	0.1
Italy	9	57.1	0.2
Netherlands	10	15.4	0.6
Finland	5	5.1	1.0
Denmark	6	5.2	1.2
Austria	11	8.0	1.4
Germany	314	81.5	3.9
Belgium	52	10.0	5.2
Sweden	118	8.7	13.6
UK	1180	58.1	20.3
• Northern Ireland	14	1.6	8.8
• Wales	36	2.9	9.2
• England	624	48.5	12.4
• Scotland	506	5.1	99.2

From 1992 to 1996, 7 countries reported 67 outbreaks caused by EHEC. 56 were reported by the UK (39 from England and Wales, 17 from Scotland) and 11 by the rest of Europe.

Data were received on 47 outbreaks. Food was the likely vehicle of transmission in 23 of the 47 outbreaks, 7 transmitted from person to person, 3 were due to animal contact, 1 was waterborne and in 13 outbreaks the mode of transmission remained unknown. In 1 Italian outbreak 3 different serotypes (O157, O111, O86) were identified.

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Table 3
Details for 46 reported outbreaks of EHEC infections or HUS in Europe 1992-1996

Year	Country	No. affected	No. with HUS	No. dead	Serotype	Likely vehicle or mode of spread
1992	Austria	9	0	0	O157	Foodborne
1992	England/Wales	5	0	2	O157	Unknown
1992	England/Wales	19	0	1	O157	Foodborne
1992	England/Wales	37	5	0	O157	Person to person
1992	England/Wales	3	0	0	O157	Foodborne
1992	England/Wales	4	0	0	O157	Foodborne
1992	France	n.a.	10	0	O111:B4	Person to person
1992	Germany	41	3	1	O157:H7	Person to person
1992	Italy	***7***	***9***	1	O111	unknown
1992	Scotland	5	1	0	O157	waterborne
1992	Scotland	5	0	0	O157	Person to person
1992/3	France	n.a.	4	1	O119:B14	Raw milk cheese
1993	England/Wales	7	3	0	O157	Milk
1993	England/Wales	9	6	0	O157	Unknown
1993	England/Wales	9	2	0	O157	Foodborne
1993	England/Wales	7	3	1	O157	Person to person
1993	England/Wales	17	1	0	O157	Beefburger
1993	England/Wales	4	1	0	O157	foodborne
1993	England/Wales	17	3	0	O157	Unknown
1993	England/Wales	5	5	0	O157	Foodborne
1993	Italy	15	14	1	O157/O111/O86	Unknown
1993	Scotland	5	3	0	O157	Person to person
1994	England/Wales	2	0	0	O157	Animal contact (cattle)
1994	England/Wales	7	4	1	O157	Animal contact (cattle, goats)
1994	England/Wales	6	1	0	O157	Foodborne, person to person
1994	England/Wales	3	1	0	O157	Foodborne, person to person
1994	England/Wales	12	2	0	O157	Foodborne, person to person
1994	France	n.a.	4	0	O103	Raw milk goat cheese
1994	Scotland	24	1	0	O157	Burger meat
1994	Scotland	100	9	0	O157	Milk
1994	Scotland	8	3	0	O157	Milk
1994	Scotland	16	n.a.	n.a.	O157	Burger meat
1994	Scotland	4	1	0	O157	Animal contact
1994	Scotland	22	1	0	O157	Cheese
1994	Scotland	16	0	0	O157	Foodborne
1995	Ireland	8-15	1	0	O157:H7	unknown
1995	Sweden	81	n.a.	0	O157	Food-unknown
1995	Scotland	5	0	0	O157	Water-and foodborne
1995/6	Germany	n.a.	28	3	O157:H-, sf*	Unknown
1996	Scotland	8	0	0	O157	Unknown
1996	Scotland	496	n.a.	19	O157	Foodborne
1996	Scotland	3	n.a.	n.a.	O157	Person to person
1996	Scotland	2	n.a.	n.a.	O157	Unknown
1996	Scotland	n.a.	n.a.	n.a.	O157	Unknown
1996	Scotland	n.a.	n.a.	n.a.	O157	Unknown
1996	Scotland	n.a.	n.a.	n.a.	O157	Unknown
1996	Sweden	10	n.a.	0	O157	Person to person

*sf sorbitol-fermenting strain of *E. coli* O157
n.a. not available

Table 4

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Trend of EHEC Infections for European Countries 1992-1996*

	1992	1993	1994	1995	1996
Belgium	n.a.	n.a.	29	38	52
Germany	36	32	**	195**	314
Sweden	0	2	3	114	118
UK	627	540	685	1138	1180

* Only countries which reported more than 20 EHEC infections in 1996
** The figures for 1994 and 1995 are combined
n.a. not available

Ammon (1997) states that 'The 200-fold difference in the rate at which European countries report EHEC infections and the fact that nearly all of the outbreaks have been reported in one country, suggest markedly varying sensitivity of data sources rather than real differences, although the possibility of real difference cannot be excluded. If all countries adopted surveillance systems, data from different countries could be compared and the value for determining trends would increase.'

'If it can be assumed that the proportion of people infected with EHEC who develop HUS (5%-10%) is relatively constant, data on the number of cases of HUS can be used to estimate the true number of EHEC infections. HUS surveillance should be adopted as well as EHEC surveillance.'

2.2.2.2 E. coli O157 in the UK, (Chapman, 1997)

Following a cluster of cases of VTEC O157 infection in Sheffield in May/June 1992, an abattoir study by PHLS showed the organism to be present in 4% of cattle at slaughter and on up to a third of carcasses from rectal swab-positive animals. VTEC O157 was isolated for the first time from a food source in the UK in May 1993.

During a 2-year surveillance study of VTEC O157 (DH-funded), faecal samples from 4800 cattle, 1000 pigs, 1000 sheep and 1000 chickens were collected over a one year period and examined for VTEC O157. Strains of VTEC O157 were isolated from 15.7% of cattle with a monthly prevalence which varied from 6 to 37%. VTEC O157 was isolated from 2.2% of sheep but not from either pigs or chickens. In the second year of the study, 5093 samples of raw retail processed meats were examined. Despite its prevalence in cattle being much higher than in sheep, VTEC O157 was isolated from 2.9% of lamb products and 1.1% of beef products, with the highest prevalence (3.8%) being in lambburgers. The study is ongoing and work is in progress to try to explain this higher prevalence in lamb products.

More recently, VTEC O157 have been isolated from farmed deer, though human cases have not been associated with this source in the UK. During on-farm investigations in cases associated with farm visits, VTEC O157 have been isolated from faecal samples from adult cattle, calves, 3 different breeds of sheep, 2 different breeds of pigs, goats and a pony.

2.2.2.3 EHEC In Sweden Since 1995 (Andersson *et al*, 1997)

Since 1988, between 0 and 3 human cases of EHEC have been reported each year. Half of the reported cases were infected with *E. coli* O157. The first cases during 1995 were reported in July. During the autumn of 1995 and the first 3 weeks of 1996, 99 confirmed cases were caused, of which 24 contracted HUS. The epidemiological investigation showed that there were at least 2 different outbreaks. Over 2200 food samples from refrigerators and from freezers in patients' homes and from stores where food had been bought were investigated for the presence of *E. coli* O157, with negative results. The case-control study did not implicate any single food item.

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Each year since 1995, about 100 indigenous cases of *E. coli* O157 infection have been reported in Sweden. One special subtype seems to have settled, especially on the west coast of Sweden. This type is also found among cattle in Sweden.

The National Food Administration surveyed EHEC in beef during a period of 10 weeks in the spring of 1996. 482 samples of imported beef (3rd countries and EU countries) and 543 samples of Swedish beef were analysed for EHEC. In Swedish meat, no *E. coli* O157 was detected. In foreign beef, one sample (0.2%) was positive for VT-producing *E. coli* O157.

Two small studies have also been performed, one on fermented meat products (60 samples) and one on cheese (14 samples). All samples were negative for *E. coli* O157.

An officially conducted prevalence study of *E. coli* O157 in cattle was initiated in April 1996 and stopped in August 1997. Faeces samples, representative of the whole population, were collected at the 16 main abattoirs producing 90% of all Swedish beef. 3,000 cattle were individually sampled during this period. 1.2% were identified as VT-producing (VT1 and/or VT2) and positive for eaeA genes. They also agglutinated for *E. coli* O157.

2.2.3 Potential Routes of Entry into Food Supply Chain

E. coli O157:H7 causes an estimated 20,000 infections and 250 deaths each year in the USA (Armstrong, 1996). Outbreaks involving acidic foods such as mayonnaise and apple cider have underscored the unusual acid tolerance of this organism. Acidic foods (defined by the US FDA's Retail Food Sanitation Code as those with a pH of less than 4.6) are generally considered to be at low risk for transmission of pathogenic bacteria, but *E. coli* O157:H7, under certain circumstances, can survive a pH as low as 2.0 and can persist for up to several weeks when inoculated into apple cider or mayonnaise (Armstrong, 1996). Leaf lettuce has also been implicated in 2 separate [USA] outbreaks in 1995. It is known that *E. coli* O157:H7 can grow on lettuce at temperatures as low as 12°C. (Armstrong et al, 1996)

When a ruminant feeds, rumen bacteria and protozoa break down the complex polysaccharides into methane and carbon dioxide, and short chain fatty acids. The fatty acids are absorbed by the animal and used as carbon and energy sources. The concentration of the fatty acids and pH of the rumen depend upon the nutritional status of the ruminant: the concentration of volatile fatty acids will be high and pH low in the rumen of a well-fed animal, and the opposite true in a starving animal. Since volatile fatty acids inhibit the growth of enteric organisms, it would be expected that the rumen fluid from a well-fed animal would contain few enteric organisms, whereas that from a poorly fed animal [*such as one prepared to go to slaughter*] might even support their growth. In well-fed animals, when Salmonella was introduced artificially in 1960s experiments, it was rapidly eliminated from the rumen fluid and could not be detected in the faeces. When food was cut off for 2-3 days, *Salmonella* would grow in the fluid and would be shed in the faeces in detectable numbers (Armstrong et al, 1996). However, there are complicating issues, as evidenced by research carried out by Russell at Cornell University (Couzin, 1997). This work indicates that grain-based cattle diets promote the growth of '*E. coli*' that can survive the acidity of the human stomach and cause intestinal illness. Cattle are generally fed starch-containing grains to increase growth rate and produce tender meat. Because the gastrointestinal tract digests starch poorly, Russell explains, some undigested grain reaches the colon, where it is fermented. When the grain ferments and acetic, propionic and butyric acids accumulate in the animal's colon, a large fraction of '*E. coli*' produced are the acid-resistant type. Russell stated that 'Grain does not specifically promote the growth of *E. coli* O157:H7, but it increases the chance that at least some '*E. coli* ' could pass through the gastric stomach of humans. The carbohydrates if hay are not so easily fermented, and hay does not promote either the growth or acid resistance of '*E. coli*'." When cattle were switched from grain-based diets to hay for only five days, acid resistant '*E. coli*' could no longer be detected. In these studies, beef cattle fed grain-based rations typical of commercial feedlots 'had 1 million acid-resistant *E.* coli' per gram of faeces, and dairy cattle fed only 60% grain also had high numbers of acid-resistant bacteria. In each case, the high counts could be explained by grain fermentation in the intestines. By comparison, cattle fed hay or grass had only acid-

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sensitive '*E. coli*' , and these bacteria were destroyed by an 'acid shock' that mimicked the human stomach.

Porter et al (1997) examined the survival of *E. coli* O157 in pond water and occurrence of *E. coli* O157 in a farm environment. The strain inoculated into sterile pond water decreased by 1 log after 5 days, then numbers remained constant for the remainder of the experiment (21 days total, incubated at 13°C, pH 8.35). However, with non-sterile pond water, numbers decreased over the 3 week incubation period, from 10^6/ml to none detected after 21 days (13°C, pH 7.79). These results suggest that *E. coli* O157 would not survive in water for long periods. However, there have been a number of outbreaks associated with drinking water/well water. In the environmental work, *E. coli* O157:H7 was only found in places on the farm with a faecal load/presence (milking parlour and slurry pit areas). No *E. coli* O157:H7 was found in soil or grass with the sampling regime used.

Faith *et al* reviewed a number of papers relating to incidence in cattle. In a Washington State survey, *E. coli* O157:H7 was isolated from 10 of 3, 570 dairy cattle (0.3%), 10 of 1,412 beef cattle (0.7%), and 2 of 60 feedlot beef cattle (0.3%). The herd prevalences of *E. coli* O157:H7 strains in dairy and beef cattle were 8.3% (5 of 60 herds) and 16% (4 of 25 herds), respectively. (Hancock *et al*, 1994)

In another survey conducted in 1991 and 1992, preweaned dairy calves in 28 states throughout the US were analysed for *E. coli* O157:H7 and 0.4% (25 of 6,894) of the calves and 1.8% (19 of 1,068) of the herds tested positive (Hancock *et al* ,1996).

The Faith *et al* survey found that 7.1% of the dairy herds were positive for *E. coli* O157:H7. The higher prevalence values found compared with the national survey and Washington study were probably due to the size of the faecal samples collected and analysed in the former studies. On subsequent visits to previously positive and negative farms, the *E. coli* O157:H7 status of 3 herds changed. These findings support the assertion that the O157:H7 status of a herd cannot be ascertained from a single test involving a limited number of cattle in a herd. During the follow-up study, it was observed that positive animals shared the same bar, pen or water, occupied a pen that previously contained a positive animal, or were located in an area that was close to a positive barn or pen.

Grouping of preweaned calves has been associated with *E. coli* O157:H7 status of herds in another study (Garber *et al*, 1995). Similarly, in a study performed with sheep, *E. coli* O157:H7 was transmitted from inoculated lambs to mothers. (Kudva *et al*, 1995). Kudva, in 1996, also found that faecal shedding was transient and seasonal, with 31% of sheep positive in June, 5.7% in August and none in November. The sheep showed no signs of disease throughout the study. Diet influenced faecal shedding – sheep apparently negative began to shed when they were removed from confinement and their feed was changed from alfalfa pellets to sagebrush and bunchgrass (Kudva *et al,* 1997).

Collectively, these results suggest that transmission among animals and contact with areas previously contaminated by animals shedding *E. coli* O157:H7 are important factors for its dissemination in a herd.

2.2.4 <u>Persistence in the Environment</u>

Work at the Centre for Applied Microbiology Research (CAMR) in the UK has focused on the survival of E coli O157 on surfaces and in the environment, and the effects of chemicals. (Maule, 1997)

Using CT/SMAC media and samples of river water incubated at 18°C, numbers of *E. coli* O157 only reached undetectable (in 500 ml) levels after 27 days. Initial inoculum was approximately $10^{7.5}$.

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Survival in cattle faeces at 18°C showed a slower and less marked decrease (<2 log decrease) in numbers even after 50 days. It had been found that survival was better at lower temperatures. Work had been carried out comparing survival at 4 and 18°C.

In cattle slurry, using strain AM-1 (isolated from the environment and not known to be pathogenic) had been found to still be present after 9 days, but thereafter declining to undetectable levels.

Maule also carried out experiments on the survival of *E. coli* O157 in soil cores surface inoculated (on grass) and allowed to permeate whilst being incubated under continuous illumination at 18°C. Samples were homogenised and tested using different media. The initial trend in all media was for numbers to decline (not linear), then to increase. At lower temperatures even better survival had been shown. However, in these tests sieved soil had been used, which would become anaerobic and waterlogged, resulting in the growth of a large number of sulphite-reducing bacteria.

In tests where recovery from the surface was by swabbing, and samples were grown on CT/SMAC at 18-20°C, the organism almost disappeared in 8-10 days. Using different media (TSBA-PS14 and TSBA 1024A) it had been shown that the organism was still present and viable (verotoxigenic) after more than 30 days when incubated at room temperature. This was probably due to these media being much less inhibitory towards other organisms. Survival had again found to be better (over 30 days) at 4°C vs. 18°C. It was only when temperatures were increased to 37°C that numbers had declined markedly.

Maule also found that Maxide (1% solution – may only be 100 ppm free chlorine) decreased numbers to log 2 (undetectable) from log 7.5 in 30 mins, whereas the control (water) had no effect even after 40 mins (personal communication, 1997).

It can be concluded from this work that survival is strong in soil, faeces and water under laboratory conditions i.e. *E. coli* O157 appears to be extremely persistent in the environment.

The effects of drying have not been studied specifically at CAMR, however, cases of infection from handling manure-contaminated vegetables would indicate that this was a vector. The strong similarities in the survival of *E. coli* O157 to Shigella are notable.

For comparison, decimal reduction times for salmonellae in soil or on crops have been calculated from data of experiments on survival, and are reported in the WRc 1998 Report (Table 5).

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Table 5

Decimal Reduction Times for Salmonellae in Soil or on Crops

Experimental Regime	D (time in days*)	Notes and Ref
Digested sludge applied to grazing land: residue on surface sampled weekly, 10-99 days after application	13	For 0-36 days after application. Initial count 920/100g. From 36-99 days, no decline, geom. Mean 4.2/100g. Netherlands (Kampelmacher & van Noorle-Jansen, 1974)
Mesophilically digested sludge applied to arable land, weekly for 9 weeks after application and furrowing into topsoil	28, 32,21	Respectively, sandy soil April-July, sandy May-July, rich loam April-June, North West England (Watson, 1980)
Sludge sprayed onto field, salmonellae isolated for 5 weeks	11	Yorkshire (Watkins & Sleath, 1981)
Raw sludge containing salmonella mixed with soil or leaching tubes, buried in soil profile to simulate injection: Clay Loam Sand	 27, 30, 19 11, 11, 13 11, 10, 8.3	Cambridge (Dickson & Tribe, 1985)
Raw sludge containing up to 5,000 salmonellae/100g injected, 120m^3/ha, into light, sandy grassland soil	4.4, 17.5	East Anglia (Andrews *et al*, 1983), July 15C; <1/100g after 7 days. February, 8-9C; <1/100g by 24 days.
S typhimurium culture added to sewage effluent pr sludge, weekly sampling of lettuce crops and soil after application: Sludge/lettuce Sludge/soil Effluent/soil Lettuce/sludge Lettuce/Effluent	 8.9 15, 4.4 12, 12 9.1, 8.8 15, 5.7	 4-8 weeks, 1976. 1975 and 1976 respectively. All data for 5-9 weeks after application, except 1976 sludge/soil – first 5 weeks only. In weeks 5-12, no further decay, geom. Mean 5-/g

* D values calculated by linear regression from the count and time data published in the references given.

Source: WRc, 1998

The rates of decay are greatest when populations in salmonellae in sludge are applied directly to vegetation, or to the surface of the soil, to dry soil, to sand rather than to clay and at summer temperatures. In all cases of surface application, the time for 90% decay did not exceed 13 days, and for sub-surface application, 32 days.

2.2.5 Importance with Respect to Food Poisoning, Occurrence on Produce

In 1973-1987, among those foodborne outbreaks [of food poisoning – USA] with an identified food vehicle reported to CDC, 2% of outbreaks and 2% of outbreak-associated cases were associated with fresh fruits and vegetables. In 1988-1991, these proportions increase to 5% and 8%, respectively. These outbreaks have raised concerns about the safety of foods, including fresh fruits and vegetables that are not processed to eliminate pathogens. (FDA/CFSAN, 1997)

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The UK Public Health Laboratory Service (Little *et al*, 1997) in 1995 surveyed 2,552 samples of these products sold at retail in England and Wales:-

- General *E. coli* was present in 13% (307 of 2,276) salads and 13% (33 of 247) of crudités sampled. 'The low incidence and low levels (<10^2 cfu/g) of *E. coli* associated with the raw, ready to eat vegetable products indicates that hygiene and/or production practices were generally good.'

- 1% of salads and 2% of crudités sampled had *E. coli* counts of 10^2 cfu or more/g, and 0.2% salads and 0.4% crudités had counts of 10^4 cfu or more/g.

- The pH of the majority of raw salad vegetables and crudités was between 5.0 and 7.0 (68% and 78%, respectively), with most having a pH between 6.0 and 7.0 (48% and 51%, respectively).

- Of the salads and crudités examined, most were grown in the UK, 45% and 47% respectively. The origin of 36% of samples was not recorded. 12% and 10% of pre-packed salad vegetables and crudités, respectively, contained products from more than one country.

- The majority of salad and crudités sampled (98%) were acceptable, 1% were unsatisfactory and 0.2% unacceptable. Of the 5 unacceptable samples, all contained *E. coli* at or greater than 10^4 cfu/g.

- No difference was found in the microbiological quality of samples from supermarkets and shops, or produced from different countries.

- Between 1992 and 1997, there were 35 outbreaks of food poisoning in England and Wales attributed to the consumption of raw vegetables or salads. At least 1,497 people had symptoms. Several large outbreaks of food poisoning associated with the consumption of raw vegetables have been reported in other developed countries. (Little *et al*, 1997)

Table 6
Outbreaks of Food Poisoning Associated with Ready to Eat Vegetables in England & Wales. 1992-February 1997

Organism	**No. Outbreaks**	**No. Ill**	**Vehicle**
Campylobacter	2	24	Salad, 1*; lettuce & tomato, 1
Cl. perfringens	1	70	Coleslaw
E. coli O157	2	302	Lettuce & tomato, 1; mixed salad, 1
Enteroagg. *E. coli*	1	7	Salad
S. typhimurium	1	7	Onion
Shigella flexneri	1	9	Salad vegetables
Shigella sonnei	2	116	Salad, 1; lettuce, 1
SRSV	8	525	Salad & coleslaw, 1; raw carrot, 1; salad, 4; watercress, 1; tomato/cucumber, 1
unknown	17	437	Lettuce, tomato, 1; coleslaw, 6; salad, 5; coleslaw & salad, 1; mushrooms (dried), 1; bean salad, 1; lettuce, 2
Total	**35**	**1,497**	

* Number of outbreaks attributed to the vehicle of infection
Number of outbreaks associated with the location: army (1), canteen (2), community (2), hall/caterer (3), hospital (3), hotel (8), private house (6), public house/bar (3), restaurant (14), other (1).
Number of outbreaks associated with faults in handling vegetables: storage (5), heat treatment (1), cross contamination (13), infected food handler (8).

Vegetable seeds may also harbour pathogens. Germination of contaminated seeds in a warm, moist environment will allow the growth of pathogens leading to the contamination of the

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vegetables. An outbreak of *E. coli* O157 in the USA, affecting 108 people, was also attributed to alfalfa sprouts grown from contaminated seeds (MMWR, 1997, 46(32)).

A comprehensive review by Beuchat (1997) on produce handling and processing found that treatment of sewage does not always yield a sewage sludge cake or a final discharge free of *Listeria* (Al-Ghazali and Al-Azawi, 1986).

The use of sewage sludge as a fertiliser could contaminate vegetation destined for human consumption. Sewage was examined at 2 month intervals in 1991 and 1992 and 84-100% was found to contain *Listeria monocytogenes* or *L. innocua*. (MacGowan *et a*l, 1994).

Ascaris ova sprayed onto tomatoes and lettuce remain viable for up to 1 month, while *Entamoeba histolytica* could not be recovered 1 week after spraying. If sewage irrigation or night soil application is stopped 1 month before harvest the produce would not likely be vectors for transmission of diseases caused by these parasites. (Rudolfs et al, 1951)

Wang and Dunlop (1996) recovered Salmonella, *Ascaris* ova and *Entamoeba coli* cysts from more than half of irrigation water samples contaminated with either raw sewage or primary-treated, chlorinated effluents. Only one of 97 samples of vegetables irrigated with this water yielded Salmonella, but *Ascaris* ova were recovered from two of 34 vegetable samples.

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3. ORGANIC MATERIALS IN AGRICULTURE

Summary

Although data on the survival of Salmonella in treated human sewage sludge may only be indicative of the behaviour of VTEC, there is evidence that contamination can be present in this material[4,5,6]. Cattle and other animals[7,8,9] have been shown to carry and/or shed *E. coli* O157, providing another potential source of contamination of the agricultural environment and food chain. In addition, under laboratory conditions, *E. coli* O157 has been shown to persist in soil cores and river water[10] for significant periods.

Further Reading

5 Beuchat & Ryu (1997)
6 Grant *et al* (1996)
7 Watkins & Sleath (1981)
8 Armstrong *et al* (1996)
9 Porter *et al* (1997)
10 Faith *et al* (1996)
11 Maule (1997)

3.1 Sewage Sludge

Sewage sludge can be defined as residual sludge from sewage plants treating domestic or urban waste waters, and from other sewage plants treating waste waters of a composition similar to domestic and urban waste waters.

Sewage sludges are produced by separation of settleable solids (primary treatment) and conversion of dissolved and colloidal solids by a biological process (secondary treatment) to metabolites such as carbon dioxide, sulphate and water and also microbial cells and residues in flocculent form (humus or surplus activated sludge) which can be separated by a secondary stage of settlement. The end products can be returned to the river or other receiving water either directly or after some further (tertiary) treatment, and a sludge containing much of the organic load of the original raw sewage. Sludges from the primary and secondary treatments are usually combined before disposal, the rates of primary sludge solids to secondary solids being about 2:1 (w/w).

Before any thickening, dewatering or treatment, sewage sludge is a putrescible, thin slurry typically with a solids content of about 2% of which 70-80% is organic matter. The volume of sludge produced will be only about 1% of the volume of raw sewage received, and the thickening and treatment of the sludge which is usual before disposal further reduce this. Sewage sludge for disposal may be in various forms according to water content, ranging from a thickened slurry (about 25% dry solids [ds] content) through dewatered 'cake' (25-35% ds) to dried pellets or granules (85-95% ds). (WRc, 1998)

3.1.1 Statutory Treatments

3.1.1.1 European

Despite the general requirements of the 1986 Directive, there is wide variation of national rules regarding the treatment of sewage sludge in the EU.

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Table 7
Legislated Treatment of Sewage Sludge in Europe and the USA

Country	Treatment
EU	Sludge must be treated before being used in agriculture. Member States may nevertheless authorise, on certain conditions, the use of untreated sludge, without risk to human or animal health, if it is injected or worked into the soil. Member States should be able to draw up more stringent provisions , which should be communicated to the Commission.
Belgium	Biological/Chemical/thermal/ long term storage or any other appropriate method
Denmark	None, but restrictions on application
Finland	Stabilisation, but not 'hygienization' of sludge required before applied to agricultural land.
France	Dewatered sludge must be ploughed into soil no later than the day after application, but derogations may be available. pH must be 6.5-8.5 (12.5 when lime applied)
Germany	No untreated may be used on agricultural and horticultural soils. 1982 Ordinance required disinfection prior to application to grassland or grazing crops. 1992 Ordinance prohibits sludge use on permanent pasture.
Greece	1982 Ordinance effectively banned use on fruit and vegetable crops. 1992 Amendment bans use on grasslands, but Länder may allow exemptions. Use on soils <pH 5 also banned.
Ireland	As in Directive
Italy	1992 Decree requires sludge to be subjected to biological, chemical or thermal treatment, or stored for a reasonable length of time, or to be subjected to any procedure to reduce its putrescible content and any hygienic problems. Application is restricted whenever it is ascertained that there is a threat to human or animal heath and/or the environment.
Luxembourg	Good Agricultural Practice must be used. In order to avoid hygienic problems, all sewage sludges must be treated prior to use in agricultural, e.g. biological, chemical, thermal long-term storage or any other appropriate method. No treatment is required if it is to be injected into the soil.
Netherlands	Sludge to be treated (biological, chemical, thermal, storage or other process) designed to destroy 'a major part' of pathogens in sludge.
Norway	Stabilisation and treatment of all sludges from treatment works larger than 5000 pe required, prior to application to agricultural land.
Portugal	Banned: use along the side of rivers and lakes; use within 100m of houses and 200m of villages and other populated areas; within 50m of wells and boreholes used for irrigation; within 100m of drinking water. Must be mixed into the ground within 2 days of application. Application must be done on deep soils to protect soil and surface ground water quality.
Spain	As in Directive: no grazing 3 weeks after application; use in gardening and fruit production banned during their vegetative cycle, except for fruit trees; not to be applied within 10 months of harvest to fruit or vegetables that may be eaten raw.
Sweden	Stabilisation is strongly recommended and in certain applications such as parks and landscaping, is mandatory
Switzerland	Stabilisation is strongly recommended and in certain applications such as parks and landscaping, is mandatory
UK	Sludge to be treated except where injected or otherwise worked into the soil. Treatments: pasteurisation, mesophilic anaerobic digestion, thermophilic aerobic digestion, composting, lime stabilisation and storage.
USA	S/s applied to land must be treated to reduce its pathogenic content and vector attraction. Sludge classified as **Class A** (applied to land with which the public is expected to be in contact) and **Class B** (public not expected to be in contact) with respect to pathogens. Different treatments set out in the Regulations (no details available) enable compliance with these requirements.

Source: WRc, 1994

Salmonellosis attributable to use of sludge in Switzerland led to a decision to require all sludge to be 'pasteurised' before application and to meet a microbiological standard. WRc (1998) states that

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this was appropriate under these circumstances since the Swiss growing seasons are shorter than in the UK, population densities, expressed as humans to available arable and pasture land, or cattle to pasture were respectively seven and eight times higher than in the UK at that time, even though the percentages of sludge disposed to land were the same in both countries.

3.1.1.2 UK-specific

Except when it is to be injected or otherwise worked into the soil so as not to cause nuisance, sludge must be subjected to biological, chemical or heat treatment, long term storage or any other appropriate process. Examples of sludge treatment processes are given in the non-statutory Code of Practice for Agricultural Use of Sewage Sludge (DETR, 1989).

Table 8
UK-Approved Sludge Treatments

Process	Descriptions
Sludge pasteurisation	Min 30 mins at 70°C or min 4h at 55°C (or appropriate intermediate conditions), followed in all cases by primary mesophilic anaerobic digestion.
Mesophilic aerobic digestion	Mean retention period of at least 12 days primary digestion in temp range 35°C±3°C or at least 20 d primary digestion in temp range 25°C±3°C followed in each case by a secondary stage that provides a mean retention period of at least 14 days.
Thermophilic aerobic digestion	Mean retention period of at least 7 days digestion. All sludge to be subject to a min of 55°C for a period of at least 4 h.
Composting (windrows or aerated piles)	The compost must be maintained at 40°C for at least 5d and for 4 h during this period at a min of 55°C within the body of the pile followed by a period of maturation adequate to ensure that the compost reaction process is substantially complete.
Lime stabilisation of liquid sludge	Addition of lime to raise the pH to >12.0 and sufficient to ensure that the pH is not <12 for a min period of 2h. The sludge can then be used directly.
Liquid storage	Storage of untreated liquid sludge for a min period of 3 months
Dewatering and storage	Conditioning of untreated sludge with lime or other coagulants followed by Dewatering and storage of the cake for a min period of 3 months. If sludge has been subject to primary mesophilic anaerobic digestion, storage to be for a min period of 14 d.

A review of the scientific basis of these processes regarding the control of pathogens was carried out under contract from the DETR by WRc (WRc, 1998) and is considered in section 5.3.1.3.

3.1.2 Microbial Quality of Sewage Sludge

No evidence is available detailing outbreaks associated with the controlled use of treated sewage. Information on the survival of microbial pathogens in the environment after application to soil/water is limited but some viruses (enterovirus) and bacteria (Salmonella) appear to survive in excess of 100 days whilst parasites may be more resilient (300-400 or more days). Survival is dependent on whether the contaminant is sprayed onto the soil or injected/ploughed in as this affects moisture and exposure to sunlight (UV). [J Sainsbury briefing paper to BRC, 1997]

In the UK, CIWEM (1995) considered that there were essentially only 2 sludge-borne pathogens that are of concern to human and animal health: Salmonellae and *Taenia saginata* (beef tapeworm). Other parasites and other foodborne pathogens were not considered. CIWEM recognised that human and enteroviruses will inevitably be present in sewage, but most will not survive for long outside their normal host. The usual treatment given to destroy pathogens will also kill most viruses and the risk of infection through the sludge route is considered to be negligible. Attempts to relate the use of sludge to viral infections have proven inconclusive, but research in this area is still continuing (Hygiene aspects related to the treatment and use of organic sludge and sanitary aspects of spreading of slurries and manures. Anon, 1992)

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According to WRc (1998), the examination of sludge for the presence of pathogenic microorganisms tends to be lengthy, tedious and imprecise. Therefore, for routine microbiological monitoring purposes it is not practicable to set numerical limits for the microbiological quality of sludge. However, the DETR Code of Practice for the Agricultural Use of Sewage Sludge, states that where there are significant discharges to sewer of wastes from animal or poultry processing plants, on veterinary advice it may be necessary as an added safety measure to analyse such sludge periodically (after treatment) in respect of its microbiological quality. It is essential that the physical parameters relevant to the control of sludge treatment processes (see table above) be carefully monitored to ensure that the processes are operating efficiently.

Grant *et al* (1996) studied the prevalence of EHEC in raw and treated municipal sewage PCR analysis of sewage concentrates showed that DNA encoding SLT II (Shiga-Like Toxin) was present in a single sample of untreated sewage and absent in all other samples tested. These results indicate that, if SLTII-harbouring organisms are present in the raw influent coming into the plant, they appear to be removed from the sewage by preliminary and/or primary treatment processes. These results suggest that EHEC strains do not constitute a significant fraction of the *E.* coli population naturally present in municipal sewage.

However, in a study by Martin *et al*, 3.4% of 'thermotolerant' *E. coli* strains isolated from untreated water (8 of 237) and 4.2% of 'thermotolerant' *E. coli* strains isolated from treated water (9 of 212) harboured DNA for SLT I and/or II. This study raises the possibility that municipal sewage represents a reservoir for EHEC in the environment because sewage contains high concentrations of *E. coli* even after conventional treatment and treated sewage effluent is often discharged into bodies of water used for recreational purposes.

Watkins and Sleath reported in 1981 that *Listeria monocytogenes* was isolated from every sample of sewage, sewage sludge, river water and trade effluent examined. In many instances the numbers isolated were higher than the salmonella counts, and on two occasions, *Listeria monocytogenes* was isolated when no salmonellas could be obtained.

The UK National Water Council (WRc, 1998) found in 1976 that there was an identifiable risk to animal health from discharge of sewage effluents and disposal of sludges, as shown by unpublished records of 37 outbreaks of salmonellosis (including 32 cattle, 2 sheep and cattle, 2 horses) and that salmonellae constituted the greatest likely source of infection to livestock.

Table 9
Counts from samples of sewage sludge

		Counts/litre	
Date	**Location**	**Salmonellas**	***Listeria monocytogenes***
29/1/79	Knostrop, Leeds	1,200	11,000
12/3/79	Blackburn Meadows, Sheffield	1,300	2,500
2/4/79	Knostrop, Leeds	7,000	800
12/2/80	Sandall, Doncaster Balby, Doncaster (Primary sludge)	16,000 1,800	16,000 1,800
25/2/80	Thorne, Doncaster (Primary sludge) Sutton	>18,000 >18,000	 >18,000

Listeria monocytogenes was isolated by enrichment at 4°C with subculture and enrichment in thiocyanate, naladixic acid broth and plating onto Tryptose Agar. The results indicated that *Listeria monocytogenes* is present in sewage and sewage sludge in considerable numbers and it survives longer than *salmonella* spp. on land sprayed with sewage sludge. (Watkins & Sleath, 1981)

Table 10
Soil samples from a field at Fishlake analysed for salmonellas and Listeria monocytogenes after spreading with sludge

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	Counts/50g soil sprayed			
	Heavily		Lightly	
Date	Salmonellas	*Listeria monocytogenes*	Salmonellas	*Listeria monocytogenes*
10/10/78	35	>180	0	>180
30/10/78	3	>180	0	80
20/11/78	0	>18	0	5
28/11/78	0	5	0	0

The high counts obtained from sewage sludge from sewage treatment works (Watkins and Sleath) gave rise to concern because of the practice of spraying this material onto agricultural land. Preliminary results indicated that *Listeria monocytogenes* could survive for longer periods than salmonellas. Subsequent detailed survival studies have shown that although salmonellas die off rapidly in sewage sludge, the numbers of *Listeria monocytogenes* may remain unaltered over an 8-week period.

Table 11
Survival of salmonellas and *Listeria monocytogenes* in sewage sludge applied to land

		Counts/100g soil of:	
Week no.	Date	Salmonellas	*Lm*
0	4/12/79	130	170
1	11/12/79	35	350
2	18/12/79	8	225
5	8/1/80	1	>180
6	15/1/80	0	>180
7	22/1/80	0	>180
8	29/1/80	0	160
Tanker sample		70	250
Unpsrayed area		0	0

Ref. Watkins & Sleath, 1981

In California, the treatment processes specified by the Water Reclamation Criteria (California Water Code, 1994) can achieve a 5-log reduction *in situ* of viruses. This level of reduction produces effluent that is accepted as being 'free' of viruses. In the Monterey Wastewater Reclamation Study for Agriculture (Sheikh et al, 1990), tests conducted over a 5 year period of over 80,000 gallons of reclaimed water that met Title 22 requirements found no viruses (Engineering Science, 1987). Virus seeding studies were conducted that verified the 5-log reduction in viruses from the treatment process. Additionally, a 99% natural die-off rate over 5 days was demonstrated under both field and laboratory conditions for the virus T99.

The NRC 1996 Report includes what it describes as 'a rough calculation' which illustrates the very low level of viruses to be expected after irrigation with reclaimed water of this quality on food crops. In the Monterey study, the median number of viruses detected in the raw wastewater influent was 8 plaque-forming units (PFUs) in 67 samples (sample size not specified), so that even without treatment, the number of viruses that might remain following irrigation is very small. To illustrate, reclaimed water is typically applied to the crop in an 'irrigation set' of 2 inches of water. In California, crops cannot be harvested for two weeks following a reclaimed water irrigation set. If a plant occupies 2 square feet, it would receive about 2.4 gal of water. Even if the treatment failed completely, and assuming all the viruses in that volume of untreated wastewater stuck to the edible part of the plant, one would expect approximately 10^{-3} PFU per plant. With treatment, the number of viruses remaining on the plant is 'essentially zero'. The study also found that a 5-log reduction in viruses occurred in soil after ten days.

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If one were to take the count for Shigella in raw sewage and digested sludge (see Table 12) and do a similar calculation, one could expect the number of Shigella cells to be approximately 0.1 and $0.5x10^3$ per plant, or 1 per 10 plants and 5 per ten thousand plants, respectively. If one were to do the same calculation for faecal coliforms using the data below, these figures would be expected to be a million-fold and a factor of 300,000 higher, respectively. None of these figures reflect potential destruction of organisms under field conditions. More formal risk assessment needs to be carried out, however, and acceptable incidences of contamination determined.

The NRC goes on to say that 'while the use of essentially pathogen-free sewage sludge or effluent would be ideal, materials of lesser sanitary quality (less treatment) can be applied in cases where direct human exposure to applied sludge or effluent is minimal. In these instances, natural decay processes in the soil would be relied on to reduce the number of pathogenic agents to safe levels, site restrictions would be required to limit public access and to allow adequate time for pathogen reduction prior to crop planting, harvesting, or domestic animal grazing.'

According to the NRC Report (1996), there is no general agreement on the numerical values used in setting microbiological standards, and they therefore vary from region to region, and country to country. Because coliforms are not always reliable indicators of the quality of reclaimed water or sludge, other indicator organisms are continually being sought. Cl perfringens is present in wastewater in large numbers and the ease and speed of detection and of the resistance of its spores to disinfection, has meant that it is considered by some to be a good indicator of how effective a treatment process has been.

Table 12

Typical Numbers of Microorganisms Found in Various Stages of Wastewater and Sludge Treatment

	Number per 100ml effluent				Number per gramme of sludge	
Microbe	**Raw sewage**	**Primary treatment**	**Secondary treatment**	**Tertiary[a] treatment**	**Raw**	**Digested[b]**
Faecal coliform MPN[c]	$1x10^9$	$1x10^7$	$1x10^6$	<2	$1x10^7$	$1x10^6$
Salmonella MPN	8,000	800	8	<2	1,800	18
Shigella MPN	1,000	100	1	<2	220	3
Enteric virus PFU[d]	50,000	15,000	1,500	0.002	1,400	210
Helminth ova	800	80	0.08	<0.08	30	10
Giardia lamblia cysts	10,000	5,000	2,500	3	140	43

[a] Includes coagulation, sedimentation, filtration and disinfection
[b] Mesophilic anaerobic digestion
[c] MPN = Most Probable Number
[d] PFU = Plaque Forming Units

Ref.: NRC, 1996

Although EU Member States should all be working to the Urban Waste Water Treatment Directive (91/171/EEC), some countries have specific limits for pathogens in their national legislation.

Table 13

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EU and USA Pathogen Limits for Sewage Sludge

Country	Pathogen Limits
EU	None set
Italy	Not more than 1000 MPN/g ds of Salmonella if used on agricultural land
Luxembourg	None, except for use on grassland and in market gardening
Netherlands	Sludge to be treated (biological, chemical, thermal, storage or other process) designed to destroy ‘a major part’ of pathogens in sludge.
Portugal	No limits set, but powers exist for monitoring content if considered necessary.
UK	Code of Practice refers to possible presence of Salmonella, beef tapeworm, potato cyst nematodes and viruses (no limits specified).
USA	**Class A:** faecal coliforms must be <1000 MPN/g ds or Salmonella <3 MPN/g ds. **Class B:** geometric mean of density of faecal coliforms in 7 samples must be no more than 2,000,000 Alternatively, the treatment works can use a process to Significantly reduce pathogens (PSRP) as defined in the Regulations.

Note: Class A = applied to land with which the public is expected to be in contact
Class B = public not expected to be in contact

Ref. WRc (October 1994) Report EC 3646

3.1.3 **Usage of Sewage Sludge**

3.1.3.1 Across Europe

During the period 1972-1990, the European Commission sponsored a concerted action on the treatment and disposal of sewage sludge. The objective of the study was to co-ordinate the research work on sludge being undertaken in the member states and in some other European countries, and to disseminate the results. A comprehensive review of this programme was published in 1992 (L’Hermite and Newman, 1992).

Directive 86/278/EEC (the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture) requires that five years after notification of the Directive, and every four years thereafter, each member state shall prepare a consolidated report on the use of sludge in agriculture. The UK first submitted its report to the European Commission in 1993 (Sludge Use in Agriculture 1990/1. Report to the European Commission under Directive 86/278/EEC. DoE). In order to provide information for the report, a major survey was conducted during 1990-1 on the production, utilisation and disposal of sludge on the UK. The survey report (DoE, 1993) showed the relative use of different disposal routes both in 1990-1 and those predicted by sludge producers for 1996 and 2005. An important finding of the survey was that soil metal concentrations on land on which sludge is used and the rates of metals addition in sludge, were well below the respective UK Regulation limits. The quality of UK sludges was also significantly better in terms of metals content than found in a survey which was carried out in 1982-3.

The main conclusions of the 1993 UK report submitted to the European Commission on the use of sewage sludge in agriculture included that three quarters of the sludge used in agriculture was treated, with 44% being digested before use. About 25% of the sludge used in agriculture is therefore untreated and is injected or worked into the soil after application.

Sludge producers intend to rely on agriculture as the principal disposal route for nearly half of the UK’s sludge well into the next century. However, a major concern for UK sewage sludge producers is the potential impact of European Commission waste legislation on sewage sludge. If sewage sludge of normal quality is to be classified as hazardous waste, it would unnecessarily constrain the number of sludge disposal options available and would reduce beneficial use. Also, in areas which are designated as vulnerable zones under the European Commission nitrates

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Directive (91/676/EEC), sludge applications will need to be reduced to meet the nitrogen limit of 170kg/ha.

In 1993, MAFF and DoE jointly commissioned independent scientific reviews of the implications for soil fertility and food safety and animal health, of the current rules (The Sludge (Use in Agriculture) Regulations 1989, IS 880) on sewage sludge applications to agricultural land, with particular reference to heavy metals. The report of the steering group on food safety and animal health concluded that there was insufficient evidence to warrant a recommendation that limits for specific heavy metals should be reduced, and that their uptake by crops was unlikely to pose a food safety problem. The group suggested that more information should be acquired on the organic constituents in sludges to ensure that more information should be acquired on the organic constituents in sludges to ensure that there is not risk to food safety or animal health. (MAFF. 1993).

In 1993, a comprehensive study of alternative uses, carried out for the DoE, concluded that the UK should develop a policy for sludge products with national quality standards which would permit their wider and safe use (WRc, 1993).

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Table 14
Sludge Disposal in European Community and USA:-

	Agric	Landfill direct	Landfill after incineration	Sea	Other beneficial	Other outlets	Total dry tonnage
EC total	2.394 (37%)	2.61 (40%)	0.725 (11%)	0.386 (6%)	0.12 (2%)	0.269 (4%)	**6.457M**
Belgium	33.2 (56%)						**59.2K**
Denmark	93K (54.4%)						**170K**
***Finland**							**170K**
France	0.5M (60%)						**830K**
Germany							**2.5M**
Greece							**48.2K**
Ireland	4.4K (12%)						**36.7K**
Italy	277K (34%)						**816K**
Lux	0.944K (12%)						**7.87K**
NL	84K (26%)						**323K**
Portugal	15K (30%)						**45K**
Spain	0.15 (50%)						**300K**
UK	0.521M (44%)	0.095 (8%)	0.083 (7%)	0.332 (30%)	0.066 (6%)	0.055 (5%)	**1.107M**
****Other**	228K (35%)	2.515 (47%)	0.642 (12%)	0.054 (1%)	0.054 (1%)	0.214 (4%)	**0.214M**
*****USA**	¤ 1.908 (36%)	2.014 (38%)	0.848 (16%)			0.53 (10%)	**5.3M**

* Not an EC Member in 1994
** unattributed quantities from EC members
*** Data from NRC 1996 Report
¤ includes turfgrass production and reclamation of surface mining areas

The initial Report does not give a further breakdown of figures. Disposal at sea in the EU will be banned from 1 January 1999.

Ref: WRc, 1994.

In the Netherlands a policy decision has been taken to incinerate sewage sludge rather than utilising it in agriculture. The basis of this decision is reportedly in order to balance inputs and outputs of contaminants in soils.

It should be borne in mind that the figures above only relate to sewage that has been treated at a sewage treatment works. The proportion of the population of each European country which is connected to such systems varies considerably, resulting in the use of septic tanks or release of untreated sewage into the environment (Table 15).

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Table 15

Percentage Population Connected to Sewage Treatment Works

Country	%
Belgium • Flanders • Walloon	 30 25
Denmark	92
Finland	N/a
France	50
Germany	62-98
Greece	34
Ireland	45
Italy	52
Lux	97
NL	88
Portugal	N/a
Spain	59
UK	85

N/a not available
Ref. WRc, 1994

Table 16

Examples of the Use/Recycling of Sewage Sludge – UK

	1990-1 %*	1994 % **	1997 %***
Re-use in agriculture	42	44	49
Incineration	7	7	9
Landfill	8	8	15
Sea deposition	30	30	17
Others	13	31	10
Total mass	**22.12Mt**	**22.14Mt**	**25Mt**

Ref.: * WRc, 1998
** WRc, 1994
*** British Retail Consortium Briefing Paper, 1997

About 25% of all sludge used on UK land is untreated (Anon, 1996). The Royal Commission on Environmental Pollution, in its report on Sustainable Use of Soil (1996), recommended that the use of untreated sewage sludge on land should be phased out to reduce the risk of pathogen spread.

The agricultural outlet is more important to UK sludge producers than any other option, and its use (relative to other outlets) is predicted to increase still further in future years. The sustainability of the outlet is therefore of crucial importance to all UK sludge producers.

The 1990-1 national survey showed that only 0.3% of agricultural land in the UK actually received sludge over a twelve month period; furthermore, it has been calculated (Anon, 1989) that sludge utilisation accounts for only about 1.2% of the total nitrogen input to agriculture from chemical fertilisers and housed livestock manure. Therefore, sludge makes a relatively small contribution to the national fertiliser budget. Since the annual quantity of sludge used in agriculture is sufficient for only about 0.3% of agricultural land, it would be expected that the demand for sludge by farmers would exceed it supply. This is the case in some regions of the country.

According to a report which reviews the rules for the application of sludge to agricultural land, approximately 60% of agricultural land in England and Wales is potentially suitable for the recycling of sewage sludge, and about 20% in Scotland. In practice, however, only about 10% of agricultural land is both within an economic transport distance of sewage treatment works and is

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potentially suitable for receiving sludge. Even then, 10% is not available, for reasons including land use controls and the important fact that not all farmers will accept sludge. In summary, sludge producers assume that overall, only 1% of UK Agricultural land is available for sludge recycling, although this percentage varies from region to region and may increase in the future should longer transportation distances for sludge become cost effective (MAFF, 1993).

MAFF, the Scottish Office Agriculture and Fisheries Department (SOAFD) and ADAS provide detailed advice and information on sludge in the UK. SOAFD has published a Code of Good Practice on Prevention of Environmental Pollution from Agricultural Activity, which includes advice on the use of sludge (Prevention of Environmental Pollution from Agricultural Activity, Scottish Office, 1993).

The WHO 1997 Consultation on EHEC recommended that crops used for raising seeds that are going to be used as sprouts should not be fertilised with animal slurry or human faecal waste, even if some form of treatment has been applied. In identifying control and prevention measures, EHEC are assumed to be present in animal and human faeces. Therefore appropriate animal husbandry practices should be adopted to minimise the spread of contaminated material on animals or on ground used for crops. Animal slurry and human faecal waste (night soil) should not be used on or near crops intended for human consumption, unless it has been adequately treated.

It is necessary to co-ordinate sludge applications in time with planting, grazing or harvesting operations. Sludge must not be applied to growing fruit and vegetable crops nor used where crops are grown under permanent glass or plastic structures. Untreated sludge must not be used in orchards or on land used for growing nursery stock (including bulbs).

3.2 Exempt Waste

The only current information available relates to the UK situation.

In the context of the DETR *Code of Practice on the Agricultural Use of Sewage Sludge*, the contents of septic tanks and sludges from secondary biological treatment such as humus sludge, surface activated sludge and residual sludge from extended aeration plants, cannot be considered to be biologically treated.

However, 'exempted wastes' as defined under the Waste Management Licensing Regulations 1994 are not covered by the Sludge (Use in Agriculture) Regulations 1989. Such exempted wastes include:-

- blood and gut contents from abattoirs
- waste food, drink or materials used in/resulting from the preparation of food and drink
- septic tank sludge
- sludge from biological treatment plants

Information derived from one UK commercial source indicates that there is currently approximately double the quantity of exempt waste going onto land, compared with sewage sludge (private communication, 1997).

Farmyard manure and waste (FYM) is being applied increasingly as slurry - a mixture of excrement, urine and bedding, etc. If it is composted this is not equivalent to pasteurisation.

The National Farmers' Union estimates the amounts of FYM applied annually in the UK are: 80M tonnes applied during farming practice, 120M tonnes by cattle during grazing. Their estimate of (human) sewage sludge quantities applied is 1-2M tonnes p.a. In addition to having its own Protocols, NFU promotes the uptake of advice such as Farm Assured Schemes and Government (voluntary) codes of practice on water, air and soil. An example of one of its 41 sectoral protocols is that for watercress which was developed through NFU. This document sets out best practices for

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growing, compliance with which is compulsory for members of the Watercress Association, which exists under NFU.

Table 17
Examples of Current Usage of Organic Wastes in UK Farming

ANIMAL/ORIGIN	USE	APPLICATION
Chicken/Turkey	Celery	Pre-planting cultivation
Cattle	Onions, iceberg lettuce	After 2 year storage in pile - pre-planting cultivation
Sheep	Grazing of rye grass. Waste applied at end of main salads/veg cropping season	When crops at full cover and approximately 10” high
Pig	Slurry applied to land for celery and lettuce crops	Pre-cultivation - injected into soil
Goats	Grazing of crop debris after harvest	

MAFF concluded in its 1997 review of Animal By-products Legislation and the Potential Impact of the Landfill Directive, that the three existing statutory instruments concerning the processing and disposal of animal by-product should be consolidated into a single statutory instrument. In addition, MAFF also consider that there is a need to make sure that GB legislation accurately reflects European legislation in this area.

The new legislation would cover:-

- Processing and disposal of animal by-products (i.e. parts of animals or poultry which are not intended for human consumption) through rendering plants; through premises such as knackers’ yards from which they will not enter the human food chain; by incineration; or by burning or burial on farm
- Processing of catering waste (e.g. leftovers from restaurants) for the feeding of pigs and poultry
- Some other points such as testing for salmonella of certain processed animal by-products, licensing and fees

The Animal By-Products (Amendment) Order 1997 has been proposed, which would no longer permit the disposal of these materials by landfill, although on-farm burial would still be permitted. MAFF states in its proposal that ‘although the landfilling of low risk unprocessed ABPs in appropriately licensed sites is not thought to endanger public health, widespread adoption of the practice could be unwelcome on environmental grounds. In addition, the Commission are elsewhere proposing a Landfill Directive which would in due course require the pre-treatment (e.g. by incineration or rendering) of all waste before burial.’ This could be expected to reduce severely the amount of biodegradable material that can be landfilled.

There are 34 rendering plants, 470 slaughterhouses and 152,000 livestock producers in the UK. The cost of landfill has been estimated at around £25/t compared with an average cost of rendering at £80/t. Slaughterhouse waste amounts to about 1 million tonnes p.a.

Information is required on types, any treatment, handling and application of these materials including farmyard manure, in the rest of Europe.

3.3 Irrigation Water

WHO Guidelines (Mara & Cairncross, 1989) for the safe use of wastewater and excreta in agriculture and aquaculture recommend that treated wastewater should contain <1,000 faecal

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coliforms/100 ml for unrestricted irrigation in agriculture and <10,000 faecal coliforms/100 ml/g for unrestricted use in aquaculture. These Guidelines were prepared before the emergence of EHEC as a serious human pathogen, especially transmission through contaminated vegetable produce.

It is recognised that it may be necessary to review these Guidelines in light of new information on EHEC as a cause of foodborne infections. The WHO 1997 consultation on EHEC recommended that crops for food should be irrigated with water that is not faecally contaminated. WHO Guidelines recommended that water is decontaminated before coming into contact with fruits and vegetables for cooling and rehydrating purposes during packing, transportation and processing. Any water used for washing and/or processing [of animals], fruits and vegetables is recommended in the 1997 WHO Consultation on EHEC to be of potable quality. (WHO, 1997)

A limit of 1000 faecal coliforms/100ml of irrigation water was recommended for use in the USA on all crops, including those to be eaten raw (NAS/NEA, 1973). No microbiological guidelines exist in the UK (MAFF communication, 1997). No microbiological guidelines were proposed in Australia for irrigation water other than those proposed for use of wastewater in irrigation (Hart, 1974). In Manitoba, the maximum acceptable concentration of faecal coliforms in irrigation water is a geometric mean of 1000/100ml, and a maximum of 2000/100ml coliforms in individual samples (Williamson, 1983). Ontario recommends that water of 'the best microbiological quality possible' be used. Guidelines of 100 faecal coliforms/100ml and 1000 total coliforms per 100ml are recommended by the Ontario Ministry of the Environment, 1984.

A recent research paper (Doyle, 1998) determined the survival of five nalidixic acid-resistant *E coli* O157:H7 strains (10^3 CFU/ml) in filtered and autoclaved municipal water, reservoir water and water from two recreational lakes, over a period of 91 days at 8,15 and 25°C. Survival was greatest in autoclaved water and least in lake water. Regardless of the water source, survival was greatest at 8°C and least at 25°C. *E coli* O157: H7 populations decreased by 1 to 2 logs by 91 days at 8°C, whereas it was not detectable within 49 to 84 days at 25°C in three of the four water sources. These studies confirmed that *E coli* O157:H7 is a hardy pathogen that can survive for long periods of time in water, especially at cold temperatures. However, direct viable counts of *E coli* O157:H7 determined by acridine orange staining remained essentially the same for 12 weeks at 25°C, whereas viable counts on tryptic soy agar plates decreased to undetectable levels within 12 weeks. Results suggest that *E coli* O157:H7 can enter a viable but not culturable state in water.

3.4 Animal Contamination including Grazing and Stray Animals

Special care should be paid to fruit and other produce to prevent the accidental contact with soil or animal faeces (e.g. fallen apples), as these have been implicated in the transmission of EHEC infections. (WHO, 1996)

When sewage sludge is used or disposed at a site, a previous US rule (Sec. 503.32(b)(5)(v) and Sec. 503. 24(l)) prohibited grazing of animals at the site in certain circumstances. The EPA has since prohibited intentional, not inadvertent, grazing of animals. The land application site restriction and surface disposal management practices that restrict public access may prevent access to the site for many types of animals depending on how public access is restricted (e.g., by a fence).

The USDA (1998), announced a $10.4M program to pay 350 farmers to stop growing crops and grazing cattle along streams feeding New York City's upstate reservoirs. The goal is to transform the banks of 165 miles of watershed streams from easily eroded cropland or pasture into pollution-filtering buffer zones of grassland and forest. Farms who abandon fields along streams will be paid a yearly fee of $100-150 an acre for the loss of the use of their land. The money is to be paid to farmers under contracts lasting 10 to 15 years. 90% of New York Coty's drinking water starts as runoff in rural hills 100 miles to the northwest where the water trickles across farmers' field and picks up undesirable silt, chemicals, and nutrients that promote algae growth, and intestinal parasites. Almost all of these contaminants are naturally removed from the water as it flows

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towards the city, but federal environmental officials have increasingly pressed the city to either bolster protection of its water at source or build a $6bn filtration plant.

4. Organic Foods

4.1 EU Regulation for Organic Foods

Regulation No. 2092/91 came into force on 22 July 1991. It applies to unprocessed agricultural crop products, to products intended for human consumption composed essentially of one or more ingredients of plant origin, and it introduces specific rules for the production, inspection and labelling of such products.

Products authorised exceptionally for use in soil conditioning and fertilisation, in accordance with the dispositions of Annex I (2) include:-

- Farmyard manure (comprising a mixture of animal excrements and vegetable matter (animal bedding)) when the need is recognised by the inspection body or inspection authority
- Dried farmyard manure and dehydrated poultry manure (when the need is recognised by the inspection body or inspection authority)
- Composted animal excrements, including poultry manure and composted animal manure (when the need is recognised by the inspection body or inspection authority)
- Liquid animal excrements (slurry, urine, etc) 'used after controlled fermentation and/or appropriate dilution' (when the need is recognised by the inspection body or inspection authority). Factory farming origin is forbidden.
- Composted household waste (only animal and vegetable waste) produced in a closed and monitored collection system, accepted by the Member State, but only up to 31 March 2002 and where the need is recognised by the inspection body or inspection authority. Maximum heavy and trace metal concentrations are given.
- Dejecta of worms (vermicompost) and insects
- Guano
- Composted mixture of vegetable matter
- Products or by-products of blood meal, hoof meal, horn meal, bone meal or degelatinised bone meal, animal charcoal, fish meal, meat meal, feather, hair and 'chiquette' meal, wool, fur, hair, dairy products.

The Regulation requires that operators who produce, prepare or import from third country, products specified by the Regulation for the purposes of marketing them, must notify the activity to the competent authority of the Member State in which the activity is carried out, e.g. UKROFS (UK Register of Organic Food Standards) in the case of the UK and they must submit the undertaking to the specified inspection system.

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4.2 UK Soil Association Standards

These Standards define Organic farming systems and lay down criteria which must be met and maintained when food products are described as Organic. Standards are based on guidelines originally established by the International Federation of Organic Agriculture Movements (IFOAM). The Standards comply with EC Regulation 2092/91 and UKROFS Standards for Organic Food Production.

Key points

- Land contaminated by environmental pollution (e.g. from factories, traffic, and sewage sludge) or by residual pesticide residues may render the holding ineligible for Organic status or require a longer conversion period, at the discretion of the Certification Committee.
- Where the land was previously used for exploitative cropping, the conversion programme must begin with a fertility-building phase.
- The soil management must ensure a regular input of Organic residues in the form of manures and plant remains to maintain the level of humus, biological activity and plant nutrients
- Recommended: a protective covering of vegetation, e.g. green manure or growing crop, to protect surface living organisms and soil structure from damage by exposure to dry conditions, heavy rain or strong winds
- Brought-in manures or plant wastes form Non-organic sources must not form the basis of a manurial programme, but should be adjuncts. The use of all plant wastes and animal manures from non-organic sources are restricted and the need for them must be recognised by the Certification Committee and receive the treatments specified before use. Details provided to the Committee should include the source of the manure, the animal species and the husbandry system used.
- An analysis of the soil and/or manure may be required by the Certification Committee, at the applicant's expense, before approval can be given for a restricted material.

Permitted

- Straw, FYM, stable and poultry manures from Organic sources preferably after being properly composted
- Slurry, urine and dirty water, from Organic sources preferably after being aerated
- Plant waste materials and by-products from Organic food processing industries

Restricted

- Straw, FYM and stable manure from Non-organic sources – after being properly composted for three months or stacked for six months
- Poultry manure or deep litter from the following organic systems – after being properly composted for six months or stacked for twelve months:-
  - Egg producing
  - Deep litter pullet rearing systems
  - Meat producing (defined by EEC Regulation 1538/91):
    - Free range
    - Traditional free range
    - Extensive indoor barn reared
- Manures from Non-organic straw-based pig production systems – after being properly composted for 6 months or stacked for 12 months
- Plant wastes and by-products from Non-organic food processing industries, Mushroom composts made from non-organic manures, Composts from organic household refuse – after being composted for 3 months or stacked for 6 months
- Animal slurry from non-organic sources – after aeration
- Dirty water from non-organic systems – applied to in-conversion land
- Processed animal products from slaughterhouses and the fish industries

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Prohibited:

- Sewage sludge, effluents and sludge-based composts
- Peat as a soil conditioner
- The use of animal residues and manures (other than processed animal products from slaughterhouses and the fish industries) from livestock systems not complying with the standards. These include:-
  - Poultry battery systems and broiler units with stocking rates over 25 kg/m^2
  - Indoor tethered sow breeding units
  - Other systems where stock are not allowed to turn through 360°, where they are permanently in the dark, or are permanently kept without bedding

Manure Management and Application

- A temperature of 60°C will facilitate the destruction of most weed seeds, pathogens, chemical residues and antibiotics and the composting process should aim to achieve this. After an initial heating the compost heap must be turned again, preferably covered and maintained for at least 3 months.
- Manure treatments, storage systems and applications are expected to conform to the Statutory Code of Good Practice for the Protection of Water
- Care must be taken when spreading manure/slurry to avoid run-off and the pollution of watercourses and ground water. Attention must be paid to the capacity of the ground to absorb the manure/slurry at the time of application. When conditions appear unfavourable and pollution seems likely to occur, application must not take place.

Recommended:-

- The storage and composting of manures indoors or under plastic sheeting to prevent leaching of nutrients during periods of heavy rainfall
- Steel and concrete slurry tanks and lagoons built to BS5502, with aeration facilities
- Applications of composted manures and aerated slurries onto fertility building crops, grassland and cultivated land in spring and summer
- Avoiding the spreading of manures within 10 metres of ditches and watercourses and within 50 metres of boreholes
- Avoiding the spreading of manure or slurry on frozen or saturated ground

Permitted:-

- The autumn/early winter applications of composted manures to grassland – only whilst nutrient uptake is actively taking place
- Applications of composted manures to green house soils – at any time
- Slurry systems without buffer storage tanks applying slurry over winter – to grasslands only when conditions are suitable

Prohibited:

- Storage systems and practices which result in the pollution of watercourses.

Mineral fertilisers and supplementary nutrients

Restricted

Approval must be obtained from the Symbol Department before use: [NOTE: incomplete list]

- Blood meal

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- Horn and hoof meals
- Meat and bone meals
- Fish, blood and bone meals – if free from non-permitted substances
- Fish meals
- Basic slag
- Sylvinite

Prohibited: [NOTE: incomplete list]

- Use of fertilisers based on slaughterhouse by-products on farms with cattle and sheep
- Fresh blood
- Guano

Grassland Management

Recommended:

- Manure applications on unimproved meadows not exceeding an average of 30kgN/a/yr or equivalent (e.g. approx. 10 tonnes FYM/a/yr)

Prohibited

- Enzyme silage additives derived from GMOs
- Pollution of water courses by silage effluent
- Grazing Organic or In-conversation livestock on non-registered land

Horticultural Crop Protection

- If crops are grown from seeds or transplants then they must be grown on a registered Organic Unit

Permitted:

- The transplants brought in must after planting be cultivated in accordance with the Soil Association Standards for a minimum period of 6 weeks before harvesting

Harvesting and Storage

Permitted:-

- Hypochlorite in solution – followed by rinsing with potable water

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5. MINIMISING THE RISKS

Summary

There is currently little guidance available on the control of VTEC in the field and little work has been carried out to determine which control measures are the most effective. Better understanding is required and effective controls need to be established at the point of entry of VTEC into the food chain, i.e. during primary production in the field, in order to break the cycle of infection. The industry needs to be involved earlier in the food chain, in agricultural practices relating to animal and human waste, working in partnership with the farming sector in the EU and rest of the world.

The VTEC Working Group recognises the significance and importance of having a Best Agricultural Practice document at European level, for example through CIAA, and recommends that this be developed.

5.1 Statutory Regulations and Codes

5.1.1 EU

EC Council Directive 86/278/EEC on the Protection of the Environment and in Particular of Soil, when Sewage Sludge is Used in Agriculture. No. L181/8, Official Journal of the European Communities, 4/7/86.

Data on organic materials usage and controls in EU Member States other than the UK is required.

5.1.2 UK

In the UK, DETR is generally responsible for sewage sludge. Revised water and soil codes are out for consultation. Sewage sludge is regulated - treatments are defined. Water companies monitor and have legal responsibilities. The UK ACMSF (Advisory Committee on Microbiological Safety of Foods) is in 1998 reviewing the efficacy of these treatments with respect to foodborne pathogens.

There is no legislation for farmyard and other organic waste. The UK Royal Commission Report expressed concern about the application of this type of material to crops but its application is 'unlikely to be relevant to farmed crops'. DETR, EA and MAFF are reviewing the situation regarding these materials.

The National Rivers Authority in England and Wales, the River Purification Boards in Scotland and the DoE in Northern Ireland, have statutory obligations to protect the quality of surface and underground waters. They have powers to prosecute those responsible for causing pollution. The are usually able to advise sludge producers and farmers where there is uncertainty about risk of causing pollution by sludge applications. In general, provided that the precautions specified in the various official codes of practice and guidelines are strictly observed, the risks of causing pollution from sewage sludge is very small, according to CIWEM (1995).

Application of Sewage Sludge

Sludge (Use in Agriculture) Regulations 1989 No. 1263, HMSO (ISBN 0 11 097263 5) as amended by the Sludge (Use in Agriculture) Regulations 1990, SI 1990 No 880, HMSO (ISBN 0 11 0033880 0).

Code of Practice on the Agricultural Use of Sewage Sludge, 1989. DoE. Revised 1996 (ISBN 185112005x). (Non-statutory)

Information on the Application of Sewage Sludge to Agricultural Land, MAFF Publications, 1996 (PB2568).

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Waste

Code of Practice for Safe Disposal of Agricultural and Horticultural Waste. 1997 (unpublished)

Special Waste Regulations 1996, SI 1996 No. 972, as amended by the Special Waste (Amendment) Regulations 1996, SI 1996, No. 2019, The Stationery Office, (ISBN 0 11 062941 8), and the Special Waste (Amendment) Regulations 1997, SI 1997, No. 251, The Stationery Office (ISBN 0 11 063881 6).

Waste Management Licensing Regulations 1994, SI 1994 No.1056, HMSO (ISBN 0 11 044056 0)

Water

Collection and Disposal of Water Regulations 1988, SI 1988, No.819, HMSO (ISBN 0 11 086819 6)

Water Act 1989, Chapter 15, HMSO (ISBN 0 10 541589 8).

Water Industry Act 1991, Chapter 56, HMSO (ISBN 0 10 545691 8).

Water Resources Act 1991, Chapter 57, HMSO (ISBN 0 10 545791 4).

Code of Good Agricultural Practice for the Protection of Water, 1998, MAFF.

Soil

Code of Good Agricultural Practice for the Protection of Soil (The Soil Code), MAFF, 1998.

Recommendation on Soil Protection, May 1992, Council of Europe, R(92)8.

General

Preventing the Spread of Plant and Animal Diseases - a Practical Guide, MAFF Publications, 1991, PB0486.

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5.2 Review of Basis of Controls

5.2.1 Sewage Sludge

Many of the EU statutory controls on the treatment and use of sewage sludge in agriculture were established before *E coli* O157 had fully emerged as a pathogen and the use of HACCP, coupled with risk analysis became widespread in the food industry.

The following are examples of reviews that the Working Group was able to obtain.

5.2.1.1 UK Royal Commission on Environmental Pollution (RCP), 1996

The RCP, in its Nineteenth Report on the Sustainable Use of Soil, published in 1996, reviewed the use of organic materials in agriculture, including their safety. It concluded that there is a potential risk to human and animal health from pathogens in animal wastes.

The RCP referred to its Seventh Report, on Agriculture and Pollution, its Sixteenth Report on Freshwater Quality, and the 1990 Badenoch report on *Cryptosporidium* in Water Supplies which quote evidence establishing livestock as carriers of pathogenic bacteria, viruses and parasites. Its Seventh Report concluded that insufficient is known at present about the effects of different sewage treatment processes to provide a sound basis for determining sludge disposal policy from the viewpoint of risks posed by pathogens'

The 1996 RCP Report found that:-

- Recent research on the fate of *Cryptosporidium* oocysts in sewage sludge concluded that mesophilic anaerobic digestion at 35°C for a period of 4 days followed by 14 days storage of the digested sludge kills oocysts (WRc, March 1993). That report recommended that higher temperature processes, including thermophilic aerobic digestion and pasteurisation should be employed if it is suspected that sewage sludge is contaminated with *Cryptosporidium* oocysts. The final version of UK Government guidance on minimising the dissemination of *Cryptosporidium* oocysts into the final environment be published as soon as possible.
- The EC Directive on use of sewage sludge in agriculture allows a derogation from the normal limit values on land which was dedicated to sludge disposal in 1986 but on which commercial crops were being grown exclusively for animal consumption; the Regulations make the use of sludge in such land and the selling of any crop grown on it conditional on advice from the Agriculture department
- it would be logical for all wastes applied to agricultural land to be subject to the same regulations but there is, at present, a lack of consistency between the rules applied to land disposal of sewage sludge and the spreading of exempted non-agricultural wastes.
- There is a need for greater co-ordination in the prevention of infection. The 1995 Badenoch Report recommended that Regulations, codes of practice and enforcement procedures for the disposal of sludges which may contain *Cryptosporidium* should be reviewed and if appropriate harmonised.' The same point would also apply to other pathogens.

5.2.1.2 US National Research Council (NRC), 1996

The NRC's Water Science and Technology Board (WSTB) in 1993 formed a committee of experts to conduct an independent study of the safety and practicality of the use of treated municipal wastewater and sludge in the production of crops for human consumption. The Committee's Report, published in 1996, concluded that:-

- If reclaimed water and sludges are to be used in the production of human food crops, particularly those that are eaten raw, then there is a chance of exposure through ingestion

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- Until a more sensitive method for the detection of salmonella in sludge is developed, the present test should be used for support documentation, but not substituted for the faecal coliform test in evaluating sludge as Class A
- EPA should continue to develop and evaluate effective ways to monitor for specific pathogens in sewage sludge
- EPA should re-evaluate the adequacy of the 30-day waiting period following the application of Class B sludges to pastures used for grazing animals
- The Part 503 Sludge Rule should be amended to more fully assure that only sludge of exceptional quality, in terms of both pathogen and chemical limits, is marketed to the general public so that further regulation and management beyond the point of sale or give-away would not be necessary

5.2.1.3 UK WRc Report for DETR (1998)

Following the Report of the RCP in 1996, the Water Research Centre (WRc) was commissioned by the DETR to review the basis of controls in the 1989 DETR Code of Practice regarding the use of sewage sludge.

The Report considered the data available in 1989 when the first DETR Code was developed, and concluded that ‘in retrospect it may seem curious that infections by enteropathogenic E coli were not considered by the Standing Committee in the two report’ which led to the establishment of the UK Code. ‘The position at the time was that these pathogens were known to be a common cause of ‘scours’ in calves and lambs and that this problem was also seen as self-contained within the agricultural environment by animal-to-animal transmission, exacerbated by intense rearing practices. There was no direct evidence of transmission through the sewage sludge route into agriculture. Conversely, there were documented cases of animal salmonellosis arising from direct contamination (see section 3.1.2).

Directive 86/278/EEC did not specify the treatments to be used, or the operating conditions needed to ensure that health effects were significantly reduced. It also left the restriction period between applying treated sludge and resuming grazing or harvesting to the Member State, provided that the minimum period of 3 weeks was observed. This allowed a degree of autonomy to Member States to take account of local conditions. A review paper (Bruce *et al*, 1990) made a case for following the decision of the US EPA (US EPA, 1984), in its sludge regulations to regard processes giving 90% (1 log) reduction in numbers of salmonella or 99% (2 log) reduction of faecal coliforms as ‘processes to significantly reduce pathogens’. In the event, those processes which appeared able to meet this criterion in UK research studies on full-scale plant and to produce an adequately stabilised sludge, were accepted as ‘‘examples of effective sludge treatment processes’ in the 1989 DoE Code. Although the EPA documentation does not provide specific data on the destruction of salmonellae, WRc states that ‘there are a number of references which demonstrate a significant reduction in numbers at temperatures above 50°C’.

The sludge Directive was implemented in the UK through the Sludge (Use in Agriculture) Regulations 1989, which were subject to minor amendments in 1990. Further Statutory constraints are given in the DoE (now DETR) 1989 Code of Practice for the Agricultural Use of Sewage Sludge (see section 5.3.3.6).

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Regarding the choice of treatments in the UK 1989 Code, WRc concludes that 'the fundamental approach was sound and will reduce risks to an acceptable level in all foreseen eventualities. However, it is possible to discern a possible oversight, in that there is nothing to prevent grass and other forage crops being sown on soil into which raw sludge has been cultivated and animals grazing before the elapse of sufficient time (e.g. 6 months) to allow infectivity of *Taenia saginata* eggs to decay. It is now also clear that a certain proportion of gastro-intestinal illness of unknown aetiology can be accounted for by infections by newly recognised pathogens, such as the rotaviruses, small round viruses, Cryptosporidium and various bacteria. There is a lack of definitive information on the survival of some of the more recently identified pathogens, e.g. *E coli* O157:H7.

Veterinary evidence from the UK (Wray and Callow, 1985) and the Netherlands (Kampelmacher and van Noorle-Jansen, 1974) shows that salmonellae can persist for many weeks in lumps of sludge on grazing land.

There are two reports of E coli O157 infection of cattle involving grazing after recent applications of farm slurry, one accidental – in which cattle invaded a silage field recently treated – and a large survey which showed association between infection in cattle and grazing with a median delay of only 10 days after application of farm waste. A single experiment (Maule, 1997) suggests that the decay rate of VTEC O157:H7 in soil is less than that of salmonellae of other coliform bacteria in sludged soils. However, this experiment was done with far higher levels than might be expected in sludge.

The Report notes that the 1989 DETR Code does not distinguish between those treatments which partially and virtually completely disinfect sludges. Of those which do, it does not consider the newer thermal processes, such as drying, endothermic treatment with lime, cement dust and pulverised fuel ash, or thermophilic anaerobic digestion.

HACCP is touched upon, noting that its adoption would require a higher level of control at the first CCP, sludge treatment, and a questioning of whether the second CCP, restrictions on the use of land after application, would be as effective as controlling risks to health. The Report concludes on this point that 'HACCP 'would point the way to a need for a higher level of sludge treatment and less reliance on land management practices to prevent disease transmission.

The Report concluded that, with the exception of sludge applied to the surface of grazing land, there is no necessity to introduce high level treatment for sludge going on to land while larger route of potential infection remain. First priority should be given to phasing out the use of untreated sewage sludge and to strengthen the current Code, for example to require 10 months between application of sludge before planting any vegetable crop, including potatoes, and harvest, unless sludge has been treated by a thermal process.

Further specific recommendations included:-

i) the secondary stage of mesophilic anaerobic digestion to provide a minimum retention period of 14 days to ensure that all sludge receives full secondary digestion
ii) mesophilic anaerobic digestion at 25$\pm$ 3°C for at least 20 days to be phased out from the Code
iii) untreated liquid and dewatered sludges to be stored for a defined minimum period of three months as a batch, without admixture or withdrawal during the storage period
iv) further research is needed on the survival of novel pathogens and viruses at low temperatures
v) introduce statutory process specifications and monitoring requirements to ensure that process descriptions set out in the Code are met – the effectiveness of treatment processes should be monitored as operated
vi) include the process of thermophilic anaerobic digestion, thermal drying and autothermic process using treatment with lime or other chemicals as 'effective processes' in the Code in order to encourage their use
vii) only sludge treated by thermal processes to be applied to the surface of grazing land. Sludges treated by other methods may be injected into grazing land: grazing may be resumed when the injection slit is healed (at least 3 weeks after application).

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viii) Sludge not treated by a thermal process must not be applied to land less than 10 months before harvest of any vegetable or fruit crop.

ix) There is a strong case for testing for the survival of pathogens other than salmonellae and *T saginata* during sludge treatment processes, e.g. Cryptosporidium, Giardia, *E coli* O157, S typhimurium DT104, viruses and other pathogens of interest, new pathogens not currently known to be present in the UK, but which are present elsewhere in the world

x) The persistence on soil and vegetation of certain pathogens likely to be introduced in applications of sludge should be monitored – more information is required in order to develop risk assessment-based strategies.

xi) Hazards posed by landspreading of farm wastes need to be defined and appropriate control measures introduced.

5.2.2 Exempt Waste

The UK RCP paid much attention to the potential risks associated with the use and disposal of exempt wastes, and concluded that:-

- to maximise the benefits and minimise the potential problems associated with the use of manures, application rates and timing must be matched to crop demand; [organic manure] storage would therefore be generally necessary over the autumn/winter period. Advice is freely available from the National Rivers Authority (NRA) Regional Offices and there are periodic campaigns by NRA and the UK consultancy ADAS to promote farm waste management plans. ADAS publishes a free guide, and has obligations to provide free advice to farmers in England and Wales, on the preparation of such plans. Although the advice is free, a consultancy fee is charged for drawing up waste management plans.
- the application of wastes in relation to crop control should be timed precisely to assist in minimising environmental damage - spring dressings are generally more efficient than autumn dressings and minimise nitrate leaching losses (it may be necessary to store wastes for up to six months)
- the Agriculture and Environment Departments ensure that DoE [now DETR] guidance reaches the appropriate constituency of farmers and landowners. Until it is available, we recommend landowners and occupiers consult existing guidance before accepting exempted wastes onto their land and seek advice from qualified commercial consultants
- the Environment and Agriculture departments review immediately the present legislation governing the spreading of wastes on land, with the aim of improving control and making regulation of the application of all wastes to land more consistent.

The RCP noted that there is no central [UK] record of exempted wastes spread on agricultural land annually. MAFF evidence to the Commission stated that land spreading of waste is not a common method of waste disposal, except for sewage sludge, dairy wastes and, occasionally, blood from slaughterhouses. However, the British Society of Soil Science considered that volumes are increasing yearly. Data indicating land application of an estimate 2,781,000 tonnes of [exempted] waste per year were obtained from the waste disposal contractors surveyed. The waste disposal industry was preoccupied with other disposal and recovery methods and did not give the land spreading of waste more than cursory consideration. The contractors responding to the survey, however, recognised the importance of the use of land for [exempt] waste management in the future.

MAFF warned in its evidence to the RCP that tightening controls and increasing costs for other [disposal] outlets might lead to the diversion of unsuitable wastes to agricultural land via the exemption route. Although NRA is a statutory consultee in England and Wales under the licensing procedure for controlled wastes, it has no role in the procedure for exempted wastes. NRA was concerned about what it described as 'effectively uncontrolled deposition on soils over vulnerable aquifers, providing loadings of alien potentially toxic substances that are available for leaching from soil to groundwater resources.' The Water Services Association agreed with NRA about the lack of control over application of exempted materials. During consultation on the Waste Management Licensing Regulations in 1994, the WSA tried and failed to persuade DoE to introduce a code of

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practice to cover these materials The WSA concluded that both regulators and operators would welcome clear guidelines in this area and that their absence encourages low cost and low quality operations.

Increasing quantities [of septic tank and cesspit wastes] are thought to be being spread on [UK] land without treatment. The RCP stated that there was a case for stronger regulation in this area, as it is vital that the disposal of septic tank and cesspit wastes do not prejudice the legitimate spreading of treated sludge from sewage works.

The RCP received data estimating the total production of compost in the UK at about 150,000 tonnes a year. It accordingly assumed that the quantity of farm waste composted is very small.

In terms of microbiological quality of organic waste, little information was available. The Ecolabel criteria for soil improvers included a requirement for fresh material that Salmonella must be absent in 25g and *E. coli* must not exceed 1,000 MPN/g (mean probable number/g).

The RCP noted that the DETR, MAFF and EA were funding production of a technical report on the application to land of the 13 types of [exempt] wastes; and hoped that it would provide the basis for published guidance which would be addressed to waste contractors, waste producers and occupiers of land, and will clarify regulatory requirements. The report would provide best practice guidance.

Kudva *et al* (1998) found that E. *coli* O157 survived for 21 months in a manure pile (7m long by 3m wide and 0.6m deep) collected from experimentally infected sheep, incubated in the open (Idaho) under fluctuating environmental conditions. The average concentrations if the cultured background flora in the manure ranged from 10^5 to 10^8 CFU/g at the start of the study. The concentrations of E. *coli* O157 recovered ranged from $<10^2$ to 10^6 CFU/g at different times over the course of the experiment.

A second *E. coli* O157-positive ovine manure pile, which was periodically aerated by mixing, remained culture positive for 4 months. An *E. coli* O157-positive bovine manure pile was culture positive for 47 days. In both cases, the detectable background flora concentrations remained 105 to 106 CFU/g for the duration of the studies. The bacterium survived at least 100 days in bovine manure frozen at –20°C or in ovine manure incubated at 4 or 10°C for 100 days, but under all other conditions the length of time that it survived ranged from 24h to 40 days. In addition, it was found that the Shiga toxin type 1 and type 2 genes in *E. coli* O157 had little or no influence on bacterial survival in manure or manure slurry. Kudva *et al* concluded that 'the long term survival of *E. coli* O157:H7 in manure emphasises the need for appropriate farm waste management to curtail environmental spread of this bacterium'.

Work at the Aberdeen University Centre for Organic Agriculture (AUCOA) with manure is focusing on the development of effective composting methods which will produce manure which is equivalent to pasteurisation (Dr C Leifert, personal communication, 1998).

Composting standards have been established in various countries, including Austria, Canada and Germany.

In Canada (Anon/Composting Council of Canada), three organisations are responsible for the development of standards and regulations for compost and composting: Agriculture and Agri-Food Canada (AAFC), the provincial and territorial governments, and the Standards Council of Canada (through the Bureau de Normalisation de Quebec - BNQ). This collective responsibility reflects government regulatory requirements of both the AAFC and provinces and territories as well as voluntary industry initiatives (BNQ). The Canadian Council of Ministers of the Environment (CCME) assists in co-ordinating provincial and territorial initiatives wherever possible.

The development of compost standards in Canada was co-ordinated by these organisations to establish and maintain 'high standards for product safety and quality while maximising uniformity, thereby facilitating industry competitiveness.' This approach resulted in a National Standard

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entitled 'Organic Soil Conditioners – Compost Act and Regulations (AAFC). The standards are based on four criteria for product safety and quality: maturity, foreign matter, trace elements and pathogens. Each standard classifies composts in different ways, reflecting usage ands type. Compost maturity is determined using several indicators, all of which are common to the three standards. Compost is deemed mature if it meets two of the following requirements:-

- C/N ratio <=25
- Oxygen uptake rate <=150mg O_2/kg volatile solids per hour; and
- Germination of cress seeds (*Lepidum sativum*) and of radish seeds (*Raphanus sativus*) in compost must be greater than 90% of the germination rate of the control sample, and the growth rate of plants grown in a mixture of compost and soil must not differ by more than 50% in comparison with the control sample.

The CCME Guidelines also identify the following criteria which may be used instead of the above to confirm maturity:-

- Compost must be 'cured' for at least 21 days; and
- Compost will not reheat upon standing to >20°C above ambient

OR

- Compost must be 'cured' for at least 21 days; and
- Reduction of organic matter must be >60% by weight

OR

- If no other determination of maturity is made, the compost must be cured for 6 months. Curing begins when the pathogenic reduction process is complete & the compost no longer reheats to thermophilic temperatures

All three standards (BNQ, CCME, AAFC) identify that the pathogenic organism content must not exceed the following limits:-

- Faecal coliforms must be <1,000 MPN/g of total solids calculated on a dry weight basis; and
- Absence of salmonella (<3 MPN/4g total solids).

Reflecting its ability to regulate and monitor processes, CCME has also identified additional process guidelines to be followed to meet pathogen limits. The process choice reflects both the feedstock in addition to the composting method used.

Within the CCME guidelines, 'if the compost does not originate from feedstock known to be high in human pathogens', either a test may be conducted to meet the limits identified above (similar to BNQ and AAFC) or the following process may be carried out:-

- Using the 'in-vessel composting method', the solid waste shall be maintained at operating conditions of 55°C or greater for three days.
- Using the 'windrow composting method', the solid waste shall attain a temperature of 55°C or greater for at least 15 days during the composting period. Also, during the high temperature period, the windrow shall be turned at least five times.
- Using the 'aerated static pile composting method', the solid waste will be maintained at operating conditions of 55°C or greater for three days. The preferable practice if to cover the pile with an insulating layer of material, such as cured compost or wood chips, to ensure that all areas of the feed material are exposed to the required temperature.

If the compost 'contains feedstock known to be high in human pathogens', it must not exceed the identified limits for faecal coliforms or contain salmonellae, and must undergo the composting process identified above or other treatment as identified by the relevant province or territory.

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Details are not given of how the various composting processes are to be monitored in the field.

5.3 Controls in the Field

A number of guidance documents on practical aspects of food safety in agriculture have been produced in 1997-8 in particular. Since there remain significant gaps in research, recommendations given are on the whole general, designed to reduce the level of contamination to which crops are exposed.

5.3.1 CODEX

In response to increasing concerns about fresh fruits and vegetables as a source of foodborne illness, the Codex Committee on Food Hygiene at its 30th session initiated work on a Code of Hygienic Practice for the Primary Production, Harvesting and Packaging of Fresh Produce, to include seed sprouting. A discussion paper on this proposed draft Code has been published for discussion at the October 1998 session of the Committee (CX/FH 97/7). This paper recommends that the proposed Code should:-

i) include water quality, use of untreated or improperly composted manure as fertiliser, use of untreated sewage sludge and animal slurry, hygienic systems including sanitation and hand washing facilities for workers in field, use of clean equipment and transportation vehicles, hygiene in packaging facilities, storage conditions (temperature and relative humidity), decontamination techniques, measures to prevent cross contamination, health of personnel and training.
ii) Be based on risk based food safety management systems where the prevention of contamination is favoured over pathogen reduction treatment
iii) Provide a general framework of recommendations to allow uniform adoption by the produce sector rather than providing detailed recommendations for specific agricultural practices, operations or commodities.
iv) Clearly link sanitary measures to food safety objectives which should reflect the appropriate level of public health protection

Work on the proposed Code is expected to begin in earnest after the October CODEX session, however, numerous questions can be expected to remain unanswered due to lack of authoritative scientific research.

5.3.2 FDA/CFSAN Guide to Minimize Microbial Food Safety Risks for Fruits and Vegetables

Both working drafts of this US Government-led Guide have been circulated to VTEC WG Members. President Clinton announced on 2 October 1997 that the Secretary of Agriculture, in close co-operation with the agricultural community, the Secretary of Health and Human Services, would issue guidance within one year on good agricultural practices and good manufacturing practices for fruits and vegetables. Much detail appears to have been drawn from the IFPA Voluntary Guidelines (see below).

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5.3.3 IFPA Voluntary Food Safety Guidelines for Fresh Produce

The US-based International Fresh Cut Produce Association in 1997 issued voluntary Guidelines, the key points of which were taken up by FDA and CFSAN in its guidance document (see 5.4.1.).

Manures and Composts

Growers are encouraged to establish and implement restrictions on the sources and use of manure. If used, fertilisers such as manures and composts need to be monitored for possible microbial pathogens. Organic producers should note that the national Organic Standards Board recommends that raw manure not be applied within 60 days of harvest.

No further details are included about manure or other organic material use, and no reference is made to work (Maule, 1997) indicating the substantial survival time of E coli O157 in soil.

Pre-cooling

See text for various ‘points of contamination’ for a range of cooling systems.

Labelling

If the produce is not intended as a ready to eat product it should be clearly labelled as such. Examples of such labelling may be as follows:-

‘wash before consuming’
‘always wash fresh produce before consuming’
‘wash before serving’
‘this product is not intended as a ready to eat product’

5.3.4 UK HSE Agriculture Information Sheet: Common Zoonoses in Agriculture,

This advice from the UK Health and Safety Executive contains:-

- Legal requirements to control the risk of zoonoses in humans
- good occupational hygiene practices to control the spread of zoonoses - personal protective equipment
- symptoms and controls for common zoonoses

This document does not mention VTEC.

Quote from HSE spokesman (Boy caught *E.* coli from farm goat, The Times, 16/7/97): “*E. coli* is incredibly easy to catch and it is necessary to scrub your hands for at least 4 minutes after touching an infected animal.”

5.3.5 Getting to Grips with *E. coli* O157, Scottish Agricultural College

Sets out information for farmers and farm workers in question and answer and guidance format.

5.3.6 UK DoE Code of Practice 1989 & ADAS-Mediated Matrix, 1998

Controls over the usage of sewage sludge are stipulated in the UK Code:-

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Table 18

Constraints on the Use of Sewage Sludge in UK Agriculture: Table A

When applied to growing crops	When applied before planting crops
Cereals, oil seed rape	Cereals, grass, fodder, sugar beet, oil seed rape, etc
Grass[1]	Fruit trees
Turf[2]	Soft fruit [3]
Fruit trees [3]	Vegetables [4]
	Potatoes [4,5]
	Nursery stock [6]

1. No grazing or harvesting within 3 weeks of application
2. Not to be applied within 3 months before harvest
3. Not to be applied within 10 months before harvest
4. Not to be applied within 10 months before harvest if crops are normally in direct contact with soil and may be eaten raw
5. Not to be applied to land used or to be used for a cropping rotation that includes the following:-
   - basic seed potatoes
   - seed potatoes for export
6. Not to be applied to land used or to be used for cropping rotation that includes the following:-
   - basic nursery stock
   - nursery stock (inc. bulbs) for export

Table 19

Constraints on the Use of Sewage Sludge in UK Agriculture: Table B

When applied to growing crops by injection*	When cultivated or injected* into the soil before planting crops
Grass [1]	Cereals, grass, fodder, sugar beet, oil seed rape, etc.
Turf [2]	Fruit trees
	Soft fruit
	Vegetables [3]
	Potatoes [3,4]

1. No grazing or harvesting within 3 weeks of application
2. Not to be applied within 6 months before harvest
3. Not to be applied within 10 months before planting if crops are normally in direct contact with soil and may be eaten raw
4. Not to be applied to land used or to be used for a cropping rotation that includes seed potatoes
5. injection carried out in accordance with WRc publication FR008 1989 ‘Soil Injection of Sewage Sludge – A Manual for Good Practice (second edition)’

The Code states that whenever liquid sludge is applied care must be taken to ensure that the sludge does not run off into roads or onto adjacent land.

ADAS recommends that application of liquid sludge should be avoided in the late autumn or winter period when soils are at field capacity and spreading must be kept at a safe distance from any watercourses. Application to cracked soils, to steeply sloping or frozen ground should also be avoided. When crops for human consumption such as lettuce, which may be eaten raw, are to be grown, only sludges that have received treatment likely to significantly reduce their pathogen content should be applied and even then they should not be applied within 12 months of sowing or

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planting the crop. In the interim it would be acceptable to grow forage crops for livestock or crops for human consumption provided they are cooked before eating. (ADAS, 1985)

Where it is proposed that kale or any similar crop is to be grown and fed directly to livestock before winter frosts, sludge should not be applied between March and August of the year the crop is to be planted [non-statutory Code of Practice for Agricultural Use of Sewage Sludge (DoE)].

In recent years the use of sludge in horticulture has been much restricted owing particularly to the legislation relating to the use of sewage sludge in agriculture, which virtually bans the use on crops grown for raw consumption. There is also a resistance on the part of retailers and consumers to horticultural crops that have been grown on sludge-amended soils. For this reason, work has been carried out in the UK, mediated by ADAS, on the development of a matrix for the use of sludge in agriculture. This matrix would significantly tighten current DETR usage rules. (See Table 20).

Table 20

The Safe Application of Sewage Sludge to Agricultural Land: The ADAS Matrix

	Untreated Sludge	**Digested Sludge**	**Advance Treated Sludge (5)**
Fruit	No	No	No (6)
Salad	No	No	No (6)
Vegetables	No	No (1)	Yes (7)(6)
Horticulture	No	No	Yes
Combinable and Animal Feed Crops	Target end date 31.12.99 (2)	Yes (3)	Yes
Grass • Silage • Grazing	Banned with effect from 31.12.98	Yes (3) Yes (3)(4)	Yes Yes
Maize • Silage	Banned with effect from 31.12.98	Yes (3)	Yes

(1) Field vegetables

Field vegetables may form part of an arable area to which digested sludge is applied, subject to:

- A period of 12 months must elapse between the application of digested sludge for the arable crop and harvest of the following field vegetable crop;
- Where the field vegetable crop may be eaten raw application must be made at least 30 months before harvest.

(2) Combinable and Animal Feed Crops

The application of untreated sludge to these crops will cease with effect from 31.12.99 with the exception, until 31.12.01 of certain combinable crops which receive further processing to minimise risk. Where a field is returning to a rotation which may include field vegetables, the periods specified in (1) above shall apply.

(3) **Digested Sludge**

The application of digested sludge to these crops will be permitted and the water industry has put in train a research programme (see Steering Group below) to provide the necessary assurances that food safety is not compromised.

(4) **Grazing**

The surface spreading of digested sludge onto grassland used for grazing shall cease with effect from 31.12.98. However digested sludge may continue to be deep injected into grassland used for grazing subject to (3) above.

(5) **Advanced - Treated**

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To include heat treated and other methods of treatment as agreed by the Steering Group.

(6) **EU Directive**
In accordance with the Regulations (SI 1989 No 1263) which implement EU Directive (86/278/EEC) and the 1996b DoE Code of Practice for the Agricultural Use of Sewage Sludge, sludge may not be applied to growing fruit and vegetable crops within 10 months of harvest.

(7) **Regulations**
In accordance with the regulations referred to in (6) above sludge may not be applied within 10 months before harvest if crops are normally in direct contact with soil and may be eaten raw.

The implementation date shall be 31.12.1998. Where the matrix allows for the continued use of sewage sludge, including advanced – treated, all applications shall be carried out in accordance with the Regulations and the 1996 DoE Code of Practice for the Agricultural Use of Sewage Sludge. A Steering Group, chaired by ADAS, has been established, made up of representatives from both the food retail and water industries together with government and regulatory bodies to detail as soon as possible the R&D requirements needed in regard to (2), (3), and (5) above. Arrangements will be made to provide from the implementation date management and monitoring of all food safety aspects of the continued use of sewage sludge in agriculture as provided by this matrix. Sludge applied to land before 31.12.98 will not be accounted for in applying the cropping restrictions set out in the matrix i.e. there shall be no retrospective liability for applications of sewage sludge made in accordance with the ADAS matrix and guidance notes. The current Code(s) and Regulations will be amended to take account of the ADAS Matrix.

5.3.7 UK DETR 'Raising the Quality' 1998

This guidance to the Director-General of the Office of Water Services (OFWAT) from the relevant UK Ministers was issued on 23 September 1998, and incorporates the main points of the ADAS Matrix.

The guidelines make the following changes to current practice:-

- Phasing out all use of untreated sewage sludge on agricultural land by the end of 2001. Earlier phase-out dates will apply to particular uses of untreated sewage sludge, including the end of 1998 for use on grass for silage and grazing and maize for silage, and the end of 1999 for certain combinable crops and animal feed crops;
- More stringent requirements for the performance of sludge treatment processes, with a distinction being drawn between conventional treatment and advanced treatment, and the introduction of performance monitoring and auditing provisions;
- Phasing out all surface application of conventionally-treated sludge to grass for grazing by the end of 1998. Subsequent applications must be injected unless the sludge has been subjected to advanced-level treatment;
- Stricter post-application controls when conventionally-treated sludge is used, including an interval of 12 months between application and harvest of field vegetables and 30 months where vegetables are eaten raw;
- Reduction of the maximum concentration of leas in soil from 300 to 200 mg/kg as a precautionary measure to limit metal accumulation in animal offal under exceptions circumstances.

Subject to consultation and parliamentary approval, the Government intends to make the necessary amendments to the statutory framework of controls and associated Codes of Practice 'as soon as possible'. The guidance states that 'Water companies should revise their sludge disposal strategies now to take account of the new requirements and make them available for scrutiny by the EA in accordance with the guidance note on implementation of the Urban Waste Water Treatment Directive'.

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5.4 Potential Decontamination Methods

5.4.1 Challenges

Microbial populations on (and in) fresh vegetables can range from as low as 10^2cfu/g to as high as 10^9cfu/g. (Nguyen-the & Carlin 1994) (Breidt & Fleming, 1997).

The hydrophobic nature of many plant surfaces may protect bacteria on these surfaces from contact with aqueous sanitising solutions. Bacteria may also be within the flesh as a result of tissue damage, or reside within otherwise health tissue (Meneley and Stanghellini, 1974).

5.4.1.1 Produce

5.4.1.1.1 WHO 1998: Surface Decontamination of Fruit & Vegetables Eaten Raw

This valuable review noted that treatments for sanitising or disinfecting raw produce are, perhaps with the exception of irradiation, not totally effective in killing pathogens. Specific findings include:-

i) Lm is generally more resistant than salmonella, E coli O157 and Shigella to sanitisers, but little is known about the efficacy of sanitisers in killing parasites and viruses on produce.
ii) The lethal effect of chlorine occurs within the first few seconds of treatment. the population of microorganisms decreases with an increase in concentration of chlorine up to 300 ppm, above which effectiveness is not proportional to increased concentration
iii) Vigorously washing fruits and vegetables with water reduces the number of microorganisms by a factor 10-100, which is often as effective as treatment with 200 ppm chlorine
iv) Treatment with chlorine dioxide, trisodium phosphate, organic acids or ozone offers potential for removing pathogens from raw produce, but it should be noted that the use of organic acids will not destroy organisms, only release tehm from the surface to some extent
v) Prevention of contamination at all points from the field to the plate, through GAP, GTP, GMO and HACCP is favoured over the application of chemical sanitisers after contamination occurs.

5.4.1.1.2 Calcium hypochlorite

The International Association of Sprout Growers (ISGA), the sprout industry trade association, is currently promoting the rapid approval by the FDA and EPA of a 20,000 ppm treatment with calcium hypochlorite to sanitise sprouts. This treatment has reportedly been devised at the University of Georgia and the University of Massachusetts, sponsored by ISGA,

5.4.1.1.3 Other Chlorine-based Treatments

Numerous alternatives for sanitising equipment can be used in a total sanitation programme, but none has as broad a spectrum of activity as chlorine. Possible uses (chlorine) in packinghouses and during washing, cooling, and transport to control postharvest diseases of whole produce were reviewed by Eckert and Ogawa, 1988.

Mazollier (1988) studied the effect of chlorine concentration on aerobic microorganisms and faecal coliforms on leafy salad greens. Total counts were markedly reduced with increased concentrations of chlorine up to 50 ppm, but a further increase in concentration up to 200 ppm did not have an additional substantial effect.

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A standard procedure for washing lettuce leaves in tap water was reported to remove 92.4% of the microflora. Including 100 ppm available free chlorine in wash water reduced the count by 97.8%. Adjusting the pH from 9 to 4.5 to 5.0 with inorganic and organic acids resulted in a 1.5 to 4.0 fold increase in microbicidal effect. Increasing the washing time in hypochlorite solution from 5 to 30 minutes did not increase microbial levels further, whereas extended washing in tap water produced a reduction comparable to hypochlorite. The addition of 100 ppm of a surfactant (Tween 80) to a hypochlorite washing solution enhanced lethality but adversely affected sensory qualities of lettuce. (Brackett, 1987)

Treatment of alfalfa seeds injected with *S stanley* (10^2 to 10^3 cfu/g) in 100 ppm chlorine solution for 10 minutes has been reported to cause a substantial reduction in population, and treatment with 290 ppm chlorine resulted in a substantial reduction compared with treatment with 100 ppm chlorine. Initial free chlorine concentrations up to 1,000 ppm, however, did not result in further reductions. Treatment of seeds containing 10-110 cfu/g of *S stanley* for 5 minutes in a solution containing 2,040 ppm chlorine reduced the population to less than 1 cfu/g. (Lund, 1983)

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