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Land application of treated sewage sludge: quantifying pathogen risks from consumption of crops

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ABSTRACT

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Aims: To predict the number of humans in the UK infected through consumption of root crops grown on agricultural land to which treated sewage sludge has been applied in accordance with the current regulations and guidance (Safe Sludge Matrix).

Methods and Results: Quantitative risk assessments based on the source, pathway, receptor approach are developed for seven pathogens, namely salmonellas, *Listeria monocytogenes*, campylobacters, *Escherichia coli* O157, *Cryptosporidium parvum*, *Giardia*, and enteroviruses. Using laboratory data for pathogen destruction by mesophilic anaerobic digestion, and not extrapolating experimental data for pathogen decay in soil to the full 30-month harvest interval specified by the Matrix, predicts 50 *Giardia* infections per year, but less than one infection per year for the other six pathogens. Assuming linear decay in the soil, a 12-month harvest interval eliminates the risks from all seven pathogens; the highest predicted being one infection of *C. parvum* in the UK every 45 years. Computer simulations show that a protective effect from binding of pathogens to particulate matter could potentially exaggerate the observed rate of decay in experimental systems.

Conclusions: The results confirm, assuming pathogens behave according to our current understanding, that the risks to humans from consumption of vegetable crops are remote. Furthermore the harvest intervals stipulated by the Safe Sludge Matrix compensate for potential lapses in the operational efficiency of sludge treatment.

Significance and Impact of the Study: The models demonstrate the huge potential impact of decay in the soil over the 12/30-month intervals specified by the Matrix, although lack of knowledge on the exact nature of soil decay processes is a source of uncertainty. The models enable the sensitivity of the predicted risks to changes in the operational efficiency of sewage sludge treatment to be assessed.

Keywords: crops, pathogen decay, risk assessment, sewage sludge, sludge treatment, soil.

INTRODUCTION

In the UK, some 520 000 tonnes dry solids (tds) of sewage sludge (1996/97) are applied to agricultural land annually (WRc 1998). Here, microbiological risk assessment (MRA) approaches are described for modelling the exposures and risks to humans from pathogens potentially present on vegetables which have been grown on land to which treated sewage sludge has been applied in accordance with the UK guidance as set out in the Safe Sludge Matrix (http://www.adas.co.uk/matrix). The Matrix specifies not only that the sewage sludge is treated prior to application to land, but also that a harvest interval is observed between application of the treated sludge and the harvesting of crops. In the case of vegetable crops (which are not eaten raw, e.g. potatoes), the harvest interval is 12 months. For ready-to-eat crops, such as salad crops and carrots this is extended to 30 months.

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The barriers

Environmental MRAs are based on the source, pathway, receptor approach and an event tree model has previously been set out for quantifying levels of salmonellas and *Cryptosporidium* oocysts on root crops from the land application of treated sewage sludge (Gale 2003). The major barriers were identified as sewage sludge treatment, and decay and dilution of the pathogens in the soil. In the UK, 54% of sewage sludge is treated by mesophilic anaerobic digestion (MAD) which is a conventional treatment process (WRc 1998). In the near to medium term, the majority of conventionally treated sludge will continue to be produced using this process, which is therefore considered here in the MRA. Under the proposed revision to the statutory controls for the agricultural use of sewage sludge (Department of the Environment 1996), conventional treatment is required to remove 2 log of pathogens. Gale (2003) demonstrated that the pathogen decay in the soil over the 12 or 30-month harvest interval is potentially huge (>20 log for salmonellas over 12 months) and could eliminate any pathogens originating from the sewage sludge. However, there is uncertainty in the extrapolation of decay data. For this reason, computer simulations are performed here to give a better understanding of the potential effect of soil particles and two-component decay.

Quantitative MRA is appropriate despite low pathogen levels on root crops

An arithmetic mean level of 0·070 salmonellas kg^{-1} root crops at point of harvest was predicted previously (Gale 2003). Monte Carlo simulations predicted that *ca* 1% of 1-kg batches of root crops contained salmonellas (generally 1–5 cells kg^{-1}). However about one in 10 000 of the 1-kg batches contained >50 salmonellas; the maximum in that simulation being 706 salmonellas kg^{-1} root crops. These levels apply to the unwashed and uncooked crops and are well below the ID_{50} for salmonellas in humans which is in the region 24 000 cells for the non-typhi salmonella dose–response data set (Table 1). The ID_{50} is the dose which when given to each and every member of a population, infects half of that population. It is not the 'minimum infective dose'. Indeed the term 'minimum infective dose' is meaningless (FAO/WHO 2003) and is, in effect, a single salmonella cell, each of which has a small but finite probability (0·0001; Table 1) of initiating infection in a human if ingested. For this reason, quantitative MRAs are appropriate even when the pathogen exposures are so low that most receptors only ingest a single pathogen cell (or particle).

Defining an acceptable risk

When the United States Environmental Protection Agency (USEPA) promulgated the Surface Water Treatment Rule for treatment of drinking water, its goal was to ensure that the population consuming water would not be subject to a risk of greater than one infection of giardiasis per 10 000 persons $year^{-1}$ (Regli *et al.* 1991); the acceptable risk to the individual consumer of infection by *Giardia* through drinking water is thus 10^{-4} per person per year. In a population of 60 000 000 persons (e.g in the UK) this would translate into 6000 *Giardia* infections per year through drinking water alone. In addition, there are other enteric waterborne pathogens to consider, and also other routes of transmission to humans, such as direct contact with animals (and animal faeces), food, and recreational activities. Indeed, risk assessments should address the 'big picture' by considering all routes, rather than single routes in isolation (Gale 2002). This raises the question of what is an acceptable risk of infection to humans in the UK through consumption of root crops, which have been grown on land to which treated sewage sludge was applied according to the Safe Sludge Matrix. To address this, the total number of persons infected annually in the UK is predicted.

The objectives of the work presented here relate to the consumption of root crops grown on land to which sewage sludge has been applied, and are to:

i quantify the exposures to humans from consumption of root crops for seven pathogens known to be present in

Table 1 Summary of parameters and models to relate risk of infection to dose ingested for the seven pathogens

Pathogen	Risk from a single particle	Model	ID_{50}	Parameters	Reference
Salmonellas (non-typhi)	0·000104	Equation (1)	24 420	$\alpha = 0{\cdot}3136$; $N_{50} = 24\,420$	FAO/WHO (2000)
Campylobacter jejuni (strain A3249)	0·0177	Equation (1)	795	$\alpha = 0{\cdot}15$; $N_{50} = 795$	Teunis *et al.* (1999)
Listeria monocytogenes‡	$4{\cdot}7 \times 10^{-6}$	Equation (1)	$2{\cdot}1 \times 10^{6}$	$\alpha = 0{\cdot}17$; $N_{50} = 2{\cdot}1 \times 10^{6}$	Haas *et al.* (1999)
Escherichia coli O157*	0·0102	Equation (1)	1130	$\alpha = 0{\cdot}16$; $N_{50} = 1{,}130$	Crockett *et al.* (1996)
Cryptosporidium parvum†	0·00418	Equation (2)	165	$r = 0{\cdot}00419$	Haas *et al.* (1996)
Giardia	0·0197	Equation (2)	35	$r = 0{\cdot}0199$	Rose *et al.* (1991)
Enterovirus (rotavirus)	0·269	Equation (1)	5·6	$\alpha = 0{\cdot}265$; $N_{50} = 5{\cdot}6$	Haas *et al.* (1993)

*Risk of illness.

†29 adults used in the study of DuPont *et al.* (1995) were selected on the basis of having no serological evidence of past infection with *C. parvum*.

‡Dose–response data for *L. monocytogenes* strain 10401 in mice.

raw sewage, namely salmonellas, *Cryptosporidium parvum*, *Listeria monocytogenes*, campylobacters, *Escherichia coli* O157, *Giardia*, and enteroviruses

ii translate these pathogen exposures into risks of infection in humans using available dose–response data

iii express the risks in terms of the number of infections annually in the UK population.

Helminth ova, although present in sewage sludge (Theis *et al.* 1978) are not considered here.

Mathematical approach: use of the arithmetic mean pathogen level

Central to the quantitative MRA is the calculation of the arithmetic mean pathogen exposure from consumption of root crops in units of counts per person per day (Gale 2003). The arithmetic mean pathogen level in a medium (e.g. raw sewage, treated sludge, or root crops) represents the total loading of pathogen in that medium. However, it gives no information on how those pathogens are dispersed within the medium. Typically pathogens are not evenly dispersed, but tend to be 'clustered' both in space and time. Campylobacters in raw sewage sludge present an extreme example of seasonal variation with greatly elevated counts in the months of May and June (Jones *et al.* 1990). The sources of variation of pathogen counts on root crops are discussed by Gale (2003) and include variation in loadings in the raw sewage (and hence the raw sludge), variation in efficiency of sewage sludge treatment, and variation in the dilution (mixing) of the sludge in the soil. Where the loading of pathogen in a medium is low (e.g. in drinking water), or where the infectivity of the pathogen is low, the arithmetic mean may be used directly in the dose–response curve (Haas 1996; Gale and Stanfield 2000). However, this simple approach begins to fail for highly infectious pathogens, or where the pathogen loadings in a medium are very high. Thus, Gale (2003) demonstrated that:

i using the arithmetic mean directly in the dose response model is worst case

ii dispersion of pathogens increases the risk to the group; while

iii concentration (or 'clustering') of pathogens minimizes the risk to the group by confining exposure to just a few individuals.

The approach adopted here is to use the arithmetic mean exposure directly in the dose–response equation. Thus, Gale (2003) demonstrated for salmonellas by Monte Carlo simulation that using the arithmetic mean directly in eqn (1) gives a very similar risk prediction when compared with that predicted by accommodating the log-normal variation in salmonella exposures. However, salmonellas are of relatively low infectivity (Table 1). *Giardia* cysts are in the order of 170-fold more infectious than salmonellas (Table 1). A Monte Carlo simulation performed for *Giardia* demonstrated that accommodating the log-normal variation decreased the predicted number of infections by only 1·3-fold compared with using the arithmetic mean directly, which is the approach adopted here. The variance used in the distribution was that for salmonellas (Gale 2003) and this factor of 1·3 would vary if other distributions were used.

MATERIALS AND METHODS

The MRA is based on the arithmetic mean values, which are abbreviated to AM with a suffix to indicate which term they refer to. The general approach is to calculate an arithmetic mean pathogen level (AM_{raw}) in the raw sewage sludge using available data, and to apply laboratory data from UKWIR (2002) for destruction of those pathogens by MAD to predict the arithmetic mean pathogen level in the treated sludge ($AM_{treated}$). The working assumption for the MRA is that soil on the crops at point of harvest may contain pathogens originating from the treated sewage sludge. Root crops were chosen for the MRA on the basis of expert advice from crop growers that they contain higher proportions of soil at point of harvest than leafy vegetables (Gale 2003). Furthermore, some root crops (e.g. carrots and radishes) are consumed uncooked. Root crops therefore present a worst case.

Receptor term: dose–response data

The negative exponential and Beta-Poisson mathematical models describing dose–response relationships for the seven pathogens are taken directly from the literature. Both models assume that the pathogens act independently, that the minimum infectious dose is one pathogen particle, and that the relationship is linear at low doses. The parameters are summarized in Table 1. Dose-response curves for non-typhi salmonellas, *Campylobacter jejuni*, *L. monocytogenes*, *E. coli* O157 and rotaviruses are Beta-Poisson and defined mathematically by:

$$p = 1 - \left[1 + \frac{N}{N_{50}}(2^{1/\alpha} - 1)\right]^{-\alpha}, \qquad (1)$$

where p is the probability of infection from an exposure of N pathogens, and α and N_{50} are parameters. N_{50} is the ID_{50} (Regli *et al.* 1991). The Beta-Poisson model for rotavirus is plotted in Fig. 1. Two dose–response models, which have been proposed for *E. coli* O157 illness in humans, are presented in Fig. 2. For the purposes of risk assessment, the more conservative of these is used (Table 1). Dose-response relationships for the protozoan parasites *Giardia instestinalis* and *C. parvum* are modelled by the negative exponential model which is expressed mathematically as:

$$p = 1 - e^{-rN}, \qquad (2)$$

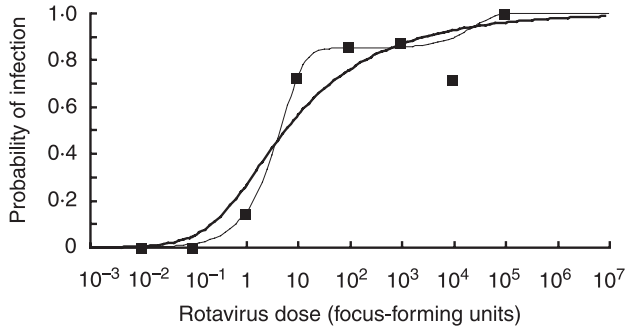

Fig. 1 Beta-Poisson dose–response curve ($\alpha = 0{\cdot}265$; $N_{50} = 5{\cdot}6$) (heavy line) fitted by Haas *et al.* (1993) to rotavirus infectivity data for adult humans (■). Two-component dose–response curve (faint line) represented by eqn (6) with $f_1 = 0{\cdot}85$, $r_1 = 0{\cdot}2$, $f_2 = 0{\cdot}15$, and $r_2 = 0{\cdot}00004$ gives a better fit to the experimental data points and is consistent with molecular biology data for noroviruses (Lindesmith *et al.* 2003)

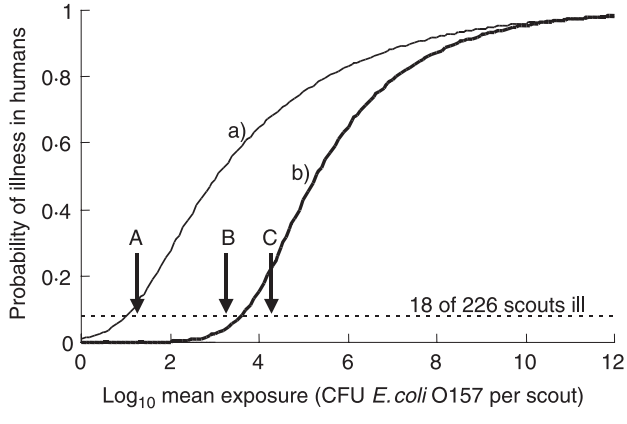

Fig. 2 Proposed Beta-Poisson dose–response curves for *Escherichia coli* O157 illness in humans – a: model of Crockett *et al.* (1996) for *Shigella* ($\alpha = 0{\cdot}16$; $N_{50} = 1{,}130$) and b: model of Powell *et al.* (2000) ($a = 0{\cdot}221$; $N_{50} = 192\,000$). In the New Deer (Scotland) outbreak 18 of 226 scouts (8% represented as dashed line) were ill with *E. coli* O157. The arithmetic mean *E. coli* O157 exposure to scouts estimated in that outbreak (Strachan *et al.* 2001) depends on the magnitude of the counts in the lamb faecal sample recording $>10^6$ CFU g^{-1} – A: $5{\cdot}0 \times 10^6$ CFU g^{-1} ; B: $5{\cdot}0 \times 10^8$ CFU g^{-1} ; C: $5{\cdot}0 \times 10^9$ CFU g^{-1} and whether further high-count samples were missed

where r is a parameter specific for the pathogen and the host population (Regli *et al.* 1991) and approximates the risk of infection after ingestion of a single pathogen particle.

Source term; calculating the arithmetic mean pathogen level in raw sewage sludge (AM_{raw})

Three approaches are used depending on the available data.

Predicting levels in raw sludge from raw sewage. Monitoring data are available for salmonellas, *L. monocytogenes*, *Giardia* cysts and *Cryptosporidium* oocysts in sewage (Watkins and Sleath 1981; Robertson *et al.* 2000). These data are used directly in MRA by constructing an event tree to model the partitioning of the pathogens from the raw sewage into the raw sludge at a sewage treatment works (Gale 2003). This is illustrated in Fig. 3 for *Giardia*. The arithmetic mean level of *Giardia* cysts in raw sewage is 16 152 l^{-1} (after correction for 85% recovery efficiency of the analytical method) of which an average of 37·7% settle into the sludge during primary treatment (Robertson *et al.* 2000). On the basis of data from Casson *et al.* (1990) for removal of *Giardia* cysts at an operational works, the risk assessment assumes a 98% removal by secondary treatment. This comprises an 80% destruction by decay, followed by a 90% physical partitioning into the sludge. According to the event tree, each 1 l of raw sewage contributes 7896 *Giardia* cysts to the raw sludge. As 100 l of raw sewage are required to make each 1 l (wet weight) of sludge (CIWEM 1996), the level of salmonellas in the wet sludge is 789 600 l^{-1}. The dry solid content (w/w) of wet sludge is 2·7% (Gale 2003). Therefore, 1 tds raw sludge contains $2{\cdot}9 \times 10^{10}$ cysts. Source term data and the predicted levels of pathogen in the raw sludge are presented in Table 2. Levels of *L. monocytogenes* in the raw sewage were back-calculated from data for levels in the settled sewage (Watkins and Sleath 1981). The arithmetic mean MPN for the settled sewage was 9006 l^{-1}. Using salmonella data as a surrogate (Gale 2003), levels of *L. monocytogenes* in the raw sewage would be 5·34-fold higher than in the settled sewage, i.e. with an arithmetic mean of 48 061 MPN l^{-1}, of which 82·9%, i.e. 39 864 MPN l^{-1} partition into the raw sludge. The calculated loading in raw sludge is $1{\cdot}5 \times 10^{11}$ tds^{-1}. At present, there are no data for *L. monocytogenes* in raw sewage sludge to validate this number.

Using monitoring data for raw sewage sludge. Jones *et al.* (1990) presented data for campylobacters in raw sewage sludge samples collected over a period of 21 months. The arithmetic mean level was 7585 MPN 100 ml^{-1} (wet weight) and accommodates the seasonal variation including two May/June peaks. Assuming sludge is 2·7% dry solids, the level of campylobacters is calculated as $2{\cdot}8 \times 10^9$ counts tds^{-1} (Table 2). Soares *et al.* (1994) presented enterovirus levels in raw sludge collected monthly over a period of 1 year. The arithmetic mean was 105 000 MPN kg^{-1} (dry weight) which is equivalent to $1{\cdot}05 \times 10^8$ counts tds^{-1} (Table 2).

Estimating the net E. coli O157 loading in sewage sludge in the UK. The main source of *E. coli* O157 in raw sewage is from sheep and cattle faeces discharged at

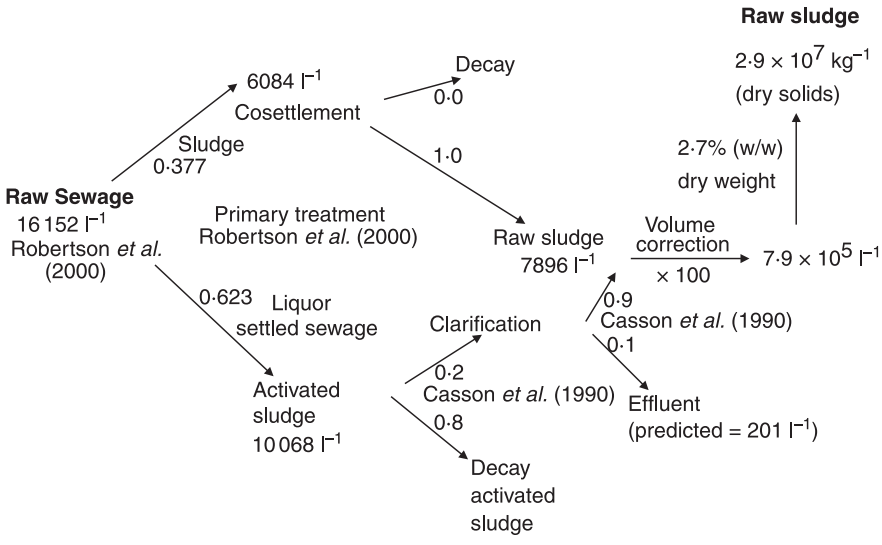

Fig. 3 Event tree to model the partitioning of *Giardia* cysts in raw sewage into raw sewage sludge

Table 2 Summary of arithmetic mean pathogen levels in raw sewage sludge used for MRA

Pathogen	Raw sewage (counts l^{-1})	Partitioning into raw sludge (%)	Reference	Raw sewage sludge (counts tds^{-1})
Salmonellas	3272	82·9*	Yaziz and Lloyd (1979)	$1·0 \times 10^{10}$
Listeria monocytogenes	48 061†	82·9§	Watkins and Sleath (1981)	$1·5 \times 10^{11}$
Cryptosporidium	494	25·9‡	Robertson *et al.* (2000)	$4·8 \times 10^{8}$
Giardia	16 152	48·9 (Fig. 3)	Robertson *et al.* (2000)	$2·9 \times 10^{10}$
Escherichia coli O157	–	82·9§	Described in text	$3·2 \times 10^{7}$
Campylobacters	–	–	Calculated from Jones *et al.* (1990)	$2·8 \times 10^{9}$
Enteroviruses	–	–	Calculated from Soares *et al.* (1994)	$1·1 \times 10^{8}$

*82·9% of salmonellas in raw sewage partitioned into the raw sludge (Gale 2003).
†Back-calculated using arithmetic level of *L. monocytogenes* in settled sewage of 9006 MPN l^{-1} (Watkins and Sleath 1981) and data for settlement of salmonellas in raw sewage as a surrogate (Yaziz and Lloyd 1979).
‡25·9% of *Cryptosporidium* oocysts in raw sewage partitioned into the raw sludge (Gale 2003).
§Salmonella data as surrogate.

abattoirs. For *E. coli* O157, there are not sufficient monitoring data available for raw sewage or sewage sludge for quantitative MRA. The approach is therefore to estimate the *E. coli* O157 loading in sewage on the basis of animal inputs from abattoirs as was done previously for bovine spongiform encephalopathy (BSE) agent (Gale and Stanfield 2001). Central to calculation of the arithmetic mean is the fact that in total 967 000 tds of sewage sludge are produced annually in England and Wales (WRc 1998).

Defra (2002) reported that 3·13 million cattle were slaughtered in 2000 at abattoirs in England and Wales, including those under the Over Thirty Month Scheme for BSE. *E. coli* O157 were isolated from 15·7% of cattle during surveillance at abattoirs in Sheffield (UK) in 1995/96 (Chapman *et al.* 1997). The number of cattle infected with *E. coli* O157 and slaughtered at abattoirs is therefore $3\,130\,000 \times 0·157 = 491\,600$ $year^{-1}$. Shere *et al.* (1998) reported levels of *E. coli* O157:H7 in heifer faeces of up to 87 000 CFU g^{-1}. A cow typically discharges 37 200 g of faeces over a 24-h period (Geldreich 1978). For the purpose of MRA, it is assumed that the gut contents contain 10 000 g of faeces at the time of slaughter. The loading of *E. coli* O157 entering abattoirs in England/Wales is therefore 491 600 cows $year^{-1} \times 10\,000$ g per cow $\times$ 87 000 CFU $g^{-1} = 4·27 \times 10^{14}$ CFU $year^{-1}$.

The Meat and Livestock Commission (Sheep Yearbook 2001) reported 19·14 million head of sheep were marketed in the England/Wales in 2000. Strachan *et al.* (2001) presented data for levels of *E. coli* O157 in faeces from sheep and lambs

during the New Deer (Scotland) outbreak. In total, faecal samples from two lambs and 12 ewes were positive for *E. coli* O157 and are assumed to represent infected sheep. Assuming that the true count in the lamb faecal sample recorded as $>10^6$ g^{-1} was 10^7 g^{-1}, then the arithmetic mean density for infected sheep is 731 000 CFU g^{-1}. Chapman *et al.* (1997) reported that 2·2% (22 of 1000) sheep were positive for *E. coli* O157. Assuming each sheep discharges 1 kg of faeces at the abattoir, then the total loading of *E. coli* O157 at abattoirs from sheep is $19{\cdot}14 \times 10^6$ sheep year^{-1} $\times$ $0{\cdot}022 \times 731\,000$ CFU g^{-1} $\times$ 10^3 g per sheep $= 3{\cdot}07 \times 10^{14}$ year^{-1}.

The combined inputs from slaughter of both cattle and sheep at abattoirs in England and Wales is therefore $4{\cdot}27 \times 10^{14} + 3{\cdot}07 \times 10^{14} = 7{\cdot}35 \times 10^{14}$ CFU year^{-1}. According to expert advice (V. Allen, Bristol University, personal communication), 5% of the faeces is lost to sewer at the abattoir. Using the salmonella model (Gale 2003) as a surrogate for behaviour of *E. coli* O157 at the sewage treatment works, 82·9% of *E. coli* O157 in the raw sewage would partition into the 967 000 tds of raw sewage sludge produced annually in England/Wales. This gives a concentration of $3{\cdot}15 \times 10^7$ *E. coli* O157 tds^{-1} of raw sewage sludge (Table 2).

Pathway term

Pathogen destruction by MAD. Arithmetic mean pathogen destruction ratios by MAD (presented both as logarithms and AM_{surv}) are summarized in Table 3. The data were obtained at laboratory scale (UKWIR 2002).

Decay and dilution of pathogens in the soil. Decay rates for pathogens in the soil are presented in Table 4. In the case of salmonellas, *L. monocytogenes* and campylobacters, decay experiments were either terminated before 6 weeks or counts remaining in the soil reached zero before the end of 6 weeks. Indeed, for campylobacters the time frame of detectable counts was just 16 days. For these pathogens, the decay times used in the MRA are extrapolated beyond the time scale of the experiments to a period of 6 weeks. Six

Table 3 Summary of net pathogen destruction ratios by mesophilic anaerobic digestion (MAD) from *n* experiments

	Operational efficiency 100%†		Operational efficiency 99%‡	
Pathogen	$\log_{10}$ (*n*)	AM_{surv}	$\log_{10}$ (*n*)	AM_{surv}
Salmonella senftenberg	4·24 (2)	0·000058	1·997	0·0101
Listeria monocytogenes	2·23 (2)	0·0059	1·800	0·0158
Cryptosporidium	2·33 (3)	0·0047	1·835	0·0146
Giardia	3·0*	0·0010	1·959	0·0110
Escherichia coli O157	3·8 (2)	0·00016	1·993	0·0101
Campylobacter jejuni	0·34 (2)	0·4571	0·335	0·4624
Poliovirus	5·19 (3)	0·0000065	1·9997	0·0100

*Data from Gavaghan *et al.* (1993).
†Data from UKWIR (2002).
‡Calculated from eqn (3) as described by Gale (2003).

Table 4 Summary of soil decay data

	log Decay on soil			
Pathogen	Experimental range (time)	Extrapolated to 6 weeks (42 days)	Extrapolated to 12 months	Reference
Salmonella	2·11 (5 weeks)	2·53	21·94	Watkins and Sleath (1981)
Listeria monocytogenes	3·0 (30 days)	4·2	36·5	Hutchinson *et al.* (2002)
Campylobacter	2·4 (16 days)	6·3	54·75	Hutchinson *et al.* (2002)
Escherichia coli O157	4·5 (50 days)	–	–	Bolton *et al.* (1999)
	1·05 (49 days)	–	7·82	Maule (1995)
Cryptosporidium	3·0 (63 days)	–	–	Hutchinson *et al.* (2002)
	1·0 (12 weeks)		4·33	Olson *et al.* (1999)
Giardia	2·78 (12 weeks)	–	12·04	Hu *et al.* (1996)
Enteroviruses	4·0 (90 days)	–	16·22	Tierney *et al.* (1977)

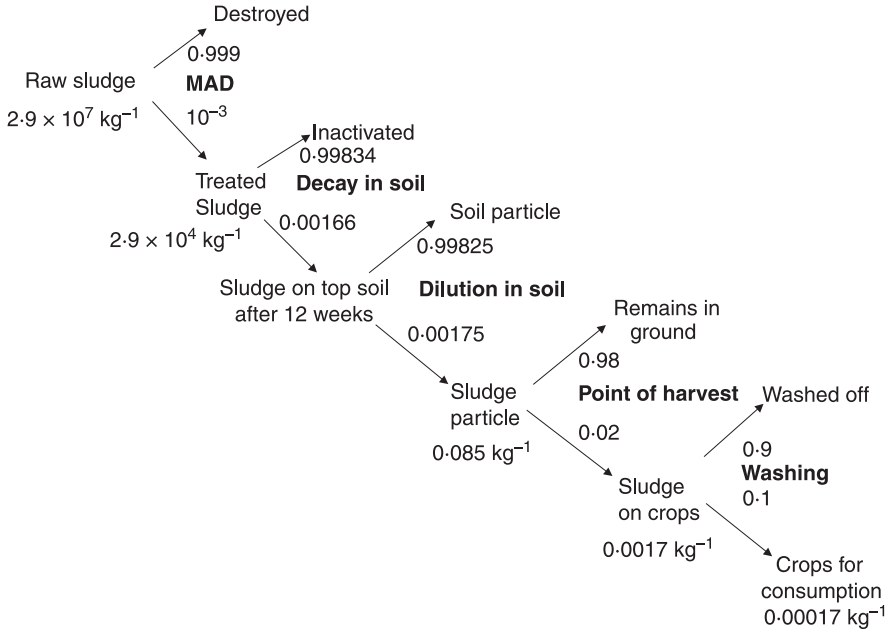

Fig. 4 Event tree for transmission of *Giardia* cysts in raw sewage sludge to root crops for consumption

weeks was chosen since this is the minimum time for germination and growth of a field-grown lettuce under UK conditions (C. Rahne, HRi, personal communication). This is a credible worst case, but it would not happen in practice because of the regulatory controls and policies of the sludge producers which ensure that growers are aware of their responsibilities. For *E. coli* O157, *Cryptosporidium*, *Giardia* and enteroviruses the time scale of the experiment was longer than 6 weeks and decay rates were used without extrapolation. Bolton *et al.* (1999) demonstrated a linear decay of 5 log units for a nontoxigenic strain of *E. coli* O157:H7 over 50 days on the surface of grazing land. Their study provides evidence supporting the extrapolation of decay curves for bacterial pathogens of up to 7 weeks. However, it should be noted that the faecal samples were spiked with cultured bacteria, and the results may not be directly applicable to environmentally stressed organisms. The decay of *E. coli* O157 in soil mesocosms (Maule 1995) also appears linear up to 7 weeks (Fig. 5). Decay of poliovirus on soil treated with sewage sludge during winter months was also linear over a time period of at least 100 days (Tierney *et al.* 1977). Under the Safe Sludge Matrix, a harvest interval of 30 months is stipulated for ready-to-eat salad crops grown on land previously dressed with sewage sludge which was treated by conventional processes (e.g. MAD). For vegetables such as potatoes, this period is 12 months, and the decay rates extrapolated to 12 months are presented in Table 4. Dilution of the sludge in the soil is calculated for an application rate of 6·57 tds sewage sludge per hectare per year tilled into a depth of 0·25 m (Gale 2003). The arithmetic mean dilution ($AM_{dilution}$) is 0·001752

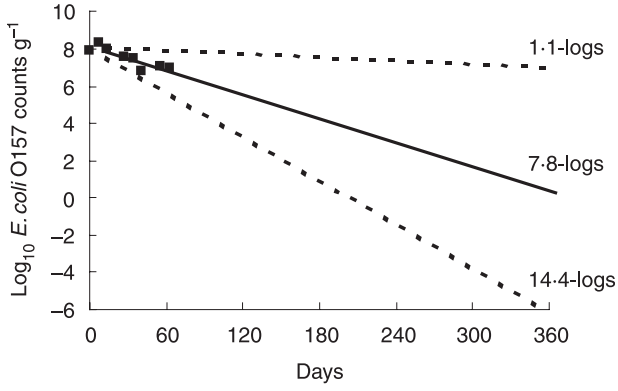

Fig. 5 Linear extrapolation of data for decay of *E. coli* O157 in soil (Maule 1995) to 12 months. 95% CIs (from method of least squares) plotted as dashed lines

and represents the probability of a root crop colliding with a sludge particle at point of harvest.

Harvesting and washing of the root crops. On the basis of expert advice from the growers, it is assumed that root crops comprise 2% (w/w) soil at point of harvest. It is assumed that washing removes 90% of the soil prior to consumption. This could either be visualized as 90% of soil being removed from all root crops, or alternatively as 90% of root crops being thoroughly cleaned and 10% being consumed unwashed.

Consumption data for root crops in Europe and the UK. Each person in the UK ingests 1986 g of vegetable and

vegetable products per week on average (Defra 2000). Thus, the total vegetable consumption in the UK is 284 g per person per day. Of this, 70 g per person per day comprise 'other fresh vegetables' including root crops such as carrots and radishes. On the basis that 50% of such crops are likely to be cooked, the risk assessment is based on each person ingesting 35 g day^{-1} of uncooked root crops. Specific data for consumption of individual root crops in the UK (UK Pesticide Safety Directorate 2001) and in Europe (GEMS/Food Regional Diets 1998) are presented in Table 5 and suggest that this estimate of 35 g per person per day is conservative.

Estimating the number of persons consuming root crops grown on land treated with sewage sludge in the UK. The British Survey of Fertiliser Practice (2000) surveyed 248 fields that grew vegetables out of a total of 4487 fields. Assuming these were randomly chosen, 5·53% of arable land is used for growing vegetables. The WRc (1998) survey found that sewage sludge was applied to 73 000 ha of land of which 59%, i.e. 43 070 ha was arable. Therefore, 5·53% × 43 070 ha = 2380 ha of land for vegetables is treated with sewage sludge. Root crop yields are typically 40 tonnes ha^{-1} for carrots and 60 tonnes ha^{-1} for potatoes (B. Chambers, ADAS, personal communication). Using an average of 50 tonnes ha^{-1}, the total tonnage of vegetable crops grown on land treated with sewage sludge is 119 025 tonnes $year^{-1}$. The total vegetable consumption in the UK is 284 g per person per day (Defra 2000) which is equivalent to 0·1035 tonnes per person per year. Thus, $1{\cdot}15 \times 10^6$ persons in the UK consume vegetable crops (all year) grown on land treated with sewage sludge. This assumes that land application of sewage sludge is independent of land use or type, and is a conservative assumption.

Table 5 Data for consumption of root crops in Europe (GEMS/Food Regional Diets 1998) and the UK (UK Pesticide Safety Directorate 2001)

	Arithmetic mean consumption (grams per person per day)	
Commodity	European (general population)	UK adult
Potatoes	240·8	132·0
Beetroot	2·0	1·6
Carrots	22·0	15·1
Parsnip	2·0	1·0
Radish	2·0	0·3
Swede	2·0	1·9
Turnips	2·0	0·9
Spring onion	1·0	–
Lettuce (for comparison)	22·5	5·7

Mathematical calculations

Calculating human exposure through consumption of root crops. The basis of the mathematical approach is described fully in Gale (2003) and is based on that set out by Slob (1994). The net removal of pathogen by the sewage sludge treatment process is represented by AM_{surv} which is the arithmetic mean of the proportion of pathogens surviving, or breaking-through, sludge treatment. For a set of n experiments:

$$AM_{surv} = \frac{1}{n}\sum_{i=1}^{n}\left(\prod_{surv}\right)_i, \tag{3}$$

where $(\prod_{surv})_i$ is the proportion of pathogens surviving treatment as measured in the ith experiment (Gale 2003). It may be shown for both log-normally and Beta-distributed variables such as $\prod_{surv}$ (P. Gale, unpublished data) that the arithmetic mean pathogen level in the treated sludge ($AM_{treated}$) is the product of the arithmetic mean pathogen level in the raw sludge (AM_{raw}) and the arithmetic mean survival of pathogen through treatment. Thus:

$$AM_{treated} = AM_{raw} \times AM_{surv}. \tag{4}$$

This is intuitively the same as saying the total number of pathogens in the treated product equals the total number in the starting material minus the total number taken out by treatment. Eqn (4) is only applicable if $\prod_{surv}$ is independent of the level of pathogens in the raw sludge. Regression analysis of data for destruction of enteroviruses at an operational sludge treatment works (Soares *et al.* 1994) showed no association between the observed log destruction of enterovirus and the log level of enterovirus in the raw sludge, thus supporting eqn (4). Indeed, if pathogens behave independently in a medium such as sludge, then there is no mechanism by which greater pathogen destruction rates could occur at higher pathogen levels. Such a phenomenon would in fact require pathogens to influence each other in some way, so promoting their death. Of greater importance for this MRA is that there is no evidence to suggest lower destruction rates by MAD at higher pathogen loadings (Horan *et al.* 2004).

Equation (4) may be extended to allow for pathogen decay and dilution in the soil, and for the washing of crops, to calculate the arithmetic mean pathogen level on the crops (AM_{crops}) at point of consumption. Thus:

$$AM_{crops} = AM_{raw} \times AM_{surv} \times AM_{decay} \times AM_{dilution} \times AM_{washing}, \tag{5}$$

where AM_{decay} is the mean pathogen decay in soil, $AM_{dilution}$ is the mean dilution factor of the sewage sludge in the soil and $AM_{washing}$ is the mean removal by washing of the crops. This is set out as an event tree for *Giardia* cysts in

Fig. 4 which uses the AM_{raw} (Fig. 3) of $2{\cdot}9 \times 10^7$ cysts kg^{-1} (dry solids). Working through the event tree in Fig. 4, AM_{crops} is calculated as:

$$2{\cdot}9 \times 10^7 \times 0{\cdot}001 \times 0{\cdot}00166 \times 0{\cdot}00175 \times 0{\cdot}02 \times 0{\cdot}1 = 1{\cdot}7 \times 10^{-4} \text{cysts kg}^{-1}\text{crops}$$

Predicting exposures and risks to humans through consumption of root crops. The daily human exposure is expressed in units of pathogen particles per person per day, and is calculated as the product of AM_{crops} and the daily crop consumption (0·035 kg per person per day). This daily human exposure is translated into a daily risk of infection by the dose–response equation (Table 1). The annual, individual risk is calculated as 365 times the daily risk. The total number of persons infected annually in the UK is calculated as the product of the annual, individual risk and the number of persons in the UK exposed to that risk through consuming those root crops grown on sludge-treated land (i.e. $1{\cdot}15 \times 10^6$ persons).

Modelling operational efficiency – 'bad days and by-pass'

A reduction in operational efficiency of a treatment process can result from 'bad-days' and/or 'by-pass' (Gale 2002). 'By-pass' can be envisaged as 'spatial variation' where certain areas *within* each batch are not treated to the full extent (Carrington *et al.* 1982), or where raw material continuously contaminates each batch of treated material (Bendixen 1999). In contrast, 'bad days' are when the overall treatment efficiency falters or fails completely for a period of time giving rise to 'temporal variation'. 'Bad days' can be minimized by HACCP, while 'by-pass' is potentially more difficult to detect, and may require more intensive monitoring (Gale and Stanfield 2000; Gale *et al.* 2002). Net removal efficiencies by MAD are calculated to simulate 99% operational efficiency (Table 3) as described previously (Gale 2003) using eqn (3). This approach in effect assumes 1% continuous by-pass, such that each batch of treated sludge has the same $AM_{treated}$, i.e. there is 'within-batch' variation in pathogen levels in the treated sludge, but no 'between-batch' variation (each batch has the same total loading). This scenario gives greater dispersion of the pathogens across the crops and is therefore worst case (see above). The effect of by-pass on the magnitude of AM_{surv} can be considerable (Gale 2002, 2003).

Simulating the decay of pathogens in soil

Simulation software was written in the 'C' programming language to investigate the potential effects of particulate-bound pathogens and two-component decay on the shapes of curves observed for the decay of pathogen levels with time in soil. There are currently no quantitative data on counts of pathogens bound to individual soil particles in decay experiments. For the purpose of the simulation, therefore, a Poisson-log-normal distribution [$\mu = -1{\cdot}66$; $\sigma = 0{\cdot}04$ ($\log_{10}$)] of *Cryptosporidium* oocyst counts was taken from Gale and Stanfield (2000) to represent the distribution of pathogen counts on soil particles. The reason for choosing a Poisson-log-normal distribution was to give a 'clustered' effect such that most soil particles have relatively few pathogens, while a small proportion of soil particles contain very high pathogen counts. This is shown in Table 6. Indeed 40 of the 11 550 particles (i.e. 0·35%) in the simulation contained >75 pathogens per soil particle, accounting for 9·8% of the total pathogens. For the purpose of the simulation of two-component decay, the pathogens on those 40 'high-count' particles are given a 10-fold slower decay rate (0·01 day^{-1}) than the pathogens on the other 'lower count' soil particles (0·10 day^{-1}) as set out in Table 6.

Table 6 Distribution of pathogen counts on soil particles and pathogen decay rates used for simulation of two-component decay in Fig. 6 (see text for details)

Number (n) of pathogens/soil particle	Frequency (f) of soil particles with n pathogens	Total pathogens ($n \times f$)	Probability of death (day^{-1})	
			Single-component decay (Fig. 6a,b)	Two-component decay (Fig. 6c,d)
1	6760	6760	0·10	0·10
2	1910	3820	0·10	0·10
3–9	2190	10 020	0·10	0·10
10–20	450	6160	0·10	0·10
21–40	150	4290	0·10	0·10
41–75	50	2430	0·10	0·10
79	10	790	0·10	0·01
90	10	900	0·10	0·01
97	10	970	0·10	0·01
98	10	980	0·10	0·01
Total	11 550	37 120		

Thus in the two-component decay simulation, 90·2% of pathogens have a decay rate of 0·10 day^{-1}, while 9·8% have a decay rate of 0·01 day^{-1}. At time $t = 0$ day, there are 37 120 pathogens on 11 550 soil particles (Table 6). The simulation proceeds by each pathogen 'throwing a die' with either 10 sides or 100 sides, each day. This is done using the random number generator. If a pathogen 'throws a one' it is dead. The total pathogen counts surviving and the total number of positive particles are summed each day and plotted. It is assumed that each soil particle gives a single colony during microbiological analysis, providing at least one pathogen remains alive on it. The process is repeated each day until all the pathogens are dead. Thus, a soil particle with 98 pathogens at $t = 0$ day, has to 'throw a one', 98 times with a 100-sided die before being eliminated. During that time it is observed as a single colony in microbiological analysis.

RESULTS

The arithmetic mean pathogen levels in the raw sewage sludge are presented in Table 2. The highest levels are predicted for *L. monocytogenes*, *Giardia* and salmonellas with $>10^{10}$ tds^{-1}. Predicted levels of *Cryptosporidium* oocysts, campylobacters and enteroviruses are lower at 10^8–10^9 tds^{-1} and *E. coli* O157 levels are lowest at $<10^8$ tds^{-1}. The predicted *Giardia* levels are 60-fold higher than for *Cryptosporidium*, reflecting the greater loadings in raw sewage and a higher proportion partitioning into the sludge (Table 2).

Predicted risks without extrapolation of soil decay data to the 12/30-month interval specified by the Safe Sludge Matrix

Arithmetic mean pathogen levels predicted on 1-kg batches of root crops at point of harvest are remote fractions (Table 7). The highest pathogen levels are for *L. monocytogenes* and *Giardia*, while the lowest are predicted for *E. coli* O157 and enteroviruses. These are calculated using the laboratory-derived removal ratios for MAD (Table 3), and from the point of view of decay in the soil, are worst case because the decay times have not been extrapolated to the full 12 or 30-month harvest intervals specified by the Safe Sludge Matrix. The predicted risks of infection to individuals are remote (Table 7) for salmonellas, *L. monocytogenes* and enteroviruses. Thus the predicted risk of infection from salmonellas is $7{\cdot}9 \times 10^{-9}$ per person per year, giving one infection in the UK population every 111 years, or 0·009 $year^{-1}$ (Table 8). The highest risk predicted is that for *Giardia* at $4{\cdot}3 \times 10^{-5}$ per person per year, which translates into 50 infections per year in the UK (Table 8). The risk of infection from *Cryptosporidium* is $4{\cdot}2 \times 10^{-7}$ per person per year (Table 7) and would give one infection in the UK population every 2 years (0·48 $year^{-1}$). The predicted risk of illness from *E. coli* O157 is remote using the decay data of Bolton *et al.* (1999) (Table 4) with one illness predicted every 11 500 years (Table 8). Using the more conservative decay data of Maule (1995) predicts one illness in the UK every 4 years, i.e. 0·24 $year^{-1}$ (Table 8).

Effect of operational efficiency

It is assumed here that the data of UKWIR (2002) obtained at laboratory scale represent the treatment plant operating at an efficiency of 100%. This raises the question of, 'What if the sludge treatment process is only 99% efficient?' Net removal rates for 1% operational failure of MAD are calculated using eqn (3) for each of the pathogens in Table 3. In general, 1% operational failure gives a net removal of 1·7–2·0 log. Thus, MAD operated at 100% efficiency destroys 5·16 log of poliovirus (Table 3) but, if operated at only 99% efficiency, such that 1% is completely

Table 7 Predicted risks of infection and pathogen levels in MAD-treated sludge, soil and on root crops. MAD-removal and soil decay rates from Tables 3 and 4 respectively

Pathogen	Pathogen levels (arithmetic mean, kg^{-1})			Exposure† (pathogen particles per person per day)	Risk of infection (per person per year)
	MAD-treated sludge	Soil (decay time)	Root crops at point of harvest		
Salmonellas	$5{\cdot}8 \times 10^{2}$	$3{\cdot}0 \times 10^{-3}$ (42 days)	$6{\cdot}0 \times 10^{-5}$	$2{\cdot}1 \times 10^{-7}$	$7{\cdot}9 \times 10^{-9}$
Listeria monocytogenes	$8{\cdot}7 \times 10^{5}$	$9{\cdot}6 \times 10^{-2}$ (42 days)	$1{\cdot}9 \times 10^{-3}$	$6{\cdot}6 \times 10^{-6}$	$1{\cdot}15 \times 10^{-8}$
Cryptosporidium	$2{\cdot}2 \times 10^{3}$	$4{\cdot}0 \times 10^{-3}$ (63 days)	$7{\cdot}8 \times 10^{-5}$	$2{\cdot}7 \times 10^{-7}$	$4{\cdot}2 \times 10^{-7}$
Giardia	$2{\cdot}9 \times 10^{4}$	$8{\cdot}5 \times 10^{-2}$ (84 days)	$1{\cdot}7 \times 10^{-3}$	$6{\cdot}0 \times 10^{-6}$	$4{\cdot}3 \times 10^{-5}$
Escherichia coli O157	5·0	$2{\cdot}8 \times 10^{-7}$ (50 days)*	$5{\cdot}5 \times 10^{-9}$	$1{\cdot}9 \times 10^{-11}$	$7{\cdot}5 \times 10^{-11}$‡
Campylobacters	$1{\cdot}3 \times 10^{6}$	$1{\cdot}1 \times 10^{-3}$ (42 days)	$2{\cdot}2 \times 10^{-5}$	$7{\cdot}9 \times 10^{-8}$	$5{\cdot}5 \times 10^{-7}$
Enteroviruses	0·7	$1{\cdot}1 \times 10^{-7}$ (90 days)	$2{\cdot}4 \times 10^{-9}$	$8{\cdot}3 \times 10^{-12}$	$1{\cdot}8 \times 10^{-9}$

*Decay data of Bolton *et al.* (1999).

†Assumes washing removes 90% of soil from root crops.

‡Illness.

Table 8 Predicted number of human infections per year in UK population: effect of operational efficiency of the MAD process and pathogen decay over a 12-month harvest interval

Operational efficiency of MAD:	100%		99%	
Decay time:	Experimental range or 6 weeks	Extrapolated to 12 months	Experimental range or 6 weeks	Extrapolated to 12 months
Salmonella	0·009	$<10^{-16}$	1·6	$<10^{-14}$
Listeria monocytogenes	0·013	$<10^{-28}$	0·036	$<10^{-28}$
Campylobacter	0·63	$<10^{-53}$	0·64	$<10^{-53}$
Escherichia coli O157*	$8{\cdot}6 \times 10^{-5}$	$<10^{-37}$	0·006	$<10^{-35}$
Escherichia coli O157†	0·24	$4{\cdot}1 \times 10^{-8}$	15·6	$2{\cdot}7 \times 10^{-6}$
Cryptosporidium parvum	0·48	0·022	1·5	0·070
Giardia	49·7	$2{\cdot}7 \times 10^{-8}$	546	$2{\cdot}9 \times 10^{-7}$
Enteroviruses	0·0021	$<10^{-15}$	3·2	$<10^{-12}$

*Decay data (4·5 log) of Bolton *et al.* (1999).
†Decay data (1·05 log) of Maule (1995).

untreated, then the net destruction is just 1·9997 log (Table 3). The exception is for campylobacters, which show poor removal by MAD (Table 3) as has been reported by Kearney *et al.* (1993). Indeed, operational efficiency of MAD has little effect on the predicted campylobacter risks (Table 8). Without extrapolation of the soil decay data to the full time intervals specified by The Safe Sludge Matrix, 1% operational failure of MAD predicts around one to three infections annually for salmonella, enterovirus, *Cryptosporidium* and campylobacter in the UK population, but over 500 infections of *Giardia* per year (Table 8). It should be stressed that this represents a sensitivity analysis conducted in the absence of data, to demonstrate the potential importance of good plant design, management and maintenance.

Effect of the 12-month harvest interval

The log decay rates for pathogens in soil as calculated by linear extrapolation to 12 months are presented in Table 4. The degree of uncertainty in these extrapolations is illustrated in Fig. 5 with data from Maule (1995) for decay of *E. coli* O157. On the basis of linear log-decay over the 12-month harvest interval specified for vegetable crops, the predicted number of infections in the UK through consumption of root crops is virtually nil, irrespective of the degree of operational failure. Even with 1% operational failure, the highest risk, that predicted for *C. parvum*, is one infection in the UK every 14 years.

Distribution of crops grown on sludge-treated fields across the UK population

The 119 025 tonnes of crops grown annually on sludge-treated fields in the UK will undoubtedly be distributed unevenly across the UK population. However, this does not affect the overall number of infections predicted because of the linear nature of the dose–response relationship at low doses (Regli *et al.* 1991). Thus, with regard to vegetables grown on sludge-treated fields, whether $1{\cdot}15 \times 10^6$ persons ingest 284 g per person per day for all 365 days of the year, or whether all 60×10^6 persons in the UK ingest 284 g per person per day for just 6·99 days of each year, the total consumption is still 119 025 tonnes of vegetables. The risk to the population as a whole from root crops is the same for both scenarios (results not shown). Indeed, the distribution of crops grown on sludge-treated land across the UK population does not influence the risk prediction. This does not allow for any protective effects to consumers from acquired protective immunity, which in the case of exposure to sludge alone, would be negligible.

Simulated pathogen decay in soil

Figure 6 presents the simulated decays over time of 37 120 pathogens in soil. In Fig. 6a, all 37 120 pathogens have the same 10% chance of death each day. The log counts exhibit a linear decay except at very low counts where stochastic events take over. Indeed in the simulation in Fig. 6a, the last remaining pathogen particle takes an additional 32 days to 'throw a one' and die. This gives a false impression of a two-component decay. However, it is not that this last remaining pathogen is any more stable than the other 37 119 pathogens; simply a matter of chance. Thus, in a game a dice, some players take longer to throw a six, than others. In Fig. 6b, the effect of having multiple pathogens on soil particles is shown. It is assumed that each soil particle gives a single colony on a membrane filter plate irrespective of how many pathogens remain alive on that particle. The observed counts initially show a plateau effect (faint line).

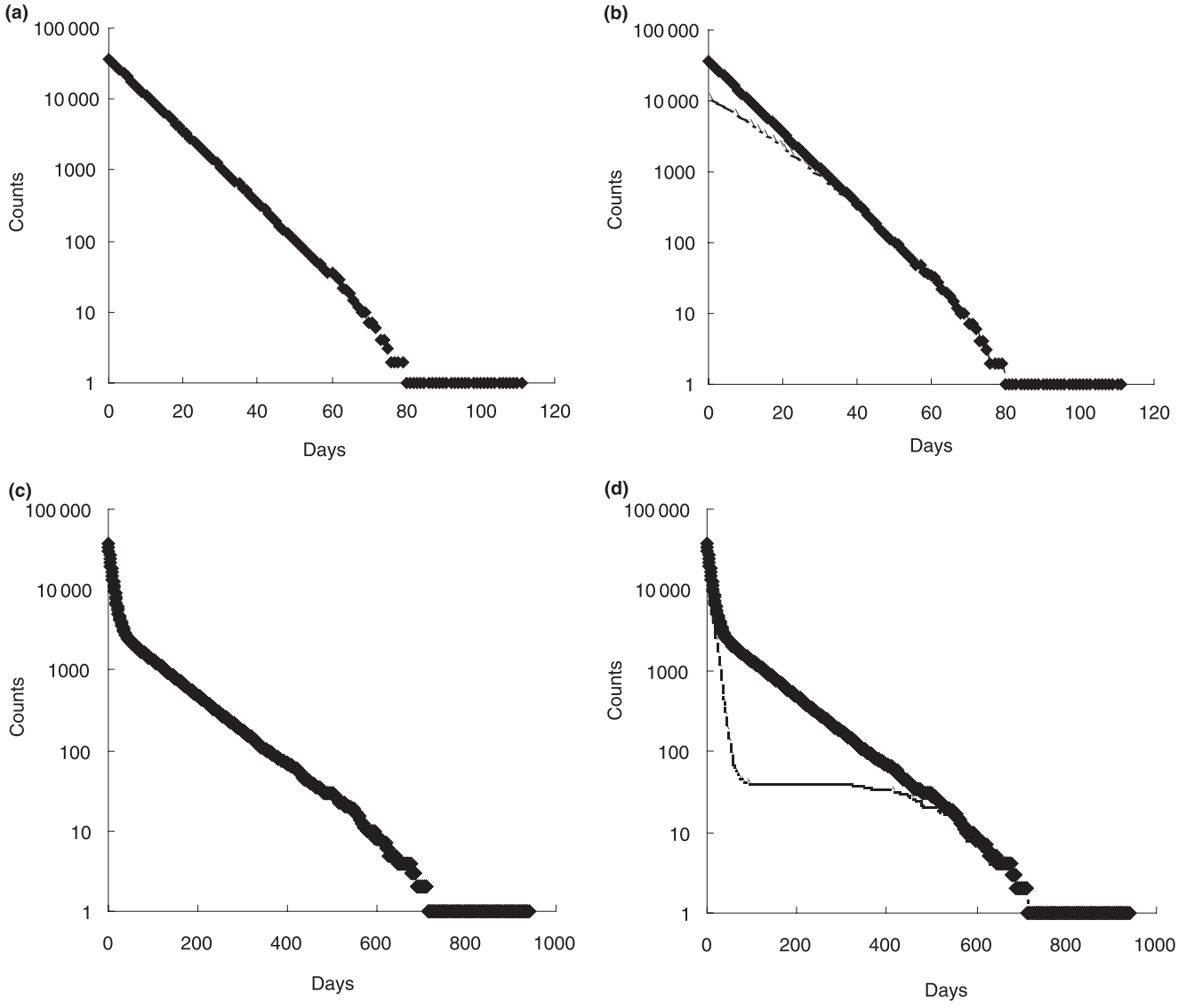

Fig. 6 Simulated decay of pathogens in soil: (a) single-component decay; (b) effect of binding to particulates on single-component decay with observed counts (faint line) and total counts (heavy line); (c) two-component decay; (d) effect of particulates and two-component decay with observed counts (faint line) and total counts (heavy line)

This is because although the total counts (and hence microbiological risk) are decaying linearly (heavy line), the total number of positive particles remains relatively unchanged with time. Such an effect has been observed for decay of *E. coli* O157 counts in manure (Bach *et al.* 2002). In Fig. 6c, 9·8% of the pathogens are 'protected' having a 1% chance of death daily (in effect pathogens have to throw a one with a 100-sided die), compared with the other 90·2% of the pathogens which have a 10% chance of death each day (they have to throw a one with a 10-sided die). This gives a two-component decay curve, with 90·2% of pathogens decaying rapidly and the remaining 9·8% much more slowly. The last pathogen takes an additional 229 days to 'throw a one' with a 100-sided die and die, again giving a plateau effect.

In Fig. 6d, the 9·8% of pathogens which decay slowly are all localized on a few soil particles with high pathogen loadings. This represents a scenario where high pathogen counts on a soil particle give a protective effect. It is interesting to note that the observed counts, reflecting the total number of positive particles, drop rapidly to a plateau of 40 counts. This is because all of the rapidly decaying pathogens are localized on particles with low counts. In contrast, the more resistant pathogens are localized on particles with high counts. In fact the more resistant pathogens (9·8% of the total pathogens) are all localized on

just 0·35% of the particles. The observed decay curve is thus moulded by the distribution of pathogens on soil particles with 99·65% of soil particles exhibiting a rapid loss of the few pathogens they contain. This gives the rapid 99·65% decrease in observed counts (faint line) in Fig. 6d, while the total counts (and hence the microbiological risk) decay more slowly (solid line).

DISCUSSION

It is estimated here that 119 025 tonnes of vegetable crops are produced annually in the UK on land to which treated sewage sludge has been applied. On the basis that the average intake of vegetable and vegetable products in the UK is 283·7 g per person per day (Defra 2000), this represents the total annual vegetable intake of $1{\cdot}15 \times 10^6$ persons in the UK (i.e. *ca* 2% of the UK population). The results presented in Table 8 confirm that the risks from pathogens in sewage sludge through consumption of root crops are, at worst low, but at best remote. Furthermore the harvest intervals stipulated by the Safe Sludge Matrix compensate for potential lapses in the operational efficiency of sludge treatment. The risk assessment here demonstrates that a 2 log reduction in pathogen levels (Table 3) achieved by sludge treatment operating at 99% efficiency is quite sufficient (providing the harvest interval is maintained as set out in the Safe Sludge Matrix). It is concluded that there is little to be gained in public health protection by attempting to improve operational efficiency of sludge treatment processes to >99%.

Qualitative assessment of the uncertainty

The mathematical approach in eqn (5) greatly simplifies quantitative MRA and eliminates the need for using complex and time-consuming Monte Carlo simulations to model the variation in pathogen exposures to individual humans. Furthermore, the approach focuses the uncertainty into the individual terms and simplifies sensitivity analysis. Thus, for example, a 10-fold increase in the net pathogen removal by sludge treatment will decrease the pathogen level in the treated sludge (and hence the risks) by 10-fold (eqn 4). This is straightforward to apply in risk assessment; the problem is determining experimentally, in the face of potentially huge 'between-batch' and 'within-batch' variation at operational scale, whether the arithmetic mean removal by treatment has indeed been improved by 10-fold. Indeed, data on 'bad days and by-pass' of processes are critical in quantitative MRA (Gale 2002). 'One problem with quoting quantitative predicted risks is that the degree of uncertainty is quickly forgotten' (see Macgill *et al.* 2001). A natural extension of calculating the arithmetic mean values for the source, pathway and receptor terms, and indeed for the risk of infection, is to calculate the uncertainty, and quote the 95% confidence intervals (CIs). Indeed, it would be greatly reassuring for a risk assessor to state with 95% certainty that the expected number of infections is between x and y annually. A formal uncertainty analysis is not undertaken here because there are not sufficient data and understanding in the source, pathway and receptor terms at present (discussed below). Indeed, there would be uncertainty in the uncertainty (Slob 1994). The uncertainty is therefore dealt with qualitatively (Macgill *et al.* 2001).

Uncertainty in the source term

Censored data, i.e. samples reported as '>' or above the upper limit of quantification, are a particular problem for quantitative MRA and limit the estimation of the arithmetic mean. In the case of the data for *L. monocyotgenes* in settled sewage, the arithmetic mean of 9006 MPN l^{-1} (Table 2) may be an underestimate because seven of 29 samples (24%) were recorded as >18 000 MPN l^{-1} reflecting all tubes turning positive in the MPN method (Watkins and Sleath 1981). The actual MPN values of these samples are unknown, but would increase the magnitude of the arithmetic mean (and hence the predicted risk) to some unknown degree. The methods for analysing Poisson data containing too numerous to count (TNTC) counts have been described by Haas and Heller (1988). Extrapolating a log-normal distribution through the 22 samples which are not censored, gives an arithmetic mean of 14 625 MPN l^{-1} (i.e. increases the predicted risks by 1·6-fold), but even this approach cannot rule out the possibility of a second very high-count distribution, arising perhaps from extreme events. For the purpose of the MRA here for *E.coli* O157, the high-count sample of lamb faeces recorded as $>10^6$ g^{-1} (Strachan *et al.* 2001) is allocated a value of 10^7 g^{-1}. It cannot be ruled out that more intensive monitoring programmes of lamb faeces during outbreaks would not have detected more 'rare but all important' samples with even higher counts. Thus counts of shiga toxin-producing *E. coli* (STEC) in human faeces in the order of 10^9 CFU g^{-1} have been reported (Paton *et al.* 1998).

Uncertainty in the pathway term

Sewage sludge treatment. Treatment of sewage sludge is well managed and controlled in the UK. There are few quantitative data on operational efficiency of sludge treatment to draw from. Therefore a sensitivity analysis is undertaken here. As a worst case a 99% operational efficiency is tested, such that sludge treatment fails completely 1% of the time, or alternatively, according to eqn (3), that 1% of the raw material continuously by-passes the treatment process. In effect, 1% operational failure (or

by-pass) reduces the net pathogen removals to approx. 2 log (Table 3), which is that required by conventional treatment under the proposed revisions to the statutory controls for the agricultural use of sludge (Department of the Environment 1996). In terms of public health protection, a 2 log reduction in risk is significant, reducing the number of infections by 100-fold.

Decay on the soil. The effect of decay over the full time interval of the Matrix potentially has a huge impact on pathogen loadings on crops (Table 8). However, it should be noted that any uncertainty in the experimental decay data is multiplied during the extrapolation. The 95% CIs for decay of *E. coli* O157 according to data of Maule (1995) extrapolated to 12 months are 1·1 log to 14·4 log (Fig. 5). There is little knowledge on the nature of the decay of pathogens in the soil with respect to extrapolation of decay data to the 12 or 30-month harvest intervals specified by the Safe Sludge Matrix. Thus, for extrapolation of decay data as in Fig. 5, it is assumed that all cells of a certain pathogen have exactly the same probability of death in the soil giving a linear reduction in the log-transformed counts over time (Fig. 6a). The stochastic nature of the decay process at low soil counts is apparent in Fig. 6a. Thus although all the pathogens have the same decay rate, a tail is observed at low counts. This is frequently observed in experimental data sets at the limit of detection (e.g. Hu *et al.* 1996), and is not necessarily indicative of a second more resistant population. The presence of a more resistant subpopulation would give nonlinear decay (Fig. 6c). This, together with the confounding effects of binding to soil particles, potentially exaggerates the decay rates reported in experimental systems as in the case of Fig. 6d where 9·8% of the total pathogens are sequestered and protected on a small number of soil particles. Linear extrapolation of the observed counts (faint line) recorded in Fig. 6d would give a hugely over-optimistic view of pathogen decay. It should be noted that the decay in total pathogen counts and hence the microbiological risk is represented by the heavy line in Fig. 6. The effect of high counts bound to, and protected by particulate could contribute to the rapid decay observed in some experimental data sets as shown by the faint line in Fig. 6d.

In the case of decay data published for *L. monocytogenes* there is conflicting information. Thus, Watkins and Sleath (1981) demonstrated little decay in soil over a period of 8 weeks. In contrast, Hutchinson *et al.* (2002) demonstrated a 3 log decay in 30 days (Table 4). Survival of *L. monocytogenes* in soil is critically affected by inoculum (i.e. initial concentration in soil), microbial competition, and soil type (Dowe *et al.* 1997). Generally a decrease in counts was observed with time, providing there was natural microbial competition. However, reducing the microbial competition by autoclaving the soil resulted in elevated counts with time. *L. monocytogenes* can grow at temperatures as low as 0°C (Rocourt *et al.* 2000), and the possibility of growth in crops, and other foods, cannot be ruled out.

Uncertainty in the dose–response (receptor term)

The dose–response relationship is an area of great uncertainty. The Monte Carlo simulation for salmonellas on 1-kg batches of root crops (Gale 2003) predicted that most of those consumers who are exposed, would ingest just one to five cells. Lack of knowledge on the nature of dose–response relationship at such low pathogen doses, e.g. co-operative or independent action, has been reviewed (Gale 2001). The general consensus is that pathogens act independently, i.e. they do not co-operate with each other at low doses (Meynell 1957; Haas *et al.* 1996). This simplifies the mathematics of risk assessment such that the arithmetic mean exposure is sufficient for risk prediction (Haas 1996). However, recent evidence of genetic recombination between oocysts of different genotype (Wastling 2002) within the host raises the possibility of generating oocysts of more virulent genotypes. This is a potential mechanism for co-operation between pathogens during infection. Furthermore, the recent discovery of small molecules for pathogen communication demonstrates a potential mechanism for pathogen co-operation, although whether this could occur in the human gut is not known. Thus, cell–cell communication is used by bacterial cells to sense their population density (Dong *et al.* 2001). This is known as quorum-sensing and, for example, controls the expression of virulence genes in *Pseudomonas aeruginosa* (Passador *et al.* 1993). Among the extracellular virulence factors produced by *P. aeruginosa* are a battery of enzymes that neutralize the host defence systems. Passador *et al.* (1993) speculate that cell–cell communication could result in a concentrated attack on the host. Co-operative effects would diminish the risks from low pathogen doses and hence the number of infections predicted here in Table 8. Indeed quorum-sensing only occurs at high doses, hence the diminished risk at low doses according to the co-operative model.

A further key area for dose–response is the issue of hyperinfectious pathogens (Merrell *et al.* 2002). Human passage of *Vibrio cholerae* O1 Inaba El Tor (responsible for cholera outbreaks in Bangladesh) enhances subsequent waterborne spread of the cholera bacterium by effectively increasing the infectivity in secondary individuals. In contrast, infection of human volunteers with cholera bacteria grown *in vitro* is difficult in the absence of stomach acid buffering. Many of the pathogens in sewage sludge are of human origin. It would seem unlikely that the hyperinfectious state could remain over the time periods specified by the Safe Sludge Matrix.

The MRAs here do not allow for acquired protective immunity within the population, which would decrease the number of infections predicted in Table 8. In the case of *C. parvum*, analysis of human volunteer data obtained by Chappell *et al.* (1999) demonstrated that the ID_{50} in volunteers with pre-existing antibody was 23-fold higher than in serologically negative volunteers. Teunis *et al.* (2002) have modelled the IgG-dependence of the dose–response for *C. parvum*. In addition there are strain differences to consider; the virulence of three distinct *C. parvum* isolates varying by over 100-fold in healthy human adults (Okhuysen *et al.* 1999). Infectivity of different strains of *L. monocytogenes* varies considerably (see Haas *et al.* 1999).

There is little information on the influence of genetic differences between individual humans on dose–response. In the case of BSE for example, polymorphism at codon 129 of the human prion protein gene affects the magnitude of the cow-to-man species barrier and hence the susceptibility of individual humans (Raymond *et al.* 1997). Therefore, on the basis of molecular biology evidence, a dose–response model for BSE in humans should comprise two components, each with a different value of r in eqn (2). In the case of rotavirus, polymorphism in the gene coding for the receptor protein (on human host cell surfaces) for rotavirus attachment could give rise to differences in susceptibility between adult humans. This has recently been demonstrated for Norwalk virus infection in humans (Lindesmith *et al.* 2003). A dimorphism in the gene coding for the receptor protein (on human host cell surfaces) for rotavirus attachment would support the use of a two-component dose–response model, which is written mathematically as:

$$p = f_1(1 - e^{-r_1 N}) + f_2(1 - e^{-r_2 N}), \qquad (6)$$

where f_i represents the fraction of the population represented by the negative exponential model defined by r_i. The sum of f_1 and f_2 is always equal to 1·0 in a two-component model. The two-component model assumes there are two human populations which differ fundamentally in their susceptibility to rotavirus infection. In the case of noroviruses, Lindesmith *et al.* (2003) reported that 29% of the study population did not express the oligosaccharide ligand required for virus binding and were not infected regardless of dose. A two-component dose–response model is plotted in Fig. 1 with 85% of the adult human population ($f_1 = 0{\cdot}85$ in eqn 6) being very susceptible to rotavirus ($r_1 = 0{\cdot}20$ in eqn 6), but the remaining 15% ($f_2 = 0{\cdot}15$ in eqn 6) being very resistant ($r_2 = 0{\cdot}00004$ in eqn 6). It is apparent that the two-component model gives a much better fit to the data than the Beta-Poisson model of Haas *et al.* (1993), and furthermore predicts risks (at low virus doses) which are 3·5-fold lower. Thus the 3·2 infections of enterovirus predicted in the UK annually (Table 8) would become <1 year^{-1}. With the publication of the initial sequencing of the human genome (International Human Genome Sequencing Consortium 2001), bioinformatics may provide a theoretical basis for explaining or predicting differences in the susceptibilities of humans to certain pathogens.

The dose–response model used here for *L. monocytogenes* is based on mouse infectivity data, albeit calibrated on data from the 1996 milk-borne outbreak of gastroenteritis in Illinois, in which *L. monocytogenes* counts of $1{\cdot}2 \times 10^9$ and $8{\cdot}8 \times 10^8$ CFU ml^{-1} were reported in two unopened milk cartons (Haas *et al.* 1999). There are no validated dose–response data to model the adverse health outcomes of *L. monocytogenes* in pregnant women and individuals whose immune system is perturbed. The risk of illness from *E. coli* O157 varies greatly depending on which of proposed dose–response models is correct (Fig. 2). For the purposes of MRA, the more conservative model of Crockett *et al.* (1996) (line a in Fig. 2) is used as a worst case for *E. coli* O157. This dose–response model has been advocated by an analysis of exposures from the New Deer (Scotland) scout camp outbreak of *E. coli* O157 (Strachan *et al.* 2001). The arithmetic mean scout exposure in that outbreak was estimated at 18 CFU per scout on the basis that a lamb faecal sample recorded as $>10^6$ g^{-1} was in fact $1{\cdot}3 \times 10^6$ CFU g^{-1}. If some 'rare' sheep faecal samples contained 10^8 or 10^9 CFU g^{-1}, then the exposures would have been much greater and consistent with lower infectivities (thus supporting line b in Fig. 2). Paton *et al.* (1998) reported STEC counts of 10^9 CFU g^{-1} of human faeces, and it cannot be ruled out that 'rare but very high-count' samples would not have been detected by more intensive monitoring of sheep faeces during the outbreak (see Gale and Stanfield 2000 and Gale *et al.* 2002 for discussion on sampling from over-dispersed distributions). Adopting the model proposed by Powell *et al.* (2000) represented by line b in Fig. 2 would predict risks 400-fold lower for *E. coli* O157 than those presented in Table 8. Data from the Morioka city (Japan) outbreak of *E. coli* O157 at a school (Teunis *et al.* 2004) also appear to be consistent with the more conservative model of Crockett *et al.* (1996). However, the arithmetic mean exposure could have been greatly underestimated due to spatial heterogeneity of the pathogen in the salad and seafood sauce lunches at the school, thus overestimating the infectivity.

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